Thromboelastographic assessment of the efficacy of rFVIIa in vitro and in vivo

Wilson, Glen

How to cite:
Wilson, Glen (2008) Thromboelastographic assessment of the efficacy of rFVIIa in vitro and in vivo, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/2132/

Use policy
The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:
  • a full bibliographic reference is made to the original source
  • a link is made to the metadata record in Durham E-Theses
  • the full-text is not changed in any way
The full-text must not be sold in any format or medium without the formal permission of the copyright holders.
Please consult the full Durham E-Theses policy for further details.

Academic Support Office, Durham University, University Office, Old Elvet, Durham DH1 3HP
e-mail: o-theses.admin@dur.ac.uk Tel: +44 0191 334 6107
http://theses.dur.ac.uk
Thromboelastographic assessment of the efficacy of

rFVIIa *in vitro* and *in vivo*

The copyright of this thesis rests with the author or the university to which it was submitted. No quotation from it, or information derived from it may be published without the prior written consent of the author or university, and any information derived from it should be acknowledged.

Mr Glen Wilson

A thesis submitted for the degree of Master of Science in

the School for Health

Durham University

09 FEB 2009

October 2008
Acknowledgements

I would like to thank my colleagues in the Biophysics and Trauma team at the Defence Science and Technology Laboratory (Dstl) for their support throughout the production of this thesis and for carrying out the in vivo studies. I would especially like to express my gratitude to Mr Chris Kenward for sharing his considerable technical expertise as well as his encouragement.

I acknowledge the time and assistance provided by Professor Pali Hungin. Thanks also to Dr Kirkman for his input into the study design and assistance with statistical analysis of results.

Financial support for this project was provided by Dstl. Reagents and training were provided in part by NovoNordisk.

Finally, I would like to thank my family, friends and colleagues for their patience and support throughout this time.
Abstract

Haemorrhage is a leading cause of death in both the military and civilian environs and blood loss from sites which are not easily accessible or do not lend themselves to direct physical compression to reduce blood loss contribute hugely to the haemorrhage mortality rate, particularly in the face of delay before surgical intervention.

A number of agents aimed at controlling non-compressible haemorrhage are currently under evaluation, including activated recombinant factor VIIa (rFVIIa), a drug injected intravenously. rFVIIa is an attractive option to control blood loss in this population of patients since the drug can be injected at any site, the effects of rFVIIa targeted to where they are required.

A number of clinical case reports and case series have demonstrated a beneficial effect of rFVIIa in trauma victims, when used as a last resort. These anecdotal reports are yet to be corroborated by adequately powered clinical trials. Animal studies have yielded conflicting results, some demonstrating a clear effect of rFVIIa on survival and others finding no benefit, or effect only on parameters such as blood product usage. Further research is required to firmly establish the efficacy of rFVIIa in trauma patients.

The in vivo animal study from which blood samples for the present study were obtained provided a model of haemorrhage followed by progressive haemodilution associated with intravenous fluid resuscitation. The main aim of the in vitro study that forms the experimental work for this thesis was to compare the ability of rFVIIa
with placebo to enhance clotting under the effects of progressive haemodilution. A further aim was to establish whether a second dose of rFVIIa under these conditions had any effect. The study utilised an established technique called thromboelastography (TEG) to compare clotting in blood samples treated in vitro with rFVIIa and placebo.
# Table of Contents

**BACKGROUND & PURPOSE**

**CHAPTER 1 INTRODUCTION**

1.1 Haemostasis

1.1.1 Blood Vessel Wall

1.1.2 Blood Flow

1.1.3 Blood Constituents

1.2 Platelets

1.2.1 Platelet Biology

1.2.2 Platelet Function and Activation: Role of the Endothelium

1.3 The Coagulation Cascade

1.3.1 Overview of the Coagulation Cascade

1.3.2 The Intrinsic and Extrinsic Pathways of the Coagulation Cascade

1.3.3 An integrated coagulation mechanism

1.3.4 Cellular control of the coagulation system

1.3.4.1 Initiation phase of the cell-based model

1.3.4.2 Amplification phase of the cell-based model

1.3.4.3 Propagation phase of the cell-based model

1.3.4.4 Summary of the cell-based model

1.4 A delicate balance

1.4.1 Fibrinolysis

1.4.2 Prevention of inappropriate thrombosis
CHAPTER 2  LITERATURE REVIEW

2.1 Disturbance of haemostasis

2.1.1 Trauma induced disturbance of haemostasis

2.1.2 Hypovolaemic shock

2.1.3 All bleeding is not the same

2.2 A military and civilian problem

2.3 Managing haemorrhage

2.3.1 The gold standard: intravenous fluid resuscitation

2.3.2 Hypertonic Saline Dextran (HSD)

2.4 Adjuncts to improve haemostasis

2.4.1 Specialist Blood Products

2.4.2 Haemostatic dressings and topical agents

2.5 Activated factor VII

2.5.1 Activated factor VII and the haemophilias

2.5.2 Activated recombinant factor VII, rFVIIa

2.5.3 Mechanism of action of rFVIIa

2.5.4 rFVIIa in trauma and surgery

2.5.5 Preclinical trauma studies of rFVIIa

2.5.6 Clinical experience with rFVIIa in trauma

CHAPTER 3  METHODS

3.1 The in vivo study

3.2 Blood Sampling

3.3 The in vitro study

3.3.1 The TEG® apparatus
3.3.2 Preparation of TEG apparatus 140
3.3.3 Preparation of TEG reagents 141
3.3.4 Running the TEG 142
3.3.5 Analysis of TEG parameters 144
3.3.5.1 Statistical Analysis 144

CHAPTER 4 RESULTS 146

4.1 The effect of in vitro rFVIIa on normal porcine blood 146
4.2 The effect of haemorrhage on the response to in vitro rFVIIa in porcine blood 149
4.3 The effect of intravenous fluid resuscitation on the efficacy of in vitro rFVIIa in porcine blood 152
4.4 Sensitivity of TEG to the effect of rFVIIa administered in vivo 155
4.4.1 Innovin activated samples 155
4.4.2 Kaolin activated samples 156
4.5 The effect of a second dose of rFVIIa in porcine blood 159

CHAPTER 5 DISCUSSION 164

5.1 The effect of in vitro rFVIIa on normal porcine blood 164
5.2 The effect of haemorrhage in vivo on the response to in vitro rFVIIa on porcine blood 165
5.3 The effect of intravenous fluid resuscitation on the efficacy of in vitro rFVIIa in porcine blood 166
5.4 Sensitivity of TEG to rFVIIa administered in vivo 170
5.5 The effect of a second dose of rFVIIa 173
5.6 TEG Activators 177
5.7 Coagulopathy and monitoring 179
5.8 TEG Successes & Limitations 181
5.9 Other applications of rFVIIa 184
5.10 Safety and cost effectiveness of rFVIIa in trauma 186
5.11 Future Research Directions 187
  5.11.1 Resuscitation Fluids 187
  5.11.2 Future directions for rFVIIa (superactive variants) 187
5.12 Conclusions 189

REFERENCES 191
Declaration

The research contained in this thesis was carried out by the author during 2005 and 2006 while a postgraduate student in the School for Health and employed by Dstl. None of the work contained in this thesis has been submitted for candidature for another degree.
BACKGROUND & PURPOSE

Trauma is the leading cause of death both on the battlefield and in those under the age of 40 in the civilian environment. Exsanguination is the second leading cause of death in all civilian trauma victims and is the leading cause of death in those found dead at the scene and those who succumb within 48 hours of injury (acute deaths). In civilian trauma victims, blood loss is non-compressible in 92% of those who die from exsanguination and occurs from severe isolated or combined injuries of the liver, heart and major vessels. In the military, haemorrhage is the leading cause of death in trauma victims on the battlefield, with the proportional mortality rate increasing with delayed evacuation. Hence, uncontrolled, non-compressible haemorrhage is a leading cause of death in both civilian and military trauma victims and is therefore a significant cause of mortality.

Surgery remains the only definitive treatment for non-compressible haemorrhage in both civilian and military environs. Therefore, it is important to minimise delay to surgical facilities and to attempt to preserve the physiology of these casualties to enable transfer. In addition to the absolute requirement for the casualty's physiology to be maintained to support life until surgical intervention, it is recognised that in those patients which do survive to reach a surgical capability, the outcome is more favourable if the development of the "lethal triad" of hypothermia, acidosis and coagulopathy has been avoided throughout the delay to evacuation. The optimum method of preserving the physiology of trauma victims in order to avoid the development of the "lethal triad" is therefore, continually being sought.
Clearly the delay in evacuation to a surgical facility may be protracted in hostile military arenas, depending on the nature and location of the conflict. For example, in Vietnam, the average time for evacuation to a surgical capability was just 25 minutes, compared to anything from one to five hours in the current Iraq conflict and up to five hours in Afghanistan. The potential delay in evacuation to a surgical capability in the combat environment serves to make even more urgent the requirement for effective non-surgical haemostasis.

While civilian practice is generally considered to involve rapid evacuation, so-called "scoop and run", prolonged evacuation times can be hugely problematic in civilian disasters, such as terrorist attacks, motor vehicle accidents and earthquakes. In these situations, the generation of mass casualty numbers is likely to overwhelm the medical services and it is inevitable that there will be delays in access to definitive medical care for some victims under these circumstances. In addition, the current tactical targets for terrorists, primarily enclosed spaces such as buildings and public transport systems, mean that entrapment is a likely complication, potentially leading to considerable delays to evacuation to hospital. As a result, as in the military combat setting, there may be significant delay to surgical arrest of blood loss in these situations.

Considerable research efforts are focused, both in the UK and abroad, on developing novel methods of non-surgical haemostasis. These methods are intended not as definitive treatments or alternatives to surgery, but as methods of prolonging the survival of casualties to enable them to reach a surgical capability. A hierarchy of haemostatic efforts exists, beginning with direct pressure and the application of
tourniquets through to the use of novel haemostatic agents. One such haemostatic agent is QuikClot®, which has received much attention in the United States of America. While reportedly efficacious, QuikClot®, along with tourniquets and other haemostatic dressings, require access to the point of bleeding in order to exert an effect. They may therefore offer significant benefit for use in uncontrolled haemorrhage from accessible regions (e.g. extremities) but are of limited effectiveness in truncal haemorrhage.

Research in the United Kingdom has therefore focussed on an intravenous drug, activated recombinant factor VII (rFVIIa or NovoSeven®). rFVIIa is injected into the bloodstream, with its action directed to the site of injury. As discussed in this thesis, the action of rFVIIa is, in theory, directed specifically to the site of injury because of the physiology underlying coagulation mechanisms. Originally developed to induce haemostasis in severe life or limb threatening bleeding in haemophilia patients, rFVIIa has since been used in a variety of clinical situations where intractable bleeding could not be arrested by other means, including platelet disorders (such as thrombocytopenia), intracerebral haemorrhage and liver disease. The first case of the use of rFVIIa in a trauma victim occurred in 1999 in the form of an anecdotal case report. rFVIIa was administered as a last resort to attempt to save the life of a young Israeli soldier who received a high velocity rifle gunshot wound causing major damage to the liver and inferior vena cava. Administration of rFVIIa resulted in a cessation of haemorrhage and his ultimate survival.

Our group at the Defence Science and Technology Laboratory (Dstl) Porton Down has recently completed a definitive study which shows that rFVIIa significantly
increases survival and reduces blood loss during uncontrolled haemorrhage in terminally anaesthetised swine. In the group of animals treated with placebo none survived for 1.5 hours after the onset of uncontrolled haemorrhage, while approximately 50% of those treated with rFVIIa survived for 6 hours. We have therefore firmly established the 'proof of principle' that rFVIIa can increase survival when administered early after the onset of uncontrolled haemorrhage.

Subsequent issues of clinical importance relate to the effectiveness of a second dose of rFVIIa in cases where bleeding has initially been arrested but subsequently restarts and also to the development of the "lethal triad" of acidosis, haemodilution and hypothermia which occur as a result of prolonged administration of resuscitation fluids prior to surgery. Specifically, correction acidosis and haemodilution are considered essential to the action of rFVIIa and feature in recently published guidelines as preconditions for the administration of rFVIIa.

The risk of re-bleeding and the deterioration of physiology increases with the delay to definitive treatment therefore these are especially important concerns in situations where there may be considerable delay in evacuating the casualty to a surgical facility. Such delays are conceivable in both the military and civilian scenarios already discussed here.

The aim of the present study was to determine whether it was possible in vitro to measure the effect of rFVIIa on coagulation. A further aim was then to assess the ability of a second dose of rFVIIa (versus placebo) administered in vitro, to enhance clotting during the combined effects of progressive acidosis and haemodilution that
accompany hypotensive resuscitation, during uncontrolled haemorrhage after treatment in vivo with a single dose of rFVIIa (or placebo). The study utilised the established technique of thromboelastography (TEG) to compare clotting in blood samples treated in vitro with a second dose of rFVIIa or placebo at various times post injury in vivo. These blood samples were taken from ongoing in vivo studies where the aim was to investigate resuscitation strategies in terminally anaesthetised pigs. In this way it was possible to study the effects of rFVIIa when added to blood samples taken serially during the evolution of an acidotic, haemodiluted state during a standardised, clinically relevant resuscitation regimen.

The results of this study will contribute to the development of guidelines determining when rFVIIa may be used to best effect to reduce blood loss and mortality in trauma victims where there is delay in achieving surgical haemostasis. In addition, it may be possible to determine whether the administration of a second dose of rFVIIa could have any therapeutic potential in terms of improved haemostasis.
CHAPTER 1

INTRODUCTION

This section will provide the reader with an overview of the processes involved in responding to disruptions to the vasculature which lead to decreased circulating volume. The role of the vessel itself as well as blood components (platelets and coagulation factors) are considered. Once the various aspects of the system have been discussed, current understanding of the coagulation system will be discussed in detail, beginning with initial principles and early understanding through to the currently accepted model and cellular control over the whole system. An understanding of the innate systems which exist to respond to blood loss is an essential basis for understanding the mechanism of action of rFVIIa and therefore form a core component of this thesis.
1.1 Haemostasis

Haemostasis is a complex process, which defines the host’s ability to cope with mechanically and disease induced blood loss as well as dysfunctions in the coagulation system. Essentially, haemostasis is the arrest of blood loss from the circulation through the formation of a thrombus. Impairment of haemostasis leads to prolonged bleeding, while excessive stimulation of haemostasis can cause inappropriate thrombus formation, resulting in the clinical condition of thrombosis. The process of the formation of a thrombus is termed thrombogenesis and the factors which influence the process of thrombogenesis were defined in 1856 by Virchow and are today known as Virchow’s Triad.27 Virchow’s Triad is represented schematically in Figure 1.

According to Virchow’s Triad, the condition of the vessel wall, the haemodynamics of blood flow and the levels of various blood constituents together form a triad which directly influences the formation, both physiological and pathogenic, of a thrombus. The following text will refer back to this triad as each of the three members of the triad are considered.

![Figure 1](image_url)
1.1.1 Blood Vessel Wall

In the normal, healthy vasculature, the vascular endothelial lining is potently anti-thrombogenic, expressing proteins that include heparin sulphate, which is a cofactor for antithrombin III (AT-III) and tissue factor pathway inhibitor (TFPI), both of which serve to inhibit aspects of the coagulation process. In addition the normal endothelium secretes prostacyclin (prostaglandin I₂; PGI₂), nitric oxide and tissue plasminogen activator (tPA). The latter has a fibrinolytic function, while the two former decrease platelet aggregation as well as mediating vasodilation. As will become clear throughout this chapter, each of these functions is anti-thrombogenic, which in the healthy vasculature is essential to prevent inappropriate platelet aggregation or activation of coagulation proteins. Were such events to occur inappropriately, thrombi may be formed which, if occurring in an artery, may occlude the vessel, leading to oxygen starvation of tissues served by these vessels. It is therefore essential that the healthy vasculature prevents thrombus formation, while maintaining a capability to support thrombus formation when required.

Damage to the vasculature, whether due to disease or mechanical trauma, initiates the processes involved in haemostasis and thrombogenesis. The primary, immediate, response of the vasculature to damage is vasoconstriction. This is a contractile response of the smooth muscle in the tunica media, resulting in a narrowing of the vessel and hence decreased blood flow to the damaged area, reducing the volume of blood loss. A simplified diagrammatic representation of the histological layers of the blood vessel wall is presented in Figure 2.
Figure 2 The histological layers of an arterial wall.

Note the presence and arrangement of smooth muscle cells in the media. This helical arrangement of smooth muscle cells enables the wall of the artery to contract and the vessel to thus constrict.

1.1.2 Blood Flow

The next aspect of Virchow's Triad to be considered is blood flow. A reduction in blood flow caused by vasoconstriction significantly improves the conditions for the formation of a primary plug across the hole in the vessel. Essentially, decreased blood flow provides less shear forces for dislodgement of forming haemostatic plugs.

1.1.3 Blood Constituents

Vasoconstriction is the initial step in the response of the endothelium and haemostatic system to vascular damage but is alone insufficient to bring about and maintain haemostasis. Two processes therefore follow vasoconstriction: platelet adhesion/activation, and blood coagulation. Together these phenomena lead to the formation of an insoluble thrombus, effectively plugging the hole in the vessel.

The primary plug formed during thrombogenesis consists principally of aggregated platelets (which are discussed in the following section). The result may be the formation of either a red or a white thrombus. A white thrombus forms in arteries and is based on adherence of platelets to exposed collagen. The growing thrombus restricts blood flow and the regional stasis of circulation then triggers fibrin formation (again harking back to Virchow's Triad, where decreased blood flow facilitates thrombogenesis). The result is a central, white thrombus, composed principally of platelets, that is then surrounded by a red thrombus. Red thrombi may be formed around a white thrombus, as detailed above, or in veins, which are lower pressure. As
blood flows at a lower pressure in veins than arteries, there is a lesser likelihood of dislodgement of the clot by the flow of blood and hence a red thrombus (composed of fibrin and trapped red cells) may be formed without requiring the initial white thrombus to slow the flow of blood.$^{30}$

A more detailed view of the roles of platelets and an in depth study of the blood coagulation cascade (the “blood constituents” in Virchow’s Triad) in delivering and maintaining effective and appropriate haemostasis are considered in some detail in Sections 1.2 and 1.3 respectively.
1.2 Platelets

Platelets play an integral role in haemostasis and as such receive considerable attention throughout this thesis. This section provides the reader with an overview of platelet biology.

1.2.1 Platelet Biology

Platelets are non-nucleated cells, produced from megakaryocytes, within the bone marrow. They have a short life span, 7-10 days, with up to approximately one third stored in the spleen. Ultrastructurally, at their simplest, platelets contain in addition to mitochondria and support structures, two forms of granules that are integral to their pro-coagulant function. A granules contain platelet derived growth factor (PDGF), thromboglobulins, fibrinogen and clotting factors V, VIII and XI. The second form of granules, termed 'dense granules', contain the metabolic energy molecules ADP and ATP in addition to 5-hydroxytryptamine (5-HT) and calcium.

1.2.2 Platelet Function and Activation: Role of the Endothelium

Despite its role in maintaining an anti-thrombogenic surface during normostasis, the vascular endothelium also plays an essential thrombogenic role in response to vascular damage. The endothelium synthesises and stores von Willebrand factor (vWF) to promote platelet adhesion; tissue factor (TF, factor III, tissue thromboplastin), promoting coagulation and plasminogen activator inhibitor 1, which reduces thrombus degradation.
Damage to the wall of a blood vessel leads to the exposure of collagen from the subendothelial layer. The exposed collagen interacts with specific glycoprotein (GpIa-lix and GPVI) receptors on the platelet membrane, causing adhesion of platelets to the damaged vessel. This is possible since the anti-thrombogenic endothelium is no longer present, revealing thrombogenic mediators in underlying layers of the vessel wall. This adherence is strengthened by von Willebrand factor (vWF), which binds glycoprotein receptor GpIb, located on the platelet. vWF secreted from the damaged endothelium and released from activated platelets acts to bridge gaps between platelet glycoprotein receptors and subendothelial collagen and stimulates further platelet aggregation.

The adhesion of platelets to the damaged vessel wall leads to their partial activation and the formation of a weak platelet plug. Central to this process are the interactions of thrombin with the GpIb receptor family, and cleavage of the platelet activated receptor 1 (PAR-1) on the platelet surface, leading to complete activation of platelets and the platelet release reaction.

During the platelet release reaction, intracellular microtubules contract, conferring a shape change in the platelet. This shape change results in exposure of negatively charged phospholipids and glycoprotein GpIIb/IIIa receptors on the activated platelet surface. Some of the negatively charged phospholipids exposed during this release reaction are metabolised, resulting in the release of arachidonic acid which is in turn catabolised to thromboxane, one effect of which is to further stimulate platelet activation and thrombogenesis. The mechanisms by which prostaglandins are
synthesised and their relevance to the haemostatic processes are considered in more detail in Section 1.3.

The GpIIb/IIIa receptors exposed during platelet activation are the means by which platelet aggregation occurs. In a similar manner to the interactions of GpIa-IIia and Gp-Ib receptors with the vessel wall and vWF, adjacent platelets aggregate through interactions between their GpIIb-IIIa receptors.

The platelet release reaction also involves the release of the contents of the α and dense granules from within the platelets. The released contents of the granules act on specific parts of the processes involved in achieving haemostasis. For example, 5-HT stimulates further platelet aggregation and increased vasoconstriction; fibrinogen is the precursor to fibrin and is involved in platelet aggregation while the clotting factors and calcium ions are required for amplification of the procoagulant signal, initiated through the coagulation cascade.

The resultant mass of aggregated platelets serves as a plug to provide temporary haemostasis, effective as an immediate measure to arrest blood loss from a damaged vessel. However, the platelet plug alone cannot withstand disruption from the flow of blood within the vessel, over the longer term. Long-term stability of the clot is conferred by incorporation of insoluble fibrin, which is produced by a series of reactions which comprise the coagulation cascade (covered in detail in the following section).
1.3 The Coagulation Cascade

1.3.1 Overview of the Coagulation Cascade

The platelet plug, generated through mechanisms detailed in the preceding sections, requires incorporation of insoluble fibrin in order to remain stable and support haemostasis. Fibrin is the ultimate product of a series of reactions which comprise the coagulation cascade.40,41

The components of the cascade are called factors which are represented by the Roman numerals I to XIII and with some notable exceptions (principally factors I & III) are present in the blood as inactive precursors, termed zymogens, which are activated by proteolysis.40,41 The Roman numerals are suffixed with a lowercase letter ‘a’ to indicate an activated form of the particular factor. The pivotal step in the coagulation cascade is the thrombin (factor IIa) mediated conversion of fibrinogen (factor I) to fibrin (factor Ia). Fibrinogen is a soluble subunit of fibrin, rather than an inactive cofactor.42 The fibrin monomers are polymerised to yield insoluble fibrin under the control of factor XIIIa (FXIIIa),43 activated from factor XIII by thrombin.44 Thrombin is produced by proteolysis from the zymogen prothrombin by the prothrombinase (Va/Xa) complex.45,46 These reactions comprise the final common pathway of the coagulation cascade,40,41,47 the point at which the traditionally viewed two-limbed cascade system merges. The steps involved in the final common pathway are summarised in Figure 3.
The Fva/Xa mediated activation of prothrombin to thrombin results in the release of platelet ADP, a potent activator of platelet aggregation while interactions of thrombin with specific protease-activated receptors (PAR) and receptors of the GpIb family lead to initiation of cellular responses leading to platelet aggregation and augmentation of inflammatory pathways.

Further to these roles, thrombin produced in the coagulation cascade stimulates the metabolism of arachidonic acid in the platelet membrane, leading to the production of a series of prostaglandins. The key, relevant, mediators include thromboxane A₂ (TXA₂) and PGI₂. While both are products of arachidonic acid metabolism, the former has the effect of stimulating thrombogenesis and vasoconstriction, while the latter inhibits thrombogenesis. In keeping with their opposing physiological roles, these two arachidonic acid metabolites each dominate under specific conditions. TXA₂ is secreted both from dense granules within the platelet on initiation of the platelet release reaction and from endothelial cells in response to changes in pressure.
and shear stress. Normal laminar blood flow, maintains shear stress and pressure which acts upon endothelial cells, downregulating release of TXA$_2$ and stimulating antithromobgenic modulators such as PGI$_2$. When shear stress and pressure reduce, as in the case of reduced blood volume due to haemorrhage, the inhibition of TXA$_2$ release is lifted and increased levels of TXA$_2$ serve to amplify the activation of platelets through a positive feedback loop whereby further TXA$_2$ is released as more platelets aggregate and undergo the platelet release reaction. Combined with mediation of vasoconstriction, this effect of TXA$_2$ serves to support the initial generation of a platelet plug at a site of vascular injury. Conversely PGI$_2$, which is synthesised in and released from endothelial cells under normal physiological conditions, is a potent vasodilator and inhibitor of platelet aggregation. It has been reported that much lower concentrations of PGI$_2$ are required to inhibit platelet-platelet aggregation than are required to inhibit platelet binding to collagen. Thus, it is believed that the PGI$_2$ produced by endothelial cells prevents inappropriate thrombus formation in the healthy vasculature but does not strongly inhibit platelet binding at sites of collagen exposure, i.e. sites of vascular damage. An increased ratio of TXA$_2$:PGI$_2$ around the site of vascular damage and hence lower shear and pressure would serve to provide a procoagulant environment, while the lower TXA$_2$:PGI$_2$ ratio around unaffected endothelial cells peripheral to the site of injury likely prevents the spread of the thrombus, localising thrombogenesis to the appropriate site.
1.3.2 The Intrinsic and Extrinsic Pathways of the Coagulation Cascade

Historically, the coagulation cascade has been viewed as consisting of two pathways which may be initiated either via exposure of collagen from the vessel walls, or through exposure of tissue factor at sites of tissue damage. These two initiating mechanisms formed the basis for the conventional representation of the coagulation cascade as two distinct pathways that converge at a common point and are classically referred to as the intrinsic and extrinsic pathways, respectively. They were so named as the latter requires the participation of substances extrinsic to the blood (namely, tissue factor which is found in layers of subendothelium and on certain other cell types), whilst the former was viewed to be completely dependent upon internal elements of the blood.

The extrinsic pathway, also termed the in vivo pathway, was viewed to be activated in response to tissue damage. Tissue damage leads to exposure of tissue factor (TF), a protein which is not normally present in significant concentrations in the blood. Tissue factor is usually buried deep in the vessel wall, specifically in the adventitia and tunica media, becoming exposed to the blood following damage to the blood vessel. The interaction of the tissue factor protein with factor VIIa (FVIIa) leads to the formation of a FVIIa/TF complex. The intrinsic pathway, or contact pathway, was viewed to be initiated by the contact of blood with a negatively charged surface. Typically, in vitro this would be glassware, while in vivo the initiator of the intrinsic pathway was believed to be collagen. The intrinsic pathway was initially viewed as being responsible ultimately for the generation of factor VIIIa (FVIIIa) and later
was determined to be responsible in fact for the generation of the FVIIIa/Ixa tenase complex.\textsuperscript{58}

As discussed in the next section, a view of the coagulation cascade as being defined by two distinct pathways converging at one common point is now largely obsolete, however the role of the FVIIIa/Ixa and FVIIa/TF complexes remain central to the generation of thrombin. Both complexes interact with factor X on the specific cell membranes leading to its activation to Fxa. The events involved in the activation of factor X, which goes on to activate factor II, are summarised in Figure 4.
Orange boxes represent those factors classically referred to as intrinsic factors, green boxes represent extrinsic factors, red boxes represent factors that are part of the common pathway and yellow boxes signify co-factors required at specific stages.

**Figure 4** Activation of factor X requires the presence of FVIIa and/or FVIIIa/Ixa/phospholipid complex

Figure 5, which follows, illustrates schematically the two pathways involved in the coagulation cascade as proposed in 1964, with refinements. This simplified representation of the traditional complex cascade will be used as a basis for the overview of the pathways, forming the remainder of this section.

The extrinsic pathway will be considered first, depicted on the right of Figure 5. Under normal physiological conditions, a small amount of FVII (approximately 4ng/ml) circulates as the activated form, FVIIa. On exposure to TF (factor III), this small amount of FVIIa binds to form a FVIIa/TF complex, which leads to further activation of FVII and the formation of more FVIIa/TF complexes which go on to activate factor X.
Orange boxes represent those factors classically referred to as intrinsic factors, green boxes represent extrinsic factors, red boxes represent factors that are part of the common pathway and yellow boxes signify co-factors required at specific stages.

