

Review Article

Recent developments in topical thrombins

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Summary

Managing blood loss is part of the surgeon's responsibility during surgical procedures, and a variety of therapeutic strategies are available to help accomplish this. Topical haemostatic agents are among the agents used to control surgical bleeding and locally arrest blood flow. Bovine thrombin is a commonly used topical haemostatic agent; however, its use has been associated with potential risks, including well-documented cases of antibody-mediated coagulopathy. This coagulopathy develops as a consequence of antibody formation directed against bovine thrombin, other bovine coagulation proteins, and their human orthologs. The fact that a coagulopathy can result in association with the use of bovine plasma-derived thrombin preparations prompted the FDA to require pharmaceutical companies to place a black-

box warning in their prescribing information for products containing bovine plasma-derived thrombin. Recently, human plasma-derived thrombin and recombinant human thrombin have been approved by the FDA with the expectation that they will be less immunogenic than the bovine-derived product. In clinical studies, purified human plasma-derived thrombin and recombinant thrombin have demonstrated equivalent efficacy and safety, with improved immunogenicity profiles compared with bovine-derived thrombin agents. Well-designed and adequately powered clinical trials should be conducted to indicate whether human thrombin products would improve the risk-benefit and cost-benefit profiles for surgeries complicated by excessive bleeding.

Keywords

Bovine, coagulopathy, haemostasis, recombinant, thrombin

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Introduction

Managing blood loss is a significant part of the surgeon's responsibilities during surgical procedures, and there are a variety of therapeutic strategies available to accomplish this. Good operative technique and anesthetic support are of paramount importance in the prevention of perioperative haemorrhage (1, 2). Appropriate pharmacologic and haematologic support for operative procedures can inhibit bleeding and support clot formation (3–5). Many blood management strategies stem from scientific insights into the haemostatic process, and pharmacologic haemostatic products have been developed based on research on the coagulation cascade.

Topical haemostatic agents are one weapon in the armamentarium of agents used to control surgical bleeding. A wide variety of topical haemostatic agents are available, including gelatin sponges, collagens, fibrin sealants, and thrombin preparations (Table 1) (3–6). Bovine thrombin is employed as a topical haemostatic agent in more than one million surgical procedures per

year. However, its use has been associated with risks, including the development of autoimmune iatrogenic coagulopathies (2). This article will review the use of topical thrombin, the risks associated with the use of bovine thrombin, and the recent development of plasma-derived human thrombin and recombinant human thrombin as potential substitutes for bovine thrombin products.

Role of thrombin in the coagulation cascade

Haemostasis is a complex cell-based process that involves vasoconstriction, platelet plug formation, fibrin formation and cross-linking, and fibrinolysis (Fig. 1) (1). Following a vascular insult, a series of self-activating biochemical feedback events occurs on cell surfaces containing exposed tissue factor or phospholipids, ultimately leading to bursts of thrombin generation (7). Coagulation is initiated by physical trauma or molecular signals that convert the normally resting endothelium into a procoagulant surface, initially manifested by the downregulation of antithrom-

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Table 1: Examples of currently available topical haemostatic agents to aid surgical haemostasis.

Mechanical haemostats
– Porcine Gelatin: Gelfoam® (Pfizer – Pharmacia) and Surgifoam® (Ethicon, J&J)
– Bovine Collagen: Avitene® and Ultrafoam™ (Davol)
– Cellulose: Surgicel®, Surgicel Fibrillar™, and Surgicel Nu-Knit™ (J&J)
– Polysaccharide Spheres: Arista™ (Medafor)
Active haemostats
– Bovine thrombin: Thrombin-JMI® (King)
– Human pooled plasma thrombin: Evithrom™ (Omrix)
– Recombinant thrombin: Recothrom® (ZymoGenetics)
Flowables
– Gelatin + Thrombin: FloSeal™ (Baxter) and Surgiflo™ (J&J)
Fibrin sealants
– Tisseel™ (Baxter) and Evicel™ (J&J)

bin III and thrombomodulin (1). Humoral coagulation activity is propagated via the traditional sequential cascade mechanism, initiated when a complex of tissue factor, a cell surface transmembrane glycoprotein, and clotting factor VIIa forms on cell surfaces or cell fragments, and then leads to proteolytic activation first of factor IX and then of factor X along the intrinsic pathway of coagulation. Only a small and probably physiologically insignificant amount of factor Xa is directly generated by the tissue factor-factor VIIa complex via the extrinsic pathway because of the down-regulatory properties of tissue factor pathway inhibitor (TFPI). Activated factor X (Xa) subsequently converts prothrombin to thrombin with the aid of the catalytic mediator, activated factor V. Alterations at the endothelial cell surface, such as changes in the degree and character of phospholipid exposure and the composition of the cell surface membrane, down-regulation of thrombomodulin, and modulation of the endothelial surface membrane availability of endogenous proteoglycans, are instrumental in the formation of a procoagulant environment. The coagulation process is amplified via platelet-mediated thrombin generation (i.e. thrombin bursts) and activation of factors V, VIII, and XI (1, 8).

