

REVIEW

Topical bovine thrombin: a 21-year review of topical bovine thrombin spontaneous case safety reports submitted to FDA's Adverse Event Reporting System

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SUMMARY

Purpose To review topical bovine thrombin spontaneous adverse event (AE) reports that were forwarded to the US Food and Drug Administration's (FDA) Adverse Event Reporting System (AERS) between January 1986 and December 2006.

Methods Forty-one spontaneous AE reports were summarized for reported AE profile and chronological reporting patterns. Each AE report was adjudicated by a hematologist for the topical bovine thrombin product that was given and the AE(s) that were reported. AEs were grouped as allergic, coagulopathy/bleeding, and all other AEs combined. Grouped AE serial analyses were carried out using successive 3-year time increments between 1986 (the year an AE report was first noted for a bovine thrombin product) and 2006 (the first full year that was available at the time of initiation of the data summary).

Main outcome measures The primary outcome measures were every 3-year trend lines for all-AE reports, all reporters, and topical bovine thrombin brand mentions for 2 AE groups of interest (allergic events and coagulopathy/bleeding events).

Results The all-AE spontaneous reporter trend showed a downward appearance for AE reporting activity that started in 1995–1998 and continued through 2004–2006. The all-AE reports trend showed two potential safety signals that could be identified serially: (1) a prominent 1989–1991 peak that was attributable to allergic events (in particular, anaphylaxis), and (2) a small 1995–2000 broad peak that was attributable in part to coagulopathy/bleeding events. Allergic events were predominantly reported with products approved prior to 1995, were not temporally associated with prior medical literature case reports, and continued to be forwarded to the FDA at low levels up to the end of this study in 2006. Coagulopathy/bleeding events were reported only with products approved prior to 1995, were temporally associated with medical literature case reports, and were not forwarded to the FDA after 2000.

Conclusions Overall, spontaneous AE reporting for topical bovine thrombin occurs at very low levels, and appears to have been decreasing since 1995. The serial reporting patterns for topical bovine thrombin are best explained as a strong safety signal for allergic events with ongoing, low level reporting, and a weak safety signal for coagulopathy/bleeding events that ceased on or before 2000. Although this descriptive trend analysis cannot measure associations or causation, the coagulopathy/bleeding signal may have been prompted by multiple, antecedent published case reports. The subsequent diminishment of signal attributed to thrombin likewise may coincide with lack of such reporting in larger follow-up clinical trials or, alternatively, in the introduction and growing market share of thrombin brands of greater purity. Currently marketed topical bovine thrombin formulations are rarely volunteered as possible causes of adverse events. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS — bovine; thrombin; safety; adverse event; surgery

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BACKGROUND

Thrombin is a serine protease that cleaves soluble fibrinogen to produce insoluble fibrin, the primary constituent of blood clots. In addition to clot formation,

thrombin also induces chemotaxis of inflammatory cells and activates endothelial cells, fibroblasts, platelets, and smooth muscle cells.¹ Direct application of bovine-derived topical thrombin to surgically traumatized surfaces to achieve hemostasis has been used therapeutically since the 1940s, and has been marketed in US by the US Food and Drug Administration (FDA) approval since the 1970s.^{2–4} Since that time, the number of surgical uses for topical bovine thrombin

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has steadily increased. While the extent of current utilization is not known with precision, it has been estimated by one author that at least 1 million patients receive topical thrombin hemostatic therapy every year in US.⁵

Since 1989, a series of published case reports and small clinical studies proposed that certain post operative adverse events and laboratory abnormalities were due to thrombin exposure during surgery.^{6–23} Investigators hypothesize that antibodies raised against the bovine preparation may cross react with human thrombin to interrupt normal pathways of blood coagulation and wound repair resulting in thrombosis, uncontrolled bleeding, shock, or death. Other early investigators argue that trace contaminants such as Factor V and Va and not thrombin *per se* are responsible for severe autoimmunization outcomes.²⁴ However, in large observational cohorts and randomized clinical trials of new fibrin formulations, no evidence was found to link pre-operative or postoperative antibody levels to an increased risk of adverse clinical outcomes.^{25–28}