Figure 5  Schematic representation of the traditional view of the coagulation cascade.
The traditional view of the intrinsic pathway has been that factor XII becomes activated to FXIIa on interaction with exposed collagen (or other negatively charged surface) in the presence of cofactors. FXIIa then goes on to activate FXI to FXIa, which further activates FIX to FIXa. FIXa, in complex with FVIIIa, goes on to activate FX. As discussed in the next section, the activation of the intrinsic pathway is now viewed to be somewhat different to that depicted here and on the left of Figure 5. The traditional cascade model has been superseded by a cell-based theory, which involves additional interactions and "cross-talk" between the intrinsic and extrinsic pathways.

1.3.3 An integrated coagulation mechanism

A key feature of the original coagulation cascade, as detailed in the preceding sections, was the existence of two distinct pathways which independently led to the activation of factor X and a final common pathway. The intrinsic and extrinsic pathways in this model did not interact before the activation of factor X, and being activated independently of one another; essentially provided two alternatives for initiation of fibrin clot generation.

Doubt as to the mutually exclusive, independent, nature of the two limbs of the coagulation cascade developed partly as a result of anecdotal evidence that VIIa/TF initiated factor IX activation as well as directly activating factor X. This posed a potential alternate mechanism for activation of FIX, independent of FXIa and the higher levels of the intrinsic limb. Activation of factor IX by the VIIa/TF complex was conclusively proven in 1977. This finding was inconsistent with some aspects
of the coagulation cascade models proposed in 1964 which specified two distinct limbs that did not combine until the activation of factor X\textsuperscript{40,41} and clearly questioned the importance of the roles of the contact factors (FXII and cofactors).

Furthermore, the original cascade model prescribed that the activation of the intrinsic pathway relied on activation of FXII to FXIIa on interaction of blood with a negatively charged surface. However, while traditional coagulation assays such as APTT are affected by deficiencies of FXII and/or its cofactors, HMWK and Kallikrein,\textsuperscript{63} they do not result in a clinical bleeding diathesis.\textsuperscript{63,66-68}

The integrity of the cascade model was further weakened on consideration of the clinical presentation of patients with FXI deficiency. Some factor XI deficient patients display no or minor bleeding disorders, while others suffer from more severe bleeding susceptibility; though still less so than patients with deficiencies of factors VIIIa or IXa.\textsuperscript{61,68-70} Were the cascade model correct one would expect that deficiencies of factors higher in the cascade would have equivalent or more significant effects than those lower in the cascade, as the lower (downstream) steps, reliant on the factors upstream, would also be unable to occur. Therefore, it should be expected that FXII or FXI deficiency would consistently produce at least as severe bleeding tendency as deficiencies of factors VIII and IX. Clinically, however, factor VIII and IX deficiencies (the haemophilias) are by far the more severe.\textsuperscript{61,68,69} The disparity between theoretical and clinical severity of bleeding diathesis therefore significantly questioned the accuracy and clinical significance of the traditional activation steps at the head of the intrinsic pathway. Clinical correlations, combined with the finding that the factor VIIa/TF complex was able to directly activate factor IX, have lead to a
general consensus in scientific community that factor VIIa/TF is in fact the major physiological initiator of haemostasis. With the FVIIa/TF complex being capable of activating FIXa and the resultant formation of the VIIa/Ixa tenase complex, the physiological relevance of FXIIa and FXIa for haemostasis in vivo was left questionable.

Following these revelations, it is now recognised that there are two key flaws in the original cascade model. One is the separation of the coagulation system into two distinct, intrinsic and extrinsic, pathways. The second is the total focus upon the coagulation proteins themselves, with no regard for the physiological surfaces involved. The modern, cell-based theory redresses these flaws, providing a more clinically relevant model of the coagulation system.

The intrinsic and extrinsic pathways are not mutually exclusive, as illustrated by the inability of one pathway to naturally compensate for deficiencies in the other. Were the two pathways truly independent of one another, the bleeding tendency seen in haemophiliacs would not occur. Haemophilia A and B patients are deficient in specific factors from the intrinsic pathway. Were the cascade proposal correct, the extrinsic pathway would be unaffected and able to lead to activation of factor X and mediate effective haemostasis, making the intrinsic pathway superfluous. Were this the case, deficiencies in “intrinsic” factors would not lead to the bleeding tendency seen. However, haemophiliacs do experience severe bleeding, leading to the oft posed question of “Why do haemophiliacs bleed?"
Rather than occurring as a result of two independent pathways, the cell-based model offers a view of the coagulation process in which proteins from both the extrinsic and intrinsic pathways are required to interact at levels higher in the traditional cascade than activation of factor X and that these interactions serve to guide the entire coagulation process, which absolutely requires components from both limbs of the traditional model to successfully lead to effective thrombogenesis.

The second flaw of the original coagulation cascade, the almost exclusive focus on the coagulation proteins, is also redressed in the cell-based model. The original cascade model implicitly suggests that the coagulation proteins drive and control the process of coagulation, requiring a generic phospholipid source as a surface at specific stages. The cell-based model of the coagulation system recognises that the coagulation protein interactions do not simply occur in the blood, but are targeted to specific cellular surfaces, namely platelets and TF bearing cells, which guide the entire process and localise procoagulant activity to appropriate sites, preventing widespread thrombosis while ensuring effective haemostasis.

### 1.3.4 Cellular control of the coagulation system

In 1991, Davie et al published an insightful review of advances in understanding of the mechanisms underlying the coagulation cascade. Assimilating evidence from the previous 25 years' literature, the group presented a refined version of the traditional coagulation cascade. In the 1991 model the extrinsic pathway was reported to be the primary initiating mechanism with the intrinsic pathway providing amplification of the procoagulant signal. It bore some similarity to the traditional cascade though also
featured several significant modifications and provides a useful stepping stone to the modern model which will form the basis of much of this review.

With the recognition that the cascade is initiated by FVIIa/TF and the knowledge that FXI may be activated by thrombin, the 1991 model accounted well for the lack of requirement for FXIIa and made provision for cross talk between the extrinsic and intrinsic limbs. However one important shortcoming, as was the case with the 1964 models, was its failure to recognise the pivotal role of cell surfaces in directing coagulation; continuing to suggest that the extrinsic pathway alone should be capable of generating sufficient thrombin to support stable clot formation.

Based on the advances in understanding of coagulation since 1964, Hoffman and colleagues proposed the modern cell-based model of coagulation in 1996. Focusing on the role of cell surfaces in directing the coagulation process, Hoffman et al redefined the stages previously covered by the cascade model into three overlapping phases: initiation, amplification and propagation.

1.3.4.1 Initiation phase of the cell-based model

The initiation phase of the cell-based model covers the early stages of the procoagulant response, from the exposure of tissue factor at the site of damage to the blood vessel, through to the activation of factors IX and X and the generation of the initial thrombin.
Using a novel cell-based model of TF-initiated coagulation, comprising unactivated platelets, monocytes (as a cellular source of TF), zymogen coagulation factors II, V, VIII, IX & X, the inhibitors TFPI and AT-III and a catalytic amount of FVIIa, Monroe et al were able to provide evidence for the sequence in which platelets and coagulation proteins interact in the initiation of clotting. In this cell-based model, initiation of the coagulation system is viewed to commence with the interaction of FVIIa and TF. 

*In vivo*, TF is exposed upon damage to the vasculature, essentially triggering the initiation phase. In the cell-based model, in line with the traditional extrinsic limb of the cascade model, the TF/FVIIa complex which results is then able to activate FX to Fxa. However, in the cell-based model, the Fxa which is generated on the surface of the TF bearing cell is restricted to that surface as inhibitors such as antithrombin III (AT-III) rapidly inactivate any free circulating Fxa in the blood. The Fxa expressed on the TF bearing cell is able, through interaction with Fva and formation of the prothrombinase complex, to activate small amounts of thrombin. Since the TF-bearing cell, as compared to the activated platelet surface, is not well suited to supporting large scale thrombin generation, there is insufficient thrombin generated through this TF/FVIIa mediated pathway to lead to generation of a viable fibrin clot.

The duration of the initiation phase is limited principally by TFPI, which is activated by Fxa formed by the FVIIa/TF complex. TFPI is a Kunitz type inhibitor which binds and forms a complex with Fxa and the FVIIa/TF complex, inhibiting the catalytic activities of both enzymes. Through the activation and actions of TFPI, the initiation phase of coagulation in the modern model is therefore self-limiting.
The extrinsic pathway as proposed by the cascade model of coagulation correctly illustrated that interaction of FVIIa with TF leads to activation of FX, in turn leading to thrombin generation. However, due to its failure to recognise the importance of physiological surfaces in the process, the cascade model misleadingly suggests that the extrinsic pathway alone is capable of generating sufficient thrombin to form a stable fibrin clot.\textsuperscript{40, 41}

The cascade model also omits a second, vital stage of the initiation phase – that is the activation by the FVIIa/TF complex of FIX to FlXa.\textsuperscript{61} The FIXa produced on the TF bearing cell is able to diffuse through the blood, relatively unaffected by AT-III and not at all affected by TFPI,\textsuperscript{77} to bind with the FVIIIa expressed on the surface of the activated platelets, forming the FVIIIa/Ixa tenase complex. The cell-based model again justifies the apparent lack of requirement for FXIIa and FXIa, as FIXa is initially activated by the TF/FVIIa complex in the cell-based model, rather than being reliant on FXIa activation.\textsuperscript{61, 72}

Figure 6 schematically illustrates the events which comprise “initiation” in the modern cell-based model of coagulation.
Orange boxes represent those factors classically referred to as intrinsic factors, green boxes represent extrinsic factors, red boxes represent factors that are part of the common pathway and yellow boxes signify co-factors required at specific stages. The purple shaded area covers those reactions occurring on the surface of the TF-bearing cell; note FIXa and thrombin are free to leave the cell surface, while FXa is not.

Figure 6  Schematic representation of the “initiation” phase of the modern cellular coagulation model
1.3.4.2 Amplification phase of the cell-based model

The amplification phase of the cell-based model deals with the actions of the small amount of thrombin generated during the initiation phase, leading to the complete activation and "priming" of platelets for the subsequent propagation phase.

In 1996, Monroe et al demonstrated that in order to support clot formation, a cell must not only express tissue factor, but also is required to be capable of supporting the assembly of the prothrombinase complex on its surface. This finding supported the view that the procoagulant signal generated on the surface of the TF-bearing cell was transmitted to the partially activated platelet via small amounts of thrombin generated on the TF-bearing cell. The binding of the small amount of thrombin generated in the initiation phase to PAR-1 on the surface of the partially activated platelet leads to degranulation of α-granules and release and activation of FV as previously detailed in this manuscript. Thrombin generated during the initiation phase also cleaves FVIIIa from vWF on the activated platelet, an essential role as FVIIIa is required for the assembly of the tenase complex and the free vWF strengthens platelet aggregation.

From their earlier work in 1994, Monroe et al were able to demonstrate that platelet activation by thrombin must occur prior to binding of activated coagulation factors to the platelet membrane. They further established that coagulation factor binding occurs in a defined sequence, commencing with binding of FVa and FVIIIa which then serve as binding sites for Fxa and FIXa, respectively.
A third occurrence during the amplification phase is the thrombin mediated activation of FXI,\textsuperscript{81} which is bound to the surface of the activated platelet. Generation of FXIa in this way is entirely independent of FXIIa.\textsuperscript{82}

Therefore, although insufficient to initiate the formation of a viable fibrin clot, the thrombin which is produced on the surface of the TF-bearing cell during the initiation phase is able, in the amplification phase, to activate nearby partially activated platelets, leading to the generation of FVa, essential for the assembly of the prothrombinase complex. In addition, the thrombin from the initiation phase leads to the cleavage of FVIIIa from the FVIII/vWF complex and the activation of platelet bound FXI. Presentation of activated forms of FV, FVIII and FXI, mediated by the small amount of thrombin from the initiation phase results in a primed platelet, optimised for propagation of the procoagulant signal.

The events which collectively form the “amplification” phase of coagulation in the cell-based model are illustrated schematically in Figure 7.
Orange boxes represent those factors classically referred to as intrinsic factors and red boxes represent factors that are part of the common pathway. The purple shaded area represents the TF-bearing cell while the pink area covers those reactions occurring on the surface of the platelet; note that small amount of thrombin generated in the initiation phase primes the platelet, leading to expression of the activated forms of the factors required on the activated platelet surface for propagation of the procoagulant signal.

Figure 7  Schematic representation of the “amplification” phase of the modern cellular coagulation model
1.3.4.3 Propagation phase of the cell-based model

The propagation phase of the cell-based model covers the events occurring on the surface of the activated platelet, from the amplification phase through to the generation of large amounts of thrombin and ultimately the deposition of fibrin.

The binding of FIXa to FVIIIa on the surface of the activated platelet, primed in the amplification phase, leads into the propagation phase of the cell-based model. Assembly of the FIXa/VIIIa tenase complex on the activated platelet surface generates a positive feedback loop in which additional FIX is activated on the platelet surface, through the action of FXIa which became expressed on the surface of the activated platelet during the amplification phase due to its activation by thrombin, generated in the initiation phase. The role of FXIa in amplifying the amount of FIXa appears to explain the clinical presentation of patients with FXIa deficiency and their less severe bleeding tendency compared to haemophiliacs since FXIa is required for continued generation of FIXa but not for initial formation of the tenase complex.

The FVIIIa/Ixa tenase complex on the activated platelet surface goes on to activate large amounts of FX. As the Fxa is expressed on the platelet surface there is no requirement for diffusion through the blood, therefore AT-III is unable to inhibit Fxa which in turn goes on to form the prothrombinase complex with Fva, also present on the surface of the activated platelet. The platelet prothrombinase complex is capable of generating much larger amounts of thrombin than that on the TF-bearing cell surface, as the platelet provides the most efficient surface for thrombin generation, resulting in the required thrombin burst. At this prime location, sufficient thrombin is
generated to cleave fibrinogen to fibrin and ultimately lead to the formation of a stable clot at the site of vascular damage.\textsuperscript{64, 77}

Figure 8 illustrates schematically the events which form the "propagation" phase, leading to the thrombin burst.
Orange boxes represent those factors classically referred to as intrinsic factors and red boxes represent factors that are part of the common pathway. The pink area represents the surface of the activated platelet; note the involvement of FIXa from the initiation phase in the formation of the tenase complex which ultimately leads to the thrombin burst that facilitates clot formation.

Figure 8  Schematic representation of the “propagation” phase of the modern cellular coagulation model
1.3.4.4 Summary of the cell-based model

The two principal products of the initiation phase, FIXa and Fxa were confirmed by Hoffman and colleagues in 1995 to have distinct roles. The group used a then novel in vitro model system, described here previously. Through varying the included components of the model system, they were able to comprehensively demonstrate that Fxa is required for the activation of platelets, but is relatively weak in terms of ability to promote thrombin generation. Conversely, FIXa was found to be far less effective in activating platelets but was a highly potent promoter of thrombin generation. From these data, the group proposed the now accepted view that the main role of Fxa produced from the TF/FVIIa complex is local activation of platelets while the role of the FIXa, as part of the FVIIIa/Ixa tenase complex, is to generate large amounts of thrombin on the activated platelets. These findings were strengthened by a further, later study from the same research group.

Figure 9 summarises the modern view of coagulation, highlighting the importance of the role of specific cell surfaces (platelets and TF-bearing cells) in the control and regulation of coagulation.
Orange boxes represent those factors classically referred to as intrinsic factors, green boxes represent extrinsic factors, red boxes represent factors that are part of the common pathway and yellow boxes signify co-factors required at specific stages. The pink shaded area represents those interactions which take place on the surface of the activated platelet, while the purple shaded area covers those reactions occurring on the surface of the TF-bearing cell; emphasising the importance of cell surfaces in directing coagulation.

Figure 9  Schematic representation of the modern day view of coagulation.
In addition to catalysing the conversion of fibrinogen to fibrin, the thrombin produced during the platelet thrombin burst is essential for stabilisation of the developing clot. For example, polymerisation of the fibrin monomers formed occurs under the control of FXIIIa, which is activated from FXIII by thrombin. Furthermore, the high levels of thrombin produced on the platelet surface are capable of, and essential for, activation of TAFI (thrombin-activatable fibrinolysis inhibitor). TAFI is activated as a result of thrombin mediated activation of FXI on the platelet surface.

As implied in the name, TAFI serves to prevent fibrinolysis, therefore preserving the structure and stability of the clot as it forms. TAFI functions through removal of terminal lysine residues of fibrin, thus removing binding sites for fibrinolytic proteases. As TAFI requires greater levels of thrombin for activation than are required for fibrin generation, it has been suggested that the haemophiliac tendency to re-bleed following initial haemostasis may be due not solely to weak clot formation, but as insufficient thrombin is produced to activate TAFI, the already weak clot would be particularly prone to fibrinolysis.

To summarise, the key consideration to yield from the modern view of coagulation is that the Fxa produced on the TF-bearing cell is not equivalent to that expressed on the surface of an activated platelet. In the cell-based model of coagulation, the cellular location of the Fxa determines its physiological function and ensures appropriate and effective thrombin generation. The inability of the extrinsic pathway alone to generate a viable, stable fibrin clot, based on the low levels of thrombin generated in that phase, provides a feasible answer to the question “why do haemophiliacs bleed?” While the extrinsic pathway is intact and functional in these individuals and despite thrombin
therefore being produced as usual on the surface of TF-bearing cells, there is insufficient thrombin produced to support the formation of a durable clot. The thrombin produced is capable, as normal, of activating platelets, however beyond this point, the 'normal' coagulation system is blocked in haemophiliacs. Haemophilia A patients lack factor VIII, while haemophilia B patients lack factor IX. In both cases, formation of the FVIIIa/Ixa tenase complex on the platelet surface is impaired and hence the required platelet thrombin burst does not occur. The result is a slowly, poorly formed clot which is not resistant to fibrinolysis, hence the haemophilic bleeding tendency. The cell-based model effectively illustrates why the deficient intrinsic factors cannot be bypassed, as Fxa produced on the TF-bearing cell is insufficient and expressed on the wrong cell surface to lead to an adequate thrombin burst.

The cell-based model proposed by Hoffman, Monroe and colleagues provides explanations for a number of discrepancies noted from the traditional cascade model. While the overall pattern of successive zymogen activation steps resulting in activated coagulation proteases still holds true in the modern model, there is a much greater emphasis on cellular influences. The consideration of the coagulation system as a whole; comprising cell surfaces, coagulation proteins, cofactors and inhibitors, has enabled a more clinically relevant model be developed. The cell-based model facilitates rational reasoning as to the pathophysiology underlying the haemophilias and the failure of the "extrinsic" system to compensate. Further, it addresses the lack of requirement for FXIIa, while enabling involvement of FXIa in the system as indicated from clinical observations. The cellular focus emphasises the inbuilt mechanisms in the system which ensure appropriate localisation of the procoagulant
signal; the involvement of specific cell types with subsequent activation as a result of indirect communication between cells, the coordinated role of inhibitors and recognition of differing levels of activity being driven by the formation of complexes. We will return to the cell-based model of coagulation in the next chapter as the mechanism of action of high dose recombinant FVIIa is considered.
1.4 A delicate balance

While it is essential that effective haemostatic processes occur when required, it is equally imperative that such processes occur only when appropriate and that they remain localised. Failure to limit haemostasis in this way may lead to thrombosis. In order to prevent this eventuality, the process of clot breakdown, fibrinolysis, is closely related to the procoagulant pathways already discussed. In addition, there are a number of inhibitory mechanisms built into the coagulation system which serve to prevent inappropriate fibrin deposition, primarily through acting upon thrombin.

1.4.1 Fibrinolysis

As essential as the appropriate formation of thrombi are to the control of haemorrhage and maintenance of haemostasis is the timely dissolution of inappropriately formed or no longer required clots. The process of destruction of a formed thrombus is termed fibrinolysis and is essentially mediated by the protease plasmin. Similar to the coagulation proteins which are cleaved when required from inactive zymogens present in the plasma, plasmin is derived from it’s inactive precursor plasminogen. Conversion of plasminogen to plasmin is directed by tissue plasminogen activator (tPA) or urokinase (uPA). The principal stimulus for tPA and uPA activation during the coagulation process is thrombin, already shown to be a powerful procoagulant, an important anticoagulant role of thrombin is hence illustrated here too.
The process of fibrinolysis, once activated, is subject to powerful positive feedback as plasmin generation further activates tPA and uPA, while the plasmin substrate fibrin binds both tPA and plasminogen, hence localising further plasmin generation.

The action of plasmin on fibrin results in the generation of soluble degradation products through cleavage. Again this process is self-propagating; plasmin degradation of fibrin leads to exposure of terminal lysine residues, which serve as binding sites for further plasminogen and tPA and ultimately increased plasmin formation. This process can be inhibited \textit{in vivo} by TAFI, which cleaves the lysine residues, preventing further binding. TAFI, generated by thrombin in the procoagulant process, therefore serves as a vital link between coagulation and fibrinolysis.

The process of fibrinolysis is of course more complex than the brief outline provided here, with additional co-factors and inhibitors involved, however for the purposes of this thesis, the essential information is provided here. Clearly, the generation of thrombin and the presence of fibrin serve not only a procoagulant role but also, paradoxically, are essential initiators and mediators of thrombolysis. Importantly, high thrombin concentrations are also associated with the activation of TAFI, which serves to \textit{inhibit} the fibrinolytic pathway, thrombin therefore fulfilling a procoagulant role once again. These apparently antagonistic actions of thrombin provide an essential mechanism for control over the interdependent systems of coagulation and fibrinolysis, with the thrombin concentration determining which process prevails.

The process of fibrinolysis is the subject of an excellent review by Cesarman-Maus & Hajjar.\textsuperscript{89}
1.4.2 Prevention of inappropriate thrombosis

In addition to a number of stages in the coagulation system which are self-regulating, thus preventing excessive thrombin and fibrin generation, several plasma proteases and endothelial bound proteins specifically serve regulatory roles. As already mentioned, the intact endothelium is potently anti-thrombogenic, by virtue of the expression of specific proteins on the surface of endothelial cells. One such protein is thrombomodulin I.\textsuperscript{63} On interaction with TM, the specificity of thrombin is altered in favour of activating Protein C, at the expense of thrombogenic, platelet activating and fibrinogen cleaving properties.\textsuperscript{91} Once activated, Protein C can form a complex with Protein S (also expressed on the surface of the endothelium) which can diffuse to the platelet, where it leads to the deactivation of any factor Va\textsuperscript{92, 93} expressed on that surface. In addition, activated Protein C is able to deactivate membrane bound factor VIIIa,\textsuperscript{93, 94} thus preventing assembly of the prothrombinase and tenase complexes on the platelet surface. As such, interaction of thrombin with TM sees the role of thrombin changed from pro-coagulant to anti-thrombotic.\textsuperscript{93}

In addition to endothelial surface expression of inhibitory proteins and the actions of thrombomodulin/protein C, there are two main plasma protease inhibitors; AT-III and TFPI, both of which have been considered earlier in this section. AT-III serves to inactivate circulating thrombin, while TFPI serves to "switch off" the initiation phase of the coagulation system. Importantly, the intact vascular endothelium also enhances the activity of AT-III through expression of the cofactor, heparan sulphate.\textsuperscript{93}
As will now be clear, the processes of coagulation, haemostasis and thrombosis are closely linked and in delicate balance. Not only do cell surfaces and coagulation proteins play vital roles but the various plasma and cell bound inhibitors are essential in maintaining control of the system. Failure of the inhibitors can lead to loss of the balance and thus conditions such as disseminated intravascular coagulation (DIC).
2.1 Disturbance of haemostasis

Inherited disorders, acquired disease and mechanical trauma may all lead to disturbance of haemostasis and resultantly excessive, severe bleeding.

Inherited disorders can affect any component of the clotting system and generally effect specific parts of the coagulation cascade, though multiple clotting factor deficiencies also occur. The most common of the inherited disorders are von Willebrand disease (vWD) and Haemophilia A & B. vWD is caused by irregularities in the quantity or action of the von Willebrand factor. Deficiencies of factor VIII leads to haemophilia A while Haemophilia B is caused by defective factor IX. Deficiencies of other factors are extremely rare (factor VII or X deficiencies 1:500 000, factor V deficiency 1:1 million). The inherited disorders in many instances do not have significant impact on everyday life, becoming problematic only in cases of bleeding, such as injury or surgery. In addition to inherited disorders, a number of common diseases such as heart disease and cancer, are associated with impaired haemostasis.

Trauma is a major cause of mortality and morbidity, both in the military and civilian arena. The mechanical damage caused by trauma can lead to damage to blood vessels and tissue and hence blood loss. This frequently occurs at sites which are not readily accessible for compression. Failure to apply direct pressure may lead to prolonged
blood loss which in turn can lead to physiological derangement (such as acidosis and hypothermia) and the initiation of a downward spiral of coagulopathy. The coagulopathy resulting from the combined effects of physical damage to the vasculature and impaired ability of the coagulation system can result in severely impaired haemostasis and potentially abelled ations.

2.1.1 Trauma induced disturbance of haemostasis

Haemorrhage is a broad term covering loss of small amounts of blood from vessels resulting in the formation of petechiae through to severe haemorrhage of significant blood volumes. While the formation of petechiae or ecchymoses, through the escape of small volumes of blood from the vasculature, may be indicative of an underlying pathology, in themselves they pose no appreciable risk of hypovolaemia.

Common understanding of the term haemorrhage includes either profuse visible blood loss from the body, for example following penetrating trauma, or internal bleeding requiring surgical correction. Either of these phenomena can lead rapidly to severe and life threatening hypovolaemia and it is this form of severe haemorrhage which is of grave concern to both civilian and military trauma clinicians, and forms the focus for this thesis.

2.1.2 Hypovolaemic shock

The hypovolaemia which by definition results from significant blood loss can lead to the clinical condition of shock. At its simplest, shock may be defined\textsuperscript{96} as:
"the response of the body to inadequate tissue perfusion and oxygenation".

Hypovolaemic shock is the most commonly encountered form of shock in the trauma victim, which may be subdivided, according to the volume of blood lost. A loss of less than 15% of circulating blood volume (CBV) is graded as Class I hypovolaemic shock, loss of 15-30% of CBV is Class II, 30-40% CBV loss is Class III and over 40% CBV loss is graded as Class IV hypovolaemic shock.

Prolonged starvation of the tissues of oxygen cannot support life therefore untreated shock will inevitably lead to cell death, followed by organ failure and ultimately death. The detection and control of haemorrhage is therefore vital in preventing or reducing the development of shock in battlefield casualties and civilian trauma victims.

A number of papers have highlighted the overwhelming contribution of haemorrhage to acute combat death. In a landmark paper, Bellamy produced a hypothetical model population of combat casualties from existing military casualty statistics from World War II and the Korean War. He convincingly makes the point that haemorrhage is indeed the single greatest threat to life on the battlefield. He went on further to assert that without adequate control of severe haemorrhage, other resuscitation methods are likely to be futile. In 2003, Champion acknowledged that the profile battlefield death had not changed over the years and that it was still dominated by haemorrhage, which accounts for around 50% of all deaths. The Battlefield Advanced Trauma Life Support Manual further emphasises the point that "restoration of adequate circulating volume [i.e. administration of intravenous (IV) fluid resuscitation] is not a substitute
for definitive treatment" and that it is therefore crucial, if possible, to stop the bleeding.⁹⁶

2.1.3 All bleeding is not the same

While the ultimate result of prolonged uncontrolled bleeding without intervention (i.e. labelle^2 ations) would be inevitable, the aetiology and resultant requirements for management of bleeding can be wide ranging. There are two principal overarching causes, illustrated in Figure 10, below.

Figure 10 Diagrammatic representation of the two principal overarching causes of bleeding and their typical results.