The final step of the coagulation cascade is controlled by the enzyme thrombin (factor IIa), a molecule that has been identified as central to thrombus development since the early work by Morawitz (9). Thrombin is a serine protease that catalyses the conversion of fibrinogen to fibrin, induces cross-linking of the fibrin clot through factor XIII activation, and activates platelets (2, 10). Under normal circumstances without vascular insult, thrombin is bound by cell-surface thrombomodulin, leading to activation of protein C and formation of the protein C-protein S complex, thus damping down thrombin generation and maintaining a nonthrombogenic environment.

Bovine thrombin as an adjunct to surgical haemostasis

Control of surgical bleeding requires good operative technique, anesthetic support, and transfusion management strategies, as

well as preserving a balance between activated clotting factors and their zymogen states, maintaining an appropriate systemic pH, and achieving mechanical control of blood flow. Topical haemostatic agents are one means of controlling bleeding, which is accessible and overt. Thrombins from multiple sources have been formulated into pharmacologically suitable topical procoagulant preparation, which can be utilised in a variety of clinical bleeding situations, including neurologic, orthopaedic, cardiac, thoracic, vascular, gynecologic, head and neck, and dental surgeries. Thrombin preparations can be used by themselves as single agents or in conjunction with other topical haemostatic treatments, such as gelatin sponges, combination matrices (e.g. gelatin plus thrombin matrix), and fibrin sealants (6, 11).

Thrombin was first utilised as a surgical haemostatic agent more than 60 years ago. Since that time, several formulations of bovine thrombin have been developed. Thrombostat® (Parke-Davis) was first approved for use as a surgical haemostatic agent in 1943. While this preparation was an effective haemostat, coagulopathies have been associated with cross-reacting antibodies that develop in response to contaminating bovine proteins such as factor V and factor Va (12). The bovine thrombin preparation Thrombogen® (Johnson & Johnson) gained Food and Drug Administration (FDA) approval for surgical haemostasis in 1986. However, this formulation was also plagued with complications attributed to contaminating proteins in the preparation. A quantitative evaluation of protein purity found that Thrombostat® and Thrombogen® contained only 28% and 24% thrombin, respectively (13). These formulations were likely to contain multiple contaminating proteins including factor V and factor X.

In contrast to Thrombostat® and Thrombogen®, the latest bovine thrombin preparation to be approved, Thrombin-JMI® (GenTrac), was found to be a relatively pure. Thrombin-JMI® is 96% thrombin, with little detectable contaminating factor V (less than 0.2 µg/ml) (13, 14). Thrombin-JMI® also contains 60-fold less galactose α 1-3galactose, a xenogeneic carbohydrate that has been shown to elicit an immune response in humans (15). Overall, Thrombin-JMI® is less reactive and elicits fewer immune responses than Thrombostat® and Thrombogen®. However, this formulation still contains protein contaminants that are stimulatory to the human immune system (13, 15). It is likely that the impurities present in all of the formulations of bovine thrombin contribute to antibody development in patients, and potentially, adverse clinical sequelae.

Bovine thrombin is used in more than one million procedures annually in the United States and worldwide (2, 16). Although it is a highly effective haemostatic agent and remains a readily available commercial product, use of bovine thrombin entails both hypothetical and realised risks, including the possible transmission of animal-based blood-borne pathogens and the stimulation of immune responses that can lead to autoantibody-induced coagulopathy (2). Published reports have documented that both single and repeated exposures to bovine thrombin-containing commercial products can trigger the development of xenogenic antibodies that cross-react with human coagulation proteins to induce coagulopathic bleeding (2, 14, 17–20). The documentation and reporting of patients' exposure to bovine thrombin during surgical procedures is suboptimal and the actual incidence of thrombin-induced complications is likely underestimated and