This contradiction in apparent risk may be due to changes in study design from a preponderance of case reports to controlled trials large enough to statistically test for differences in post-surgical events. Differences in safety signals may also be driven by relative market shares of competing bovine products if formulations are disparate. Indeed, SDS Page gels reveal that the purity of commercial thrombin preparations vary. THROMBIN-JMI[®] (Jones Pharmaceutical) is 96.0% pure α and β thrombin subunits with a factor V concentration of less than 0.2 $\mu\text{g/ml}$ of factor V.^{29,30} THROMBIN-JMI, introduced to market in 1995, is also the exclusive component of the new fibrin glues and sealants reinstated as products by the US Food and Drug Administration (FDA) in 1998–2000.^{31–33} Thrombinar (Armour), also introduced fairly recently in 1991, likewise yielded a single band of gel consistent with thrombin.³⁴ In contrast, Thrombostat[®] (Parke-Davis; circa 1944) and Thrombogen[®] (Johnson and Johnson; 1989) are only 28.1 and 23.8% pure, respectively.³⁰ Multiple contaminating proteins measuring 20–205 kDa were demonstrated, including bands that were similar in size to factor Va light and heavy chains.^{30,35} Factor V concentration in these older commercial preparations can therefore be as high as 100 $\mu\text{g/ml}$. Recently, a filtration method for Factor V has been available that reduces Factor V and its fragments to levels beyond the limit of detection for a Western blot assay ($\sim 100\text{ ng/ml}$).³⁶

Extrapolating population safety estimates from published literature is subject to errors. Clinical trials may be powered to detect efficacy but may lack the size to detect rare but serious events. Conversely, safety

rates derived from case reports may over represent harm because only the rare and exceptional complications will be noticed by a physician and published by an editor. Specifics of thrombin exposure is rarely, if ever, reported.³⁷ Independent of published literature, sources of voluntary reporting such as the FDA's Adverse Event Reporting System (AERS) database can also be used to assess perceived safety risks with the advantage of more complete formulation reporting. In US, the spontaneous AE reports are forwarded to the FDA by healthcare practitioners (physicians, pharmacists, nurses, and other healthcare professionals), consumers (patients, relatives and non-healthcare interested parties), or other reporters (e.g., lawyers and healthcare organizations). Although the quality of spontaneously forwarded case reports is variable, they have frequently identified important safety signals that were not found in other ways, and are particularly well suited for finding previously unsuspected immunologic phenomena with unusual presentations and/or rapid onset.³⁸

In order to supplement the safety reporting from published case reports and trials, we analyzed spontaneous case safety reports for topical bovine thrombin that were forwarded to the FDA's AERS database prior to, during, and after the onset of the coagulopathy/bleeding safety signal. The purposes of this paper are to stratify this additional safety signaling for topical bovine by brand to provide a new perspective on the conflicting results from published aggregate data.

METHODS

The FDA's legacy case reporting database, Spontaneous Reporting System (SRS), contains AE case data from 1969 to October 1997. The FDA's current case reporting database (AERS) contains AE case data recorded since November 1997, at which time an updated database format was implemented. Individual case reports received by the FDA are processed into AERS/SRS, where they can be subsequently retrieved and analyzed by safety evaluators as potential safety signals.³⁸ Individual case safety reports that are received by the FDA are subsequently released to the public *via* US Government Freedom of Information procedures on approximately a quarterly schedule. Entry of AE reports into the FDA's surveillance system does not require a causality assessment procedure and does not necessarily imply that any product mentioned on the AE report contributed to the event(s) that were indicated by the reporter.

For this project, an AERS/SRS data extract up to a date of entry of 31 December 2006 was obtained for all

AE reports in the combined SRS/AERS database in which any bovine thrombin product was mentioned as a suspect medication (Drug Logic; Reston, Virginia). Following the removal of reports in which bovine thrombin was intravenously administered, 76 topical bovine AE reports from multiple manufacturers were identified from the FDA's electronic database for which the FDA was able to provide 71 MedWatch source documents. The FDA indicated that the other five MedWatch forms, all of which had a date of entry prior to 1995, had been lost during transfer of data from legacy to current locations, and were therefore unavailable for analysis. Of the 71 MedWatch reports, 41 were forwarded spontaneously and 30 came from the medical literature. The 41 spontaneous AE reports were independently assessed by two physicians (surgeon, hematologist)¹ who adjudicated each report for medical diagnosis and exposure to specific topical bovine thrombin product(s). Bovine thrombin exposures were classified as Thrombostat[®], Thrombogen[®], Thrombinar[®], Possible Thrombin JMI[®], Definite Thrombin JMI[®], or not specified. Medical diagnoses were identified and classified as allergic, coagulopathy/bleeding, or other types of adverse reactions. Concordance between the two physician assessors for medical event identification and classification was 100%. Patients could have ≥ 1 identified medical diagnoses. Pertinent time series assessments were performed using counts of reports (i.e., number of patients), counts of reporters (i.e., number of individuals forwarding ≥ 1 case), or counts of drug mentions (i.e., number of particular drug exposures in a set of case reports). Report counts rather than reporting rates were used because reliable denominator estimates for topical bovine thrombin use have never been published.

