A Safety Review of Topical Bovine Thrombin–Induced Generation of Antibodies to Bovine Proteins

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ABSTRACT

Background: Topical bovine thrombin has been used to accelerate attainment of hemostasis in the surgical setting for >60 years, and its immunogenicity has been widely reported. Although the development of antibodies is inherent in the introduction of any non–self-therapeutic protein such as bovine-sourced thrombin, there are questions about the relationship between the presence of antibodies to constituents of the therapeutic protein preparation and the occurrence of clinically relevant adverse events (AEs).

Objective: This review examines the proposed mechanisms for the immunogenicity of topical bovine thrombin preparations and summarizes available evidence from randomized clinical trials, observational studies, and case reports to explore possible relationships between the reported immunogenicity of topical bovine thrombin and the occurrence of AEs.

Methods: A search of MEDLINE (1966–August 2008) for studies published in English was conducted using the Medical Subject Heading terms *surgery*, *antibodies*, and *hemorrhage*, as well as equivalent key words for *bovine*, *adverse events*, and *thrombin*. For inclusion in the review, studies had to report clinical or laboratory safety data for patients exposed to topical bovine thrombin during surgery.

Results: The evidence suggests that patients with repeated perioperative exposure to topical bovine thrombin have a 3- to 10-fold greater risk for development of antibodies to topical bovine thrombin than do patients with no history of surgery-related exposure to this agent. Early case reports associated the development of anti–bovine protein antibodies with bleeding and/or thrombotic complications. However, in one prospective, randomized controlled trial comparing topical bovine thrombin with topical recombinant human thrombin, 99.5% of patients in each treatment arm developed postoperative AEs. In another, 54% and 55% of patients in the respective treatment arms developed postoperative AEs. In a prospective, randomized controlled trial that compared topical bovine thrombin and plasma-derived human thrombin, 95.5% of patients in each treatment arm developed postoperative AEs.

Conclusions: Repeated perioperative exposure to topical bovine thrombin may increase both the prevalence and titers of antibodies to ≥1 protein contained in nonhomogeneous topical bovine thrombin preparations. However, the evidence reviewed does not support a definitive association between preoperative or postoperative generation of anti–bovine protein antibodies and an increased risk of AEs in surgical patients treated with topical bovine thrombin. (*Clin Ther*. 2009; 31:679–691) © 2009 Excerpta Medica Inc.

Key words: surgical hemostasis, topical thrombin, recombinant proteins, immunogenicity, adverse events.

INTRODUCTION

Topical bovine thrombin has been used to accelerate attainment of hemostasis in the surgical setting for >60 years.[1] It is used alone or in combination with other drugs to control perioperative surface bleeding in cardiovascular, vascular, neurologic, orthopedic, gynecologic, and transplant surgeries.[2–5] Because the use of topical bovine thrombin is often undocumented in patients' medical records,[6] it is difficult to accurately estimate the risk for adverse events (AEs) associated with clinical use of this agent. One study estimated that at least 1 million surgery patients in the United States are treated with topical bovine throm-

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bin each year.[7] Exposure, particularly repeated exposure, of surgical patients to bovine thrombin preparations may result in the generation of anti–bovine protein antibodies that may also cross-react with some human coagulation factors,[6–21] as most bovine thrombin preparations are not homogeneous.[21,22] Generation of antibodies in patients treated with animal plasma–derived, human plasma–derived, or recombinant therapeutic proteins is a well-recognized phenomenon.[23–25]

Exposure of patients to topical bovine thrombin may lead to the generation of antibodies directed against ≥1 constituent of the preparation. Up to 70% of patients exposed to bovine thrombin more than once may develop antibodies to several of the proteins present in nonhomogeneous topical bovine thrombin preparations.[4] It is not possible to accurately quantify antibody generation in surgical patients exposed or reexposed to bovine thrombin, however, as the methods used for this purpose are generally not comparable across studies. Furthermore, the purity of bovine thrombin preparations can differ markedly, with the bovine thrombin content varying from >90% to <30%.[21,22] Estimated qualitatively, the factor V/Va content in topical bovine thrombin preparations can also vary widely.[6,11–16,20–22,26–28] Bovine factor V, a highly immunogenic constituent, was likely to be present in topical bovine thrombin preparations in clinical use before 2000. Up to 50% of patients treated with topical bovine thrombin containing bovine factor V/Va develop anti–bovine factor V/Va antibodies that may also cross-react with and inactivate human factor V/Va.[8,10–16,26–28] In published reports, the purity and/or concentrations of antigens and the specificity of the antibodies used in immunoassays have generally not been well described.[6,18,28] Nonetheless, studies in patients exposed/reexposed to bovine thrombin have reported the generation of neutralizing and nonneutralizing antibodies that have varying affinities for bovine factor V/Va, bovine prothrombin, bovine thrombin, and bovine factor X, and may cross-react with the corresponding human clotting factors.[6–9,12,15,20,21]

Differences in the amino acid sequences of human and bovine clotting factors may result in the development of antibodies to bovine clotting factors in surgical patients exposed/reexposed to bovine thrombin. For example, the amino acid sequences of bovine and human thrombins differ by 36 residues. There are also minor differences in the patterns of *N*-glycosylation of human and bovine clotting factors.[29–31] The xenogeneic Forssman disaccharide found in some bovine proteins also contributes to the immunogenicity of bovine thrombin preparations in humans.[21,27,32] Bovine glycoproteins containing the Forssman antigen elicit immune responses in all patients exposed/reexposed to this antigen, which is normally synthesized by all nucleated mammalian cells except those of humans, apes, and Old World monkeys.[27,32] Exposure of patients to bovine thrombin preparations containing galactose-α-1-3-galactose increases titers of the anti-Forssman antibodies that are normally found in most human plasma and are directed against this xenogeneic disaccharide.[15,21] The lack of the Forssman disaccharide in bovine thrombin may explain the relatively low frequency of anti–bovine thrombin antibodies (<10%) in the plasma of patients exposed to topical bovine thrombin, as this disaccharide is highly immunogenic in humans.[15,16,21,27,33] In addition to inducing the generation of anti-Forssman antibodies, bovine coagulation proteins may induce the generation of anti–bovine clotting factor antibodies that cross-react with human clotting factors (ie, autoantibodies to human coagulation factors) by breaking self-tolerance.[34,35] Among factors that may interact with ≥1 constituent of topical bovine preparations to favor the breaking of self-tolerance are the generalized increased inflammation associated with major surgery and the transfusion of blood products.[34,35]

This review examines the proposed mechanisms for the immunogenicity of topical bovine thrombin preparations and summarizes available evidence from randomized controlled trials comparing the safety profile of topical bovine thrombin with placebo or recombinant human thrombin (rhThrombin), observational studies, and case reports to explore possible relationships between the reported immunogenicity of topical bovine thrombin and the occurrence of AEs.

METHODS

A search of MEDLINE (1966–August 2008) for studies published in English was conducted using the Medical Subject Heading terms *surgery*, *antibodies*, and *hemorrhage*, as well as equivalent key words for *bovine*, *adverse events*, and *thrombin*. All English-language abstracts and papers describing studies, case reports, or case series in surgical patients treated with topical bovine thrombin that reported the incidence of antibodies and/or AEs were included in the review.