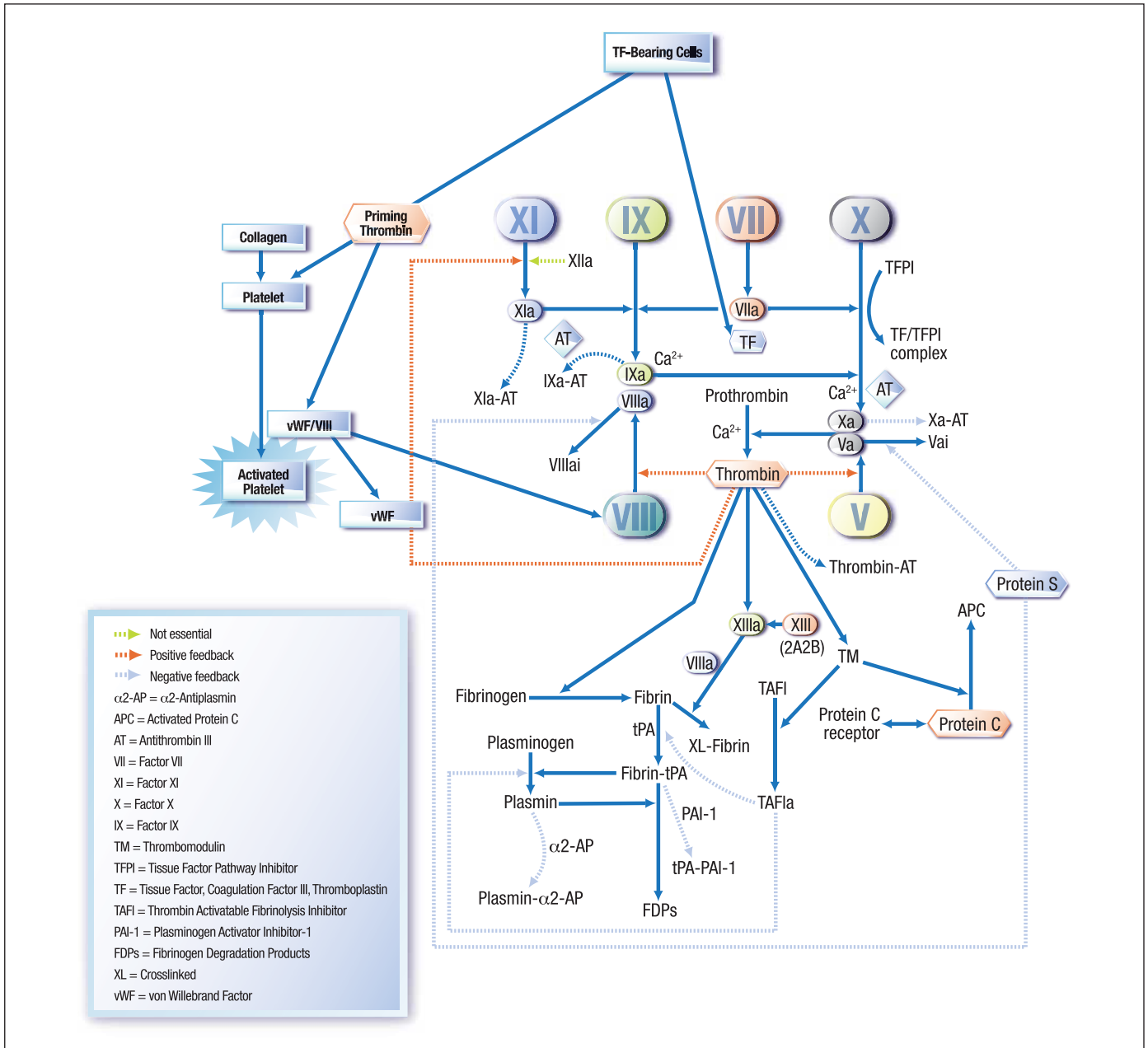


Figure 1: Haemostasis and the coagulation cascade.

underappreciated. Nevertheless, when formulating a risk-benefit analysis for any of the broad array of surgical procedures (Table 2) that are likely to involve the use of haemostatic products containing bovine thrombin as an active ingredient (21), the potential for antibody-induced coagulopathy should be considered.

Recently, the FDA approved topical haemostatic products containing human plasma-derived thrombin or recombinant human thrombin. The FDA approved pooled human plasma-derived thrombin prepared using a refined purification method (Evithrom™, Omrix Biopharmaceuticals Ltd., Israel) (Table 3) in 2007 and in 2008, recombinant human thrombin (Recothrom®, ZymoGenetics, Seattle, WA, USA) received FDA approval as a topical haemostatic agent. These human thrombin preparations have demonstrated comparable efficacy and reduced immunoge-

nicity relative to the original first-generation marketed bovine thrombin products (2, 7).

Risks associated with bovine thrombin

Transmission of disease

Although rigorous donor screening programs have been mandated by the FDA to ensure the safety of donated blood, any human plasma-derived pharmaceutical carries the potential risk of transmitting blood-borne pathogens (22, 23), particularly those lacking a lipid envelope (e.g. parvovirus B19, hepatitis A, prions, etc.), which are not susceptible to currently utilised viral attenuation techniques. The potential for the transmission of species-specific pathogenic viruses and prions also complicates

the safety of bovine plasma-derived products, given that adequate screening and attenuation/elimination techniques for such pathogens in bovine blood are not always applied and are not 100% effective (24).

Table 2: Procedures that may result in initial patient exposure to bovine thrombin[†] [21]. [†]Clinical context: It is important for surgeons to recognise the various types of procedures that may have resulted in a patient's initial exposure to bovine thrombin.

Surgical specialty	Procedures likely to use bovine thrombin
Vascular and cardiothoracic surgery	<ul style="list-style-type: none"> – Carotid endarterectomy – Vascular grafts – Coronary artery bypass grafts – Valve surgery – Abdominal aortic aneurysm – Haemodialysis access – Lower extremity revascularisation – Transplantation
Neurosurgery	<ul style="list-style-type: none"> – Craniotomy – Spine/back procedures – Peripheral nerve – Neurovascular
Orthopaedic surgery	<ul style="list-style-type: none"> – Joint replacement – Bone grafts – Osteotomy – Open reduction, internal fixation – Laminectomy – Back/spine procedures
Burn/trauma surgery	<ul style="list-style-type: none"> – Wound debridement – Skin grafts – Solid organ injury
Abdominal/pelvic surgery	<ul style="list-style-type: none"> – Liver resections – Cholecystectomy – Bowel anastomoses – Partial nephrectomy – Retroperitoneal operations – Total abdominal hysterectomy – Transplantation

Table 3: Timeline of developments related to thrombin formulations.