RESULTS

The demographic and clinical characteristics of the 41 spontaneous topical bovine thrombin AE reports are provided in Table 1. When age was reported the mean age was 51.1 years, and the median age was 50 years. When gender was reported 17 (73.9%) AE reports involved males and 6 (26.1%) involved females. The most common outcome was Other/Important Medical Event ($n = 13$, 31.7%), followed by hospitalization ($n = 9$, 22.0%). The most commonly reported AE was anaphylactic or anaphylactoid reactions ($n = 19$), followed by infections ($n = 5$), coagulopathies

Table 1. Demographic and clinical characteristics of 71 topical bovine thrombin adverse event case reports

Age (Years) ($n = 22$)	
Mean age when known	51.1
Median age when known	50
Gender when known (No, %) ($n = 23$)	
Male	17 (73.9)
Female	6 (26.1)
Clinical outcome (No, %) ($n = 41$)	
Death	7 (17.1)
Hospitalization	9 (22.0)
Disability	0 (0.0)
Recovered	7 (17.1)
Other/important medical event	13 (31.7)
Unknown	5 (12.2)
Reported adverse events (No, %) ($n = 41$)	
Anaphylactic/anaphylactoid reaction	19
Infection	5
Coagulopathy	3
Medication error	3
Bleeding	2
Disseminated intravascular coagulation	2
Lack of efficacy	2
Respiratory arrest	2
Allergic reaction	1
Cauda equina syndrome	1
Pain	1
Reflex sympathetic dystrophy	1
Stroke	1
Reported topical bovine products (No, %) ($n = 41$)	
Thrombostat [®]	16
Definite Thrombin-JMI [®]	7
Possible Thrombin-JMI [®]	6
Thrombogen [®]	6
Not Specified	5
Thrombinar [®]	1

($n = 3$), and medication errors ($n = 3$). There were two bleeding events and two instances of disseminated intravascular coagulation. The most commonly reported topical bovine thrombin product was Thrombostat[®] ($n = 16$), followed by definite Thrombin-JMI[®] ($n = 7$), possible Thrombin-JMI[®] ($n = 6$), and Thrombogen[®] ($n = 6$).

Table 2 shows the chronology of topical bovine thrombin marketing in US. In order of approval year, the available, new topical bovine preparations were Thrombostat[®] (1940's), Thrombogen[®] (1989), Thrombinar[®] (1991), and Thrombin JMI[®] (1995).

The chronological pattern in the FDA's SRS/AERS Database for all spontaneous case reports combined

Table 2. Dates of approval for topical bovine thrombin products

Topical bovine thrombin product	Date of US marketing approval
Thrombostat [®]	1940's
Thrombogen [®]	1989
Thrombinar [®]	1991
Thrombin-JMI [®]	1995

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**Spontaneous Reports Only
FDA AERS Database: 1986–2006**

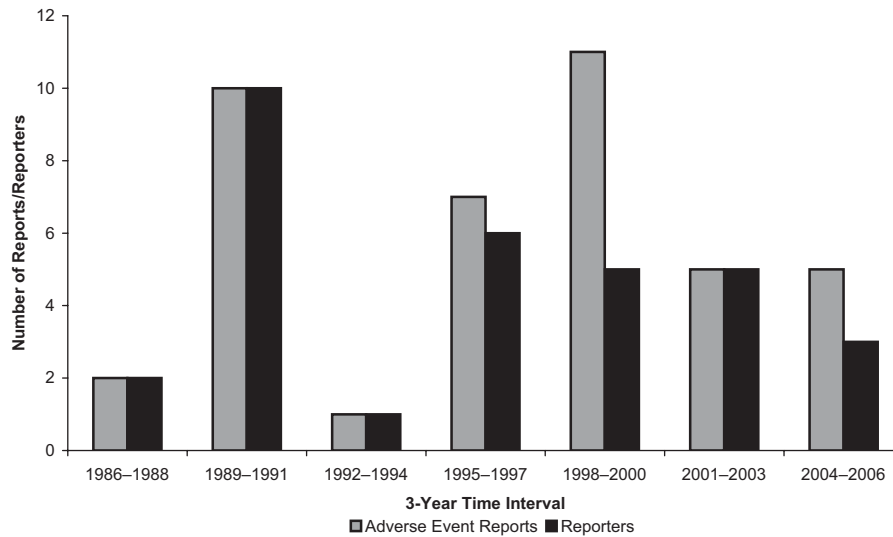


Figure 1. All adverse event reports

and all reporters combined is presented in Figure 1 by 3-year intervals. Overall, spontaneous reports following the use of topical bovine thrombin exhibited a peak of 10 reports forwarded by 10 reporters in the 1989–1991 time interval, which was followed by a second, broader peak in the 1995–1998 (seven reports forwarded by six reporters), and the 1998–2000 (11 reports forwarded by five reporters) time interval. Five AE

reports were filed in the FDA's AERS Database during both of the most recent 3-year time periods (2001–2003; 2004–2006). However, only three reporters sent the five spontaneous reports that were recorded in 2004–2006, which produced a progressive, downward shape for the reporter curve between 1995 and 2006.