Mechanical damage to blood vessels as can be directly caused by physical trauma is often repairable by surgical intervention, with resultant restoration of haemostasis. By contrast, coagulopathic bleeding results from impaired thrombin generation²⁶ and is therefore not surgically correctable.
The causes of impaired thrombin generation leading to coagulopathy can be multifactorial and include:

- **Consumption** of platelets and clotting factors as the coagulation system makes futile attempts to restore haemostasis thus depleting reserves of blood constituents.\(^{97, 98}\)

- **Hypothermia** and **acidosis** can both contribute significantly to the development of coagulopathy.\(^{98, 99}\) Deteriorating coagulopathy, acidosis and hypothermia serve to exacerbate one another in an often “lethal triad” or “vicious cycle”.\(^{100, 101}\)

- **Dilution** of blood coagulation proteins and platelets is a major concern due to intravenous fluid resuscitation.\(^{98}\)

- **Dysfunction** of already depleted supplies of platelets and coagulation factors can be encountered as a result of ongoing coagulopathy.\(^{98}\)

- **Inappropriate activation of the fibrinolytic system** can further exacerbate the coagulopathy and lead to further consumption of blood constituents and may see the patient deteriorate into the clinical condition of DIC.\(^{98}\)

In the haemorrhaging trauma victim, coagulopathy develops rapidly, as the coagulation system is strongly activated in an attempt to restore haemostasis. A recent study demonstrated that coagulopathy was present in a significant proportion of severely injured patients on time of admission to the Emergency department and prior to significant intravenous fluid administration\(^{102}\) and that early coagulopathy is a predictor of mortality.\(^{103}\) In cases of massive haemorrhage where surgical control of the bleeding will ultimately be required to repair the damaged vessel, the futile action of the coagulation system in attempting to restore haemostasis quickly depletes
reserves of platelets and coagulation factors. Should the patient survive to reach a surgical capability, the underlying consumptive coagulopathy can result in continued diffuse blood loss despite surgical control of the primary site of injury. Ongoing bleeding and seeping may occur due to the impaired ability of blood to form viable clots due to lack of the vital procoagulant constituents. If the underlying coagulopathy is not corrected, then the patient may exsanguinate, despite surgical control.

Further complications may be encountered in the trauma patient as the development of shock can lead to worsening hypothermia and acidosis which have each been shown to adversely affect coagulation capabilities. Starvation of oxygen to tissues, due to a decreased circulating volume, is a major cause of acidosis in the haemorrhaging trauma patient. Intravenous fluid administration is advocated in the haemorrhaging patient where no surgical correction is possible in the short term, in order to increase circulating volume in an attempt to avoid or limit the development of tissue hypoxia and shock. However, as the majority of intravenous fluids, such as normal saline, add volume but not platelets or coagulation factors, they cause haemodilution, diluting further the already sparse blood constituents in clear fluid. This reduction in concentration of coagulation factors and platelets causes impaired coagulation in itself, however fluid administration also increases the risk of development of hypothermia. Administration of inadequately warmed fluids can decrease the core body temperature to levels at which coagulation enzymes cannot function thereby further exacerbating the coagulopathy.

Current clinical management of coagulopathy centres around blood component replacement therapy. While this can prove effective, in order for it to be effective it is
necessary to identify the deficient part(s) of the coagulation system to ensure that appropriate product(s) are administered. In addition, blood component replacement therapy is dependent upon availability of blood products. While this may seem obvious, such products can be relatively expensive and are considered scarce resources. Particularly in the military scenario, such products often may simply be unavailable. Despite these logistical considerations, a recent review has suggested that for serious injured, coagulopathic trauma patients who are in shock, plasma products should be considered the optimal resuscitation fluid currently available.
2.2 A military and civilian problem

In civilian practice, asphyxiation is the most common cause of death in trauma victims found dead at the scene and in over half of those who succumb within 48 hours of injury. Haemorrhage is also the leading cause of mortality on the battlefield, accounting for around 50% of all deaths, around 80% of which are due to uncompressible haemorrhage. While severe haemorrhage is approximately equally deadly in both military and civilian environs, there are some significant differences between combat deaths and those in the civilian environment. Firstly, due to the protracted timescales to evacuation which are frequently encountered in the military, compared to those generally encountered in civilian arenas in both the UK and USA, there are likely to be proportionally more patients which exsanguinate prior to evacuation to hospital. Secondly, the mechanism of wounding is very different in the military, compared to civilian setting, particularly in the UK. Clearly, the vast majority of cases of severe haemorrhage in the military are due to penetrating trauma, while in UK civilian practice, blunt trauma such as that often suffered by victims of road traffic collisions is far more prevalent. There is perhaps less disparity between civilian and military arenas in the USA, where gun crime, and resultant penetrating trauma, accounts for a far greater proportion of trauma cases and deaths in American society than in the UK.

Trunkey presented data in 1983 indicating that, in the urban civilian environment, trauma deaths follow a trimodal distribution. This may be considered to represent Immediate deaths, which are non-salvageable (e.g. decapitation), Early deaths, some of which are potentially salvageable (e.g. severe haemorrhage) and Late deaths,
generally caused by sepsis and multiple organ failure. Figure 11 is adapted from this paper and illustrates the timescale over which each of these peaks of death occurs.

Figure 11  The trimodal distribution of deaths following trauma.

Adapted from Trunkey, 1983

Given the delayed evacuation times frequently encountered in the military, the trimodal distribution proposed by Trunkey\(^4\) may shift to bimodal, with a slurring of peaks one and two (immediate and early deaths).\(^{108}\) In the hostile military environment, or indeed following a major disaster in the civilian arena, due to delayed access to adequate medical care, injuries which under normal conditions are potentially salvageable, may be fatal. Essentially this would increase the number of deaths seen in the first few hours following injury.

In addition, it has been reported that in geographically diverse civilian areas, a shift from the classic trimodal distribution of trauma deaths to bimodal may also be anticipated.\(^{109,110}\) Meislin et al\(^{109}\) reported a peak of deaths at 0-60 minutes, corresponding to "immediate" deaths and a peak of deaths 24-48 hours following
injury. In this paper, in contrast to Trunkey, the second peak contained by far the highest number of deaths, with fewer deaths in the first 60 minutes. The second peak appears to represent a prolonging of “early deaths”, defined by Trunkey as occurring around 2 hours after injury. The authors of the paper suggest that these changes may in some part be due to delayed access to medical treatment due to increased distances to hospital and also to the type of trauma encountered. Previous papers which had supported the trimodal distribution of trauma deaths\textsuperscript{4, 5} saw approximately equal incidence of penetrating and blunt trauma while this paper saw a significantly greater frequency of blunt trauma.\textsuperscript{109} The study catchment area used by Meislin et al was Pima county in Arizona, USA which consists of both suburban and rural regions, much like the UK. The bimodal distribution of trauma deaths reported by Demetriades et al\textsuperscript{110} was similar to that of Meislin et al, except that the second peak occurred earlier, more akin to the times quoted by Trunkey. The catchment area used by Demetriades et al was Los Angeles County Southern California, USA, a largely urban area. A higher proportion of penetrating trauma was seen in this study compared to that seen by Meislin et al, possibly accounting for the more rapid occurrence of the second peak, and perhaps making the data less representative of rural areas of the UK where penetrating trauma is uncommon and evacuation time to a surgical facility may be prolonged.

It should be noted that this bimodal distribution of trauma deaths is different from that reported in the military in which it is the first and second peaks which are reported to merge. These conflicting models of trauma deaths effectively illustrate the point, regardless of the model used, the differences between which are likely due to resource availability and regional conditions, that there are a group of patients who are
potentially salvageable, should adequate and timely medical care be available. These patients are represented by the second peaks of the trimodal model and civilian bimodal models. While the military model appears to indicate that such an intermediate group does not exist, it is likely that improvements in acute medical care may see the slurring of early deaths into the "non-salvageable" peak reversed, with more patients surviving to reach definitive care.

The early deaths are therefore a dynamic group, which may survive up to 48 hours, or slip virtually into immediate deaths in the absence of timely treatment, but also may be salvaged if effective control measures were available to prolong the window of opportunity to evacuate such casualties to a medical facility. Much research, including the subject of this thesis, is focussed upon minimising the early deaths, through seeking methods which serve to prolong the time over which a subject may remain viable prior to reaching definitive medical care.
2.3 Managing haemorrhage

The obvious response to haemorrhage to anyone trained in basic first aid would be application of direct pressure on the wound, to reduce blood loss. This method of control is often sufficient to prevent loss of considerable blood volume from, for example, an extremity injury. Such cases are termed compressible haemorrhage and bleeding from such sites should be controllable, even in the battlefield scenario, and haemorrhagic shock avoided by appropriate and timely application of direct pressure to arrest blood loss.

Where haemorrhage cannot be controlled through application of pressure and/or conventional wound dressings, the term non-compressible haemorrhage is used. In these cases it may be beneficial to utilise a tourniquet to stem the flow of blood upstream from the point of haemorrhage. This will reduce blood flow to the damaged blood vessel and therefore decrease blood loss. While their use is often advocated, tourniquets have been associated with a myriad of risks including nerve injury and development of tissue necrosis in tissues downstream of the tourniquet.\textsuperscript{111,112}

Tourniquets are also limited in their use to points at which they can be applied upstream of the injury. In general, tourniquets can therefore only be used in (severe) limb injuries.

Non-compressible truncal haemorrhage is far more difficult to manage, since it is not possible to arrest the bleeding by application of pressure or mechanical reduction of blood flow through the use of tourniquets. Examples of non-compressible haemorrhage would include abdominal, thoracic or junctional/truncal (i.e. at the joints
of the limbs and the neck with the trunk) bleeding. Perhaps unsurprisingly then, some 92% of cases which exsanguinate do so as a result of non-compressible haemorrhage.\textsuperscript{5}

Severe non-compressible haemorrhage overwhelms the innate haemostatic processes detailed previously, with potentially lethal consequences if unresolved. Where application of pressure, conventional dressings and tourniquets is not feasible, there is currently no effective method of achieving haemostasis in these cases, until surgical facilities are available to definitively arrest the blood loss. As such, the key to successful treatment and survival of severe uncontrollable haemorrhage is currently the timely transfer of the patient to a surgical capability. In the urban civilian environment this is often achievable however in isolated rural areas and particularly on the battlefield, rapid transfer/evacuation is not always possible. The same is true of major incidents involving the public, such as a terrorist attack or train crash, where limited emergency services are overwhelmed by the number of casualties.

In the face of non-compressible haemorrhage where transfer to a surgical facility is not possible in the immediate term, IV fluid resuscitation is at present the only realistic acute measure available to attempt to limit deterioration of hypovolaemic shock. While administration of IV fluids can be life saving, there has been much debate concerning the risks involved in its use and which fluids are the best to use; leading to the ongoing crystalloids versus colloids and hypotensive versus hypertensive resuscitation debates.
2.3.1 The gold standard: intravenous fluid resuscitation

There is a wealth of literature on the cost-benefit ratio of isotonic intravenous fluid resuscitation and while it is outside the remit of this thesis to cover the debate in any great detail, there are a number of salient points which should be noted, particularly from the military point of view, but true also in the civilian pre-hospital arena to some degree.

Considering briefly the potential problems of fluid resuscitation in the military environment specifically; in order to adequately administer appropriate volumes of standard infusion products (typically normal saline or Hartman's solution), the Combat Medical Technician (CMT) is required to carry large volumes that may be quickly depleted when treating severe haemorrhage. In addition to the logistical issues of carrying sufficient fluid without severely adversely affecting performance of personnel, there exists the potential to (unintentionally) cause or worsen a developing coagulopathy in the casualty.

While an increase in blood pressure is desired in order to facilitate increased perfusion of tissues and thus avoid deterioration to shock, a number of papers have indicated that a rapid increase mean arterial pressure (MAP) is associated with higher mortality rates. This is not a recent concept, and indeed in 1918 Cannon et al warned "Hemorrhage in a case of shock may not have occurred to a marked degree because the blood pressure has been too low and the flow too scant to overcome the obstacle offered by the clot. If the pressure is raised before the surgeon is ready to check any bleeding that may take place, blood that is sorely needed may be lost". This warning
was echoed in 1965 by Shaftan and associates who demonstrated in a canine arterial haemorrhage model that hypotensive resuscitation produced a more favourable outcome than normotensive, stating that in the normotensively resuscitated group "The still-liquid contents [of the clot] could be seen to enlarge, pulsate and finally break through at one corner to produce rebleeding". The authors went on to state that haemostasis is dependent not only on the formation of an initial clot across the damaged area of the blood vessel, but also upon secondary blood pressure depression and that the clot may be dislodged whenever the blood pressure was increased.

A more recent paper which addressed this issue was that of Bickell and colleagues in 1991. Anaesthetised swine were subjected to aortotomy and subsequently either normotensively resuscitated with lactated Ringers solution (to MAP 80mmHg) or not resuscitated. The volume of haemorrhage and mortality were both significantly increased in the group of animals which were resuscitated with lactated Ringers solution, compared to the unresuscitated controls. Indeed, resuscitation to MAP 80mmHg was associated with 100% mortality while all animals in the control group survived. The model was specifically designed to be potentially survivable, to enable establishment of the proof of principle that aggressive fluid resuscitation to normotensive pressures could be associated with a deleterious effect on survival and volume of haemorrhage. Bickell and associates showed that the infusion of lactated Ringers solution actually re-initiated bleeding which had already stopped and suggested the causality for this may be three-fold. Firstly, the authors suggested that the rapid infusion of fluid was likely to have mechanically disrupted a recently formed thrombus, facilitating re-bleeding. Secondly, the addition of clear fluid to the circulation caused a profound haemodilution which through decreasing the viscosity of
the circulating volume, according to the Bernoulli equation, would increase the velocity of the flow of the blood, further destabilising any formed thrombus. In addition to direct effects on the integrity of the thrombus, haemodilution causes a decrease in the oxygen carrying capacity of the blood, leading to impaired cellular function.

Kowalenko et al built on the work of Bickell and associates in 1992. Again, anaesthetised swine were haemorrhaged then subjected to a resuscitation regimen, however in this study, the haemorrhagic insult was designed specifically to be fatal in the majority of untreated cases. Animals were randomised to be resuscitated to MAP 80mmHg, 40mmHg, or non-resuscitated. Survival was not significantly different between those animals in the 80mmHg group and the non-resuscitated group, however survival was significantly higher in the hypotensively resuscitated (MAP 40mmHg) group, compared to no resuscitation and normotensive resuscitation. Survival rates in the hypotensively resuscitated group demonstrate that the model was potentially survivable, with treatment. Despite being a potentially survivable injury, in this severe haemorrhage model, normotensive resuscitation conferred no detectable decrease in mortality. Kowalenko and colleagues suggested two potential mechanisms which may account for the failure of normotensive resuscitation to improve survival; one being an increased volume of intraperitoneal haemorrhage and the other being haemodilution and resultant decreased oxygen carrying capacity of the circulating volume. The authors suggested that the increase in intraperitoneal haemorrhage may be partly due to an increase in transmural pressure caused, according to LaPlace’s Law, by an increase in vessel size due to the infused fluid. Also suggested to contribute to the increase blood loss seen with normotensive resuscitation was an increase in flow
across the damaged vessel surface (Poiseuille’s Law). Both of these factors, as suggested by Bickel et al., may lead to disruption of a thrombus.

A rat study conducted by Capone and associates provided further evidence that attempts to restore normal blood pressure prior to achievement of surgical control in a severe tail haemorrhage model led to increased blood loss, haemodilution and mortality. The authors of this study noted that animals which were resuscitated to a mean arterial blood pressure (MAP) of 80 mmHg had profound haemodilution and transient hypervolaemia, compared to animals which resuscitated to MAP 40 mmHg, or control animals which received no resuscitation. Again, the authors attributed the increased mortality rate in the normotensively resuscitated group to disruption of the clot, caused by a combination of decreased blood viscosity, increased blood pressure and increased pulse pressure. The potential role of the increase in pulse pressure was initially hypothesised by Stern and associates in a study which had utilised a near-fatal model of aortic haemorrhage in swine which were resuscitated to MAP 40, 60 or 80 mmHg. Survival was found to be significantly lower in the group resuscitated to 80 mmHg compared to either of the other groups, while volume of intraperitoneal haemorrhage was significantly higher in this group.

In addition to the potential for dislodgement of a clot by infusion of IV fluids to restore normal blood pressure, if adequate temporary haemostasis had not been established prior to administration of IV fluids, then they would likely have little long term beneficial effect, simply increasing the volume and rate of fluid loss from the site of injury. Furthermore, IV fluids carried by ‘far-forward’ medical personnel will simply provide volume expansion of the circulating intravascular fluid, offering
neither increased oxygen carrying capacity nor procoagulant capability, indeed
diluting the precious limited remaining platelets and coagulation factors.

In the military arena, if immediate evacuation of a casualty with uncompressible
haemorrhage is possible, then fluid administration should not delay transfer. However
if there is a delay in evacuation, administration of IV fluids is sanctioned. The delay
to evacuation often encountered on the battlefield compounds the limitations of IV
fluid resuscitation, potentially leading to the loss of more potentially salvageable
injuries over a protracted timescale.

The civilian situation is similar, with recommendations of the National Institute for
Clinical Excellence (NICE) stating that pre-hospital initiation of fluid replacement
therapy is generally not advocated and that in cases where it is deemed necessary it
should only be used en route to hospital and not delay arrival.

2.3.2 Hypertonic Saline Dextran (HSD)

As a means to address the logistical issues involved in carrying sufficient volumes of
traditional fluid resuscitations, hypertonic volume expanders such as HSD have
received much attention from the military medical community as a small volume
alternative.

The rationale behind the use of hypertonic plasma volume expanders lies in the high
salt concentration of such solutions, drawing water from the cells of the body through
osmosis. The result is an increase in the extracellular fluid volume through
redistribution of intracellular fluid. Theoretically, therefore, effective restoration of an adequate circulating volume may be attained from just a small volume infusion.

In 1984, Nakayama et al. published preliminary results from experiments in which sheep were bled to a MAP of 50mmHg, then resuscitated using either normal saline (around 1600 ml) or hypertonic saline (around 200 ml). They demonstrated that, despite its’ small volume, hypertonic saline had increased MAP to levels comparable to those achieved using the higher volume of normal saline. Plasma volume had also been significantly increased in those animals receiving hypertonic saline, by around twice the volume infused suggesting there had indeed been redistribution of intracellular fluid to the vasculature.

HSD is the volume expander currently receiving the most attention, based on encouraging results from a number of studies. HSD is composed of 7.5% saline and 6% dextran 70. It is administered as a small volume as required to maintain a MAP of 80mmHg, with a maximum infusion of 250ml. The BATLS resuscitation protocol is then followed once the course of HSD has been administered.
2.4 Adjuncts to improve haemostasis

While standard intravenous fluid resuscitation remains the gold standard for the shocked casualty in the absence of a surgical capability, there are severe limitations to its use and alternatives are actively being sought. Improved acute term care of casualties suffering from severe haemorrhage could significantly reduce the number of early deaths and prevent potentially salvageable injuries – such as a severe haemorrhage – from becoming inevitable deaths. Given the incidence of severe haemorrhage and hypovolaemic shock as cause of death both on the battlefield and in acute civilian care, there has been much emphasis on research into methods of improving haemorrhage control.

Several adjuncts to haemorrhage control have been considered and continue to be assessed. The most prominent of these are briefly considered in this section.

2.4.1 Specialist Blood Products

In the face of continued blood loss in theatre, despite ‘damage control’ surgery having taken place, the principal tools available to the surgeon are specialist blood products.

Three main products are available – fresh frozen plasma (FFP), cryoprecipitate and platelets. Fresh frozen plasma and cyoprecipitate are preparations derived from whole blood donations. They provide a mechanism through which coagulation factors can be administered as part of the volume expansion, thereby increasing the circulating volume while avoiding dilutional coagulopathy. Considering the overview of
coagulation, discussed in the introduction of this thesis, it should be clear why the administration of platelets and/or coagulation factors may improve the ability of the body to generate an effective thrombus and therefore mediate haemostasis.

While such products can effectively restore haemostasis in a number of cases, it is not always clear which product should be used and whether their use will actually help. With every transfusion there remains, an albeit small, risk of infection in addition to the relatively more frequently encountered complications of transfusion such as adverse anaphylactic or allergic reactions and blood group antibody-antigen reactions.\textsuperscript{122}

Furthermore, particularly in the field in the military arena, these specialist blood products may simply be unavailable and even in the civilian environment should be considered scarce, valuable, resources. As they are tools of the surgeon, they are invariably available only once the casualty reaches a surgical facility. As already discussed, there are instances, particularly in the hostile military environment, when methods of controlling haemorrhage are required much sooner than a surgeon can tend to the casualty.

2.4.2 Haemostatic dressings and topical agents

To provide an alternative to tourniquets for controlling blood loss in cases of severe non-compressible haemorrhage and to improve the efficacy of fluid resuscitation, through preventing any further circulating volume loss, a range of novel haemostatic dressings and topical agents have been the focus of intense research over recent years,
One of the main focuses of the reviews is Quikclot (mineral zeolite), a novel haemostatic agent, developed and marketed by Z-Medica (Wallingford, Connecticut, USA) and approved by the FDA for external use in May 2002. It is composed of zeolite granules, a derivative of volcanic rock, which is porous and strongly hydrophilic. The product is a powder (described recently in the media as 'sand'), which is poured directly onto a wound and since the mineral structure is hydrophilic, fluid is adsorbed. The result is a highly concentrated population of platelets and coagulation factors at the wound site and therefore, theoretically, increased clotting kinetics.

In a swine model of severe mixed arterial and venous haemorrhage, Quikclot produced a statistically significant improvement in both volume of blood loss and survival, decreasing mortality from 83% in the untreated group to 0% in the Quikclot group. These findings were supported in a further study in 2004 in which Quikclot was shown to be more effective in improving survival and reducing volume of blood loss in a swine model of Grade V liver injury, compared to a standard gauze dressing.

The first *in vivo* use of Quikclot in humans was reported in 2004. A 22 year-old male suffering multiple gunshot wounds continued to suffer considerable haemorrhage which was not responsive to the surgical measures employed. As coagulopathy developed, the decision was taken to use Quikclot as a "last resort" to attempt to save life. Bleeding ceased at each wound site to which Quikclot was applied and the patient went on to make a full recovery. This study demonstrated the apparent ability of Quikclot to establish haemostasis in a severe bleeding episode in a coagulopathic patient.
While this is an encouraging report, it must be remembered that the Quikclot was administered in the operating theatre, as an adjunct to surgery. Administration of Quikclot to internal injuries such as those reported by Wright et al\textsuperscript{17} would simply not be feasible on the battlefield or in the civilian prehospital scenario. A further limitation to the use of Quikclot, which has prevented its more widespread use in the trauma field, is that the absorption of water results in the release of a large amount of heat from the substance itself. This exothermic reaction, which has been shown to reach temperatures over 90 degrees Celsius,\textsuperscript{16,17} has been reported to cause burn injuries in humans,\textsuperscript{127} however the incidence of such iatrogenic injuries has been relatively low and must be considered in context, as the product manufacturer claimed in April 2006 that Quikclot "is credited with saving at least 200 lives during the Iraqi war and Afghanistan operations".\textsuperscript{18}

Further haemostatic dressings which received much attention in the recent reviews were the poly-N-acetyl glucosamine (p-GlcNAc) based chitosan and chitin dressings. Chitin is a fully acetylated form of p-GlcNAc, while chitosan is the deacetylated form of p-GlcNAc.\textsuperscript{125}

The chitin dressing, Rapid Deployment Hemostat (RDH) has undergone various modifications along the process of development, with three different versions reported in the literature. The current version has been shown to be efficacious in some recent animal studies but is yet to be tested alongside the other dressings discussed here and is not yet available commercially.\textsuperscript{125} As a result, it has not been used in theatre in Iraq or Afghanistan, and will not be discussed in further detail here.
The chitosan dressing HemCon™ (HemCon Medical Technologies Inc, Portland, Oregon) has been shown in a number of animal studies to be efficacious. In a swine model of severe liver injury, the dressing was shown to improve survival from 29% in the control (standard gauze dressing treated) group to 88%. In a swine model of severe aortic haemorrhage, HemCon™ initially stopped arterial bleeding in 71% of cases, compared to 0% with the standard dressing. However, rebleeding occurred resulting in ablatedations after a mean time of less than 1 hour, with all animals having died within 102 minutes. In the same study, the fibrin sealant dressing (discussed in more detail shortly) performed more favourably.

In 2004, a study was published which directly compared the efficacy of HemCon™ and Quikclot® in a large animal model of severe haemorrhage. In concordance with the 2003 study by Alam et al, Quikclot® was shown to be associated with 100% survival, compared to 0% in the “no dressing” group and 43% in the standard field dressing group. Again, the use of Quikclot® was associated with an increased wound temperature in this study. HemCon™ showed mixed results in this study, proving either very effective, or completely failing due to lack of adherence. The authors stated that each of the HemCon™ dressings looked identical with no obvious imperfections and were all derived from the same batch. They suggest that this is likely a quality control/manufacturing issue which they claim should be easy to resolve; though they acknowledge that in a pilot study for this experiment, the entire batch of HemCon™ failed to adhere. The overall survival rate for the HemCon™ group in this study was 71%.
Recent data published on the use of the HemCon™ bandage in Afghanistan and Iraq reported a success rate of 97% in 64 cases where the dressing was used in an attempt to achieve cessation of bleeding or improvement in haemostasis.\textsuperscript{131}

A third haemostatic dressing, the fibrin sealant dressing, has also been the focus of much research effort. The efficacy of this dressing was compared with that of HemCon™ in a study conducted by Kheirabadi and associates.\textsuperscript{129} Using an aortic injury in a swine model, the fibrin sealant dressing was shown to be 100% effective in achieving initial haemostasis (compared to 71% with HemCon™) and while rebleeding occurred in all cases treated with HemCon™ resulting in a mean survival time of less than 1 hour, all but one of the six animals treated with the fibrin sealant dressing survived the full study duration of 96 hours, with a mean survival time of over 80 hours.

In a further study, the fibrin sealant dressing was compared with Quikclot®, HemCon™ and a standard gauze dressing in a swine model of severe arterial haemorrhage.\textsuperscript{132} Contrary to previously discussed papers, neither Quikclot® nor HemCon™ were found to confer haemostatic benefit in this study, with no animals surviving in either group. The fibrin sealant dressing was associated with 67% survival. The authors noted that, though not reaching significance, the HemCon™ dressing appeared to decrease the bleeding rate and increase the time to abelled ations by 20 minutes with no adverse effects, compared to Quikclot® which conferred no benefit whatsoever and was again noted to be associated with an intense exothermic reaction. This study was limited through the fact that the dressings were applied within 45 seconds of injury and free bleeding, a timescale clearly not

69
applicable to the vast number of cases in either military or indeed civilian environs. Furthermore, the dressings were applied through a surgical access point, directly to the wound. Again, it is highly unlikely that direct access to the point of vessel damage would be available in the trauma setting. Therefore, particularly in the case of the HemCon™ and fibrin sealant dressings, as agents which require direct access to the point of bleeding, as compared to Quikclot which could conceivably be poured into a wound site, the relevance of these findings to the trauma setting must be questioned. The timescale is clearly not realistic and the requirement for access to the point of injury means that these dressings are unlikely to be applicable in a number of cases of non-compressible haemorrhage. This point is made clear by Pusateri who stated that the dressings tested were only likely to be useful in cases where the bleeding sites were accessible to buddy aid.¹³³

Both HemCon™ and Quikclot® have been granted FDA approval and both have been deployed by the U.S. military in the ongoing conflicts in Iraq and Afghanistan. The fibrin sealant dressing, though having shown promise in studies such as those described here, requires testing in clinical trials in order to obtain FDA approval.¹³² There are also availability issues, and it is considerably more expensive than the other dressings (up to $1000 per dressing, compared to $10 for Quikclot® and $100 for HemCon™).¹²⁵

It is evident from reviewing the published literature on the range of haemostatic dressings that there is no single ideal option. Efficacy, cost, availability and safety issues mean that the risk-benefit as well as cost-benefit ratios must be weighed up. Quikclot® has proven effective in the majority of studies, is cheap, readily available
and does not appear to vary in efficacy between batches. However, Quikclot® is the only agent to have been shown to have any adverse effect. HemCon™, while having no adverse effects and being commercially available and relatively cheap, appears to vary in efficacy between batches, though has been shown to be effective in clinical use in current military operations. The fibrin sealant dressing has proven effective in comparative studies with the other dressings, but lacks FDA approval, is not readily available and carries a large unit cost. A further significant limitation of all of these advanced haemostatic dressings is that they require a degree of access to a point of bleeding, which is often difficult or simply not feasible in the trauma patient. A requirement therefore exists for an agent which is capable of mediating effective haemostasis when the site of haemorrhage is inaccessible. An intravenous agent which was directed to the site of vascular damage would seem a promising candidate and one such agent, activated recombinant factor VII, is discussed in detailed in the following section.
2.5 Activated factor VII

2.5.1 Activated factor VII and the haemophilias

Clotting factor VII is an integral part of the coagulation system. Minute amounts of activated factor VII, FVIIa, circulate in the bloodstream of physiologically normal individuals. Interaction of FVII(a) with tissue factor, exposed at the site of injury, leads to the activation of factor X and the initiation of the final common pathway through the traditionally viewed “extrinsic” pathway, as discussed previously. The notation FVII(a) is used here as there is much debate in the literature as to whether FVII is activated on interaction with TF or if only true FVIIa is able to form an active complex with the protein. Possible activators of FVII in vivo are suggested by different groups to include not only tissue factor, but also factor VII-activating protease (FSAP) factor Xa, factor Ixa, the FVIIa/TF complex and thrombin.\textsuperscript{134, 135}

While there is no clear consensus on the definitive activator of FVII in vitro, the key concept from the point of view of this thesis remains that the FVIIa/TF complex leads to initiation of coagulation.