Year	Milestone
1940	First clinical use of bovine thrombin
1943	Approval of Thrombostat [®] (Parke-Davis)
1970	FDA “grandfathered” bovine thrombin
1978	Feinstein (Feinstein 1978) report of 12 patients with acquired factor V inhibitors
1982	FDA approval of Thrombinar (Armour Pharmaceutical Co.)
1986	FDA approval of Thrombogen [®] (Johnson & Johnson Medical, Inc.)
1995	FDA approval of Thrombin-JMI [®] (GenTrac, Inc)
1996	Black box warning for bovine thrombin formulations
2007	FDA approval of pooled human plasma thrombin (Evithrom [™])
2008	FDA approval of recombinant thrombin (RECOTHROM [®])

Prions are small, infectious, proteinaceous particles devoid of nucleic acids. They have been identified as analogs of a protein called PrP^C that is normally found predominantly on the surface of neurons, and at lower levels on circulating leukocytes (24, 25). Bovine spongiform encephalopathy (BSE) (24) and the related human disease, variant Creutzfeldt-Jakob disease, are examples of transmissible spongiform encephalopathies believed to be caused by prions. Current FDA guidelines prohibit the use of bovine-derived materials from countries with documented incidence of BSE, so the risk of infection from bovine thrombin is comparatively low (26). Although no documented cases of source host-to-recipient prion transmission by commercially available pooled plasma-derived human or bovine thrombin-containing products have been reported, studies employing experimental animal models suggest that such transmission can potentially occur (27, 28). Fractionation techniques and nanofiltration can decrease the quantity of prions in plasma-derived products, but there is currently no way to completely eliminate the potential risk (29).

Antibody development and immunogenic coagulopathy

When animal-derived proteins are utilised as therapeutic agents, host antibodies against the xenogenic protein may develop and subsequently block the pharmacologic benefits of the therapy, especially upon repeated exposure (30). For example, the administration of porcine plasma-derived factor VIII concentrate to treat bleeding complications in haemophiliacs with alloantibody inhibitors stimulated anamnestic immunologic responses in approximately 30% of recipients. Titers of both anti-human and anti-porcine factor VIII antibodies increased, frequently to levels that neutralised the clinical haemostatic benefits of the replacement factor VIII therapy (31, 32). The therapeutic use of bovine thrombin, which shares 70% structural homology to human thrombin (33), has also been associated with the development of antibodies in the human recipient. These antibodies are directed against bovine thrombin and contaminants in the bovine thrombin preparation, but some antibodies may also cross-react with the recipient's native coagulation proteins (predominantly factor V, thrombin or both) (12, 34–38).

The ability of bovine thrombin to elicit an antibody response was first reported in 1989 (39), Flaherty et al. observed prolonged thrombin times in four patients who had been treated with bovine thrombin during surgery. The authors were able to affinity purify antibodies specific for bovine thrombin from the patients' serum and demonstrate that these antibodies were able to prolong the thrombin time of normal plasma. Zehnder and Leung (40) added to these initial findings when they reported that a patient exposed to bovine thrombin during cardiac surgery developed a severe bleeding diathesis. The patient's factor V activity was 1% of normal due to antibodies to bovine factor V that cross-reacted with the patient's endogenous protein.

Since these initial findings, a number of studies and reports have published evidence documenting the incidence of anti-bovine fibrinogen, anti-bovine thrombin, and anti-bovine factor V antibodies following exposure to bovine thrombin (12, 35–38, 41). Due to the structural similarities of bovine and human coagulation proteins, the bovine antibodies may be cross-reactive to the human proteins (41), or patients may develop human coagulation-protein antibodies (13, 34, 41).

A study by Ortel et al. presented the first prospectively collected clinical data defining the immune response in individual patients exposed to bovine-derived thrombin (Thrombogen®) during cardiac surgery (34). The authors observed that 94.3% of their patients manifested an immunologic response to Thrombogen®, becoming seropositive with elevated antibody titers against one or more bovine coagulation proteins. The majority of patients developed antibodies specific for bovine factor V (80.7%) and bovine factor Va (90.7%), suggesting the presence of contaminating bovine proteins in the thrombin preparation. Regardless of the purity level, patients are at risk for developing cross-reactive antibodies, as 20.5% of patients also developed antibodies to bovine thrombin (34).

Of greater concern was that 51% of the individuals exposed to bovine-derived thrombin in this study also developed antibodies cross-reacting with their own endogenous human coagulation proteins (34). Furthermore, 33% of the patients who had prior exposure to bovine thrombin had detectable preoperative antibodies to two or more bovine antigens (34). Concerns about the persistence of these antibodies is illustrated by a report of nine patients who developed coagulopathies after a second exposure, the initial exposure had occurred as many as 12 years prior and of the four patients who developed coagulopathic bleeding there was one mortality in which the coagulopathy was considered a significant contributory factor (42). Furthermore, in another report, three years after a known exposure to bovine thrombin, a patient developed severe epistaxis subsequent to a second exposure (37). In this patient, the authors were able to demonstrate that not only did the patient develop antibodies against bovine thrombin, but that they interfered with both the coagulant and anticoagulant functions of thrombin. They also found that their patient had developed antibodies to factor V and fibrinogen after a second exposure to bovine thrombin.