Figures 2–4 show the chronological pattern of drug mentions in AE reports of allergic events, coagulo-

**Spontaneous Report Mentions By Drug Product
FDA AERS Database; 1986–2006**

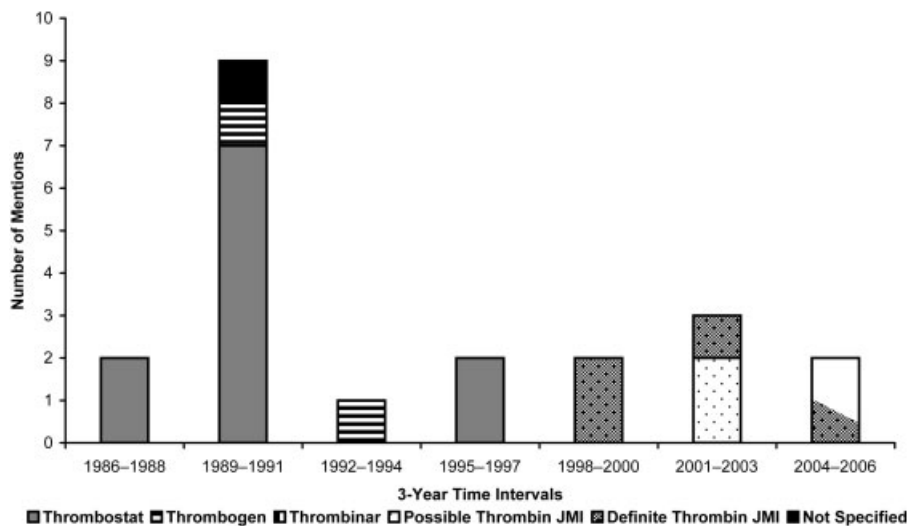


Figure 2. Allergic reactions

TOPICAL BOVINE THROMBIN SAFETY REPORTS

Spontaneous Report Mentions By Drug Product FDA AERS Database; 1986–2006

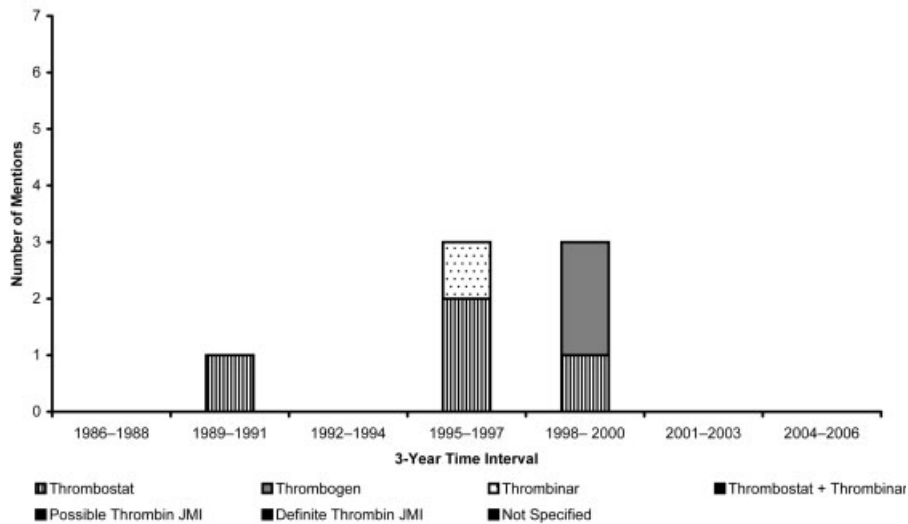


Figure 3. Coagulopathy and bleeding events

pathy/bleeding events, and all other events, respectively. Allergic drug mentions (Figure 2) showed a sharp peak in the 1989–1991 time interval, which corresponded to the early report/reporter peak seen in Figure 1. In the 1992–1994 time interval, allergic drug mentions dropped to one, following which they remained in the 2–3 range for the rest of the study. In order of first appearance, the specific product exposures associated with allergic events were Thrombostat[®] ($n = 11$),

Thrombogen[®] ($n = 2$), not specified ($n = 1$), definite Thrombin JMI[®] ($n = 5$), and possible Thrombin JMI[®] ($n = 2$).