Over the last few decades, preparations of FVIIa have been used increasingly to treat bleeding disorders; initially primarily the haemophilias. Haemophiliacs are deficient in, or have alloantibodies (inhibitors) to, either factor VIII or factor IX (haemophilia A and B respectively). Haemophiliacs with deficiencies of these factors can be treated effectively through administration of the deficient factor concentrate. However, in patients with inhibitors to factors VIII or IX, replacement therapy is often ineffective as the inhibitors also act on the infused factor. Such patients require treatment which
circumvents the inhibited step of the "intrinsic" pathway and initial approaches to
treatment in these cases involved administration of prothrombin complex
concentrates. Such treatment had mixed success and a safer, more efficacious
option was sought.

In 1979, it was shown that clotting factor concentrates contained high concentrations
of FVIIa and it was hypothesised that this may be the primary active agent. Since it
was viewed to be the intrinsic system which was blocked in haemophilia patients with
inhibitors, attention turned to the possibility of utilising the extrinsic pathway,
specifically FVIIa, to mediate clotting. FVIIa was considered a potentially ideal
choice as it was understood to require interaction with tissue factor, exclusively at the
site of injury, to activate factor X and therefore iatrogenic systemic thrombosis should
not be a concern.

The first use of human FVIIa as a treatment in haemophiliacs with inhibitors appeared
in the peer reviewed literature in 1983, though there had been an earlier
unsubstantiated use reported in 1981. In the landmark 1983 paper, two haemophilia
A patients with inhibitors to factor VIII were administered human FVIIa concentrate
to control bleeding. Both patients responded to the treatment with no ill effect noted.
While the mechanism of action of FVIIa in these and subsequent cases was not clear,
the effectiveness was remarkable, proving FVIIa to be an effective treatment for
haemophiliacs with inhibitors to factors VIII or IX.
2.5.2 Activated recombinant factor VII, rFVIIa

Human factor VII was first isolated in the early eighties and was subsequently produced by recombinant technology. Initial studies provided evidence to suggest that rFVIIa produced from baby hamster kidney cells is safe and very similar to plasma-derived human FVIIa. These findings made rFVIIa an attractive candidate as an alternative source for FVIIa; as plasma contains only trace amounts of FVIIa, making it difficult to collect adequate amounts and there are inherent risks of viral transmission in the use of any proteins derived from human plasma.

The first reported clinical use of rFVIIa was published in 1988. A haemophilia A patient with inhibitors to factor VIII successfully underwent surgery with minimal perioperative blood loss, no post operative bleeding and no thrombotic events in a protocol where rFVIIa was used to provide cover. This paper provided the first anecdotal evidence for rFVIIa as a safe, effective treatment in the control of bleeding. Following this initial report, there have been a multitude of papers endorsing the use of rFVIIa in the treatment of haemophilia patients with inhibitors to factors VIII or IX and the drug is now an established treatment for such patients.

Hedner coined the phrase “universal haemostatic agent” in 1998, referring to multiple reports of successful use of the drug not only in haemophiliacs but also in patients with a range of bleeding disorders including Glanzmann’s Thrombasthenia, thrombocytopenia and factor VII deficiency.
Recombinant factor VIIa is currently manufactured by NovoNordisk, marketed as NovoSeven®. NovoSeven® is licensed in the USA for the treatment of congenital haemophilia with inhibitors to factors VIII or IX. In the EU it is additionally licensed for use in cases of acquired haemophilia, factor VII deficiency and Glanzmann's thrombasthenia. In addition to these licensed uses, NovoSeven® has in recent years seen a number of cases of 'off-label' emergency usage.

2.5.3 Mechanism of action of rFVIIa

An earlier section outlined why haemophiliacs bleed. This section will now consider how infused FVIIa is able to stop bleeding in haemophiliacs.

Based on the interaction of FVIIa with TF, it was not clear how, through addition of an excess of FVIIa, there could be an increased procoagulant signal, as it is viewed that TF, and not FVIIa, is the limiting agent in vivo. This was particularly perplexing as high doses of FVIIa (far higher than the dose required to saturate TF binding sites) had been required to elicit a response in haemophilia patients; yet once all TF binding sites were occupied, it was not clear how the infused high-dose FVIIa could be acting. The mechanism of action of rFVIIa was far from clear when it was first used in the 1980's and still remains a point of contention.

In 1990, Rao & Rapaport published a paper which attempted to elucidate the mechanism of action of infused FVIIa in haemophiliacs, proposing that infused FVIIa may be acting through a non-physiological, TF-independent mechanism. They demonstrated that exogenous FVIIa was capable of shortening the clotting time of haemophilic plasma and that this effect could not be blocked by anti-tissue factor
antibodies; indicating that FVIIa was acting through a non-TF dependent mechanism. This was confirmed using an assay system containing only FVIIa, FX, calcium and phospholipid, with no TF source. Despite the lack of TF, FVIIa proved capable of activation of FX in this system. Removal of phospholipid from the assay system, however, prevented activation of FX by FVIIa. On addition of TF to the phospholipid-containing system, there was no reported difference between levels of FX activation with or without TF. In the same paper, Rao & Rapaport also noted that the enzymatic efficiency of the FVIIa/phospholipid complex was significantly lower than that achieved through FVIIa/TF complex formation. This finding provided compelling evidence to potentially explain the requirement for high doses of FVIIa. It was interpreted from these findings that the mechanism of activation of FX by high-dose FVIIa was phospholipid-dependent but TF-independent.

The conclusion of Rao & Rapaport was supported by a later study from Hoffman et al in which it was clearly demonstrated that human monocytes were able to support the generation of Fxa on their surface, independent of the presence of TF. Interestingly, they reported in the same paper that endothelial cells could not support Fxa generation, demonstrating that a generic phospholipid source is not sufficient to support initiation of coagulation, implying instead that specific cell surface characteristics may be required.

Consistent with and adding to the earlier work and hypotheses of Rao & Rapaport and indeed their own work, Monroe et al provided evidence in 1997 that high dose FVIIa was able to directly act upon activated platelets independently of tissue factor. Using the cell-based model described previously, Monroe et al
demonstrated that FVIIa was able to bind to activated, but not unactivated, platelets. This binding was found to be entirely independent of TF. Although the affinity of FVIIa for activated platelets was found to be lower than that for TF, the FVIIa/activated platelet interaction nonetheless formed an active complex which was able, ultimately, to mediate thrombin generation directly on the surface of the activated platelet.

In later studies, Monroe and associates demonstrated that the amount of Fxa generated on the surface of activated platelet in the absence of TF, FVIII and FIX but in the presence of rFVIIa was significantly less than that when the intrinsic factors were present, however at high doses of rFVIIa (resulting in plasma FVIIa concentrations which approximate to those found in haemophiliac patients treated with rFVIIa), thrombin generation approached normal levels seen in the cell-based model of coagulation. The ability of rFVIIa to facilitate even limited Fxa generation in the absence of FVIII or FIX allows understanding of how pharmacological doses of rFVIIa are able to compensate for the deficiency of the intrinsic factors in haemophiliacs. At high doses, rFVIIa was able to act directly on the activated platelet, leading to the expression of Fxa on the surface of the activated platelet – the correct surface to generate the required thrombin burst.

The findings of Monroe et al in 1997 explained both the requirement for high doses of rFVIIa and the localisation of effect, avoiding systemic thrombosis. As activated platelets are found specifically at the site of injury, the matter of localisation was addressed while the lower enzymatic activity of FVIIa with platelets (compared to TF-bearing cells) also accounted for the higher dose dependency. This proposal also
offers a rational explanation for the efficacy of rFVIIa in thrombocytopenic patients, as high levels of rFVIIa have been shown to significantly increase the amount of thrombin generated on a given number of platelets, thereby maximising thrombin generation from a limited platelet population. Notably, contrary to the proposals of Monroe and colleagues, a TF-dependent model was able to account for the localisation effect but, for reasons already discussed, could not explain the high doses necessary, while the proposal by Rao & Rapaport of interaction of fVIIa with generic phospholipid, explained the high dose requirement but not the localisation effect.

Despite the mounting evidence for a TF-independent mechanism provided by these authors, work published in 1993 provided directly opposing evidence, suggesting that the mode of action of rFVIIa was very much dependent on TF. Following injection of high dose (50ug/kg) rFVIIa into healthy chimpanzees, ten Cate et al had measured a considerable increase in plasma levels of FIXa and Fxa as well as prothrombin activation fragments. Crucially, injection of monoclonal antibodies to TF in one group of chimpanzees had resulted in loss of effect of subsequently administered rFVIIa, indicating that the drug indeed requires TF to function.

Aside from the clear conflict with other published reports, it is alarming that administration of rFVIIa to healthy animals resulted in generation of factors Ixa and Xa, indicating potential inappropriate activation of coagulation. As these animals were uninjured, there should theoretically have been no significant exposure of TF nor should there have been upregulated expression of phospholipid as there was no requirement for activation of the coagulation system in these healthy animals. Ten Cate et al suggested that, since their results suggested that TF was essential for rFVIIa
activity, in their model at least, physiologically expressed TF must be interacting with rFVIIa and that this interaction may represent competitive binding of rFVIIa and zymogen FVII. The authors ultimately concluded that the rFVIIa in these experiments was acting upon TF to increase basal coagulation (often termed “idling” of the coagulation system). It is possible, therefore, that the mode of action of rFVIIa in these uninjured animals may well be TF dependent, in so far as the action of the drug is limited and not comparable to that seen when administered to the bleeding patient who may express increased levels of phospholipid source as a trigger for coagulation, with which rFVIIa may interact. Nevertheless, in 1996 Rao & Rapaport, who had been early advocates of a TP-independent mechanism, reported that they were sufficiently satisfied by this data to shift position, convinced that the mode of action of rFVIIa in haemophiliacs was TF-dependent, involving competition between zymogen FVII and rFVIIa.154

The TF-dependent mechanism was further championed in 2000, as van’t Veer et al reported the outcome from an in vitro model which indicated that rFVIIa acted by overcoming inhibition of TF binding by unactivated FVII.155 They found that the lag time to initiation of coagulation was increased in their model on addition of plasma levels of zymogen FVII and that this lag time shortened on addition of rFVIIa. Van’t Veer et al had used a model system in which relipidated TF-containing synthetic phospholipid vesicles were used in place of platelets; a system which was criticised by Hoffman & Monroe in their response to this hypothesis.156 Hoffman & Monroe found no effect of zymogen FVII in extending the lag period in their previously described cell-based model, suggesting that this discrepancy may be an artefact of the model system used by van’t Veer et al. Hoffman & Monroe used cell-
associated TF in their in vitro model while van’t Veer and associates used synthetic phospholipid vesicles; leading Hoffman & Monroe to question the validity of model systems utilising synthetic sources of TF. Notably, van’t Veer and colleagues made the observation that the inhibitory effect of zymogen FVII was evident only in the presence of low concentrations of tissue factor (less than or equal to 20pmol/L), a concentration far lower than that likely to be encountered in patients with damage to the endothelium. This means that the inhibitory effect of zymogen FVII, if extant, may be largely irrelevant in the trauma patient, and therefore for the subject of this thesis.

In 2001 Friedrich et al reported the results of a study looking at the potential of rFVIIa to overcome excessive inhibition of the TF/FVIIa pathway by novel anticoagulants in the form of inhibitors to the TF/FVIIa complex. Healthy human subjects were pre-treated with the inhibitor in the presence of either rFVIIa or placebo in a double-blind randomised study. Those subjects which received the inhibitor and placebo were found to have various impaired coagulation parameters (including prolonged prothrombin time and reduced thrombin generation). These were largely overcome in subjects which received the inhibitor in combination with rFVIIa rather than placebo, demonstrating that rFVIIa was effective even in the absence of TF. A further phase of the study involved subjects being treated with rFVIIa alone (no TF inhibitor). From comparison of coagulation parameters between those receiving rFVIIa alone and those receiving rFVIIa and the TF inhibitor, the authors were able to determine that rFVIIa thrombin generation potential was decreased by around 25% in the presence of the TF inhibitor. This clearly suggests that the mode of action of rFVIIa is in part dependent on TF, but importantly that up to 75% of its activity appears to be TF-independent.
Unfortunately, the authors were unable to confirm that all TF sites had been blocked by the inhibitor, weakening the strength of this evidence somewhat.

In 2003, Butenas et al published results of a study from which they concluded that rFVIIa acted through a TF-dependent mechanism.\textsuperscript{158} Using an \textit{in vitro} model system with non-activated platelets and additional phospholipid, the authors noted no notable effect of rFVIIa on thrombin generation unless TF was added. It is somewhat confusing that the authors concluded from this that the mode of action of rFVIIa was TF dependent. One could argue that this study has shown only that \textit{initiation of coagulation} requires TF - an already well-documented fact. Nowhere in the reviewed literature has it been suggested that rFVIIa is capable of activating an uninitiated system in the absence of TF. Indeed were this the case, then systemic thrombosis would be a grave and likely effect of rFVIIa administration \textit{in vivo}. However, Butenas and colleagues cite this evidence as their rationale for concluding that rFVIIa acts to restore haemostasis in a TF-dependent reaction. While this statement is true insomuch as that TF is required to initiate coagulation (with or without exogenous rFVIIa), it is a misleading interpretation of their data to suggest that the mode of action of rFVIIa is TF dependent. One would be forgiven for concluding that this work provides little new information, simply supporting the already accepted fact that rFVIIa is unable to exert an effect on unactivated platelets and that the increase in thrombin generation seen on addition of TF to the assay is most likely a result of TF-mediated initiation of the system, with rFVIIa exerting its therapeutic effect largely on the resultantly activated platelets. The authors of the paper suggest that the presence of generic phospholipids in the assay system should have been capable of supporting rFVIIa mediated thrombin generation if the action of the drug was TF-independent.
In their response to this paper, Monroe and Roberts\textsuperscript{159} again challenged the use of phospholipids as a replacement for platelets and suggested that it is inappropriate to extrapolate these \textit{in vitro} findings on the lack of effect of rFVIIa on phospholipids to any potential lack of \textit{in vivo} effect on platelets. In a subsequent response to this challenge,\textsuperscript{160} Mann & Butenas vehemently stood by their model, emphasising that it included platelets and that it was therefore physiological. Again, one should challenge this astounding statement. Mann & Butenas note in this response, that the whole blood was "minimally altered" suggesting that the platelets were not activated and no detail on the state (or indeed presence) of platelets in the synthetic model was provided. The presence of unactivated platelets and the absence of tissue factor does not make this a physiological system representative of the procoagulant state, as already discussed here. In this response, Mann & Butenas seem to suggest that the fact that rFVIIa cannot activate unactivated platelets in a resting system somehow proves that the therapeutic mechanism of action of rFVIIa is TF dependent.

In another paper published in the same year, Butenas and colleagues again presented results which they asserted were proof that the mode of action of rFVIIa was TF-dependent.\textsuperscript{161} An important point of difference between this and their earlier report is that in this paper it is emphasised that, in the absence of TF, pharmacological doses of rFVIIa were unable to generate detectable levels of thrombin generation on the surface of \textit{activated} platelets. This finding clearly adds some weight to their claim of a requirement for TF in the mechanism of action of pharmacological doses of rFVIIa; but is in direct contrast to evidence provided by other researchers.\textsuperscript{151} Commenting on this paper, Lisman & de Groot\textsuperscript{162} suggested that the failure of rFVIIa to mediate
thrombin generation on the surface of the activated platelets may be a result of the non-physiological method of activation employed by Butenas et al.

Weighing up the evidence from the published literature, and considering the clinical efficacy of rFVIIa in haemophiliac and other patients with impaired thrombin generation, a TF-independent mechanism of rFVIIa action seems the most plausible. This is not to say that TF is not important, indeed vital for the initiation of coagulation. However, once the first thrombin is generated and platelets become activated, it would seem reasonable that TF would cease to be principally influential – having served its role in both initiating and localising the subsequent thrombin burst on the recruited activated platelets. Combined with the knowledge that pharmacological doses of rFVIIa are able, independently of TF, to mediate thrombin generation directly on the surface of activated platelets, it seems wholly reasonable that beyond the initiation phase, high-dose rFVIIa may act in a primarily TF-independent manner.

A recent review indicates that the prevailing view of the mechanism of action of high dose rFVIIa does favour a principally TF-independent role, which essentially overrides the propagation phase. Rather than focussing on interaction of rFVIIa with TF, current evidence indicates that rFVIIa acts directly on the activated platelet, via low-affinity non-physiological binding. This concept is based on assessment of the body of evidence presented here, and specifically on the ability of high-dose rFVIIa to mediate thrombin generation directly on the surface of the activated platelet, even in the absence of the tenase (FVIIa/Ixa) complex.
In 2001, a study was published which demonstrated that high-dose rFVIIa was capable of increasing the initial rate (but not maximal) of thrombin generation and decreasing the lag time for platelet activation. These findings are in accordance with the reported beneficial role of rFVIIa in thrombocytopenic patients, as their limited platelets therefore would be activated more quickly due to the rapid thrombin generation. Initial studies by Wolberg and associates in 1999 suggested that the initial rate, rather than the total amount or maximal level, of thrombin generation may be the most important determinant of the structure and integrity of the resultant fibrin clot.

The evidence provided by Kjalke et al that rFVIIa appears to increase the initial rate of thrombin formation is therefore very encouraging in terms of the potential for stable clot formation. The apparent ability of rFVIIa to increase the amount of thrombin generated in the initial minutes may be vital in facilitating the timely development of a haemostatic plug in a range of bleeding situations, including trauma induced haemorrhage. Importantly, this study provides evidence that rFVIIa may be expected to be efficacious even in the presence of thrombocytopenia, a likely complication of traumatic haemorrhage due to the development of dilutional coagulopathy. Clearly, early administration of rFVIIa would be expected to reduce the amount of blood loss and hence development of coagulopathy, as a clot would be formed earlier. The findings of this thesis, and published data which are discussed in more detail later, support the theory that early administration of rFVIIa may confer considerable benefit over late administration.

Further support for the generation of more stable clots in the presence of rFVIIa was provided by Lisman and colleagues in 2002 as it was demonstrated that clots formed
in the presence of rFVIIa were more resistant to fibrinolysis.\textsuperscript{167} Lisman \textit{et al} used an \textit{in vitro} model system involving plasma from haemophilia A patients. They demonstrated that rFVIIa led to a significant prolongation of clot lysis times of plasma from haemophilia A patients and that this effect was completely reversed by addition of an inhibitor to TAFI. In addition, this study demonstrated that rFVIIa consistently produced a significant reduction in clot formation time, which is attributed to competitive binding overruling the inhibitory effect of zymogen FVII.

The authors concluded that rFVIIa brings about haemostasis in haemophilia by facilitating an increased speed of clot formation and, through activation of TAFI, the formed are better protected from fibrinolysis. An important note from this study is that the effect of rFVIIa on downregulation of fibrinolysis was found to be dependent on the concentration of TF and the authors therefore suggested that the role of rFVIIa in activating TAFI may be particularly significant in situations where there is a large expression of TF, such as in the case of severe vascular injury.

In 2003, a study was published which specifically assessed the permeability of fibrin clots formed from FVIII or FIX deficient plasmas and the effect of rFVIIa on permeability in these situations.\textsuperscript{168} They found that clot permeability increased as the concentration of FVIII or FIX decreased and also importantly that therapeutic levels of rFVIIa were able to completely normalise clot permeability. They therefore proposed that a complete fibrin burst, not seen in the haemophilias in the absence of rFVIIa, is required for the formation of an effective fibrin clot, with a tighter fibrin network, which may be more resistant to fibrinolysis. These findings lend further support to the concept that rFVIIa leads to the formation of more stable fibrin clot structures.
A detailed study of the effects of rFVIIa on the structure of the fibrin clot was published in 2005. The authors employed an in vitro coagulation model of the haemophilia B condition, known to have a delayed onset and rate of clot formation and impaired clot structure and stability. The results clearly demonstrated that rFVIIa was able to decrease the lag to onset, and increase the rate, of clot formation. The study also found both a significant decrease in the delay to onset of thrombin generation and a significant, dose-dependent, increase in the rate of thrombin generation when rFVIIa was added to FIX deficient plasma. Through assessment of clot turbidity, the authors of this study suggested that haemophilia B conditions led to the formation of abnormal, deficient, fibrin clots while addition of rFVIIa was credited with generation of more normal, improved structured, clots. Scanning electron microscopy (SEM) was enlisted to confirm these observations. SEM of clots derived from FIX deficient plasma allowed visualisation of an altered clot structure with notably thicker fibres than found in a normal clot. SEM of clots produced in FIX deficient plasma in the presence of rFVIIa found the structure to be similar to that found in normal clots, with thinner fibres than seen in the FIX deficient plasma. The authors cited a previous study in which it was shown that clots containing thicker fibres were more susceptible to fibrinolysis. To determine whether clots formed in the presence of rFVIIa really were more resistant to fibrinolysis than those formed in FIX deficient plasma in the absence of rFVIIa, susceptibility of the clot to fibrinolytic enzymes was assessed. rFVIIa was found to produce clots more resistant to fibrinolysis, compared to haemophilic plasma. Notably, TAFI was shown to have no effect on the generation or lysis of clots in this model system, which the authors suggest may be due to differences in the model systems used by different research
groups and acknowledge that activation of TAFi is likely to be another aspect to the haemostatic role of rFVIIa.

The authors of this study noted that rFVIIa had far more pronounced dose-related effects on thrombin generation than on fibrin clot formation, suggesting therefore that the factors governing clot formation are more complex that simply the onset and rate of thrombin generation. The differences seen in clot structure in the presence and absence of rFVIIa suggests that a full thrombin burst, present in rFVIIa treated haemophilia B plasma, plays an essential role in determining the structure and integrity of the clot.

A further potential mechanism by which rFVIIa may serve to explain the therapeutic effects of rFVIIa in patients with both normal and low platelet counts was suggested by Lisman et al in 2005. Using a flow model, the authors were able to effectively illustrate that rFVIIa was associated with an increase in both platelet adhesion and activation.¹⁷⁰

In reality, it is likely that rFVIIa acts both in a TF-dependent manner, assisting in and possibly augmenting the initiation of coagulation; and a TF-independent manner, directly on the platelet surface. Based on the published data, it would appear that, provided endogenous FVII(a) is able to bind TF, coagulation will be initiated and platelets activated. The main effect of pharmacological doses of rFVIIa under these conditions would be directly on the surface of activated platelets, independent of TF. The published literature that has supported a TF-dependent role has generally employed blockade or inhibition of TF or the TF/FVIIa complex.¹⁵³, ¹⁵⁷, ¹⁵⁸

Considering our current understanding of the coagulation system, with an absolute
requirement for TF/FVIIa for initiation in vivo, the blockade of this complex shows nothing of the mechanism of action of pharmacological doses rFVIIa. With the initiation phase blocked, rFVIIa would not be able to exert any effects which usually occurred subsequently to initiation, since the required activated platelets would not have been generated in the initiation phase. Conversely, when endogenous levels of FVIIa, in complex with TF, initiate coagulation, platelets are activated and the required substrate for exogenous rFVIIa is therefore provided. The result is a huge thrombin burst on the platelet surface. As a result of the increased thrombin generation in the presence of rFVIIa, a well-structured clot with tight fibres results, which is more resistant to fibrinolysis.\(^{168}\) In addition to the innate fibrinolytic resistance of the clot conferred by the enhanced structure, the increased thrombin generation in turn leads to maximal expression of TAFI and FXIII, which serve to further limit fibrinolytic potential.\(^{167}\) The result is a strong clot, formed in a timely manner, which is resistant to fibrinolysis.\(^{163}\)

In 2003, Meng and colleagues produced an important paper looking at the effects of acidosis and hypothermia on the efficacy of pharmacological doses of rFVIIa.\(^{171}\) As already discussed, these are both commonly encountered complications in the trauma victim and therefore an understanding of their likely effects on rFVIIa efficacy is particularly important. Using in vitro models, the authors were able to provide evidence that hypothermia had little overall effect on the efficacy of rFVIIa. Conversely, a decrease in pH from 7.4 to 7.0 resulted in over 90% reduced efficacy of rFVIIa in one model system, representing the TF-independent system, and a 60% reduction in the other model, representing the TF-dependent system. The authors concluded that, based on this limited in vitro data, one may expect rFVIIa to be
effective in hypothermic cases, but that efficacy may be reduced by increasing levels of acidosis.

To illustrate the importance of the effect of acidosis on haemostasis, the European recommendations on the use of rFVIIa state that acidosis should be corrected before treating with rFVIIa.\textsuperscript{172}

2.5.4 rFVIIa in trauma and surgery

Generally, the developing coagulopathy in trauma is threefold. One aspect of what is essentially a vicious cycle is that there is a lack of procoagulant signalling, due to consumptive and dilutional coagulopathy. A further component of the cycle is that, due again to dilution and consumption of platelets, a thrombocytopenic state develops. Completing the cycle, there is an increase in activity of the fibrinolytic system, due to release of high concentrations of various proteolytic enzymes which lyse the forming fibrin thrombus.\textsuperscript{97}

rFVIIa is able, to some extent, to compensate for each of these contributing factors.\textsuperscript{173} Through direct action upon the activated platelet, rFVIIa is able overcome a lack of coagulation proteins FVII, FVIII, FIX and FXI,\textsuperscript{149,152} supplies of which may have been exhausted either through dilution, leakage or consumption. A low platelet count, with similar aetiology, may be overcome as rFVIIa has been shown in thrombocytopenic patients to not only increase thrombin generation on the activated platelet surface but also to increase the rate of activation of platelets, thus essentially
increasing the concentration of activated platelets in the affected area.\textsuperscript{164} The increase in fibrinolysis may be overcome, as already detailed, through activation of TAFI.\textsuperscript{90}

Since its introduction in 1988, rFVIIa has seen a multitude of uses beyond its initially envisioned role. It is beyond the scope of this thesis to provide a detailed review of the range of clinical uses of the drug, therefore the remainder will focus on the reported uses of rFVIIa in controlling major hemorrhage in surgical and trauma patients.

In 1999, Kenet \textit{et al} presented the first reported off-label use of NovoSeven® in a trauma patient.\textsuperscript{24} The subject of the report was 19 year-old Israeli soldier who had sustained a high-velocity rifle wound which had perforated the inferior vena cava. Surgical intervention and repeated packing of the site failed to control the bleeding which was confounded by worsening coagulopathy and hypothermia. With a rate of blood loss of 500ml/min, the outcome was considered to be inevitably fatal. Given the dire prognosis, a decision was made to attempt a final, potentially life saving, measure and a single 60 ug/kg dose of rFVIIa was administered. Within 10 minutes of injection, coagulation parameters were markedly improved and blood loss fell to just 10-15ml/min, though oozing from wound surfaces continued. A second 60ug/kg dose of rFVIIa was administered one hour after the first. Following this second dose, oozing completely stopped and coagulation parameters returned to normal ranges. There was no incidence of re-bleeding in this patient.

Also in 1999, White \textit{et al} reported successful use of recombinant factor VIIa in two patients suffering intractable intra-abdominal hemorrhage following surgery.\textsuperscript{174} In
both cases, patients experienced persistent heavy bleeding following abdominal surgery and in one case showed no response to treatments such as tranexamic acid and desmopressin. Both patients received a total of 2,900ug/kg doses of recombinant factor VIIa in final attempts at controlling the bleeding. There was an immediate cessation of bleeding and decrease of PT measurements in both patients. Neither patient required any further blood product treatment and one made a full recovery. The other died from multiple organ failure that was unlikely to have been attributable to administration of recombinant FVIIa.

