LETTERS TO THE EDITOR

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

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

Palabras clave: Triptasa, Mastocitos, Fibrinogeno, 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 activity 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 hTryptase-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]. Lys575 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**