Coagulopathy/bleeding drug mentions (Figure 3) showed a small, broad peak in the 1995–2000 time interval that corresponded to the second report/reporter peak seen in Figure 1. No spontaneous drug mentions of coagulopathy/bleeding were recorded in FDA's AERS/SRS database between 2000 and 2006. In order

Spontaneous Report Mentions By Drug Product FDA AERS Database; 1986–2006

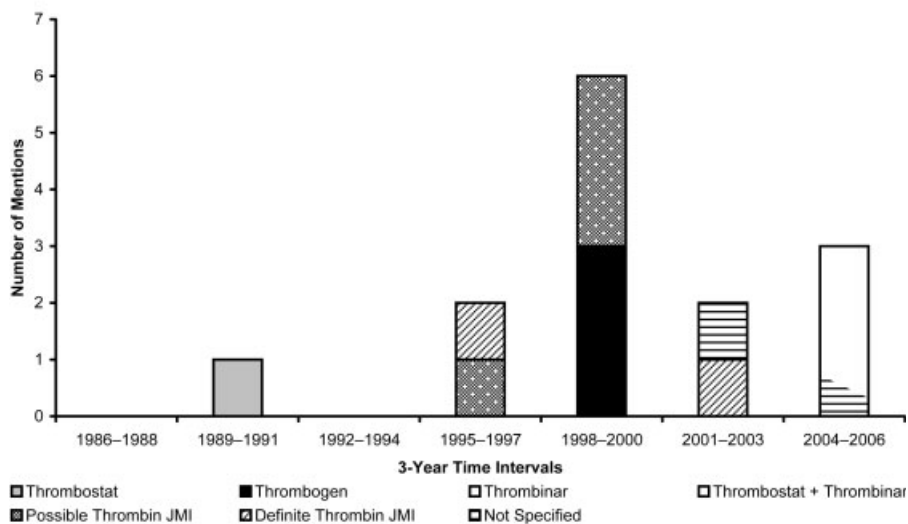


Figure 4. All other events combined

of first appearance, the specific product exposures associated with coagulopathy/bleeding events were Thrombostat[®] ($n=4$), Thrombinar[®] ($n=1$), and Thrombogen[®] ($n=2$).

Drug mentions for all other events (Figure 4) showed a peak in the 1998–2000 time interval that corresponded to the second report/reporter peak seen in Figure 1. Those medical events that were reported more than once in this group of AE reports were: infections ($n=5$), medication error ($n=3$), lack of efficacy ($n=2$), and respiratory arrest ($n=2$). In order of first appearance, the specific product exposures associated with all other events were Thrombostat[®] ($n=1$), possible Thrombin JMI[®] ($n=4$), definite Thrombin JMI[®] ($n=2$), Thrombogen[®] ($n=3$), and not specified ($n=4$).

DISCUSSION

Simultaneous examination of the trend lines for all AE reports and all reporters demonstrated four major features of spontaneous AE topical bovine thrombin reporting in FDA's AERS database: (1) a difference in the chronological AE report *versus* AE reporter pattern since 1995, (2) a generalized decrease in spontaneous reporter activity since the 1995–1998 time interval, (3) a prominent increase in the number of spontaneous AE reports and reporters that were received by FDA's AERS database in 1989–1991, and (4) a second, less prominent increase in the number of AE reports/reporters in 1995–2000. Of the two overall trend lines for topical bovine thrombin reporting (AE reports *vs.* AE reporters) the reporter trend is more indicative of true generalized AE reporting activity because it corrects for the multiple same site reporting bias and therefore more directly measures the recognition of potentially important case reports by the spontaneous reporter population.³⁹ An examination of the reporter trend line indicates that the already low level reporting activity seen in the 1995–1997 time interval likely declined even further between 1998–2000 and 2004–2006.

Figure 2 demonstrates that the 1989–1991 peak was due to a spontaneous safety signal for allergic reactions. Most of the reported allergic events were consistent with anaphylaxis, and all but one was attributable to either Thrombostat[®] or Thrombogen[®]. Since then, the FDA's AERS database has received ≤ 3 allergic reaction reports every 3 years on an ongoing basis, and the specific product profile is consistent with the newest topical bovine thrombin product that is sold in US at any given time (Thrombostat[®], then Thrombogen[®] up to 1997, then Thrombin-JMI[®] after

1997) (see Table 2). Signal strength for the topical bovine thrombin and anaphylaxis safety signal appears to be strong. The association is supported by a large number (relative to overall reporting) of AE reports over a limited time span, ongoing, low level AE reporting activity of similar events up to the present time, and the lack of multiple reports per reporter, which can create false positive signals.³⁹ This pattern of spontaneous reports is often seen when rare events are discovered in an AE reporting database, following which information is disseminated to the prescribing population through labeling and other means. The reporting of such events is usually mitigated in subsequent time periods due to a combination of risk management steps (e.g., changes in product manufacture) and a decrement in AE reporting as the event is perceived as less novel.