While the Ortel study (34) reported that 94% of the cardiac patients in their study developed antibodies in response to bovine thrombin exposure, the incidence rates of antibody reported in the literature range anywhere from 10% to 90% (20, 34, 36, 41). This variability is likely due to the type of surgery, the history of previous bovine thrombin exposure, and the purity of the bovine thrombin preparation. For example, a comprehensive literature review found that factor V antibodies develop in 40–66% of cardiac surgery patients in contrast to only 20% of neurosurgery patients (20). In addition, if patients have had prior exposure to bovine thrombin, they are eight times more likely to develop antibodies to bovine thrombin or other coagulations factors upon subsequent exposures (36).

The availability of different topical thrombin preparations is a probable explanation for the wide range antibody incidences reported. The bovine thrombin preparations vary in the amounts of thrombin, prothrombin and factor V. Thrombostat® and Thrombogen® are only 20–30% thrombin, in contrast to Thrombin-JMI® which has been shown to be 96% pure thrombin (13). Thrombostat® and Thrombogen® contain significant amounts of contaminating factor V, which is significantly reduced, but still present in Thrombin-JMI® (13). Even the relatively pure formulation of Thrombin-JMI® has been shown to elicit antibody responses. In a study of individuals undergoing cardiac, hepatic, iliac, and general surgery who were exposed to Thrombin-JMI®,

29% developed antibodies to bovine thrombin (13). Of these patients, 30% also developed antibodies to human thrombin. However, the number of patients positive for human thrombin antibodies was not statistically different from the control patients who were treated with InStat® collagen-absorbable haemostat without thrombin.

Despite extensive efforts, it has been virtually impossible to purify bovine thrombin to the point of complete freedom from other contaminating coagulation factors, and immune-mediated coagulopathies would be expected to occur to some degree even with exposure to highly purified products derived from pooled bovine plasma (34). Recently, additional purification processes directed at reducing contaminants have been developed for the preparation of bovine thrombin. Bovine thrombin subjected to newer chromatographic and membrane filtration purification steps is approximately 20 times purer and contains approximately 60 times less factor Va than unpurified bovine thrombin products (43). While the new purification steps appear to reduce the levels of factor V in this bovine thrombin preparation, the clinical advantages of this reduction, but not total elimination, remain unknown.

Despite the increased purity of the bovine thrombin preparation, it is likely that patients will continue to develop anti-bovine thrombin antibodies due to the bovine, and therefore “foreign”, origin of the product. In fact, a recent phase 3 study comparing thrombin of human or bovine origin found that patients receiving human thrombin did not develop human thrombin antibodies, while 10/126 patients receiving bovine thrombin developed anti-bovine thrombin antibodies (44). Out of these 10 patients, three seroconverted to anti-human thrombin antibodies. In contrast to the 7.94% of patients who developed anti-bovine thrombin antibodies in this study, it is well documented that 20%–40% of patients develop anti-bovine thrombin antibodies in response to exposure to topical bovine thrombin preparations (13, 34, 36, 37, 39, 45, 46). This difference is likely related to methods of detection and reagents used in the study.

A serological analysis of patients treated with a surgical haemostat containing a purer form of bovine thrombin, Thrombin-JMI®, found that 48% of patients demonstrated reactivity against bovine thrombin (13). Furthermore, greater than 80% of the patients with anti-bovine thrombin antibodies had prior surgical procedures during which they were likely exposed to bovine thrombin. Similar results were also found by Ortel et al. (34). These authors found that 4–8 weeks after surgery, 47% of patients had detectable antibodies to bovine prothrombin and 20.5% had detectable antibodies to bovine thrombin. Significant preoperative levels of antibodies to bovine antigens were also detected. This further demonstrates the persistence of anti-bovine antibodies due to previous bovine thrombin exposure, which may result in an increased cumulative risk for adverse events. It is advisable that patients, who have been or are highly likely to have been exposed to bovine thrombin once, avoid subsequent exposure to bovine thrombin.

In addition to issues of purity, information regarding the commercial formula of bovine thrombin, as well as a quantitation of thrombin exposure is not always reported in studies evaluating immune reactivity. In a recent literature review of 37 papers on surgical patients, only 59.5% of papers listed the com-

mercial source of thrombin. Further, only 19% reported bovine thrombin exposure in units (47). Confirmation of bovine thrombin use can be difficult and an extensive review of patient records is often needed to confirm exposure (20).

Clinical events

Due to the variability in purity, as well as incomplete information on the thrombin formulation utilised, it difficult to sort out adverse outcomes that may be related to particular thrombin preparations. Despite this difficulty, the link of antibody development to clinical sequelae in patients has been well documented. A number of reports have demonstrated that exposure of bovine thrombin can lead to adverse clinical sequelae such as prolonged in-vitro clotting times, haemorrhagic complications, thrombosis, or even anaphylaxis (12, 20, 34, 38, 40, 47–49). However, the majority of these reactions are likely to be the result of the less pure bovine preparations, Thrombostat® or Thrombogen®.

Antibodies to bovine thrombin may be associated with impaired factor V clotting. In one case study, factor V clotting was reduced to 9% of normal with the inhibitor to bovine thrombin persisting for one year (35). In addition, 11/24 cardiac surgery patients and 2/10 neurosurgery patients exhibited prolonged thrombin time and low factor V clotting activity (105 to 60% of normal) that was attributed to the presence of inhibiting antibodies (35). In a comprehensive literature review, Streiff and Ness found that 33% of patients developed coagulopathies related to bovine thrombin-associated antibodies (20). Antibodies to coagulation factors can lead to life-threatening bleeding, and in 6% of these patients, the bleeding was fatal. Antibodies to thrombin, fibrinogen, and factor V can also result in severe epistaxis, prolonged coagulation tests, and decreased plasma factor V in response to bovine thrombin exposure (37).

