Mast Cell-Derived hTryptase-β Functions as a Potent Anticoagulant by Proteolytically Damaging Fibrinogen

A Prieto-García,[1] MC Castells,[2*] RL Stevens[2*]
[1]*Allergy Service, Hospital Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain*
[2]*Department of Medicine, Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, USA*
[*]*Joint senior authors*

Key words: Tryptase. Mast cell. Fibrinogen. Fibrin. Coagulation.

Palabras clave: Triptasa. Mastocitos. Fibrinógeno. Fibrina. Coagulación.

In their interesting case report, Moreno-Ancillo et al [1] describe a patient with systemic mastocytosis who had a life-threatening bleeding abnormality. Coagulation tests, including activated partial thromboplastin time (APTT) and prothrombin time (PT), revealed unusually long clotting times. Based on data reported in earlier studies by Samoszuk et al [2], the authors tentatively concluded that the patient's bleeding abnormality was most likely due to the anticoagulant activity of heparin exocytosed from the large numbers of mast cells (MCs) in tissue.

The tetramer-forming β tryptases derived from the *TPSAB1* and *TPSB2* genes on human chromosome 16p13.3 are stored in the secretory granules of MCs that are ionically bound to heparin-containing serglycin proteoglycans (SGPGs). The murine orthologs of these tryptases are mouse MC protease (mMCP)-7/Tpsab1 and mMCP-6/Tpsb2. Some low-molecular-weight oligosaccharides that are commercially prepared from isolated heparin containing SGPGs and glycosaminoglycans have anticoagulant activity in vivo and in vitro by hindering the formation of fibrin via 2 antithrombin-dependent mechanisms. These oligosaccharides prevent factor Xa–dependent generation of thrombin. They also catalyze the ability of the protease inhibitor SERPINC1/ATIII to inactivate thrombin. Based on these and other pharmacologic data, it was assumed that the primary pathway by which MCs physiologically prevent the accumulation of fibrin and fibrin-platelet clots in tissues is via exocytosed heparin. It was therefore surprising when investigators subsequently discovered that heparin-deficient mice (caused by targeted inactivation of the N deacetylase-N-sulfotransferase-2 gene) did not accumulate more fibrin in their tissues in a passive cutaneous anaphylactic reaction than heparin-sufficient wild-type mice.

In 2012, Prieto-García et al [3] reported a beneficial role for the tetramer-forming tryptases exocytosed from activated MCs in fibrin accumulation and blood coagulation, namely, by proteolytically damaging fibrinogen. MC degranulation, in association with vasodilation, induces tissue edema and the accumulation of large amounts of fibrinogen and other plasma proteins at the site of inflammation. The MC-restricted serine protease hTrytpase-β was shown to proteolytically damage the α-chain of fibrinogen, thereby inducing strong anticoagulation activity at the tissue site [3].

As previously shown for recombinant and naturally occurring mMCP 7 [4], the α chains of mouse and human fibrinogen were preferentially cleaved by mMCP 6 and hTryptase-β in vitro and in vivo, even if the digestion reactions were carried out in the presence of the numerous protease inhibitors in blood and serum [3]. Lys^{575} was identified as the preferred cleavage site at the C terminus of the fibrinogen α-chain. The anticoagulant action of hTryptase-β prolonged thrombin-dependent clotting time in human plasma samples, as occurred in the case reported by Moreno-Ancillo et al [1]. In support of the in vitro data, fibrin deposits were markedly increased in the skin of tryptase-deficient mice that had been subjected to the passive cutaneous anaphylactic reaction, in contrast to observations in heparin-deficient mice.

It is now known that the primary function of the heparin glycosaminoglycans covalently attached to serglycin is to package varied positively charged proteases in MC secretory granules [5,6]. When the MCs in the skin and at other connective tissue sites are activated, most of their exocytosed protease-heparin SGPG complexes remain intact for hours in extracellular matrices [7]. Eventually, these macromolecular complexes are endocytosed by macrophages and other nearby cells [7,8] and then destroyed in their lysosomes. Thus, very few—if any—MC-derived heparin glycosaminoglycans or their oligosaccharides remain to bind to any SERPINC1, factor Xa, or thrombin that could be present in an inflamed tissue. In their study, Prieto-García et al [3] showed that when plasma samples were exposed to human or mouse tetramer-forming tryptases, clotting time was much longer than with heparin alone. It was therefore concluded that the prominent anticoagulant factor present in the protease-SGPG complexes exocytosed from activated MCs is the tryptase component rather than the heparin component.

In their in vitro study, Samoszuk et al [2] concluded that the anticoagulant activity of a recombinant hTryptase-β–heparin complex was probably due to its heparin component because the activity was inhibited by protamine and heparinase. It is now known that heparin SGPGs are essential in maintaining the enzymatic activity of hTryptase-β. Thus, an explanation of their protamine/heparinase data is denaturation of hTryptase-β. In the alternative anticoagulation mechanism described by Prieto-García et al [3], exocytosed tetramer-forming tryptases proteolytically damage fibrinogen before this plasma protein can be converted to fibrin by thrombin [9], thereby changing the paradigm of how MCs hinder fibrin deposition and blood coagulation internally.