In contrast, the peak in spontaneous AE reports for coagulopathy/bleeding events (coagulopathy ($n=2$), bleeding ($n=2$), and disseminated intravascular coagulation ($n=2$)) was not recorded until 1995–1997. All of the spontaneous AE reports of coagulopathy/bleeding were attributable to older topical bovine thrombin products (Thrombostat[®], Thrombogen[®], and Thrombinar[®]), and, after 2000, no spontaneous coagulopathy/bleeding reports were entered into the FDA's AERS database. In comparison to anaphylaxis, the signal strength for topical bovine thrombin and coagulopathy/bleeding appears to be weak. The topical bovine thrombin and coagulopathy/bleeding safety signal demonstrates a small number of AE reports (relative to overall reporting) over a wider reporting interval, a lack of coagulopathy/bleeding reports after 2000, a multiple same site reporting effect (two of the six AE reports originated from the same reporter), and signal content that is dispersed over more than one biological mechanism (e.g., both coagulopathy and disseminated intravascular coagulation were described by reporters).

The complete disappearance of AE reports for post-topical bovine thrombin coagulopathy/bleeding events after 2000 is in marked contrast to the anaphylaxis signal, and suggests that coagulopathy/bleeding either represented a false signal or was a true safety signal that resolved as a result of a mitigating factor. One well known influence that frequently stimulates spontaneous reporting and could have contributed to a false spontaneous reporting signal is publicity.⁴⁰ While the stimulation of spontaneous reporting to AE reporting databases usually involves mass media publicity, it is also known to occur as a result of 'micropublicity' from sources such as medical literature publications.⁴¹ A previous analysis of literature case safety reports for

topical bovine thrombin showed that 11 case reports in 5 publications, and 37 case reports in 8 publications, occurred in the 1989–1992 and 1992–1994 time intervals,²⁵ respectively, the two 3-year intervals that were immediately antecedent to the spontaneous coagulopathy/bleeding signal. We conducted an additional review of the 1989–1992 and 1992–1994 publications (data not shown) that indicated that the majority of case reports (10 of 11 = 90.0% and 28 of 37 = 75.7%, respectively) for both of these time periods described coagulopathy, bleeding, or both. Given this temporal sequence, it seems reasonable to conclude that ‘micropublicity’ due to case report publications could have acted as a stimulus for the spontaneous AE reporting that was later recorded in the FDA’s AERS Database. As noted previously, the literature case report safety signal for coagulopathy/bleeding was not confirmed using aggregate data collection methods, and may have been false.

Another plausible explanation for the lack of coagulopathy/bleeding AE reports after 2000 is the increasing market share of purer formulations of bovine thrombin as described in the background.^{29,30,34–36} This explanation is supported by the observation that the lack of spontaneous AE case reports of coagulopathy and bleeding is not due to the absence of AE reporting after 2000 because reports of both allergic and other kinds of events continue to be forwarded to the AERS database.

In 2007 and 2008, two new topical non-bovine thrombin preparations—Evithrom[®] (Omrix) and Recothrom[®] (Zymogenetics)—were authorized for US marketing by the FDA.^{42,43} Because these human recombinant thrombins were introduced into the market after this analysis, the impact of these products on the safety profile of exogenous thrombin is yet unknown. To date, only a few clinical trials directly compare new human recombinant thrombin to current standard bovine formulations. Two adequately controlled clinical studies in which topical bovine thrombin was compared to an active control (Chapman’s 2007 study of Recothrom[®] vs. topical bovine thrombin and Doria’s 2008 study of Evithrom[®] vs. topical bovine thrombin) showed higher rates of antibody induction in patients who were exposed to bovine thrombin compared to either Recothrom[®] or Evithrom[®], but no comparative qualitative or quantitative safety profile differences.^{44,45} Further, given the weak and diminishing signals currently detected for bovine thrombin, the need for a new and safer substitute has not been clearly established in this analysis.

CONCLUSION

An analysis of the content and chronology of spontaneous AE reports for topical bovine thrombin showed

KEY POINTS

- Few (41) topical bovine thrombin spontaneous adverse event (AE) reports were submitted to the US FDA’s Adverse Event Reporting System between 1986 and 2006.
- A peak of topical bovine thrombin spontaneous AE reports was seen at the 1989–1991 (mostly allergic drug mentions) and the 1998–2000 time interval (mostly coagulation/bleeding mentions).
- Unlike allergy mentions, no coagulation/bleeding events were volunteered after 2000, suggesting that these events may be due to the influence of published case reports and/or changes in thrombin formulation.

a long term decline in all AE reporting activity since 1995 and two potential safety signals—allergic reactions and coagulopathy/bleeding. The signal strength for allergic events was strong and was not temporally related to previously published case reports. In contrast, the signal strength for coagulopathy/bleeding events was weak, and may have been stimulated by previously published case reports that were not verified using other data sources. The absence of any spontaneous coagulopathy/bleeding events since 2000 suggests that, even if this is a true safety signal, it may have been based on manufacturing/purification techniques that are no longer applicable to topical bovine thrombin products.