In 2000 Vlot et al reported the successful use of rFVIIa in a post-surgical bleeding patient.\(^{175}\) The patient, a 59 year-old male, underwent three instances of surgical intervention and was administered tranexamic acid and octreotide, yet the bleeding persisted. Despite no pre-existing coagulopathy, rFVIIa was administered. Dosage in this case was again 90ug/kg every two hours, however this patient received doses in this time frame up to 21 hours. The patient’s requirement for blood product fell dramatically in the 16 hours post administration of the first dose of rFVIIa and, following eventual embolisation of the vessel, no re-bleeding occurred. As the patient in this case received doses of both tranexamic acid and octreotide relatively closely to administration of rFVIIa the authors were unable to exclude the possibility that these drugs contributed to the noted cessation of bleeding. It is also notable that on transportation of the patient, prior to embolisation of the vessel, rFVIIa administration was stopped and re-bleeding occurred. It is unfortunate that it is not possible to discern whether this re-bleeding was due to transportation or withdrawal of the drug. Further encouraging outcomes following administration of rFVIIa in patients with surgical complications were reported in 2000.\(^{176,177}\) Laffan & Cummins\(^{176}\) reported
that two patients were recovered from persistent surgical bleeding by administration of 80-90 ug/kg of rFVIIa followed by a background infusion containing low dose rFVIIa following arrest of blood loss. Blood loss dramatically reduced in both patients soon after administration of the drug, allowing their discharge from the intensive care unit. Al Douri et al.\textsuperscript{177} reported the successful use of a 30ug/kg dose of rFVIIa in patients suffering excessive uncontrollable bleeding or oozing during or after undergoing heart valve replacement surgery. Similar to the previously described cases, Al Douri and colleagues reported marked decreases in blood loss in the period following rFVIIa administration, without any adverse effects.

\textbf{2.5.5 Preclinical trauma studies of rFVIIa}

As a result of the initial anecdotal reports of successful use of rFVIIa in trauma and intractable surgical bleeding, combined with a considered appreciation of the understood mode of action of the drug, the first prospective, blinded, controlled animal study involving rFVIIa in trauma was undertaken and presented in 2001.\textsuperscript{178} The study involved 10 crossbred swine, which were subjected to an isovolaemic, hypothermic haemodilution; representative of the coagulopathic state commonly encountered in trauma patients. Once this imposed coagulopathy was established, animals were subjected to liver trauma, equivalent to a grade V liver injury, which carries a 50\% survival rate in humans. The liver injury was induced by cutting through the liver with a modified surgical tool and allowing the animal to bleed freely for 30 seconds. The blood lost during this time was collected by suction and in pre-weighed sponges. Measurement of the amount of blood collected was recorded. Depending on their treatment group, animals received either a 180ug/kg dose of rFVIIa or placebo (saline
control) after the 30 second free bleeding phase. Packing was used in both groups and experimenters were blinded to the treatment groups. After a further five minutes, intravenous fluid resuscitation was initiated, with the goal of achieving pre-injury mean arterial blood pressure. The endpoint of the study was survival to 60 minutes post injury, or death, whichever occurred first.

Martinowitz et al reported that all animals in both groups survived the full 60 minute post injury period, though this was most likely due to the rapid and considerable gauze packing deployed 30 seconds after the injury in both groups. This packaging was deemed necessary to prevent 100% fatality from the model, however it had the effect of meaning that neither group were truly ‘untreated controls’ and therefore any effect seen in the rFVIIa group cannot be considered to be solely due to the drug, limiting any conclusions to the use of drug as an adjunct to packing. Furthermore, as it transpired that 100% of animals in both groups survived, this liver packing may have prevented the detection of any potential effect of rFVIIa on survival. Statistical analysis of the results revealed that there was significantly (46%) less blood loss in the group treated with rFVIIa than in the group that did not receive the drug. There was not found to be any difference in the volume of resuscitation fluid required between the two groups. In terms of laboratory tests, the rFVIIa treated group were found to have a significantly shortened prothrombin time compared to the group which did not receive the drug, indicating that coagulation was occurring more quickly in the presence of rFVIIa. Interestingly, there was no detected effect of rFVIIa treatment on thromboelastography (TEG) parameters.
Taking the lack of effect seen on TEG parameters with the lack of any change in other
measures of systemic activation of coagulation in the rFVIIa treated group,
Martinowitz and colleagues stated that “there was no identifiable laboratory evidence
of systemic activation of the clotting cascade by rFVIIa”. This statement is
misleading, suggesting to the reader that if alterations in TEG parameters had been
found, this would have indicated widespread clotting. This is not the case. TEG
measures the potential of the blood to clot, but localisation in vivo is determined by
local expression of tissue factor and/or activated platelets. The presence of changes in
TEG would have indicated that, if TF was expressed (i.e. if vascular damage had
occurred) or if activated platelets had accumulated, increased clotting kinetics would
likely have been seen at that site. The failure of Martinowitz and colleagues to detect
any significant change in TEG parameters would indicate that rFVIIa had not in fact
increased the coagulant potential of the blood at all. More likely is that there may
have been some kind of flaw in TEG protocol, which is not described in detail at any
point in the paper. Selection of a particularly potent activator of coagulation (e.g.
Celite or Kaolin) may have excessively activated the TEG reaction so that no rFVIIa-
mediated increases in activity were detectable. There is no reference to the delay in
analysis of the TEG samples or the method of sampling or storage. All of these
factors may have led to artefactual activation of coagulation within the samples,
masking any differences between groups. As the paper does not provide any of the
detail required, it is unfortunately not possible to determine whether there was or was
not an effect of rFVIIa and what if anything was masking this effect.

Despite the uncertainties regarding the effect of rFVIIa in the laboratory tests and the
obvious limitations imposed by having only 60 minute post injury period, this paper
clearly showed a significant effect of rFVIIa, despite hypothermia and haemodilution, as an adjunct to gauze liver packing in decreasing the volume of blood loss, when both rFVIIa and gauze packing are applied early.

A further animal study utilising anaesthetised swine to assess the efficacy of rFVIIa following severe liver injury was published in abstract form in 2001 and later in full. Unlike the study from Martinowitz et al., in which coagulopathy was induced prior to generation of a grade V liver injury, this work involved noncoagulopathic pigs. The drug (either 150ug/kg rFVIIa or buffer) was administered 30 seconds after induction of liver injury but, unlike the study discussed above, this was the only haemostatic therapy administered throughout the study, with no simultaneous packing of the liver. Following administration of the drug, animals were resuscitated to their baseline MAP, which was maintained with repeated infusions of lactated Ringer's solution for up to 2 hours after injury, beyond which point animals were humanely killed. No rationale was provided by the authors for their selection of a timescale of 2 hours for this study, though it may have been selected to be relevant to anticipated civilian evacuation times, or may be attributable to the anticipated high mortality rate from a severe liver injury with rFVIIa as sole therapy. Despite the apparent severity of the model, all animals survived in both the rFVIIa and control groups, thus it was not possible to discern any effect of rFVIIa on survival. Notably, there were found to be no significant differences between treated and untreated animals in terms of mean arterial pressure over the time course of the study, volume of blood lost or volume of lactated Ringer's solution required to maintain the baseline blood pressure.
Clearly, these findings are quite different from those of Martionowitz et al, in which rFVIIa was shown to have a significant beneficial effect in terms of reducing the volume of blood loss. It is likely that the stark difference in results of the two research groups would have been caused by the significant differences in the studies. Most prominent were the use by Schreiber et al\textsuperscript{179} of rFVIIa as sole therapy, compared to adjunctive therapy with liver packing, in noncoagulopathic, as opposed to coagulopathic pigs, in the case of Martinowitz et al. It is also perhaps worthwhile to note that the dose of rFVIIa used by Schreiber and colleagues was lower than that used in the study by Martinowitz and associates, potentially indicating a dose reliance response. Schreiber et al suggest a further possible cause of the difference in results may be that animals in the study by Martinowitz et al were severely hypotensive at the time of liver injury, thereby reducing blood flow in the liver and thus potentially reducing the overall effect that rFVIIa would be required to have compared to that in normotensive conditions.

Several of the differences between the studies of Martinowitz et al\textsuperscript{178} and Schreiber et al\textsuperscript{179} were addressed in a further paper published by Schreiber et al, in 2002.\textsuperscript{180} In this study, a grade V liver injury was induced in hypothermic, coagulopathic, anaesthetised swine as an adjunct to liver packing. The study design was therefore very similar to that which had been used by Martinowitz et al in 2001, in an attempt to investigate the potential causes of the differences between this study and the earlier paper by Schreiber et al. In addition to using coagulopathic animals and adjunctive therapy, this study also looked at the potential of a dose response on rFVIIa efficacy, splitting animals into three treatment groups wherein animals were randomised to receive either
buffer or rFVIIa (180ug/kg or 720ug/kg). Drug administration and packing of the liver took place 30 seconds after the injury.

Since the study design used in this study was closer to that of Matinowitz et al, the results obtained were far more comparable too. Groups which received rFVIIa (both doses) had a significantly higher MAP and significantly lower volume of blood loss than those that received buffer control. There was also a decreased volume of fluid resuscitation (almost half) required in treated groups compared to controls, though this was not significant due to large variations within the groups. Neither overall survival nor survival time differed significantly between the three groups. This is particularly important as no groups achieved 100% survival in this study, with 60, 70 and 80% survival in the control, 180ug/kg rFVIIa and 720ug/kg rFVIIa groups respectively. Therefore, in this study the opportunity did exist for rFVIIa to have exerted an effect on overall survival or survival time.

There were no significant differences between the two rFVIIa treated groups in any of the outcome measures, indicating that the drug does not respond with greater efficacy to doses higher than 180ug/kg, in this model. There was however a significantly higher measured factor VII clotting activity in the group treated with the higher dose of rFVIIa. Schreiber and associates suggested that the lack of effect of the increased dose, despite increased factor VII activity, may be due to saturation of ‘the system’.

Based on current understanding of the mode of action of high dose rFVIIa, the lack of exposed tissue factor would be unlikely to prevent increased efficacy of rFVIIa as the drug is known to primarily act directly on activated platelets, through a tissue-factor independent mechanism. Therefore, either the rFVIIa binding sites on the platelets
must be saturated, or some/all of the zymogen factors II, V, VIII, IX, X must have been depleted, preventing further assembly of coagulation apparatus. This is in keeping with the finding in the same paper that there was no evidence of additional thrombin generation (through thrombin-antithrombin complex assay) in the higher dose treatment group. This level of detail on the mechanisms underlying dose response of rFVIIa is not addressed in the paper.

The overall conclusion from the study corroborates the findings of Martinowitz et al in 2001, that 180ug/kg rFVIIa as an adjunct to liver packing is able to significantly reduce the volume of blood loss and increase MAP following grade V liver injury in coagulopathic swine.

The paper suffers slightly from the fact that a number of variables were changed in this model compared to the initial studies from this group\textsuperscript{179} and Martinowitz et al.\textsuperscript{178} including the use of coagulopathic animals, the use of gauze packing as an adjunct to rFVIIa and 180ug/kg and 720ug/kg doses of rFVIIa as opposed to 150ug/kg rFVIIa. While these limitations do not detract from the overall conclusion from this valid study, the fact that such a number of variables were changed simultaneously makes it impossible to definitively determine which parameter(s) were primary influences on the differences noted in their studies on non-coagulopathic swine compared to this paper and the findings of Martinowitz et al.

A further two prospective, randomised controlled trials involving anaesthetised swine were reported in 2002,\textsuperscript{181,182} both from the same research group as the initial controlled animal study.\textsuperscript{178,182} In both trials, animals received Grade IV liver injuries, wherein haemorrhage was initiated by avulsion of the left median lobe of the liver.
The application of a Grade IV, rather than Grade V, liver injury enabled one of the limitations of the initial study to be overcome, removing the requirement for gauze packing of the liver and thus making rFVIIa the sole treatment. Both studies compared the effectiveness of a single rFVIIa dose of 180 ug/kg compared to placebo, while the paper from Jeroukhimov et al also reported on a higher dose of rFVIIa (720 ug/kg). In both studies, a 10% decrease in mean arterial blood pressure (equivalent to Grade III haemorrhagic shock) was the trigger point for administration of the drug. The time elapsed from injury to this trigger point was 30 seconds or less in both studies. Lynn et al continued the study for just one hour following injury while in the study reported by Jeroukhimov et al, animals were allowed to survive for up to two hours.

Lynn et al saw a significant decrease in MAP in the placebo group following haemorrhage and also in placebo compared to rFVIIa treated groups at each time point tested following administration of the drug. Prothrombin time was found to be significantly shortened in those subjects which had received rFVIIa, compared to placebo controls. A decrease in volume of blood loss in the rFVIIa treated group and an apparent decreased mortality was noted, with none of the rFVIIa treated animals dying in the hour study period, while 43% of the placebo treated animals died within this time. However, neither the differences in volume of blood loss nor mortality were found to be statistically significant. This is possibly due to the small number of animals used in the groups and the short duration of the study.

Within the first hour of the study reported Jeroukhimov and associates, mortality stood at 50% in the placebo group, 25% in the 180ug/kg rFVIIa dose group and 0% in the
720ug/kg rFVIIa dose group. In the second hour, one further animal died and this was from the 720 ug/kg rFVIIa dose group. Survival in the 720ug/kg rFVIIa group was significantly higher than placebo at both the one hour and two hour stages. There was no statistically significant difference between survival in placebo and lower (180ug/kg) rFVIIa groups. Similarly, volume of blood loss in the higher dose rFVIIa (720ug/kg) group was significantly lower than that in those receiving placebo, while there was no statistical significance in the differences between placebo and 180ug/kg dose rFVIIa groups. Prothrombin time was significantly shortened in both groups which received rFVIIa, compared to placebo controls.

Sondeen and colleagues published the results of a further model of severe haemorrhage in anaesthetised swine in 2004. This study differed from all previous studies in that it involved a pure arterial insult, (rather than combined liver injury), which was achieved through creation of a 2.0mm aortotomy. The aim of the study was to determine whether rFVIIa was able to increase the blood pressure at which rebleeding occurred in a high-pressure arterial haemorrhage, an important feature in many trauma patients. Designed to establish proof of principle, this study was not designed to be clinically representative and as such animals were pre-treated with rFVIIa (180ug/kg or 720ug/kg) or placebo, 5 minutes before arteriotomy. 5 minutes after the haemorrhage resuscitation was undertaken with lactated Ringer’s solution at a set rate. The blood pressure was monitored and the MAP at which re-bleeding occurred was noted. The study showed that rFVIIa significantly increased the MAP at which re-bleeding occurred compared to animals which received placebo and this effect did not appear to be dose-dependent. The authors also reported a decreased haemorrhage volume in the rFVIIa treated animals. While the decrease in
haemorrhage volume did not reach statistical significance, there was a significant increase in an adverse metabolic consequence (elevated lactate) in the control group, and a trend toward lower base excess in this group. The study was not designed to detect effect on survival and as such there was no statistical significance, but there was a trend towards an increase in survival time and in number of animals surviving, which may be dose-dependent. An important conclusion which can be drawn from this study is that pre-treatment with rFVIIa in non-coagulopathic swine permits resuscitation at a higher blood pressure, by increasing the stability of the clots. The risk of re-bleeding and the volume of re-bleed haemorrhage is therefore reduced. These findings are supported by the work of He et al and Lisman et al, who demonstrated the formation of a stronger clot, more resistant to fibrinolysis in the presence of rFVIIa in in vitro studies.

A further swine model of severe haemorrhage designed to test the efficacy of rFVIIa in non-coagulopathic swine was published in 2005. Part of the study was an ex vivo dose-escalation investigation, in which animals were uninjured. This aspect of the study is discussed elsewhere in the thesis. The dose of in vivo rFVIIa was increased gradually to 720ug/kg after which, the injury phase commenced. In the injury phase, animals, which had been effectively pre-treated with 720ug/kg rFVIIa (or placebo), were subjected to severe haemorrhage, created by laceration of the liver. No packing of the liver or resuscitation was administered and animals were monitored for a maximum of 60 minutes post-injury. rFVIIa was found to have no significant effect on volume of blood loss, survival time or overall number of survivors, with 33% of animals surviving 60 minutes in the rFVIIa treated group compared to 0% in the control group. rFVIIa was found to significantly increase 30 minute post-injury MAP.
compared to placebo. This finding is complimentary to that of Sondeen and colleagues,\textsuperscript{183} and supports the theory that rFVIIa may promote the formation of stronger, more resistant clots. In their discussion of this study and suggesting why rFVIIa may not have reduced blood loss, Pusateri \textit{et al}\textsuperscript{184} suggested that rFVIIa may be efficacious in improving haemostasis only in the presence of coagulopathy; noting differences in outcome from the various animal models discussed in the literature. While this is an interesting concept, it is in stark contrast to the general pattern of response seen in clinical studies, described shortly.

In 2005, Klemcke and colleagues published the results of a further swine model of liver injury,\textsuperscript{185} in an attempt to efficiently evaluate the efficacy of rFVIIa, given the conflicting results obtained from the previous studies. They suggested that their use of a longer observation period of 4 hours and larger group sizes of 18 animals per group would enable more definitive interpretation of the data. Essentially reproducing the initial study by Martinowitz and colleagues,\textsuperscript{178} this study used a grade V liver injury preceded by the artificial creation of coagulopathy through haemodilution and induction of hypothermia in this model. Similar to previous studies, rFVIIa (180ug/kg or 720ug/kg) or placebo was administered 30 seconds after liver injury as an adjunct to packing. The study found no effect of rFVIIa on survival time, percent survival or blood loss; while laboratory measures of coagulation were significantly improved in the presence of the drug. While only data on survival time to 4 hours were reported, the authors claimed that significance similarly was not reached when analysed at 1, 2, or 3 hours. The authors concluded that in this model, at the doses used, rFVIIa was ineffective and expressed some concern that \textit{in vitro} measures of coagulation were indicative of efficacy given the lack of \textit{in vivo} efficacy. An interesting finding from
this study was that, while not all animals became acidotic during the study, those that did were not found to respond any less well than those which were not acidotic. This suggests that, at least to pH 7.1, rFVIIa may retain efficacy. This finding is in contrast to the in vitro evidence provided by Meng and colleagues,\textsuperscript{171} previously discussed, which suggested that efficacy of rFVIIa may be expected to decrease with increasing acidosis.

A further study looking specifically at the efficacy of rFVIIa in reducing blood loss in non-coagulopathic swine was published in 2007.\textsuperscript{186} Animals were subjected to a multiple injuries, including laceration of the liver and, 15 minutes post-injury, received 120ug/kg rFVIIa as a single dose, or placebo. Animals received intravenous fluid resuscitation and were observed for 2 hours. The study demonstrated a significant decrease in volume of blood loss in animals treated with rFVIIa, compared to controls, importantly demonstrating that rFVIIa has the capacity to act in the absence of imposed pre-existing coagulopathy. Notably, there was a longer (15 minute) delay to administration of rFVIIa than that used in previous studies, meaning that a coagulopathic state may have developed in response to the trauma to a greater extent in this study than in the other non-coagulopathic models discussed here. Due to the small group sizes, no effect on survival was noted.

Recognising the reported clinical efficacy of rFVIIa from case reports in the literature but acknowledging the absence of convincing evidence for improving survival in randomised controlled trials, our group at Dstl Porton Down conducted a study in swine, which was published in 2007.\textsuperscript{25} The aim was to determine whether, in a large animal model of severe incompressible arterial haemorrhage (previously described by
Sondeen et al.\textsuperscript{183} treatment with rFVIIa (at a single dose of 180\textmu g/kg) could improve survival and reduce volume of blood loss. This study used the longest observation period yet reported, monitoring animals for up to 6 hours post-injury. This duration was selected as it represented a realistic timeline over which rFVIIa may be relied on prior to evacuation in the military environment. A further aspect of this study was to determine the effects of hypotensive versus normotensive resuscitation on the efficacy of rFVIIa in this model. Survival time was found to be significantly prolonged overall with rFVIIa, and specifically in hypotensively resuscitated animals which received rFVIIa compared to similarly resuscitated animals which received placebo. No significant effect of rFVIIa compared to placebo was found on survival in animals which were normotensively resuscitated when analysed separately. In addition to demonstrating improved survival times, this study found an increase in number of animals surviving to both 2 and 6 hours in those treated with rFVIIa, compared to controls. A significant decrease in volume of blood loss was also noted in animals treated with rFVIIa (regardless of resuscitation strategy) compared to controls.

This study clearly demonstrated efficacy of rFVIIa, alongside hypotensive resuscitation, in improving survival and decreasing blood loss in a severe arterial haemorrhage model, over a clinically relevant timeline. This was the first study which directly considered and tested the effects of resuscitation regimen on efficacy of rFVIIa, arguably providing a more clinically relevant model than those which involve immediate liver packing, or absence of any form of resuscitation. The main limitation of the study comes from the arguable pre-treatment of subjects with rFVIIa (the drug was administered midway through the haemorrhage protocol), clearly detracting from the claim of clinical relevance. It was necessary in this initial proof-of-principle study
to “pre-treat” due to the lack of experience with this model and the use of two different resuscitation regimes. Based on the results of this initial study, further work is currently underway focussing on the efficacy of rFVIIa and hypotensive resuscitation in a similar aortic injury model, with rFVIIa administration 5 minutes after injury. The results of this study will provide more firm evidence on the efficacy of rFVIIa and hypotensive resuscitation in a truly clinically relevant model.

It is apparent in reviewing the ten published animal studies of rFVIIa in traumatic haemorrhage that there exists some contradictory outcomes. Some studies have shown a significant beneficial effect of rFVIIa on survival, while the majority have not. A higher proportion of studies found a significantly decreased volume of blood loss in rFVIIa treated animals, but again this was not consistently reported.

While the varied results of the pre-clinical animal studies, all of which have been conducted in anaesthetised swine, may appear disheartening, consideration of possible explanations for the differences permits a more positive outlook. Considering the apparent lack of effect on survival first, it should be noted that a number of studies used very small group sizes, and/or were not designed to consider survival as a primary outcome. In other studies, there was no or limited scope for a beneficial effect of rFVIIa to be demonstrated as there was high survival in the control group. The two studies in which rFVIIa was shown to improve survival were in clinically relevant models, where there was no artificially induced coagulopathy, while the one remaining study where rFVIIa was found to be ineffective was conducted on swine in which coagulopathy had been artificially imposed prior to
drug administration. It is notable that in previous incarnations of this model, high survival had been noted in the control groups, while survival was considerably lower across all groups in this study. The reasons for this difference are not clear but may be due to the extended observation period. Over the 4 hour study period, the imposed coagulopathy may have had considerable deleterious effects on the general physiology of the animals.

When considering the overall findings of the studies in non-coagulopathic animals in this way, it is reasonable to state that there is mounting evidence the rFVIIa is able to significantly increase survival times and number of animals surviving to at least 6 hours. The situation is less clear in coagulopathic animal studies, where the confounding factor of more general adverse physiological effects of the imposed coagulopathy on the experimental animal is difficult to mitigate.

When considering the efficacy of rFVIIa in terms of reducing the volume of blood loss, it is necessary to exclude some studies in which this was not a primary outcome measure. The main rationale for exclusion was that in both studies, animals were pre-treated with rFVIIa. This could be viewed as a bias toward efficacy and inclusion in the analysis alongside those studies where rFVIIa was administered after injury would result in a combination of significantly heterogenous studies, weakening the observations.

The majority of the remaining studies, including two of the three with coagulopathic subjects, did demonstrate a decreased volume of blood loss in animals treated with rFVIIa compared to controls. One of the studies which did not find a significant
effect of rFVIIa on volume of blood loss\textsuperscript{181} (but did note a trend toward significance) was perhaps too severe a model as rFVIIa was used as the sole haemostatic intervention, with no resuscitation administered. Only one other paper, which came from the same research group, used such a model\textsuperscript{182} and while rFVIIa was found to have a significant effect on volume of blood loss in this study, significance was only seen with a higher dose of rFVIIa than used by Lynn \textit{et al} (and a number of the other studies detailed here). The reasons for the failure of rFVIIa to decrease the volume of blood loss in the two remaining studies\textsuperscript{178,179} are less clear; however the fact remains that in the majority of studies, rFVIIa has been shown to be effective in reducing the volume of haemorrhage compared to placebo.

Other considerations from the ten published animal studies, which it is beyond the remit of this thesis to discuss in detail, include the variability in evidence for a dose-response relationship and the clear message from all ten studies that there has been no apparent increased risk of systemic thrombosis.

\subsection*{2.5.6 Clinical experience with rFVIIa in trauma}

Following the report by Kenet \textit{et al}\textsuperscript{24} of the young Israeli soldier and based on the results of the first controlled animal trial, outlined above, the Ethical Committee of the Israeli Ministry of Health approved the use of rFVIIa in patients suffering massive, life-threatening haemorrhage as a result of trauma or surgery. Under this approval, six further patients (in addition to the Israeli soldier) received rFVIIa in Israel and Denmark between June 1999 and January 2001. Martinowitz \textit{et al} presented the outcomes of this cohort of trauma patients treated with rFVIIa.\textsuperscript{187} The group comprised three males, all of whom had suffered penetrating injuries and three
females, all of whom had sustained blunt injuries. Their ages ranged from 17-45. In their analysis, Martinowitz and colleagues included the case of the 19 year-old Israeli soldier previously reported by Kenet et al. bringing the study cohort to seven. None of the patients had responded to surgical intervention or replacement therapy and all became coagulopathic. Patients received their first dose of rFVIIa between 4 hours and 30 days of time of injury. Initial doses ranged from 40-120ug/kg; four patients were given a second dose (range 60-80 ug/kg) and two of these received a third dose (60 or 80 ug/kg). No information was provided in the paper as to the timing of subsequent doses, however the author states that diffuse bleeding ceased “within 5 to 15 minutes after administration of one to three doses”. Requirement for blood products dropped markedly in all patients, from a mean of 36.5 packed cell units to just 2 units. Similarly, PT and APTT measurements shortened toward normal following administration of rFVIIa. Four of the seven patients survived, one died at the end of surgery and two died four weeks after rFVIIa administration. The patient who died during surgery had been severely coagulopathic, hypothermic and acidotic for 14 hours prior to administration of rFVIIa while the other two deaths were due to sepsis and liver failure.

A perceived risk of thromboembolic complications, due to potential widespread TF exposure, in trauma patients has led to the use of rFVIIa being contraindicated in trauma. Given the lack of evidence of thromboembolic complications in the clinical case reports and the animal studies, and the better understanding of the mechanism of rFVIIa, Martinowitz et al. argues that this exclusion criteria appears now to be misguided and should be reviewed. Martinowitz and colleagues go on to recognise that an important obstacle to overcome in the use of rFVIIa in trauma patients is the
development of a dosing regime. There were a range of doses used in the seven patients in the paper; the determining factor presumably being the integrity of the coagulation system at time of administration. Importantly in one case included in this paper, the efficacy of the first dose was probably hampered by a critically low platelet count, while Martinowitz et al noted that the second dose, given after additional blood components, proved more effective. This finding is in keeping with the modern view of the coagulation cascade, with the coagulation proteins requiring adequate concentrations of activated surfaces on which their reactions may occur. Further clarification is also required on administration and timing of second and subsequent doses of rFVIIa.

In 2002, Martinowitz added a further 12 critically ill haemorrhaging trauma patients to the cohort, making a total of 19, all treated with rFVIIa to control intractable bleeding.\(^\text{188}\) Given the wide-ranging aetiology of traumatic injuries, the group was highly heterogeneous, comprising members of both sex, with an age range of 25±17 and a mixture of blunt and penetrating injuries. All had been treated using all available conventional methods of haemorrhage control prior to use of rFVIIa. Patients were haemodiluted, acidotic and hypothermic at the time of rFVIIa administration. The initial dose was 129±89ug/kg, with up to a further two doses required in some patients. The overall total dose required was 195±113ug/kg. A marked decrease in blood loss was achieved within 20 minutes of administration of the drug in 79% of patients. Four patients did not respond to rFVIIa and exsanguinated within 24 hours. All but two of the responders ultimately survived, with the two deaths occurring as a result of multiple organ failure and sepsis after 1 week, placing overall survival at 68% for the series. As a retrospective, uncontrolled analytical study
it is obviously not possible to state which of these patients would have survived had rFVIIa not been used, however a survival rate of 68% in severely injured, coagulopathic trauma patients may be indicative that rFVIIa treatment has been beneficial in some of these patients. The authors noted that the use of other haemostatic treatments had failed to yield haemostasis prior to administration of rFVIIa, however were noted to become effective following drug administration, leading to the proposal that rFVIIa may be suited as an adjunct to other methods of haemostatic control.

A further clinical case was presented in 2002, as O’Neill et al reported the first off-label use of recombinant factor VIIa in a trauma patient in the USA. Their case was that of a 24 year-old female, suffering from severe haemorrhage from multiple stab wounds including a grade III liver injury and a vascular extremity injury. Despite multiple attempts at surgery and embolisation, the patient continued to rebleed from the liver and developed the “lethal triad” – acidosis, hypothermia and coagulopathy. On subsequent surgical exploration, there was no evidence of missed injury or arterial bleeding; indicating coagulopathic bleeding. At 45 hours post injury, the patient had exhausted local blood product supplies and there was little scope for further surgical intervention. A decision was taken following consultation between surgeons, the haematologist and the transfusion medicine team, to administer a single dose of recombinant factor VIIa in a final attempt to save life. O’Neill et al report that almost immediately after the single dose of 90ug/kg, all external signs of bleeding ceased. Subsequent monitoring of haemostatic parameters and blood chemistry revealed vital parameters returning back toward normal, with complete resolution of the prothrombin time. Most significantly, there were no further episodes of recurrent haemorrhage. 3
days after administration of rFVIIa, an abdominal washout was performed and it was discovered that the surgical field remained dry, with no re-bleeding on removal of the liver packing. Although the patient ultimately died as a result of septic shock, this was attributed to multiple postoperative infections and there were reported to be no adverse events occurring as a result of administration of rFVIIa.