The anticoagulant activity of hTryptase-β explains bleeding episodes in patients with anaphylaxis or mastocytosis,

as reported by Moreno-Ancillo et al [1]. In support of this hypothesis, bleeding of skin blisters and gastrointestinal bleeding have been reported in pediatric patients with diffuse cutaneous mastocytosis [10]. In addition, women occasionally present with menstrual-like bleeding shortly after they experience an anaphylactic reaction [11]. Finally, prolongation of APTT or PT has been reported in patients with systemic mastocytosis or anaphylactic shock [12,13]. Bleeding episodes are not a frequent clinical feature of patients with anaphylaxis or mastocytosis, most likely because the amount of the fibrinogen in the circulation that is proteolytically damaged by hTryptase-β rarely becomes life-threatening. In that regard, the concentration of fibrinogen in the blood is ~2 mg/mL. Thus, even if 50% of the fibrinogen in the blood of a mastocytosis patient is proteolytically damaged by hTryptase-β, there might not be any observable decrease in clotting time. Nevertheless, mastocytosis and anaphylaxis should be included in the differential diagnosis of patients presenting with unusual bleeding and prolonged clotting times.

The data reported in the study by Prieto-García et al [3] raise the possibility that recombinant hTryptase-β might be a safer and more efficient anticoagulant in humans than heparin-derived oligosaccharides. However, more relevant to the case report by Moreno-Ancillo et al [1], the presence of the 2-7–kDa peptides released in blood, urine, and/or blister fluid when hTryptase-β cleaves the α-chain of fibrinogen might be of diagnostic value in the identification of patients with mastocytosis, anaphylaxis, or MC activation syndrome.

Funding

The authors received a grant (AI059746) from the National Institutes of Health and a grant from The Mastocytosis Society.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Moreno-Ancillo A, Gil-Adrados AC, Domínguez-Noche C, Jurado-Palomo J. Bleeding and shock in a 44-year-old woman with systemic mastocytosis. J Invest Allergol Clin Immunol. 2013;23:504-21.
2. Samoszuk M, Corwin M, Hazen SL. Effects of human mast cell tryptase and eosinophil granule proteins on the kinetics of blood clotting. Am J Hematol. 2003;73:18-25.
3. Prieto-Garcia A, Zheng D, Adachi R, Xing W, Lane WS, Chung K, Anderson P, Hansbro PM, Castells M, Stevens RL. Mast cell restricted mouse and human tryptase-heparin complexes hinder thrombin-induced coagulation of plasma and the generation of fibrin by proteolytically destroying fibrinogen. J Biol Chem. 2012;287:7834-44.
4. Huang C, Wong GW, Ghildyal N, Gurish MF, Šali A, Matsumoto R, Qiu WT, Stevens RL. The tryptase, mouse mast cell protease 7, exhibits anticoagulant activity in vivo and in vitro due to its ability to degrade fibrinogen in the presence of the diverse array of protease inhibitors in plasma. J Biol Chem. 1997;272:31885-93.
5. Stevens RL, Fox CC, Lichtenstein LM, Austen KF. Identification of chondroitin sulfate E proteoglycans and heparin proteoglycans in the secretory granules of human lung mast cells. Proc Natl Acad Sci USA 1988;85:2284-7.
6. Humphries DE, Wong GW, Friend DS, Gurish MF, Qiu WT, Huang C, Sharpe AH, Stevens RL. Heparin is essential for the storage of specific granule proteases in mast cells. Nature 1999;400:769-72.
7. Ghildyal N, Friend DS, Stevens RL, Austen KF, Huang C, Penrose JF, Šali A, Gurish MF. Fate of two mast cell tryptases in V3 mastocytosis and normal BALB/c mice undergoing passive systemic anaphylaxis: prolonged retention of exocytosed mMCP-6 in connective tissues, and rapid accumulation of enzymatically active mMCP-7 in the blood. J Exp Med. 1996;184:1061-73.
8. Huang C, Friend DS, Qiu WT, Wong GW, Morales G, Hunt J, Stevens RL. Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. J Immunol. 1998;160:1910-9.
9. Mosesson MW. Fibrinogen and fibrin structure and functions. J Thrombosis and Haemostasis. 2005;3:1894-904.
10. Kettelhut BV, Metcalfe DD. Pediatric mastocytosis. J Invest Dermatol. 1991;96:15S-8S.
11. Gonzalo-Garijo MA, Pérez-Rangel I, Alvarado-Izquierdo MI, Pérez-Calderón R, Sánchez-Vega S, Zambonino MA. Metrorrhagia as an uncommon symptom of anaphylaxis. J Investig Allergol Clin Immunol. 2010;20:540-1.
12. Sucker C, Mansmann G, Steiner S, Gattermann N, Schmitt-Graeff A, Loncar R, Scharf RE, Stockschlader M. Fatal bleeding due to a heparin-like anticoagulant in a 37-year-old woman suffering from systemic mastocytosis. Clin Appl Thromb Hemost. 2008;14:360-4.
13. Lombardini C, Helia RE, Boehlen F, Merlani P. "Heparinization" and hyperfibrinogenolysis by wasp sting. Am J Emerg Med. 2009;27:1176

Manuscript received January 22, 2014; accepted for publication February 14, 2014.

Alicia Prieto-García
Allergy Service, Hospital Universitario Gregorio Marañón
Dr. Esquerdo, 46
28007 Madrid, Spain
E-mail: aliprietog@gmail.com