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REFERENCES

1. Chambers RC, Leoni P, Blanc-Brude OP, Wembridge DE, Laurent GJ. Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1. *J Biol Chem* 2000; **275**: 35584–35591.
2. Jackson MR, MacPhee MJ, Drohan WN, Alving BM. Fibrin sealant: current and potential clinical applications. *Blood Coagul Fibrinolysis* 1996; **7**: 737–746.
3. Alving BM, Weinstein MJ, Finlayson JS, Menitove JE, Fratanoti JC. Fibrin sealant: summary of a conference on characteristics and clinical uses. *Transfusion* 1995; **35**: 783–790.
4. von Oppell UO, Zilla P. Tissue adhesives in cardiovascular surgery. *J Long Term Eff Med Implants* 1998; **8**: 87–101.
5. Lawson JH. The clinical use and immunologic impact of thrombin in surgery. *Semin Thromb Hemost* 2006; **32** (Suppl 1): 98–110.

6. Flaherty MJ, Henderson R, Wener MH. Iatrogenic immunization with bovine thrombin: a mechanism for prolonged thrombin times after surgery. *Ann Intern Med* 1989; **111**: 631–634.
7. Ortel TL, Charles LA, Keller FG, et al. Topical thrombin and acquired coagulation factor inhibitors: clinical spectrum and laboratory diagnosis. *Am J Hematol* 1994; **45**: 128–135.
8. Rapaport SI, Zivelin A, Minow RA, Hunter CS, Donnelly K. Clinical significance of antibodies to bovine and human thrombin and factor V after surgical use of bovine thrombin. *Am J Clin Pathol* 1992; **97**: 84–91.
9. Spero JA. Bovine thrombin-induced inhibitor of factor V and bleeding risk in postoperative neurosurgical patients. Report of three cases. *J Neurosurg* 1993; **78**: 817–820.
10. Israels SJ, Israels ED. Development of antibodies to bovine and human factor V in two children after exposure to topical bovine thrombin. *Am J Pediatr Hematol Oncol* 1994; **16**: 249–254.
11. Cruickshank MK, Black J, Wall W. Development of a factor V and thrombin inhibitor following partial hepatic resection and the use of topical thrombin concentrate. *Clin Lab Haematol* 1994; **16**: 187–190.
12. Dorion RP, Hamati HF, Landis B, Frey C, Heydt D, Carey D. Risk and, clinical significance of developing antibodies induced by topical thrombin preparations. *Arch Pathol Lab Med* 1998; **122**: 887–894.
13. Winterbottom N, Kuo J, Nguyen K, et al. Antigenic responses to bovine thrombin exposure during surgery: A prospective study of 309 patients. *J Appl Res* 2002; **2**(1): 1–11.
14. Ortel TL, Mercer MC, Thames EH, Moore KD, Lawson JH. Immunologic impact and clinical outcomes after surgical exposure to bovine thrombin. *Ann Surg* 2001; **233**: 88–96.
15. Chouhan VD, De La Cadena RA, Nagaswami C, Weisel JW, Kajani M, Rao AK. Simultaneous occurrence of human antibodies directed against fibrinogen, thrombin, and factor V following exposure to bovine thrombin: effects on blood coagulation, protein C activation and platelet function. *Thromb Haemost* 1997; **77**: 343–349.
16. Sarfati MR, Dilorenzo DJ, Kraiss LW, Galt SW. Severe coagulopathy following intraoperative use of topical thrombin. *Ann Vasc Surg* 2004; **18**: 349–351.
17. Berruyer M, Amiral J, Ffrench P, et al. Immunization by bovine thrombin used with fibrin glue during cardiovascular operations. Development of thrombin and factor V inhibitors. *J Thorac Cardiovasc Surg* 1993; **105**: 892–897.
18. Diez-Martin J, Sikkink RA, Gilchrist GS, Bowie EJ, Fass DN. Development of anti-bovine thrombin antibodies following neurosurgical procedures. *Br J Haematol* 1990; **74**: 369–370.
19. Muntean W, Zenz W, Finding K, Zobel G, Beitzke A. Inhibitor to factor V after exposure to fibrin sealant during cardiac surgery in a two-year-old child. *Acta Paediatr* 1994; **83**: 84–87.
20. Adams JD, Jones S, Brost BC. Development of antibodies to topical bovine thrombin after abdominal hysterectomy. A case report. *J Reprod Med* 2001; **46**: 909–912.
21. La Spada AR, Skalhegg BS, Henderson R, Schmer G, Pierce R, Chandler W. Brief report: Fatal hemorrhage in a patient with an acquired inhibitor of human thrombin. *New Engl J Med* 1995; **333**: 494–497.
22. Carroll JF, Moskowitz KA, Edwards NM, Hickey TJ, Rose EA, Budzynski AZ. Immunologic assessment of patients treated with bovine fibrin as a hemostatic agent. *Thromb Haemost* 1996; **76**: 925–931.