A further retrospective analysis of a cohort of patients with life-threatening haemorrhage was reported in 2003.\textsuperscript{190} In this series, all non-haemophiliac Australian patients who received rFVIIa to control major haemorrhage prior to August 2002 were included. The study consisted of 21 patients, again of heterogeneous nature in terms of patient and injury characteristics. All were administered rFVIIa as a last resort in the control of life-threatening bleeding and persistent coagulopathy, only once conventional transfusion and surgical options were exhausted. Given the retrospective nature of the study, there were a range of doses (30-180ug/kg; median 100ug/kg) and delays to administration (4.35-168 hours; median 12 hours) across the study group. 18 of the 21 patients responded to rFVIIa, with a marked cessation of bleeding, with an overall study survival rate at 30 days of 76%. Transfusion requirements significantly reduced in the 24 hours following administration of rFVIIa. As there were no control groups in the study, again it is not possible to determine whether rFVIIa definitively improved survival however, as was the case in the cohort reported by Martinowitz \textit{et al.},\textsuperscript{188} the high survival rate in these extreme, apparently moribund, clinical cases and significantly decreased reliance on transfusion fluids provides strong indicative evidence that rFVIIa may provide an improved survival profile.
The first report on use of rFVIIa in the management of uncontrolled haemorrhage from UK centres was published in 2003 as a retrospective analysis of 40 patients from the NovoSeven extended-use data collection system. At the time of publication this represented the largest group of patients to have been treated with rFVIIa for major haemorrhage. The patients included in the report were treated at one of thirteen hospitals each of which had submitted data on all non-haemophilia patients that had been treated with rFVIIa between February 1999 and December 2003. The main findings of the report were that bleeding stopped or decreased in 80% of patients and there was a significant decrease in blood product requirement following administration of rFVIIa. However while rFVIIa was found to be effective in stopping or decreasing blood loss, there was a notably high mortality rate of 57.5% across the study. The majority of these deaths occurred more than 24 hours after rFVIIa was administered and were attributed to sepsis and/or multiple organ failure (MOF), though rFVIIa did decrease or stop blood loss in those who died as a result of sepsis or MOF. There were seven acute deaths, caused by continued bleeding, suggesting rFVIIa had no efficacy in these patients. As a retrospective analysis, the study suffers from drawing on a wide variability between patients and there was wide degree of variation in dosage of rFVIIa, ranging from as little as 15ug/kg up to 180ug/kg. Interestingly O'Connell et al found no dose response relationship to efficacy or thromboembolic complications. In addition to the varied injuries and dosing regimes, some patients also received one or more of various antifibrinolytic agents, clotting factor concentrates and surgical interventions. The influences of these variables on outcome cannot be discerned from effects of rFVIIa.
In 2003, Dutton *et al* presented case reports of five trauma patients who received rFVIIa in their hospital in 2001.\(^{192}\) Again, as a retrospective analysis, there were a range of injuries and severity of bleeding, delays to administration of rFVIIa, dosage and confounding factors across the cases. Three of the patients responded well to rFVIIa (doses 144, 80 & 100 ug/kg) with prompt cessation of bleeding. These patients ultimately recovered and represent the survivors in the series. The other two patients did not respond well, with a slowing of bleeding in one case and a minimal effect in the other. These determinations of clinical effect of rFVIIa on bleeding were clearly subjective. Both of these non-survivors were severely acidotic with highly negative base excess (-28.3 & -18) and had significantly elevated serum lactate levels (27.6 & 14.6) at the time of administration of rFVIIa. In the three patients that responded well these biochemical parameters were less deranged and particularly, the patients were less acidotic.

Dutton *et al* suggest that the underlying acidosis may have been the cause of failure of action of rFVIIa, though it is also acknowledged that both patients had sustained essentially lethal initial injuries, indicating there was little hope of salvage with or without haemostatic agents. Dutton and colleagues stressed that was that there was an important need for research into levels of acidosis beyond which rFVIIa may be ineffective.

Dutton *et al* added a further 76 cases to these initial 5 in 2004,\(^{193}\) all treated at their centre between 2001-2003. The resultant case series of 81 coagulopathic trauma patients was the largest to have been reported at that time. rFVIIa recipients were matched with trauma patients who did not receive the drug. As a retrospective
analysis, there were a number of confounders in the data, not least a wide-ranging trauma aetiology. Other variations occurred in terms of dose of rFVIIa administered as well as delay to administration of the drug (maximum 37 days from admission). Despite the confounders, rFVIIa was effective in reversing coagulopathy in 75% of patients and there was an overall survival to discharge rate of 42%. The authors additionally report that some reduction of the rate and volume of haemorrhage was observed in all patients on administration of rFVIIa, with visible new clot formation.

The rFVIIa group had a higher overall mortality rate than controls; though the authors found group sizes too small to acceptably match the control to rFVIIa subjects, leading one to question how meaningful this finding was. Overall, rFVIIa did lead to a reduction in coagulopathic haemorrhage in the majority of cases.

rFVIIa was indicated only in the presence of persistent coagulopathic bleeding, after receipt of 10 units of RBC, 8 units of plasma and a phresis unit of platelets, without clinical effect. In those patients with haemorrhagic shock, a dose of 100ug/kg was employed and a dose of 50ug/kg for other causes. A second dose of rFVIIa was given in some cases.

20 patients did not respond to rFVIIa and died within a few days of admission due to irreversible haemorrhagic shock. 34 of the remaining 61 patients (56%) survived to hospital discharge. Of the remaining 27 patients that died despite responding to rFVIIa, traumatic brain injury accounted for 17 cases and multiple organ dysfunction syndrome for the other 10. The authors found that PT improved in all patients.
regardless of whether they went on to respond to rFVIIa or not and therefore questioned the clinical relevance of this measure in assessing the efficacy of rFVIIa.

The case series was confounded by the inclusion of specific factor deficiencies and traumatic brain injury patients in the overall analysis. However, the authors did provide some data specifically for those patients in haemorrhagic shock and there was no appreciable difference in survival in this subset of patients compared to the overall rate. The authors suggested that the majority of the patients in this subset who did not respond to rFVIIa were already in irreversible haemorrhagic shock before administration of the drug and that efforts should be made to identify markers of futile administration in such patients. The potential effects of acidosis, hypothermia and haemodilution are discussed in this paper but were considered in more detail in a dedicated paper the following year,\textsuperscript{194} which is reviewed shortly.

The authors noted an increased requirement for a second dose of rFVIIa in patients receiving the drug for acute haemorrhagic shock, compared to patients receiving rFVIIa for other indications. Dutton and colleagues suggested that this may be due to the timing of administration, relative to achievement of surgical control of the haemorrhage. Acknowledging that there is some evidence that rFVIIa may be efficacious even when given before surgical control, is achieved, they note that it is possible that administration during active fluid resuscitation may result in a rapid washout of rFVIIa. This would necessitate repeated dosage in order to maintain plasma levels and hence efficacy.
A further cohort of 13 patients who received rFVIIa for the control of acute, uncontrolled life-threatening bleeding were reported by Mayo et al in 2004.\(^2\) All were treated at the Tel Aviv Medical Center in Israel and received rFVIIa (90-120ug/kg) only once all other means to stop bleeding were exhausted. 9 of the 13 patients lived to at least 15 days after rFVIIa administration and bleeding significantly reduced (subjectively) or stopped in 8 of the 13 cases. The authors noted a reduced use of blood products following use of rFVIIa, but although this was evident, statistical significance was not reached due to the wide range of values and small number of cases. Of particular interest from this study was the observation that those patients which responded to rFVIIa were far less severely coagulopathic than the non-responders. Survival was found to be significantly higher in those patients which responded to rFVIIa compared to the non-responders, and therefore this may be extrapolated to state that survival was increased when coagulopathy at time of administration of rFVIIa was minimised. This finding led the authors to recommend that in order to optimise the effect of rFVIIa, it is necessary to correct coagulopathy with transfusion of blood products prior to administration and warn that, based on evidence from this study, that when coagulopathy is severe, rFVIIa may be rendered ineffective. The requirement for replacement of platelets and coagulation factors in order to attain rFVIIa efficacy is in keeping with the proposed mechanism of action of high-dose rFVIIa.

Contrary to the growing number of encouraging case reports and case series supporting the life-saving potential of rFVIIa in severe bleeding in trauma patients, Clark et al produced a damning report in 2004 asserting that “last-ditch” use of rFVIIa in patients with massive haemorrhage is ineffective.\(^3\) They retrospectively analysed
the outcome of 50 patients with intractable bleeding who were transfused with more than 10 units of packed RBC within a 24-hour period (therefore meeting their definition of massive haemorrhage) during 2002. 10 of these patients had received rFVIIa (90μg/kg, repeated every 2 hours if clinical effect was seen), having received at least 15 units of packed RBC with continued haemorrhage despite conventional interventions, with no foreseeable cessation of bleeding prior to drug administration.

There was an overall mortality rate of 20% at 24 hours and 34% at 7 days. The authors noted that rFVIIa had initially ceased or reduced bleeding in 60% of cases but the mortality rate in the rFVIIa treated group was higher than the controls, standing at 40% at 24 hours and 70% at 7 days. Severe coagulopathy was present in 70% of cases of rFVIIa use, compared to 42% overall across both study groups. The four cases which showed no response to rFVIIa had severe coagulopathy and died within 6 hours of rFVIIa administration. All of the patients which responded to rFVIIa administration survived for several days.

The usual limitations of a retrospective analysis applied in this case series. Specifically, the cause of acute blood loss was wide ranging, with only 7 of the 50 cases being due to traumatic injury (stabbing). Use of blood products prior to rFVIIa administration varied as did the severity of coagulopathy. The authors found that severe coagulopathy was associated with a significant increase in mortality at 7 days, with a trend toward significance evident at 24 hours, compared to less coagulopathic cases. It is therefore important to note that 70% of patients that received rFVIIa were severely coagulopathic, compared to only 35% of patients who did not receive rFVIIa. rFVIIa was truly used as a “last-ditch” effort in these cases which were, by the authors
definition, less likely to survive than the untreated “controls”. It may seem that
efficacy of rFVIIa was unfairly measured in this paper, however if one considers only
the severely coagulopathic patients, 7 of which received rFVIIa and 14 of which did
not, survival rates at both 24 hours and 7 days were far higher in untreated patients
than those which received rFVIIa. This said, the six patients which did respond to
rFVIIa, survived for at least 3 days after administration of the drug. It would therefore
seem reasonable to suggest that those patients which did not respond to rFVIIa were
simply unsalvageable, with too severe a coagulopathy in place.

It is interesting that rFVIIa was able to bring about cessation or reduction of bleeding
in 60% of cases, indicating that clot formation was initially mediated. However, the
authors report that this effect was not sustained, presumably necessitating a further
dose of rFVIIa. A likely explanation for the failure of prolonged haemostasis may be
severe haemodilution, with levels of platelets and factors too low to enable a full
thrombin burst. This may have had adverse effects on clot structure and
resistance to fibrinolysis through failure of TAFI activation. Rapid washout, as
suggested by Dutton et al., may have also contributed to the requirement for
additional doses of rFVIIa. The authors suggest that higher doses or shorter intervals
between doses of rFVIIa may have demonstrated more evidence of efficacy, though it
would seem likely that administration of the first dose of rFVIIa earlier, before the
patient was so severely coagulopathic, may offer the greatest opportunity to see
efficacy.

A case series published in 2006 also failed to demonstrate a favourable effect of
rFVIIa in trauma patients, with the authors concluding that rFVIIa “was not helpful
in controlling bleeding in trauma patients”. There were only three trauma cases included as part of a larger review of rFVIIa use and there was no detail provided on the clinical condition of the patients prior to drug administration, therefore it is not possible to determine whether these cases were also unsalvageable cases, with rFVIIa being used as a “last-ditch” attempt. By contrast, two case series presented during the same period reported a favourable outcome of rFVIIa use in trauma patients in what could be considered “last-ditch” use.198,199 A number of chart reviews have been published since 2005, reporting positive outcomes of rFVIIa use in institutions throughout Europe, the United States of America and Canada.200-203

Benharash and colleagues200 reported the outcome of 15 patients who received rFVIIa between November 2003 and December 2004 at the Harbor-UCLA Medical Center, California following development of severe life-threatening haemorrhage. rFVIIa was reported to have been used only when it was considered that the patient was at risk of death from abeelloned ations, essentially as a “last-ditch” effort. A single dose (ranging from 90-120ug/kg) of rFVIIa was administered, followed by a second dose (ranging from 60-90ug/kg) if a transient response had been noted. 80% of patients were reported to have responded to the drug, with partial or complete haemostatic response. Those patients which did not respond to the drug died within 48 hours. It is claimed that the expected mortality rate for all patients was 100%, therefore the authors assert that rFVIIa was shown to have a significant effect on survival, despite the lack of a control group (the authors of this paper, similar to Dutton et al, had been unable to adequately match the rFVIIa subject to untreated controls). There was found to be a significant decrease in the number of blood products administered following rFVIIa administration and there was a significant improvement in laboratory
coagulation parameters. This study demanded that rFVIIa was administered only in the presence of haemorrhagic shock, leading the authors to suggest that an even greater efficacy may have been noted had the drug been administered earlier, before development of hypothermia and acidosis.

McMullin et al. reported a resolution of coagulopathic bleeding in 94% of patients and a significant decrease in transfusion requirements following administration of the drug. Rizoli and colleagues produced a review of some 242 trauma patients, 38 of which received rFVIIa. This was the first retrospective study from which it was possible to draw conclusions regarding effect of rFVIIa on survival from the data. Despite rFVIIa recipients being more acidic than controls who did not receive the drug, in multivariate analysis rFVIIa was shown to improve 24-hour survival and there was a strong trend toward an increase in overall survival in the rFVIIa recipients, compared to those patients who did not receive the drug.

Thus, a range of clinical experiences with rFVIIa in trauma have been reported over the last several years, some showing efficacy in terms of survival, others in terms of transfusion requirements and yet others showing no beneficial effect of the drug in trauma patients. It has been suggested that there may be a sub-population of trauma victims in whom rFVIIa will not be effective, due to overwhelmingly deranged underlying physiology.

In 2005, Stein et al. published a paper which sought to define potential markers of futility of administration of rFVIIa. Using the 81 clinical cases published by Dutton et al. in 2004, Stein and colleagues attempted to establish determinants of futility of rFVIIa administration, with a view to producing guidelines on appropriate use of
rFVIIa. From the total of 81 cases, the authors looked specifically at the cohort of 46 of these patients who had suffered acute haemorrhagic shock. 26 of these cases responded to rFVIIa ("responders") while the remaining 20 either transiently responded or did not respond ("non-responders"). Stein et al compared the characteristics of responders to non-responders, using both Student’s t-test and stepwise logical regression. Both statistical analyses found serum lactate, prothrombin time and revised trauma score to differ significantly between the two groups. The authors then went on to use binary recursive partitioning to exhaustively search all possible methods of subdividing independent variables in order to determine which of the variables were independent predictors of futility of rFVIIa use. This analysis resulted in the generation of a classification and regression tree (CART), which showed revised trauma score and prothrombin time to be independent predictors. A prothrombin time at administration of rFVIIa of more than or equal to 17.6 seconds was placed at the top of the CART. In patients with a promthrombin time of less than 17.6, a revised trauma score of over 4.09 was found to be the next more significant predictor of futility of use of rFVIIa. These recommendations, along with the negative predictor of serum lactate levels greater than 13mg/dl, had already been reported in a review from the same group which was published before this analysis. The degree of increase in serum lactate is a marker of a developing acidosis, which was shown by Meng et al to be associated with potential loss of efficacy of rFVIIa.

The findings from this study, which showed elevated serum lactate to be a predictor of futility of rFVIIa administration, adds further evidence to that provided by Meng et al on the negative effects of acidosis on the action of rFVIIa. Stein and colleagues also noted that there was a trend toward an increase in efficacy of rFVIIa in patients
who had received more platelets prior to rFVIIa administration. This finding therefore supports the proposed TF-independent action of high dose rFVIIa directly on activated platelets.

The authors concluded that the predictive nature of prothrombin time, revised trauma score and serum lactate suggests that there are some patients with metabolic derangements too severe to reverse, even if the bleeding can be attenuated; making the use of rFVIIa in such patients futile. The work of Clark et al\textsuperscript{196} in 2004 supports this theory, since “last-ditch” use of rFVIIa was shown to be ineffective. The overall recommendation of Stein and colleagues was that, while not contraindications, profound haemorrhagic shock or profound metabolic acidosis should provide a guide to rational therapy, aiding in the identification of a sub-population of patients who do not appear responsive to rFVIIa.

Martinowitz and colleagues built on the work of Stein et al, publishing in 2005 a set of comprehensive guidelines for use of rFVIIa in uncontrolled bleeding.\textsuperscript{26} The authors were clear that these were intended as suggestive guidelines only, until more definitive advice could be produced based on evidence from much needed randomised controlled trials. The guidelines were developed based on analysis of published animal studies, clinical case reports and series and 36 cases from the authors’ own hospital. The first part of the paper presents the data from these 36 cases with a cessation of bleeding being achieved in 72% patients and a survival rate of 61%. Compared to expected outcome the authors’ described this as favourable. Similar to the findings of Stein et al\textsuperscript{194} and Meng et al,\textsuperscript{171} this study reported that acidosis had a significant deleterious effect on the efficacy of rFVIIa while hypothermia had no significant effect. It is
interesting to note that 9 of the 10 patients who did not respond to rFVIIa died of
abnormalities within 15 hours, further supporting the suggestion that rFVIIa had
markedly improved survival among those in which it had any effect. Of those patients
that did respond to rFVIIa there were only 4 deaths all of which occurred after 6 days
or more due to sepsis or multiple organ dysfunction syndrome. There was a
significant decrease in blood product requirement in responders compared to none
responders. There was also a significant normalisation of laboratory parameters in
responders.

The remainder of the paper was given over to the presentation of the authors' recommended guidelines for the use of rFVIIa in uncontrolled haemorrhage. They suggest that rFVIIa is indicated in any salvageable patient suffering from massive uncontrolled haemorrhage that has failed to respond to appropriate conventional measures. The use of the drug is absolutely contraindicated in unsalvageable patients while recent history of thromboembolic events is suggested as a relative contraindication. Based on the proposed mechanism of action of pharmacological doses of rFVIIa it is advised that 8-10 units of packed red blood cells should be given in conjunction with the drug to maximise the potential efficacy.

Perhaps the most important recommendations outlined in the guidelines are the conditions that should be met prior to administration of the drug. In keeping with the proposed mechanism of action of the drug and experience both clinically and from the animal studies it is advised that blood component therapy should have been employed to establish fibrinogen levels of at least 50 mg/dl and platelet levels of at least 50 000 X 10^9/l. Furthermore, based on data from the cases presented in their paper and
supported from data published elsewhere, the authors suggest correction of the pH to at least 7.2 prior to drug administration and, although normal body temperature should be restored wherever possible, rFVIIa retains its activity in the presence of hypothermia which therefore should not limit its use. A dose of approximately 120ug/kg is recommended, with a range of 100-140ug/kg quoted.

Over the last two years, numerous other groups have attempted to produce guidelines or determine methods of predicting the response of bleeding in non-hemophiliac patients to rFVIIa (though not specifically in trauma), and the role of clinical scoring systems has received considerable interest. One such paper suggested a poor SOFA (sequential organ failure assessment) score and failure to respond to the first dose of rFVIIa are predictors of futility of administration of rFVIIa. The data used to arrive at this conclusion were derived from 18 patients treated with rFVIIa at Addenbrooke’s Hospital, where the drug was found to have a poor efficacy with a mortality rate of 66.6% and active bleeding at the time of death in 8 of these 12 cases. The authors found no evidence that additional doses of rFVIIa, in the absence of clinical response to the first dose, conferred any significant benefit. These apparently disheartening results should be considered alongside the fact that 11 of the 12 patients which died had organ failure at the time of rFVIIa administration, with a very poor prognosis and, presumably, significant physiological and metabolic derangements. The other patient who died had severe thrombocytopenia at the time of rFVIIa administration. Therefore, although survival was poor and repeated doses of rFVIIa were generally ineffective in the patients included in this series, it is perhaps unfair to extrapolate this finding to trauma patients generally, as it is hoped that rFVIIa administration would be considered prior to development of organ failure. It is
acknowledged that this paper confirms the potential futility of rFVIIa administration in moribund patients with established organ failure; however one must conclude that it is not reasonable to draw any further general conclusions from the effect (or lack thereof) of rFVIIa in these particularly ill patients. It is acknowledged by many authors that determination of predictors of response or futility of rFVIIa in trauma patients is an area which requires more research attention.\textsuperscript{194, 208, 209}

In 2005, a consensus guideline on the off-label use of rFVIIa was published in the United States of America,\textsuperscript{210} based on review of the published literature by a panel of experts. The conclusion regarding the use of rFVIIa in trauma was far less detailed than that provided by Martinowitz et al, with rFVIIa being deemed “appropriate” for use in severe multiple trauma, where there is ongoing bleeding and coagulopathy despite surgical intervention and at least 10 units of blood in a six hour period. Despite these loose guidelines, they are to date the only published advice from the United States of America. European guidelines were published in 2006 and will be discussed in due course.\textsuperscript{172}

The first two, and to date only, randomised clinical trials on the use of rFVIIa in trauma patients were published in a single paper in 2005.\textsuperscript{211} The studies were essentially identical, except that one was concerned with blunt trauma while the other looked at penetrating trauma. Both were multi-centre international trials involving patients from 32 different hospitals in 8 different countries. Severely traumatised patients became eligible and were enrolled in the trials after receiving a 6\textsuperscript{th} unit of red blood cells within a four hour period. Enrolled patients were randomised to receive either 3 injections of rFVIIa or placebo. The first injection of rFVIIa was given, at a
dose of 200ug/kg, immediately after transfusion of the 8\textsuperscript{th} unit of red blood cells. The second and third injections, both 100ug/kg doses, followed at 1 and 3 hours after the first dose. The main outcome measure for both studies was the number of red blood cell units transfused during the 48 hours following the first dose of rFVIIa. In order to measure this outcome without adding bias from early deaths, patients were included in the analysis only if they survived a minimum of 48 hours. In blunt trauma patients who survived for a minimum of 48 hours, rFVIIa significantly reduced the number of red blood cell units required and the need for massive transfusions. No significant effects of rFVIIa on transfusion requirements were found in the penetrating trauma series. The authors suggest that the main reason for the lack of significance in the penetrating trauma trial was that these patients required only approximately half as many red blood cell units as the blunt trauma patients and that the penetrating injury study therefore had a lower power for detection of a reduction in transfusion requirement.

Whilst no significant effect of rFVIIa on survival or length of stay in intensive care were found in the blunt or penetrating trauma trials, positive trends in favour of rFVIIa were observed for these endpoints. The authors stressed that the lack of statistical significance for these outcome measures was to be expected as the studies were not powered for the endpoints. The authors noted that rFVIIa was efficacious despite the hypothermia present across the series. Little can be determined regarding the effects of severe acidosis as none of the groups has pH less than 7.2.

In 2006, further post-hoc analysis of data presented by Boffard \textit{et al} was published, focussing specifically on coagulopathic patients.\textsuperscript{212} This paper ultimately adds little to
the findings from the original publication and was likely triggered by criticism which was levelled at the authors' interpretation of the analyses in that study.\textsuperscript{213} In the absence of a consensus definition of coagulopathy, for the purposes of this paper patients were considered to be coagulopathic if they had "an ongoing bleeding that required the use of transfusion with FFP and RBC units at a ratio of 1 or more units of FFP for every 4 units of RBCs, and/or the use of FFP with whole blood, and/or transfusion of platelets, and/or the transfusion of cryoprecipitate". Due to the obvious resultant reduction in cohort size, patients from the penetrating and blunt trials were pooled together in this analysis. Based on this definition, 136 patients were included in the analysis, 60 of which had been treated with rFVIIa.

rFVIIa was found to reduce 48-hour transfusion requirements, irrespective of whether the analysis included all patients or just those that survived at least 48-hours. This is likely to have been the desired outcome from this post-hoc reanalysis of the trial data, as the restriction in the original paper of this finding to those patients which survived at least 48 hours had been commented upon.\textsuperscript{213} This reanalysis of the data also enabled the authors to demonstrate a significant decrease in the risk of developing MOF or ARDS in the rFVIIa treated patients, compared to placebo controls. The authors concluded that coagulopathic patients appeared to be a group particularly likely to benefit from rFVIIa therapy. This suggestion appears to be at loggerheads with the suggestion by Clark et al\textsuperscript{196} and Stein et al\textsuperscript{194} that efficacy of rFVIIa may be limited in severely coagulopathic patients. It is likely that the resolution of this conflict lies in defining how profound a coagulopathy is being referred to; it is reasonable to assume that to some (unknown) point, the more coagulopathic a patient.
the more potential rFVIIa has to improve the situation, but beyond this unknown threshold, the patient is too sick to be able to respond to the rFVIIa.

The findings from the RCT\textsuperscript{211} were supported in 2005 as 101 trauma patients, 29 of which received rFVIIa, were presented.\textsuperscript{214} The dose used in this study was lower than that reported by other groups, just 40ug/kg. Nevertheless the rFVIIa group had a significantly lower transfusion requirement than the control group. The authors again reported no significant effect of rFVIIa on survival. It is interesting to note however that patients who received rFVIIa survived at a significantly lower pH than those in the control group. The authors therefore challenged the findings of Meng et al\textsuperscript{171} and, though not directly, the recommendations of Martinowitz et al,\textsuperscript{26} stating that acidosis by itself should not preclude the use of rFVIIa. Pusateri & Park published a review of rFVIIa use in trauma in 2005\textsuperscript{215} in which they stated that rFVIIa increased total circulating FVIIa levels 100-fold, highlighting the fact that it is therefore difficult to suggest at what point acidosis may inhibit the beneficial action of rFVIIa to a clinically relevant degree. They further suggest that, bearing in mind the difference in degree of effect of acidosis noted in the two models employed by Meng et al, the apparently disparate effects of acidosis on efficacy of rFVIIa may be dependent to some degree on which mechanism, that is TF-dependent or TF-independent, of action of rFVIIa predominates in the given situation.

An analysis of outcomes reported to the rFVIIa extended-use database was presented in 2006.\textsuperscript{216} The main inclusion criteria were nonhemophilic patients who had experienced a massive bleed (defined as at least 14 units of packed RBCs within 4 hours) and subsequently received rFVIIa. 45 patients met the inclusion criteria, and
both trauma and surgical cases were included. rFVIIa was reported to be effective in stopping or markedly reducing blood loss in 93% of cases. A significant decrease in both transfusion requirement and rate of blood loss was noted following administration of rFVIIa. There was no control group to which survival rates could be compared in this analysis, therefore effect on survival was determined through comparison of observed mortality to predicted mortality, derived from clinically employed scoring systems. rFVIIa was found to significantly increase survival compared to predicted rates in trauma patients, but not in surgical patients. The authors of this study suggest that the apparent increased efficacy of rFVIIa compared to that found in the randomised controlled trial may be due to differences in the patient characteristics between the two studies. rFVIIa was given in the randomised controlled trial after the 8th unit of packed RBCs, regardless of whether surgical control had been achieved, while patients reported in this study were not given rFVIIa until at least 14 units of packed RBCs had been given and surgical control had been achieved.

The study suffered from a number of limitations, inherent in retrospective analyses. Not least were the variable doses of rFVIIa used as well as the number of doses given. In addition, the causes of blood loss were multifactorial in a vastly heterogenous population. The use of predicted mortality rates, as opposed to a control group, means that the apparent improvement in survival seen in this study cannot be ratified.