Even if contaminating factor V was completely removed from bovine thrombin preparations, there remains a significant risk of clinical sequelae associated with the development of anti-thrombin antibodies. Upon exposure to bovine thrombin, bleeding is the most common complication associated with anti-thrombin antibodies (50). The presence of anti-bovine thrombin antibodies has been associated with significant complications such as thrombosis, mild-to-severe epistaxis, fatal haemorrhage, prolonged coagulation tests, and delayed haemostasis (37, 45, 46, 50). The delayed haemostasis appears to be particularly relevant in patients undergoing cardiac surgery (44). Furthermore, the homology between bovine and human thrombin may result in the generation of not only anti-bovine thrombin antibodies, but also anti-human thrombin antibodies. Cross-reactivity to human thrombin may occur in as many as half of the patients who develop antibodies to bovine thrombin products (50), leading to significant impacts on the normal function of the coagulation cascade.

In an analysis of case reports and case series, Crean et al. (47) found that 59% reported 23 adverse events related to bovine thrombin exposure in 44 patients. An analysis restricted to those studies that listed the source of bovine thrombin found that all formulations resulted in post-surgical bleeding related complications such as excessive bleeding, epistaxis, and excessive oozing in a variable number of patients (Thrombin-JMI®: 1/6 pa-

tients; Thrombostat®: 5/6 patients; Thrombostat®, Thrombinar®, or Thrombogen®: 5/16 patients; and Thrombinar®: 1/3 patients) (47). Unfortunately, only 59% of the reports demonstrated prolonged clotting time by mixing case and normal sera; of these, only 30% confirmed that the inhibition was due to the presence of antibodies specific for coagulation proteins. An analysis of the data from cohort studies found that 38–94% of patients raised an antibody response to bovine thrombin (47). While complications were common, most of the larger studies were unable to conclusively correlate bovine thrombin exposure and antibody development to adverse outcomes (47).

In contrast to these studies, Ortel et al. (2001) found that patients with antibodies to multiple bovine proteins were five-fold more likely to experience complications (34). Antibodies were more likely to correlate with complications if the patients had elevated antibodies to coagulation proteins prior to surgery; this is likely due to prior bovine thrombin exposure (34). In addition, coagulation abnormalities were most common in patients that developed antibodies to human coagulation proteins (34). By 4–8 weeks after surgery, 36% of patients had abnormal coagulation test results (multiple abnormal test results, isolated prolonged prothrombin times, or isolated prolonged activated partial thromboplastin times). Of these patients, 56% had elevated antibodies to human coagulation proteins. Postoperative complications included 27 incidences of haemorrhagic complications, 12 of thromboembolic complications, 21 wound complications, and five deaths. While there was no association between elevated anti-coagulation protein antibody levels after surgery and an increased risk for an adverse outcome, there was a significant increase in risk for those patients with elevated antibody levels to two or more bovine proteins prior to surgery. Of the 15 patients who had prior surgeries and were also positive for preoperative bovine antibodies, 73% had adverse events. This is in contrast to only 33.8% of patients without prior surgeries and detectable antibodies at baseline. This study strongly suggests that if a patient has been previously exposed to bovine thrombin, it may be safer to use a human product in additional applications.

While clinical awareness of bovine thrombin-associated coagulopathies has increased over the last few years and has stimulated publication of numerous case reports and clinical studies, it is difficult to determine whether a specific patient population, a particular type of surgery, or a distinct manner of application of the haemostatic product increases the likelihood of antibody development and acquired coagulopathy. It is clear that many of the cases of acquired coagulopathy have occurred following major surgery (51), particularly cardiovascular surgery, and have predominantly affected older males (over the age of 65) (19, 20, 49). One report suggested that the presence of anti-thrombin antibodies may not become clinically apparent until long after exposure to the bovine-derived thrombin haemostatic agent (19). The age-related manifestation of the immunologic response probably reflects the fact that older patients are more likely to undergo cardiac surgery rather than indicating a particular immunologic susceptibility and response to bovine thrombin among older individuals (52). In fact, the phenomenon of clinically significant bleeding secondary to bovine thrombin exposure is not unknown in even very young children, commonly those with congenital cardiac malformations requiring multiple procedures (18, 53).

Post-exposure bleeding has been reported in children two years and younger, with the youngest case reported in a child three months of age following a second surgical intervention.

Another feature of the immunogenic coagulopathy is that most cases of bleeding problems caused by bovine thrombin exposure appear to occur after repeated exposures to bovine thrombin. However, instances of severe haemorrhagic complications following a single exposure to bovine thrombin during cardiac surgery have also been reported (14). There are also published reports of acquired coagulopathy in patients undergoing non-cardiac surgeries such as spinal and gynecologic procedures in which topical bovine thrombin was used as a haemostatic agent (17, 18, 54).