23. Schoenecker JG, Hauck RK, Mercer MC, Parker W, Lawson JH. Exposure to topical bovine thrombin during surgery elicits a response against the xenogeneic carbohydrate galactose alpha1-3galactose. *J Clin Immunol* 2000; **20**: 434–444. Evithrom package insert; September 2007.
24. Streiff MB, Ness PM. Acquired, FV inhibitors: a needless iatrogenic complication of bovine thrombin exposure. *Transfusion* 2002; **42**: 18–26.
25. Clark J, Crean S, Reynolds MW. Topical bovine thrombin and adverse events: a review of the literature. *Curr Med Res Opin* 2008; **24**: 2071–2087.
26. Winterbottom N, Kuo J, Nguyen K, et al. Antigenic responses to bovine thrombin exposure during surgery: a prospective study of 309 patients. *J Appl Res* 2002; **2**: 1–11.
27. Ortel TL, Charles LA, Keller FG, et al. Topical thrombin and acquired coagulation factor inhibitors: clinical spectrum and laboratory diagnosis. *Am J Hematol* 1994; **45**: 128–135.
28. Crean S, Michels SL, Moschella K, Reynolds MW. Bovine thrombin safety reporting: an example of study design and publication bias. *J Surg Res* 2008 [Epub ahead of print].
29. Kajitani M, Ozdemir A, Aguinaga M, Jazieh AR, Flick JT, Antakli T., Severe hemorrhagic complication due to acquired factor V inhibitor after single exposure to bovine thrombin product. *J Card Surg* 2000; **15**: 378–382.
30. Nelson PA, Powers JN, Estridge TD, et al. Serological analysis of patients treated with a new surgical hemostat containing bovine proteins and autologous plasma. *J Biomed Mater Res* 2001; **58**: 710–719.
31. Mintz PD, Mayers L, Avery N, Flanagan HL, Burks SG, Spotnitz WD. Fibrin sealant: clinical use and the development of the University of Virginia Tissue Adhesive Center. *Ann Clin Lab Sci* 2001; **31**: 108–118.
32. The CoStasis Multi-center Collaborative Writing Committee. A novel collagen-based composite offers effective hemostasis for multiple surgical indications: results of a randomized controlled trial. *Surgery* 2001; **129**: 445–450.
33. Hong YM, Loughlin KR. The use of hemostatic agents and sealants in urology. *J Urol* 2006; **176**: 2367–2374.
34. Rapaport SI, Zivelin A, Minow RA, Hunter CS, Donnelly K. Clinical significance of antibodies to bovine and human thrombin and factor V after surgical use of bovine thrombin. *Am J Clin Pathol* 1992; **97**: 84–91.
35. Fastenau DR, McIntyre JA. Immunochemical analysis of polyspecific antibodies in patients exposed to bovine fibrin sealant. *Ann Thorac Surg* 2000; **69**: 1867–1872.
36. Terrab A, Pawlak D, Spaay P, Happensteadt D, Fareed J. Factor V removal from bovine thrombin used as a topical hemostat in surgery: improved purity through a new manufacturing process. (Abstract). *Thromb Haemostasis* 2007; **5** (Suppl): P-M-071.
37. Crean SM, Michels SL, Reynolds MW. Exogenous bovine thrombin as a biomarker of exposure and outcome. *Expert Rev Mol Diagn* 2008; **8**: 651–661.
38. Weaver J, Willy M, Avigan M. Informatic tools and approaches in postmarketing pharmacovigilance used by FDA. *AAPS J* 2008; **10**: 35–41.
39. Clark JA, Klinecicz SP, Berk B. Calculation of the probability of multiplicities in two cell-occupancy models: implications for spontaneous reporting systems. *Drug Inf J* 1999; **33**: 1195–1203.
40. Clark P, Lough M, Whiting B. Rhabdomyolysis and listeria monocytogenes. *Scott Med J* 1989; **34**: 503.
41. Lydick E, Blumenthal SJ, Guess HA. Learning to see: twenty years of renal adverse experience reporting with Indocin. *J Clin Res Pharmacoevidem* 1990; **4**: 183–189.
42. Evithrom package insert; September 2007.
43. RecoThrom package insert; May 2008.
44. Doria C, Fischer CP, Wood CG, Li PM, Marra S, Hart J. Phase 3, randomized, double-blind study of plasma-derived human thrombin versus bovine thrombin in achieving hemostasis in patients undergoing surgery. *Curr Med Res Opin* 2008; **24**: 785–794.
45. Chapman WC, Singla N, Genyk Y, et al. A phase 3, randomized, double-blind comparative study of the efficacy and safety of topical recombinant human thrombin and bovine thrombin in surgical hemostasis. *J Am Coll Surg* 2007; **205**: 256–265.