Following the publication of the results of the first two randomised controlled clinical trials of rFVIIa in trauma, and the publication of guidelines on use of rFVIIa by the Israeli Multidisciplinary rFVIIa Taskforce in 2005, a set of European guidelines
were published in 2006. The guidelines rank evidence according to standard critical appraisal criteria, therefore any evidence drawn from the randomised controlled trial is considered in preference to case series. As a result, the main focus of the guidelines is upon blunt trauma. As the RCT found no statistically significant beneficial effect of rFVIIa in penetrating trauma, it is stated that use of the drug in cases of penetrating trauma cannot be recommended. General recommendations made state that every attempt should be made to control bleeding by conventional means before resorting to rFVIIa use, which will be effective only once sources of major bleeding from damaged vessels have been stopped. This statement could be challenged by a number of the published animal and human cases in which rFVIIa has been used successfully as a sole haemostatic agent, with other methods of haemostatic control either having been applied, or been effective.

While the Israeli guidelines laid down a series of preconditions which should be met before considering rFVIIa use, the European guidelines suggest only that “efforts should be made to achieve” similar parameters to those already detailed from the Israeli guidance. In addition to targets for fibrinogen, platelets and pH, the European guidelines also quote a target haematocrit of more than 24%.

A significant departure from the Israeli guidelines can be seen in the rFVIIa dose recommended in the European guidance. Here, 200ug/kg of rFVIIa, followed by two doses of 100ug/kg, as used in the RCT, are advised. The reason for this difference is due to the fact that the European guidance is based on the RCT, details of which were not available when the Israeli guidelines were composed. The authors of the European guidelines note that the possibility remains that lower doses may be as effective and emphasise that the second and third doses should only be used if clinically indicated.
Further advice on the appropriate use of rFVIIa in trauma was published in 2007, as part of the European guideline on management of bleeding following major trauma. In this advice, rFVIIa is indicated only in blunt trauma patients, with no avocation of use in penetrating trauma. The advice from the previously discussed 2006 guideline, pertaining to use only when other options have failed, as well as dosage advice, is unchanged. Again, while it is recommended to correct pH and restore fibrinogen and platelet levels, there is no instruction that this is a precondition which must be met before using rFVIIa.

rFVIIa has been used successfully for control of blood loss in a number of intra and post operative bleeding patients, which have been the subject of numerous comprehensive reviews. A number of other case reports on the use of rFVIIa in trauma patients have appeared in the literature since the early part of this decade, including use in pulmonary bleeding and case reports of life-saving efficacy in unusual situations, such as prolonged coagulopathic bleeding following traumatic injury in a Jehovah’s Witness.

Although considerable progress has been made in understanding the mechanism of rFVIIa, and use of this knowledge to begin to develop guidelines for the most appropriate use of the drug, a number of points still require clarification. These were summarised succinctly by Mohr et al., and include determination of appropriate timing of administration and dosage, how many blood products should be transfused prior to administration, development of tighter indications and contraindications and determinants of futility of use. In addition, conclusive beneficial effect on survival remains to be demonstrated in a randomised clinical trial, and data needs to be
continually collected and assessed for evidence of the safety or dangers of rFVIIa in trauma.

If one thing is clear from the vast amount of literature published on the use of rFVIIa in trauma over the last eight years, it is that further research is needed; specifically, randomised controlled trials sufficiently powered to detect effects on mortality. Two such RCTs are reported to be currently underway, and the results are awaited with anticipation.
3.1 The in vivo study

The study which forms the experimental work for this thesis was conducted in vitro, using blood samples obtained from an in vivo study which ran simultaneously. The in vivo study was conducted on Large White pigs, and the salient points are described here.

Following induction of surgical anaesthesia, the left carotid artery, both internal jugular veins, femoral arteries, and veins were cannulated. Blood samples for the in vitro study were drawn from the cannula placed in the left femoral artery. The bladder was cannulated and the spleen removed to prevent autotransfusion. A wire was then surgically placed in the aorta and the abdomen was closed. Animals were maintained on intravenous anaesthesia throughout the surgical period and for the duration of the study. Physiological parameters were measured using the PowerLab (AD Instruments, Australia) system.

Once surgically prepared, the experimental phase was entered, with animals randomised to receive either rFVIIa (at a dose of 180ug/kg) or placebo. All members of the team conducting the experiment were blinded to the randomisation to prevent experimenter bias. After a stabilisation phase, a controlled haemorrhage of 30% blood volume was initiated by a computerised pump, which drew blood from the femoral veins. At the end of the controlled haemorrhage, the wire placed in the aorta was pulled, creating an aortotomy (4-5mm) and uncontrolled haemorrhage. After a five-
minute shock phase, animals received either rFVIIa or placebo and intravenous fluid resuscitation was commenced in all animals. IV fluid resuscitation was continued until a systolic blood pressure of 80mmHg was reached, and was re-initiated when systolic blood pressure fell below this level.

The primary end point of this study was survival time, and number of animals surviving to six hours. Animals that survived six hours were killed without recovery from anaesthesia by an overdose of sodium pentobarbitone (80mg/kg Euthatal, Meriel, Princes Risborough, UK).
3.2 Blood Sampling

At defined time points throughout the study arterial blood samples were obtained from the indwelling cannula placed in the left femoral artery. Before each blood sample was taken, approximately 10ml of ‘waste’ blood was drawn from the cannula to clear the dead space. In excess of 3ml of arterial blood was then drawn from this line into a fresh syringe, to undergo \textit{in vitro} analysis.

Blood samples were collected at the following time points:

1. Baseline – drawn at the end of the surgical phase
2. Pre Haemorrhage – drawn immediately prior to initiation of haemorrhage
3. Post Haemorrhage – drawn after the haemorrhage, before administration of rFVIIa or placebo
4. Post Drug – drawn immediately following administration of rFVIIa or placebo
5. 10 minutes – drawn 10 minutes after the administration of rFVIIa or placebo and the start of IV fluid resuscitation
6. 20 minutes* – drawn 20 minutes after the administration of rFVIIa or placebo and the start of IV fluid resuscitation

*innovin activated samples only

The timing of blood sampling through the \textit{in vivo} study is shown in Figure 12 which schematically illustrates the experimental protocol.
Figure 12  Schematic representation of the experimental protocol, illustrating the timing of blood sampling
3.3 The *in vitro* study

The subject of this thesis is the *in vitro* clotting analysis, specifically thromboelastography (TEG®), performed on blood samples taken during the animal study described above. TEG® is a real-time method of assessing the kinetics of clot formation in whole blood. Paired blood samples were taken at each time point, one being treated *in vitro* with a (potentially second) dose of rFVIIa and the other being used as a control, treated only with placebo to maintain equivalent volume.

3.3.1 The TEG® apparatus

The TEG® System was first developed in 1947, by Professor Helmut Hartert. Since then it has undergone several modifications to reach its current form, manufactured today by Haemoscope Corporation, Illinois, USA. There follows a brief overview of the theory behind TEG®.

The TEG® apparatus comprises the TEG® labelled (Haemoscope Corporation, Illinois, USA), which consists of two channels that operate independently of one another, and TEG® analytical software (TAS™ Version 4; Haemoscope Corporation, Illinois, USA). A blood sample is added to a pre-warmed TEG® cup in each channel; the temperature of each channel can be individually controlled and for the present study were fixed at 37°C. A channel consists of a torsion wire, connected to a static pin and a cup holder with heating element. The TEG® system is illustrated in Figure 13.
The pin becomes immersed in the blood as the cup is raised and at this point the analysis software is started. The cup oscillates through an arc of 4°45' with 10 seconds duration. As the blood in the cup begins to clot, the fibrin produced binds to the pin causing the pin to oscillate with the clot. The movement of the pin is transmitted through the torsion wire to the TEG® software which produces a graphical representation of the pin’s movement. An example of this representation is shown in Figure 14 and an explanation of the abelled parameters follows.
The flat line at the beginning of the trace in Figure 14 represents the stationary phase of the pin, prior to clot formation and fibrin production. The parameter labelled R represents the duration of this stationary phase up until the point at which the first fibrin forms and binds to the pin. Graphically, binding of fibrin to the pin is shown by the splitting of the flat line, as the pin begins to oscillate with the clot. The point at which the initial fibrin forms is illustrated in Figure 14 by a vertical green line.

The gradient of the split lines represents acceleration of the pin as more fibrin binds and the clot develops further. The kinetics of clot formation is represented by the parameters K and Angle. K is a measure of the time taken to reach a given level of clot strength (amplitude of 20mm), this point is represented in Figure 14 by the vertical blue line. Angle provides an indication of how quickly fibrin is produced and therefore is a measure of how quickly the clot becomes stabilised. It is derived as the angle of the centre of the trace (dashed line) and the tangent of the line originating at R (dotted line).

The influence of the rotating cup is seen on the TEG only once the clot is sufficiently developed to cause the pin to be bound with the walls of the cup. At this stage the torque of the cup then affects the movement of the pin; the stronger the clot the more closely aligned the speed of cup and pin rotation. The maximum amplitude (MA) of the trace represents this relationship and therefore reflects the ultimate peak strength of the clot.

Considering the main aim of the thesis, to determine the effect of \textit{in vitro} rFVIIa on the coagulation process, specific TEG parameters were selected for analysis. It has already been explained that rFVIIa is understood to act by augmenting the initiation of
coagulation. R-time, defined as the time until the initial fibrin formation, has been selected as one parameter which may therefore be likely to demonstrate an effect, should rFVIIa exert one. Both K-time and Angle are measures of the kinetics of clot formation, from R-time onwards. Angle is influenced by fibrin build up and is cited as a measure of clot strengthening and therefore fibrinogen level, whereas K-time is a measure of the speed at which the clot reaches a defined level of strength and reflects a more generic level of clot kinetics. It has therefore been determined that K-time is likely to be more representative of changes in kinetics ameliorated by rFVIIa and this parameter will be analysed rather than Angle. Finally, since there have been claims in the literature that rFVIIa may lead to the formation of increased strength “superclots”, the parameter MA, which is a measure of the ultimate peak strength of the fibrin clot, will be analysed to determine whether rFVIIa has any effect on the strength of the clot ultimately formed.

3.3.2 Preparation of TEG apparatus

Before each trial, prior to any analyses being carried out, the TEG analysers were checked for balance and baselines, in accordance with manufacturer’s guidelines. Quality control samples were also processed at regular intervals to ensure there was no drift on the machines. Shortly before each sample was due, plastic TEG cups were placed in the columns to be used. The cups were then automatically heated to 37°C and maintained at that temperature throughout.
3.3.3 Preparation of TEG reagents

TEG buffer was produced as outlined in Table 1, below.

| 20mM Hepes 4.76 g (Sigma H3375) |
| 140mm NaCl 8.18g (MERCK 6404) |
| 2% BSA 20g (SIGMA 7906) |

- Hepes and NaCl dissolved in approximately 750 ml ion exchanged water
- pH adjusted to 7.4 using HCl
- The volume is made up to 1L with ion exchanged water
- 2% BSA is added (100g/ml)

Table 1 The composition of 1L TEG Buffer

Once produced, buffer was decanted in 10ml measures and stored at -80°C until required. Buffer was used as the \textit{in vitro} placebo treatment and also as vehicle for the rFVIIa. rFVIIa was provided by Novo Nordisk, Denmark at a concentration of 600ug/ml in 10 ul measures. rFVIIa vials were also stored at -80°C until required.

Shortly before use the buffer and rFVIIa were defrosted. The rFVIIa vial was diluted with 57ul of buffer.

Two activators were used throughout this study. Tissue Factor (Innovin) was the main reagent used, selected for sensitivity. Innovin is a source of recombinant human tissue factor and as such acts upon the extrinsic system, leading to a physiological activation of coagulation. The other activator used in this study was Kaolin (Haemoscope, Chicago, Illinois, USA; marketed by Medicell Ltd, London, UK). This reagent works on the intrinsic system and as such initiates coagulation through the non-physiological contact pathway.
Innovin stock (Dade Behring; marketed by Sysmex UK Ltd, Milton Keynes, UK) was frozen at -80°C until required, at which point it was defrosted and diluted 1:100 (5 ul Innovin stock in 495 ul of buffer). The 1:100 solution of Innovin, stored on ice, was stable for the duration of the experiment. Shortly before the arrival of the blood sample, the 1:100 solution was further diluted (40 ul in a further 432 ul of buffer). This solution was designated X and stored on ice, where it remained stable for 2 hours.

Kaolin (Haemoscope, Chicago, Illinois, USA; marketed by Medicell Ltd, London, UK) was provided in a ready for use formulation, while it was necessary to create premix vials of Innovin.

At each time point, two premix vials of Innovin were prepared. The first contained 29ul of rFVIIa and 29ul of X; the other contained 29ul of buffer, again mixed with 29ul of X, as a control. All reagents were added to their respective premix vials immediately prior to the addition of blood.

3.3.4 Running the TEG

At each time point, one Kaolin vial and the two Innovin premix vials, prepared as detailed in the previous section, were used. Innovin activated blood samples were used throughout the study to assess the efficacy of rFVIIa versus placebo, while kaolin samples were included in one aspect of the study to allow comparison of the two activators. Samples activated with Kaolin were not treated in vitro with rFVIIa or buffer. The analysis of samples is illustrated in Figure 15.
Once drawn, the syringe of blood was gently agitated while being transferred to the TEG suite, to prevent stasis of the blood. The time taken to reach the TEG suite was less than one minute.

On arrival in the TEG suite, 1 ml of blood was added to each of the vials, which were gently inverted a number of times to ensure complete mixing of blood and vial contents. Immediately following the inversions, 360 ul of the activated blood was pipetted into the relevant TEG channel. The cup was raised to meet the pin and the TEG analyser was started.

Each TEG channel was allowed to run at least until MA was reached and for a maximum of 120 minutes.
3.3.5 Analysis of TEG parameters

The three TEG parameters discussed earlier in this section, R-time, K-time and MA, were each measured in order to answer a series of five questions, which form the subsections of the Results section.

1. What is the effect of in vitro rFVIIa on normal porcine blood?
2. Does haemorrhage modify the effect of rFVIIa in porcine blood?
3. What is the effect of intravenous fluid resuscitation on the efficacy of in vitro rFVIIa in porcine blood?
4. Is TEG sensitive to the effect of rFVIIa administered in vivo?
5. What is the effect of a second dose of rFVIIa in porcine blood?

Due to the nature of the questions being asked, different data sets were used for different questions. The number of animals included in each analysis is quoted for each question, along with the reasoning for selection of the data set.

3.3.5.1 Statistical Analysis

All data were analysed using a 2 way analysis of variance (2 way ANOVA, SPSS v10), unless indicated otherwise. Where appropriate (indicated in the text) a log-transformation of the data was performed before analysis. A value of P<0.05 was considered statistically significant. All data are reported as mean±SEM unless indicated otherwise. The use of SEM is a standard form of expressing data spread and is frequently employed in scientific peer review journals. It is thus appropriate for
this thesis. Each statistical tool employed is appropriate for the analysis of the data studied and it is clearly stated in the text which analysis is performed for each data set.
4.1 The effect of *in vitro* rFVIIa on normal porcine blood

The complete data set (a total of 50 animals) was used in analysis of R-time and MA in this section. Due to technical failure of the TEG software, K-time was not recorded for six animals, making a total of 44 animals included in K-time analysis in this section. The blood used in this section was withdrawn from the animal before the onset of haemorrhage (see Methods section, Figure 12) and was activated with innovin (see Methods section, Figure 15).

rFVIIa added *in vitro* to normal porcine blood resulted in a significantly lower R-time compared to that found after the addition of the same volume of buffer to paired samples of blood (Figure 16, $P<0.001$, paired t test). rFVIIa also reduced K-time significantly (Figure 17, $P<0.001$, paired t test) but had no significant effect on MA (Figure 18, $P=0.43$, paired t test) in the same samples.
Figure 16  The effect of *in vitro* rFVIIa compared to placebo on R-time in baseline blood samples. Mean value ±SEM.

Figure 17  The effect of *in vitro* rFVIIa compared to placebo on K-time in baseline blood samples. Mean value ±SEM.
Figure 18  The effect of *in vitro* rFVIIa compared to placebo on MA in baseline blood samples. Mean value ±SEM.
4.2 The effect of haemorrhage on the response to *in vitro* rFVIIa in porcine blood

Two animals from the complete data set did not survive to the Post Haemorrhage timepoint and a further four were excluded since they had been given interventions that were outwith the protocol of the current study. A total of 44 animals were therefore included in the analysis of R-time and MA in this section. Again, due to failure of the TEG software K-time was not recorded in some cases resulting in 38 animals being included for K-time analysis in this section.

The blood used in this section was withdrawn from the animal before the onset of haemorrhage (Pre Haemorrhage) and immediately after haemorrhage, prior to administration of rFVIIa or placebo *in vivo* (Post Haemorrhage) (see Methods section, Figure 12). Blood samples were activated with innovin (see Methods section, Figure 15).

Both haemorrhage and rFVIIa produced significant reductions in R-time (Figure 19, P<0.001 in each case). Although the reduction in R-time induced by rFVIIa (compared to buffer) appears smaller after haemorrhage than before (Figure 19), this difference was not statistically significant (P=0.384). Thus, rFVIIa produced a significant reduction in R-time at both timepoints and the absolute value of R-time was lowest in the presence of rFVIIa after haemorrhage.

A similar effect was seen on K-time, which was also significantly reduced by both haemorrhage and rFVIIa (Figure 20, P<0.001 in each case). Again, the reduction in
K-time induced by rFVIIa appeared less after haemorrhage, compared to pre-haemorrhage (Figure 20) and this difference was statistically significant (P=0.001). A further post hoc test (Tukey) indicated that rFVIIa did indeed significantly reduce K-time both before and after haemorrhage. Consequently, as was seen with R-time, the absolute value of K-time was smallest in the presence of rFVIIa after haemorrhage.

Neither haemorrhage nor rFVIIa had significant effect on MA in the same samples (Figure 21, P=0.0584 & P=0.742 respectively).

Figure 19  The effect of haemorrhage on the efficacy of in vitro rFVIIa compared to placebo on R-time. Mean value ±SEM.
Figure 20  The effect of haemorrhage on the efficacy of *in vitro* rFVIIa compared to placebo on K-time. Mean value ±SEM.

Figure 21  The effect of haemorrhage on the efficacy of *in vitro* rFVIIa compared to placebo on MA. Mean value ±SEM.
4.3 The effect of intravenous fluid resuscitation on the efficacy of \textit{in vitro} rFVIIa in porcine blood

Only animals which received placebo \textit{in vivo} were included in this analysis (see Section 3.1). Limiting the analysis to only this group of animals, and further to those surviving 20 minutes of resuscitation resulted in a total of seven animals being included in the analysis for this section.

The blood used in this section was withdrawn from the animal immediately after haemorrhage, prior to administration of placebo \textit{in vivo} (Post Haemorrhage) and after 20 minutes of intravenous fluid resuscitation (20 mins resusc.) (see Methods section, Figure 12). Blood samples were activated with innovin (see Methods section, Figure 15).

Due to the significantly increased variance of the data at longer R and K-times during resuscitation (compared to pre-resuscitation) statistical analysis was conducted on log-transformed data.

20 minutes of IV fluid resuscitation produced significant increases in R-time (Figure 22, P=0.005) and K-time (Figure 23, P=0.015) and reduction in MA (Figure 24, P=0.044). \textit{In vitro} rFVIIa significantly decreased the K time (Figure 23, P=0.033) and there was no significant difference in the pattern of response to \textit{in vitro} rFVIIa between the two timepoints (Figure 23, P=0.085); thus \textit{in vitro} rFVIIa (compared to buffer) significantly decreased K-time both before and after resuscitation.
Conversely, *in vitro* rFVIIa had no significant effect on R-time (Figure 22, \( P=0.138 \)). This finding may be a result of the small number of animals included in this analysis, as R-time was found to be significantly affected by *in vitro* rFVIIa in the previous section, where the number of subjects was larger.

rFVIIa had no significant effect on MA (Figure 24, \( P=0.917 \)).

![Figure 22](image)

*Figure 22*  The effect of haemorrhage and 20 minutes of IV fluid resuscitation on the efficacy of *in vitro* rFVIIa compared to placebo on R-time. Mean value ±SEM.
Figure 23 The effect of haemorrhage and 20 minutes of IV fluid resuscitation on the efficacy of *in vitro* rFVIIa compared to placebo on K-time. Mean value ±SEM.

Figure 24 The effect of haemorrhage and 20 minutes of IV fluid resuscitation on the efficacy of *in vitro* rFVIIa compared to placebo on MA. Mean value ±SEM.
4.4 Sensitivity of TEG to the effect of rFVIIa administered in vivo

Inclusion criteria for animals in this section required survival 10 minutes of resuscitation and continuous fluid infusion over that 10 minute period. As a result, four animals were included in the in vivo placebo group and seven in the in vivo rFVIIa group. Due to the very small number of animals included, reliable values for MA could not be obtained for this section, and the parameter has therefore not been reported.

The blood used in this section was withdrawn from the animal immediately after haemorrhage, prior to administration of placebo in vivo (Post Haemorrhage) and after 10 minutes of intravenous fluid resuscitation (10 mins resusc.) (see Methods section, Figure 12).

As an additional limb to this question, two activators – innovin and kaolin – were studied simultaneously (see Methods section, Figure 15). The results are considered firstly for innovin, followed by kaolin activated samples.

4.4.1 Innovin activated samples

Resuscitation led to a significant increase in R-time (Figure 25, P=0.016). After 10 min of resuscitation R-time appears shorter in the group given rFVIIa intravenously immediately before resuscitation compared to those given placebo (Figure 25). However the effects of in vivo rFVIIa did not attain statistical significance (P=0.125,).

A similar pattern was seen in K-time (Figure 27), although in this case neither the
effects of resuscitation nor drug treatment attained statistical significance (P=0.073 and P=0.175 respectively). It is possible that the lack of significance could be due to the very small number of animals used here.

4.4.2 Kaolin activated samples

The results obtained with kaolin activated blood samples were similar to those seen with innovin activation. Resuscitation led to a significant increase in R-time (Figure 26, P=0.011) and although there appears to be a trend for R-time to be lowered in the presence of rFVIIa after 10 minutes of resuscitation (Figure 26), in vivo rFVIIa had no significant effect on R-time (Figure 26, P=0.548).

Unlike the pattern seen with innovin activation, kaolin activated samples showed that there was a significant effect of resuscitation on K-time (Figure 28, P=0.001). No significant effect of in vivo rFVIIa was seen on K-time (Figure 28, P=0.115).
Figure 25  R-time of innovin activated blood samples taken following
haemorrhage in animals prior to in vivo treatment and after 10
minutes of resuscitation following in vivo administration of rFVIIa
or placebo. Mean value ±SEM.

Figure 26  R-time of kaolin activated blood samples taken following
haemorrhage in animals prior to in vivo treatment and after 10
minutes of resuscitation following in vivo administration of rFVIIa
or placebo. Mean value ±SEM.
Figure 27  K-time of innovin activated blood samples taken following haemorrhage in animals prior to *in vivo* treatment and after 10 minutes of resuscitation following *in vivo* administration of rFVIIa or placebo. Mean value ±SEM.

Figure 28  K-time of kaolin activated blood samples taken following haemorrhage in animals prior to *in vivo* treatment and after 10 minutes of resuscitation following *in vivo* administration of rFVIIa or placebo. Mean value ±SEM.
4.5 The effect of a second dose of rFVIIa in porcine blood

Inclusion criteria for animals in this section required survival to at least 10 minutes of resuscitation and continuous fluid infusion over that 10 minute period. As a result, four animals were included in the *in vivo* placebo group and seven in the *in vivo* rFVIIa group. Due to the very small number of animals included, reliable values for MA could not be obtained for this section, and the parameter has therefore not been reported. It is also acknowledged that due to the small numbers of samples involved there are serious limitations to the interpretation of the statistical data, which should therefore be taken ‘for indicative purposes’ only.

The blood used in this section was withdrawn from the animal immediately after haemorrhage, prior to *in vivo* administration of intravenous drug (Post Haemorrhage) and after 10 minutes of intravenous fluid resuscitation following intravenous drug administration (10 mins resusc.) (see Methods section, Figure 12). Blood samples were activated with innovin (see Methods section, Figure 15).

Figures 29 & 30 illustrate the effects of *in vitro* rFVIIa or placebo on R-time. The intended treatment groups in Figure 29 should be identical, as no *in vivo* drug had been given at that timepoint. Figure 29 suggests that *in vitro* treatment of blood samples with rFVIIa leads to a reduction in R-time in both intended treatment groups.

Figure 30 illustrates that R-time increased in both *in vivo* groups following haemorrhage and 10 minutes of resuscitation, compared to their absolute values immediately after haemorrhage (Figure 29). Figure 30 also illustrates that *in vivo*
administration of rFVIIa lead to a shortening of R-time, compared to in vivo administration of placebo.

Figure 30 would appear to show that in vitro treatment of blood samples with rFVIIa was associated with a reduction in R-time in those animals that received placebo in vivo. However, in animals that received in vivo rFVIIa, this effect was lost, with no apparent effect of in vitro rFVIIa in this group. This may be because the rFVIIa administered in vivo had maximally reduced R-time, however the effects of in vivo rFVIIa did not reach statistical significance (P=0.149).

Figures 31 & 32 illustrate the effects of in vitro rFVIIa or placebo on K-time. The intended treatment groups in Figure 31 should be identical, as no in vivo drug had been given at that timepoint. Figure 31 suggests that in vitro treatment of blood samples with rFVIIa leads to a reduction in K-time in both intended treatment groups.

Figure 32 illustrates that K-time increased in both in vivo groups following haemorrhage and 10 minutes of resuscitation, compared to their absolute values immediately after haemorrhage (Figure 31). Figure 32 also illustrates that in vivo administration of rFVIIa lead to a shortening of K-time, compared to in vivo administration of placebo.

Figure 32 would appear to show that in vitro treatment of blood samples with rFVIIa was associated with a reduction in K-time in those animals that received placebo in vivo. As was the case with R-time, this effect was lost in those animals that received in vivo rFVIIa. Unlike the case of R-time, the relative effect of in vitro rFVIIa
compared to placebo on K-time, was significantly affected by *in vivo* rFVIIa (P=0.040).

In order to further investigate the apparent lack of significant effect of a second dose of rFVIIa, the effect of *in vitro* rFVIIa on the absolute values of R-time and K-time was compared to the effect of *in vitro* placebo. *In vivo* drug had no significant effect on either R-time (P=0.129) or K-time (P=0.517), while resuscitation significantly affected the effect of *in vitro* rFVIIa on both R-time (P=0.001) and K-time (P=0.019).
Figure 29  The effect on R-time of *in vitro* rFVIIa and placebo following haemorrhage in animals to be treated *in vivo* with rFVIIa or placebo. Mean value ±SEM.

Figure 30  The effect on R-time of *in vitro* rFVIIa and placebo following haemorrhage and 10 minutes of intravenous fluid resuscitation in animals treated *in vivo* with rFVIIa or placebo. Mean value ±SEM.
Figure 31  The effect on K-time of *in vitro* rFVIIa and placebo following haemorrhage in animals to be treated *in vivo* with rFVIIa or placebo. Mean value ±SEM.

Figure 32  The effect on K-time of *in vitro* rFVIIa and placebo following haemorrhage and 10 minutes of intravenous fluid resuscitation in animals treated *in vivo* with rFVIIa or placebo. Mean value ±SEM.
CHAPTER 5
DISCUSSION

1.1 The effect of *in vitro* rFVIIa on normal porcine blood

The present study demonstrated the efficacy of rFVIIa in increasing the coagulation kinetics in normal porcine blood. Significant decreases were noted in both R-time and K-time, indicating the rFVIIa shortened the time to initial fibrin formation, and increased the rate at which clot formation occurred once coagulation had been initiated. The lack of any effect on MA (maximum amplitude of the clot) suggests that, in normal blood samples, there is no difference in the strength of the clot formed in the presence of rFVIIa, compared to untreated samples.

In plain terms, rFVIIa appeared to facilitate faster clot formation but there was no difference in the ultimate strength of the clot in the presence of rFVIIa, compared to placebo.

While some research groups have found a more stable clot to be formed in the presence of rFVIIa, this is purported to be largely due to resistance to fibrinolysis. The fact that in the present study, no beneficial effect of rFVIIa on clot strength was found does not disagree with these previous studies, since MA is a measure of ultimate clot strength, rather than strength in the sense of resistance to fibrinolysis.
1.2 The effect of haemorrhage *in vivo* on the response to *in vitro* rFVIIa on porcine blood

Following the haemorrhage phase of the *in vivo* study, thromboelastographic analysis of blood samples taken during the shock phase demonstrated that haemorrhage itself reduced the time to initial fibrin formation (shortened R-time) and increased the speed of clot formation once coagulation had commenced (shortened K-time). Despite the apparent pro-coagulant effect of haemorrhage itself seen in this study, addition of rFVIIa was found to further significantly reduce both R-time and K-time. Furthermore, the absolute values of both R-time and K-time were lowest in those post haemorrhage samples that had been treated *in vitro* with rFVIIa. These findings indicate that early administration of rFVIIa may therefore confer significant benefit in haemorrhage control.