Thromboembolic complications have also been recognised in association with the use of topical thrombin haemostatic agents, particularly of bovine origin. A post-hoc analysis of the study by Chapman (55) indicates that patients who developed anti-bovine thrombin antibodies experienced a non-significantly higher number of thromboembolic events compared with those who did not develop anti-bovine thrombin antibodies. Similar observations were made for those exposed to recombinant thrombin. Furthermore, there was no difference in the incidence of thromboembolic events (approximately 6%) between the bovine and recombinant thrombin cohorts (56). There is a possibility that exogenously applied thrombins of any origin could be absorbed into the systemic circulation and activate coagulation *in vivo*. It is also possible that hypercoagulable complications could have been precipitated by the formation of autoantibodies against one or more of the coagulation and phospholipid components of the prothrombinase complex, perhaps similar to the pathophysiologic mechanism(s) by which hypercoagulability occurs in the context of the antiphospholipid antibody syndrome (57).

The diagnosis of bovine thrombin-induced coagulopathy or other antibody-associated complications may be underreported

due to the lack of commercially available assays and the delay in time between exposure and presentation of symptoms. Table 4 summarises the published cases of bovine thrombin-induced coagulopathy reported since 2002. These cases have appeared since the aforementioned remanufacture of bovine thrombin was undertaken in 2001 to reduce the levels of bovine-related contaminants in the product, such as factor V. The actual incidence of anti-bovine thrombin antibody formation associated with bovine-derived haemostatic products has been difficult to ascertain, since only clinically recognised, significant bleeding events will trigger a laboratory evaluation. There are no commercial assays for antibodies to bovine thrombin, factor V, or prothrombin, and the diagnosis is conjectural, based on clinical context and *in vitro* coagulation laboratory results indicating the presence of circulating cross-reacting neutralising inhibitory antibodies directed against human thrombin and/or other human clotting factors, primarily factor V. Clinical suspicion may be obscured and delayed because bleeding is not uncommon during or following major surgery and because other more common genetic and acquired bleeding disorders such as mild von Willebrand disease and qualitative platelet disorders must be excluded. Coagulation factor inhibitors should be suspected in postoperative patients who present with simultaneously abnormal PT and aPTT, particularly if these parameters do not normalise with the administration of vitamin K or fresh-frozen plasma (FFP). Idiopathic coagulopathy may not always be linked to exposure to bovine thrombin due to these confounding factors.

Once a coagulopathy has developed, there are no established treatment protocols. Case reports have included a variety of treatments with variable results. Platelets have been utilised as a source of factor V replenishment for those patients who have developed inhibitors to it (19, 61). Vitamin K supplementation and FFP have been used as therapeutic adjuncts to reinforce the vitamin K-dependent factors rather than as a treatment of the coagulopathy itself (19, 49, 58), and intravenous immunoglobulin

Table 4: Case reports of bovine thrombin-induced coagulopathy since 2002. FV = factor V.

Patient	Surgery type	Coagulopathy	Bleeding complication	Preparation used	Citation
84-year-old male	Axilobifemoral bypass; exploratory laparotomy	↓ FV ↑ FV inhibitor	Gross haematuria	Unspecified	Sarfati 2004 [19]
64-year-old male	Spinal fusion	↑ FV inhibitor	Retroperitoneal haematoma	Unspecified	Kapoor 2005 [58]
31-year-old male	Placement of ventricular assist device	↑ FV inhibitor	Epistaxis	Unspecified	Kirkeby 2005 [52]
74-year-old male	Coronary artery bypass graft	↑ FV inhibitor (human & bovine)	No bleeding; extended ICU stay	Thrombin-JMI®	Lawson 2005 [59]
78-year-old female	Vertebroplasty	↓ FV ↑ FV inhibitor	No bleeding	Unspecified	Shah 2005 [60]
38-week-old male	Shunt replacement & atrial septectomy	↑ FV inhibitor	Petechial rash	Unspecified	Savage 2007 [53]
11-year-old female	Multiple open cardiac procedures	↑ FV inhibitor (human)	No bleeding	Unspecified	Savage 2007 [53]
16-month-old female	Pulmonary angioplasty	↓ FV ↑ FV inhibitor	Excessive intraoperative bleeding	Unspecified	Crow 2007 [18]

has demonstrated efficacy in some patients in order to achieve faster results than immunosuppressive therapies with steroids (53, 60). Plasmapheresis is an option that has been explored, though its transient nature may require multiple rounds and has not always met with success (40, 58). Experience with factor VIIa supplementation is limited and in those cases reporting its use, results have been generally poor (52, 58).