Neither haemorrhage or *in vitro* rFVIIa had any significant effect on the MA, indicating that the maximum clot strength was unaffected by either influence.
1.3 The effect of intravenous fluid resuscitation on the efficacy of in vitro rFVIIa in porcine blood

Following 20 minutes of continuous intravenous fluid administration in the in vivo study, both R-time and K-time were significantly increased meaning that the velocity of initial fibrin formation, and the kinetics of clot formation thereafter, were impaired. This finding is in keeping with current haemodynamic theory, which cites haemodilution due to intravenous fluid resuscitation as a major contributory cause of coagulopathy.

Following fluid resuscitation, in vitro treatment of blood samples with rFVIIa had no significant effect on R-time, while in vitro rFVIIa did lead to a significant decrease in K-time. The decrease in K-time after 20 minutes of intravenous fluid resuscitation illustrates that rFVIIa was still able to increase coagulation kinetics, despite haemodilution and physiological derangement. As the pattern of effect on K-time both prior to and after fluid administration was not significantly different it can be stated that, at least insofar as it is assessed by K-time, the procoagulant efficacy of rFVIIa was not significantly impeded by early fluid resuscitation. This preservation of efficacy may have significant implications in supporting the early use of the drug in trauma victims. Although R-time was not significantly reduced in the presence of in vitro rFVIIa following 20 minutes of fluid resuscitation, the absolute value of R-time at this timepoint was lower in the presence of in vitro rFVIIa, compared to placebo.

The lack of effect of in vitro rFVIIa on R-time may be due to the small number of animals and resultant impact of biological variation, since the preservation of efficacy in reduction of K-time does suggest that rFVIIa has the potential to confer some
procoagulant effect, even in this severe model of haemorrhage, shock and haemodilution. The apparent reduction in efficacy of \textit{in vitro} rFVIIa on R-time following intravenous fluid resuscitation may alternatively be due to a number of influences, including the diluting effect of clear fluids, which increase volume but effectively serve to wash out clotting factors and platelets already in scant supply. A further possible cause of the reduction in efficacy of \textit{in vitro} rFVIIa after intravenous fluid resuscitation may be the clinical condition of the subject, and particularly developing acidosis.\textsuperscript{171, 225} It is unlikely, however, that in the present series acidosis was a major contributor to the development of coagulopathy unresponsive to rFVIIa. Due to the severity of the model and the limited number of animals per group, it was necessary to perform this analysis after only 20 minutes of fluid resuscitation (only 25 minutes post haemorrhage). As such, it is highly unlikely that a significant acidotic state could have developed and exerted such an effect on coagulation within this time frame. This point is a major limitation of the present study, and future studies in this area would benefit significantly from selection of a less severe model in which acidosis may develop prior to the occurrence of a significant number of deaths.

Other studies have investigated the effects of haemodilution on coagulation parameters, some utilising TEG as a method of measuring these effects. One such paper found that progressive haemodilution was indeed associated with impaired coagulation as measured by thromboelastography.\textsuperscript{226} This study however employed a method of artificially diluting blood samples from healthy volunteers, meaning that the derived response to dilution may not have been representative of the physiological response to \textit{in vivo} haemodilution. The present study has the obvious advantage of utilising a physiologically relevant \textit{in vivo} method of achieving haemodilution. The
limitation, on the other hand, is that this physiological response would be influenced by a potentially wide range of other factors, such as inflammatory response and the effects of acidosis and hypothermia, as well as other systemic events. As such, in the present study, it is not possible to state with any certainty whether the effects on coagulation seen after 20 minutes of intravenous fluid resuscitation were due to haemodilution alone, or a combination of other effects.

One study published in 2007\textsuperscript{166} found that early administration of rFVIIa was associated with a significant reduction in transfusion requirement compared to that seen following late administration of rFVIIa. In this retrospective study, "early" was defined as administration of rFVIIa before transfusion of more than 8 units of blood, while "late" was defined as administration of rFVIIa after more than 8 units of blood had been transfused. Transfusion requirements were used as a proxy measure of the clinical condition of the patient, though the study found no significant difference in actual mortality rates between early and late recipients of rFVIIa. The lack of significant effect on survival however is unsurprising, as the number of patients included in both groups was small and within the early and late administration groups there was a considerable range of time delays before which rFVIIa administration occurred. In addition, the nature of retrospective series such as this means that there was inevitably a heterogeneous range of injury severities included in each group. These confounders mean that reaching statistical significance on such an absolute variable as survival was not to be expected. Further research into the effect of early versus late administration of rFVIIa on mortality is very much needed, and the findings from the present \textit{in vitro} study may provide some useful scope from which to base future research directions.
Haemorrhage and 20 minutes of intravenous fluid resuscitation significantly decreased the MA in the placebo, but not rFVIIa, treated samples. However, due to the small animal numbers and biological variation, the effect of rFVIIa compared to placebo at this time point did not reach statistical significance. The fact that there was no significant decrease in MA in those samples treated with rFVIIa suggests that, under the pathophysiological conditions (haemorrhage, shock and haemodilution) which were developing in the in vivo model, rFVIIa may have some protective role in improving clot strength. Thromboelastographic analysis is unable to provide any information on what this protective role may be, however it would seem reasonable to suggest that the previously discussed anti-fibrinolytic effect of rFVIIa, essentially making a clot less permeable and thus more resistant to dislodgement, could be a factor in this finding.
1.4 Sensitivity of TEG to rFVIIa administered in vivo

In order to determine the sensitivity of TEG to rFVIIa administered *in vivo*, standard TEG analysis, with no *in vitro* administration of rFVIIa was undertaken. Paired blood samples were drawn, one activated with innovin and the other with kaolin. Innovin and kaolin are two commonly used TEG activators and are discussed more fully in Section 5.6.

With innovin activation R-time was significantly increased following 10 minutes of intravenous fluid resuscitation, supporting the hypothesis that in this model, fluid resuscitation impairs coagulation by increasing the time taken to initiate clotting. No significant effect of *in vivo* rFVIIa on R-time was detected, though there was a clear trend for R-time to be decreased in those animals that had received rFVIIa *in vivo*. The lack of statistical significance is highly likely to be due to the very small number of animals included (n=4 in placebo group), and it is notable that the absolute value for R-time after 10 minutes of intravenous fluid resuscitation was lowest in the rFVIIa treated animals.

A similar pattern was seen with K-time, except that for this parameter, neither *in vivo* rFVIIa nor time were found to have a significant effect, though again there was a clear trend toward a reduction in K-time in the animals which had received rFVIIa *in vivo*.

Very similar findings were made in those samples activated with kaolin. In terms of R-time, identical patterns were found with *in vivo* drug having no effect while there was a significant increase in R-time by 10 minutes of fluid resuscitation. Again there
was a non-significant trend for R-time to be lowered at the 10 minutes of resuscitation
timepoint in those animals that had received \textit{in vivo} rFVIIa.

K-time was not significantly affected by \textit{in vivo} drug, though with kaolin activation
the effect of 10 minutes intravenous fluid resuscitation was found to significantly
increase K-time. Again, there was a trend toward a decrease in K-time in those
animals that received rFVIIa \textit{in vivo}, though it is notable that this trend appeared to be
more discrete with kaolin activation than that seen with innovin activation in paired
samples.

In the present study therefore, it appears that innovin and kaolin activation of blood
samples yielded broadly similar patterns of results. It should be noted however that
the absolute values derived for each parameter was much lower in the presence of
kaolin, compared to innovin. As such the difference between any two values appears
less in the presence of kaolin, as the scale of values is essentially compacted. For
example, looking at the absolute values of R-time after 10 minutes of intravenous fluid
resuscitation, the difference between intravenous placebo and intravenous rFVIIa is
688 seconds in innovin activated samples (Figure 25) and 303 seconds in kaolin
activated samples (Figure 26). While this does not affect the overall pattern of results
in this series, it is possible that the compression of the scale seen with kaolin activation
may mean that in some situations, discrete differences between groups may be
masked.

Since the overall pattern seen with both activators matched in the current series, and
given the more physiologically relevant method of activation being attributed to
innovin, as well as the issues of scale (discussed above), the sole use of innovin activation throughout the other sections of the present study is justified.

A reasonable conclusion to draw from this aspect of the study would be that, regardless of which activator was used, it is not possible to state whether any effect of \textit{in vivo} rFVIIa could be detected. This is due to the small number of animals included in the \textit{in vivo} groups, the necessity of which has already been detailed. Based on current understanding of coagulation and the mechanism of action of rFVIIa, as well as findings from the \textit{in vitro} studies and trends seen following \textit{in vivo} administration of rFVIIa, it is likely that there would be a detectable effect of \textit{in vivo} rFVIIa and that if animal numbers were increased statistical significance could be reached.
1.5 The effect of a second dose of rFVIIa

There are a number of conceivable instances, in both civilian and particularly military environs, where there may be considerable delay to definitive surgical haemostasis being reached. In such instances, even if rFVIIa had been administered early, achieving initial haemostatic control, the possibility exists that the clot may be dislodged and rebleeding may occur. This would be of particular concern if the patient was moved, or intravenous fluid resuscitation is undertaken. In such situations, the temptation may exist to utilise a second dose of rFVIIa to regain or strengthen temporary haemostatic control.

The efficacy of a second dose of rFVIIa was tested in vitro in the present study. Animals were split according to their in vivo treatment group (rFVIIa or placebo) and paired samples were then treated in vitro with rFVIIa and placebo. In those animals that had received rFVIIa in vivo, the rFVIIa treated blood sample represented a second dose of rFVIIa.

In order to assess whether a second dose of rFVIIa had any effect on coagulation, it was necessary to determine whether the change produced by in vitro rFVIIa was any different prior to in vivo rFVIIa compared to after in vivo rFVIIa. In order to answer this question, the difference between the effect of in vitro placebo and in vitro rFVIIa was measured and compared as follows:

- the effect of in vivo rFVIIa was measured through comparison of the two in vivo groups
the effect of resuscitation was assessed through comparison across
timepoints

Following haemorrhage, but prior to *in vivo* administration of either placebo or rFVIIa, samples treated *in vitro* with rFVIIa had shorter R-time and K-time than those treated *in vitro* with placebo. The increase in R-time and K-time seen following 10 minutes of continuous fluid resuscitation is in accordance with earlier aspects of this study, which found that fluid resuscitation was associated with a worsening of coagulation parameters.

The *in vitro* treatment of blood samples with rFVIIa had no significant effect on R-time, suggesting that, under the conditions in this model, a second dose of rFVIIa had no beneficial effect on coagulation as assessed by R-time. Unlike R-time, *in vivo* drug did significantly affect the response of K-time to *in vitro* rFVIIa.

The lack of a statistically significant of either resuscitation or *in vivo* drug on the effect of *in vitro* rFVIIa on R-time may be due to the very small number of animals included in this part of the study, or may be a true reflection (i.e. there may actually be no effect to be detected). It is not possible from this study to determine which of these possible explanations is correct, and further research is required to assess fully the efficacy of a second dose of rFVIIa. Repeating the present *in vitro* study, alongside an *in vivo* study with a lower early mortality rate would be one method of assessing the effect of a second dose.
In order to attempt to confirm whether the apparent lack of efficacy of a second dose of rFVIIa is a genuine finding (i.e. that the rFVIIa administered in vivo has improved coagulation to maximal effect so no further improvement could be made) or is an artefactual finding, due to the small number of animals available for inclusion in this analysis, the results were reanalysed in another way. In the reanalysis, the absolute values of R-time and K-time in the presence of in vitro rFVIIa were compared across the two timepoints.

When the data were analysed in this way, 10 minutes of intravenous fluid resuscitation after intravenous drug administration was found to significantly affect both R-time and K-time, reflecting the fact that both parameters were significantly increased following intravenous fluid resuscitation. This finding is in agreement with earlier findings in the present study.

Neither R-time nor K-time were significantly affected by in vivo treatment group, suggesting that in vitro rFVIIa appeared to be exerting no significant effect. Nevertheless, it is notable that the absolute values of both R-time and K-time were lowest in those samples which were treated in vitro with rFVIIa taken from animals which had received in vivo rFVIIa. Further research is required to determine whether this observation would reach statistical significance with a larger group size.

In addition to the requirement for a larger group size, it would be beneficial if a larger study employed an in vivo model that would allow a larger delay before administration of the second dose of rFVIIa. In the present study it was necessary to select a short time window since it was essential that all animals included in the analysis had
received fluids continuously up to the point of measurement. At later time points, a significant number of animals had ceased to receive intravenous fluid resuscitation therefore would have had to be excluded from this analysis. The in vivo model also had a high level of early mortality, particularly in the in vivo placebo group, meaning that attempting the second dose analysis at a later timepoint would have reduced animal numbers to a level which was too low to facilitate any meaningful comparative analysis. While a delay of only 10 minutes between doses of rFVIIa may be unrealitic in real terms, it was therefore necessary in this model system. A direct consequence of this may be that the coagulation status of the animals at this time following injury was not sufficiently deranged to enable any significant effect of rFVIIa be indicated. As discussed above, it is not possible to discern whether the lack of efficacy of a second dose of rFVIIa may be due to physiological factors such as this, or be artefactual, due to variation as a result of small animal numbers.
1.6 TEG Activators

Thromboelastographic analysis may use either native or artificially activated blood samples. In the absence of an activator, fresh whole blood samples are placed in TEG cups and analysed. Use of fresh blood samples may appear attractive since no exogenous agents are added, thus minimising the potential for human error and artefactual effects; however fresh blood samples take a considerable time to clot and suffer a considerable degree of variation between duplicates. For these reasons, it is common practice for activators to be used to initiate coagulation in TEG analysis. There are a number of different activators which are employed, such as collagen, celite, kaolin and innovin, each of which exert different effects upon blood samples.

Activators such as kaolin, celite and collagen are highly potent, acting upon the contact pathway of coagulation. As such, the initiation of coagulation in their presence is far more rapid than that seen with native blood, or indeed with activators such as innovin, which act upon the tissue factor pathway. Given our current understanding of the underlying mechanisms of coagulation, with the tissue factor pathway being responsible for initiation of the system, innovin is generally considered to represent a more physiological activation than, for example kaolin. It is for this reason that innovin was used as the main activator throughout this thesis. The difference between innovin and kaolin activation on paired blood samples was investigated in Section 4.4.

Over recent years, a number of studies have been published in which TEG has been used specifically to study the efficacy of rFVIIa in restoration of haemostasis. One
such study was conducted by Pusateri and colleagues and published in 2005, involving a parallel in vivo study which has been discussed previously in this thesis.

In the in vitro aspect of the study, the investigators used two different activators, collagen and porcine thromboplasin (tissue factor), in order to assess the efficacy of increasing doses of rFVIIa on normal porcine blood. Both activators showed that R-time and K-time were shortened and MA was increased in the presence of rFVIIa. In addition, the maximum velocity of clot formation was increased with rFVIIa dosing when porcine thromboplastin, but not collagen, was used to initiate the reaction.

These findings are contrary to those of Martinowitz and colleagues, who found no effect of rFVIIa on TEG parameters in their study. They also worked with porcine blood, however the TEG activator was not defined in this paper therefore it is feasible that an inappropriate method of activation may have been employed, effectively masking any effect that there may have been. No details were provided of the TEG protocol employed, therefore it is also possible that there may have been a fundamental error in their analysis.

The relevance of this distinction between TEG activators is that contact activators such as kaolin and collagen produce a marked activation of blood compared to “physiological” activation by tissue factor. As a result, coagulation tests activated by contact activators are less sensitive to discrete changes and may mask, for example, the procoagulant effect of rFVIIa.
1.7 Coagulopathy and monitoring

The potential severity of coagulopathy in trauma victims, and the contribution of the triad of acidosis, haemodilution and hypothermia to its development, was considered in the literature review. While coagulopathy may be recognised on visual examination through non-surgical bleeding and oozing from wounds and the vasculature, selection of effective management strategies is rarely straightforward. The relative contributions of the constituent members of the triad to the developing coagulopathy are notoriously difficult to discern and thus the replacement of the deficient blood component must often be performed blind; a costly and arguably inefficient process.

Traditional laboratory assays used to assess coagulopathy, principally prothrombin time (PT) and activated partial thromboplastin time (APTT), for many decades have been the primary tools used to guide management of non-surgical bleeding. These tests however utilise platelet poor plasma, and thus do not consider the cellular interactions underlying the haemostatic process. As such, they may not provide the complete picture of the mechanism of derangement in the coagulopathic patient. A further limitation of traditional laboratory assays such as PT and APTT is the time delay encountered between the drawing of a sample to yielding a result. As the clinical condition of a haemorrhaging patient is dynamic, in a constant state of flux, the result obtained from a sample drawn and analysed with some delay may bear little resemblance to the actual condition of the patient by the time the result is obtained.

An alternative means of assessing the haemostatic status of a coagulopathic patient is available in the form of thromboelastography (TEG). The theory of TEG was
discussed in the methods section. Briefly, TEG overcomes the limitations of the traditional laboratory assay, producing a real-time result, which is dependent on all of the components of the coagulation system. The principal parameters reported by TEG (R-time, K-time and MA) are each affected to a greater or lesser extent by specific constituents of the coagulation system. The R-time, representing the time to initial fibrin formation, is largely sensitive to availability of activated clotting factors. K-time, a surrogate marker of coagulation kinetics, is affected mainly by fibrinogen levels and function, and to a lesser extent platelets; while MA is a measure of ultimate clot strength, and is most significantly affected by platelets, with fibrinogen having a lesser effect.
5.8 TEG Successes & Limitations

In addition to monitoring coagulation in trauma patients, there are an abundance of reports in the literature of other clinical scenarios in which TEG has proved invaluable. TEG is now often included in routine use during cardiac and hepatic surgery\textsuperscript{228, 229} where it has repeatedly been found effective in guiding blood product selection through real time monitoring of the coagulation status of the patient.\textsuperscript{230-232} A further clinical area in which TEG is gaining in popularity is obstetric anaesthesia.\textsuperscript{229}

Despite these encouraging reports, TEG has not yet become a standard method of assessment of coagulation status in the wider clinical setting. Aside from the lack of published clinical trials and the lack of robust quality assurance data, a major limitation of the technique is that if fresh blood samples were to be run, this would necessitate a number of TEG machines to be available in a number of locations throughout a standard hospital; an expensive prospect, as well as a labour intensive one as the machines must be calibrated before use. Logistically, the use of native blood samples is made still more problematic by the fact that native blood samples must be run within a few minutes of being drawn.\textsuperscript{233, 234} Clearly, in some clinical as well as experimental scenarios, such a tight time constraint may be simply unfeasible.

An alternative approach is to use citrated blood samples, however a period of stabilisation of the sample must then be allowed, and calcium must be artificially replaced when the sample is to be run. In addition a number of investigators have suggested that citrate storage of TEG samples may not be an entirely reliable method, as it has been associated with the generation of erroneous results.\textsuperscript{233, 235-237} Whether
citrate storage does affect TEG analysis of blood samples is yet to be conclusively
determined, although several groups have demonstrated that citrated samples require a
stabilisation period of 30 minutes prior to being recalcified. Another group however reported erroneous results associated with citrate storage up to 60 minutes.
Furthermore, the duration of citrate storage over which the sample may be stable after
the initial period of instability remains to be defined, with different research groups
reporting development of hypercoagulability after different periods of prolonged
citrate storage of blood samples. A number of advocators of the use of
citrated blood samples have suggested that the process may be used successfully,
provided clear operating procedures are defined to minimise the scope for artefacts to
be introduced. Others have recommended that citrated blood is inappropriate for
use in trials on in vitro haemodilution. To date, there have been no formal
recommendations in terms of development of a standard protocol for citrate storage of
thromboelastography samples, with different groups employing different stabilisation
periods and durations of storage.

All of the published studies that have compared citrated samples to native blood have
been concerned with the stability of the sample in citrate storage, all acknowledging
that the results obtained from citrated blood samples are numerically different from
those obtained with fresh blood samples. The difference between TEG
parameters for citrated and fresh blood for a given sample may or may not have any
impact on the pattern of results obtained, however it does clearly demonstrate that the
process of citrate storage itself has an effect on the blood sample. Two groups of
investigators have demonstrated that the overall effect of citrate storage is a trend
towards hypercoagulability, compared to fresh blood samples. It is therefore
conceivable that, as was the case with contact activators, citrate storage may mask
discrete changes in TEG parameters that could otherwise have been measured.

In order to remove the opportunity for artefacts of storage to confound the results of
the present study, and to maximise the chance of detecting any changes should they
exist, fresh blood samples were used throughout all TEG analyses reported in this
thesis.
5.9 Other applications of rFVIIa

rFVIIa has been shown to be a safe and effective treatment in haemophilia patients with inhibitors, with efficacy rates of up to around 90% reported. Early experience with rFVIIa in haemophiliacs, and a description of its mechanism of action in such patients, has already been discussed (Section 2.5) and the drug is now well established as a conventional part of treatment of this group of patients.

rFVIIa has increasingly seen use in the management of other causes of impaired thrombin generation, including platelet disorders such as Glanzmann’s thrombasthenia and thrombocytopenia. It has been shown that rFVIIa increases the initial rate of, though not total, thrombin generation. It is thought that the resultantly increased platelet aggregation and activation mediated by rFVIIa explains the efficacy of rFVIIa in the presence of lowered levels of platelets. Essentially, rFVIIa appears to maximise the output of the limited number of functional platelets in conditions where platelet number or function is impaired, compensating for the deficiency to some extent.

Some studies have shown potential efficacy of rFVIIa in the treatment of liver disease and associated impaired synthesis of vitamin-K dependent coagulation factors. The haemostatic effect in such patients is likely largely attributable to the rFVIIa mediated upregulation of TAFI, an inhibitor of fibrinolysis. rFVIIa has been used successfully operatively and perioperatively to reduce transfusion requirements in various types of surgery, including not only liver transplantation, but also during obstetric and cardiac procedures. The encouraging case reports of rFVIIa efficacy as a
last resort method of arresting uncontrolled bleeding in surgical patients led the authors of the European guidelines on the use of rFVIIa as an adjunctive treatment for massive bleeding to include the recommendation that rFVIIa be considered for massively bleeding surgical patients where other treatments have proven ineffective. Similar recommendations were made regarding the use of rFVIIa in the treatment of life-threatening post-partum haemorrhage. Randomised, placebo-controlled trials to assess the efficacy of rFVIIa in these areas are however required before its use can be routinely recommended in such cases.

In addition to being used increasingly to control overt bleeding in scenarios typified by those discussed above, the potential prophylactic role of rFVIIa in preventing bleeding in elective surgery has also been addressed. One randomised, double-blind placebo-controlled trial found rFVIIa to significantly reduce perioperative blood loss in patients undergoing retropubic prostatectomy. The role of rFVIIa in prevention of bleeding in the non-bleeding patient warrants further investigation.

A further clinical area in which rFVIIa is currently being investigated is the management of intracerebral haemorrhage. rFVIIa was found to limit haematoma growth and decrease mortality in a double-blind placebo-controlled trial. It is however necessary to note that rFVIIa use in that study was associated with a significantly increased incidence of arterial thromboembolic events (principally myocardial ischemic events and cerebral infarction). The frequency of all serious thromboembolic events was not significantly increased in the rFVIIa treated group. The safety of rFVIIa specifically in trauma is considered in the next section.
1.8 Safety and cost effectiveness of rFVIIa in trauma

While the published clinical experience with rFVIIa in trauma is encouraging in that a signal of increased thromboembolic risk has not been noted, the data are insufficient to state that there is no risk. In addition to further clinical studies to determine the efficacy of rFVIIa in trauma patients, prospective controlled studies to assess fully the safety of the drug for this indication are also urgently required. A review of the safety of FVIIa in all published and unpublished clinical reports up to 2004 was recently published, which concluded that overall the drug appeared to be relatively safe, with a 1-2% incidence of thrombotic complications.\(^{251}\) An earlier review on the safety of rFVIIa quantified the risk of serious adverse events associated with rFVIIa therapy to be less than 1% and suggested that the majority of cases may be due to underlying pathology, rather than the rFVIIa itself.\(^ {252}\)

In addition to safety considerations, a further contentious issue influencing the likelihood of more widespread use of rFVIIa is the cost of the product (currently a 1.2mg vial costs £634.81 plus tax in the UK).\(^{253}\) A recent study examined the lifetime cost-effectiveness of rFVIIa for the control of bleeding specifically in blunt trauma victims.\(^ {254}\) Based on their model, the authors concluded that for this indication, rFVIIa may be a cost-effective option for the UK National Health Service. Further evidence, in terms of efficacy, safety and cost-effectiveness will however be required before rFVIIa becomes accepted as a standard part of the arsenal available to clinicians in the management of traumatic bleeding.
1.9 Future Research Directions

1.10 Resuscitation Fluids

Returning briefly to the effects of haemodilution, there have been considerable research efforts examining the relative effects of different resuscitation fluids on coagulation. The ultimate goal of such studies would be to determine which (if any) of the available products, including crystalloids, colloids and hetastarches, are associated with less negative effects on coagulation. One such study concluded that there may be some benefit to utilising balanced electrolyte solutions, such as Ringer's, compared to saline, though much more research is required before firm recommendations may be drawn from studies of this type.

1.11 Future directions for rFVIIa (superactive variants)

Despite the relatively recent launch of rFVIIa, work is already well underway in the development of so-called superactive variants. A novel analogue of rFVIIa (V158D/E296V/M298Q-rFVIIa, NN1731) has undergone development in recent years and has shown promising results in a study published in 2007. Blood samples were taken from patients with severe haemophilia A and were then treated with either NN1731, rFVIIa or buffer as a control prior to TEG analysis. The authors reported that NN1731 was associated with more rapid coagulation as measured by TEG than that seen with rFVIIa. Evidence was also presented which suggested that NN1731 increased not only the kinetics of clot formation but also the stability of the formed clot. A further study, also published in 2007, followed a similar protocol, utilising an alternative system for the measurement of the effect on clot structure and platelet
function. This study is reported to have found greater platelet function and clot structure in the presence of NN1731, compared to either rFVIIa or buffer. In addition, the authors reported less variability in response with NN1731 than that seen with rFVIIa, with nine compared to four of ten patients having normalised coagulation parameters, respectively. The development of novel, superactive, rFVIIa analogues may further increase the scope for the use of these products in the management of severe acute bleeding in trauma victims. Further research, particularly in an in vivo model, is required and anticipated in order to assess the potential and efficacy of novel rFVIIa superactive variants, such as NN1731.
1.12 Conclusions

This study showed that rFVIIa significantly enhanced coagulation kinetics, compared to placebo, when added *in vitro* to normal porcine blood samples. The early effect of haemorrhage was also a significantly increased rate of clot formation. rFVIIa added *in vitro* to this blood further enhanced coagulation.

20 minutes of intravenous fluid resuscitation significantly impaired coagulation. The impairment of coagulation kinetics (but not initial velocity of clot formation) could be reversed by *in vitro* treatment of blood samples with rFVIIa. These findings suggest that early administration of rFVIIa may confer haemostatic benefit, even following severe haemorrhage and intravenous fluid resuscitation. While the SEM demonstrates a wide spread of data around the sample mean in a number of figures, the data still displays a clear trend which is appropriate for analysis and interpretation. Further studies should aim to decrease the variation.

Trends toward improved coagulation kinetics were noted in those animals which had received rFVIIa *in vivo*, however due to small group sizes, significance was not reached. In assessment of the effects of a second dose of rFVIIa (the second dose being administered *in vitro* following an *in vivo* first dose), some parameters showed enhanced clotting while other showed impaired clotting. These changes were not statistically significant and the implications cannot be evaluated without increasing the numbers of animals to establish which changes are significant.
Future research directions should include larger animal numbers and/or an alternative model, with a lower early mortality rate (i.e. matched resuscible patients) to allow evaluation of rFVIIa over a longer time period. Any new analogues of rFVIIa should be similarly assessed to see whether any confer particular advantages.
REFERENCES


90. Mosnier LO, Lisman T, van den Berg HM, Nieuwenhuis HK, Meijers JCM, Bouma BN. The defective down regulation of fibrinolysis in haemophilia A can be restored by increasing the TAFI plasma concentration. *Thrombosis and Haemostasis*. 2001;86:1035-1039.


209


219


256. Sorensen B, Persson E, Ingerslev J. Factor VIIa analogue (V158D/E296V/M298Q-rFVIIa) normalises clot formation in whole blood from patients with severe haemophilia A. British Journal of Haematology. 2007;137:158-165.