Development of human thrombin for topical haemostasis

With the recent availability of two new human thrombin products, the incidence of coagulopathy related to the use of bovine thrombin is expected to decline. In 2007, a human pooled plasma-derived purified thrombin product was approved by the FDA for use as a topical adjunctive aid to achieve haemostasis when standard surgical techniques proved ineffective (44). This human thrombin preparation was treated with a solvent detergent and was nanofiltered to virtually eliminate blood-borne viral contaminants. In a phase 3 prospective, randomized, double-blinded, controlled clinical trial conducted at 22 centers in the United States, 305 patients were assigned to receive either bovine-derived or human plasma-derived thrombin for oozing or mild bleeding that could not be controlled by other surgical techniques in the context of elective cardiovascular, neurologic, or general surgical procedures (44). The primary efficacy endpoint was achievement of adequate haemostasis at 10 minutes (min) after the pre-selected thrombin agent was applied with a Surgifoam® absorbable gelatin sponge (Ethicon, Inc. /Johnson & Johnson, Somerville, NJ, USA); secondary endpoints were achievement of haemostasis at 6 and 3 min. Both groups had over 90% efficacy at 10 min and over 70% efficacy at 3 min (44). Few adverse events occurred with either product, although pruritus and “procedural complications” were more commonly seen with the human-derived thrombin product (44). Four antibody markers were tested in each patient: those for bovine thrombin, bovine factor V/Va, human thrombin, and human factor V/Va (44). Only 3.3% of patients exposed to the human plasma-derived thrombin developed antibodies to at least one of the antigens, compared with 12.7% of the patients treated with bovine plasma-derived thrombin (44). In the bovine thrombin group, 7.9% of the patients developed antibodies to bovine thrombin and 9.5% developed antibodies to bovine factor V/Va (44). While this level of antibody response in the bovine thrombin group was lower than one would expect, it is possible that the initial 24 hour titer test and the subsequent five-week follow-up were suboptimal time-points for detecting maximum antibody production. Antibodies usually develop gradually over a period of days following antigen exposure, reach a plateau within 2–3 weeks, and then begin to decline in a relatively short period of time. More appropriate time-points may have yielded a higher incidence of bovine thrombin-induced antibodies. A few patients treated with bovine thrombin (number not specified) had antibodies that cross-reacted with human thrombin, but none had antibodies that cross-reacted with human factor V/Va (44). None of the patients in the study who were treated with human thrombin developed detectable antibodies to either human thrombin or to human fac-

tor V/Va (44). There were no reports of bleeding in either group.

The second human product is a genetically engineered recombinant thrombin, expressed in a Chinese hamster ovary (CHO) cell line, transfected with human thrombin DNA. The CHO product is a single-chain precursor form of thrombin, which is then proteolytically cleaved into a two-chain version and further purified by chromatography. The highly purified product is then subjected to solvent detergent treatment and nanofiltration to remove any residual contaminants. This topical haemostatic agent was approved by the FDA in January 2008, based in part on the results of clinical trials that demonstrated comparable efficacy of this product with bovine thrombin and a significant decrease in immunogenicity (55). A phase 3, randomised, double-blind comparative study of recombinant thrombin versus bovine-derived thrombin as surgical adjuncts to absorbable gelatin sponge to achieve topical haemostasis (55) enrolled 411 patients who underwent a broad variety of procedures, including liver resection, spinal surgery, vascular surgery, or dialysis access surgery. The primary efficacy endpoint was the incidence of haemostasis within 10 min after application of study drug. Results were comparable between treatment groups, with a 95.1% success rate for bovine thrombin and a 95.4% success rate for recombinant human thrombin (55). Both treatments were similarly efficacious across the various surgical settings, although a non-statistically significant trend toward faster time-to-haemostasis was noted in favor of recombinant human thrombin (55). Forty-three patients (21.5%) exposed to the bovine-derived thrombin product developed anti-bovine thrombin antibodies, compared to three patients (1.5%) exposed to recombinant human thrombin who developed anti-human thrombin antibodies (55). It is not clear whether any of these three individuals had prior exposure to bovine thrombin. A post-hoc analysis of the potential association of antibody formation with adverse events indicated that those who developed antibodies to bovine thrombin had an increased incidence of bleeding, thromboembolic events, hypersensitivity reactions, and abnormal aPTTs compared with patients in the bovine thrombin group who did not develop antibodies (55). However, the authors noted that confidence intervals for the study were broad and overlapping and that underlying patient comorbidities, concomitant medications, and blood product usage confounded interpretation of the findings (55). Nevertheless, the superior immunogenic profile of recombinant human thrombin compared with bovine thrombin warrants consideration of the preferential use of homologous human thrombin products in the clinical setting (16).

Conclusions

As an adjunct to surgical haemostasis, the use of bovine-derived thrombin products has been beneficial in many complex surgical scenarios. However, the immunogenic character of bovine-derived thrombin products has also resulted in a number of well-documented cases of antibody-mediated coagulopathy. The development of antibodies against bovine thrombin and closely associated coagulation proteins, and the subsequent cross-reactivity of those antibodies against human coagulation proteins in the recipients, prompted the FDA to require black-box warnings on the package inserts for these bovine plasma-derived thrombin

products. The incidence of immunologic coagulopathy and its consequences in terms of bleeding and hypercoagulability emphasised the need for an alternative, less immunogenic thrombin preparation.

Purified human plasma-derived thrombin and recombinant thrombin have recently been approved for use as topical adjunctives to achieve surgical haemostasis. Clinical trials have demonstrated that these human thrombin-based products possess equivalent efficacy and safety, with an improved immunogenic-

ity profile compared with bovine-derived thrombin agents. It remains to be seen whether the improved immunogenicity profiles of human thrombin products relative to bovine thrombin will translate to improved safety profiles in the clinical setting, although only years of clinical experience will confirm this. In the meantime, data should be collected prospectively to determine whether human thrombin would improve the risk-benefit profile for surgeries complicated by excessive bleeding.

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