Comparison of Anti-inflammatory Activity of Human and Bothrops jararaca snake Antithrombin

Morais-Zani, K.¹,², Torquato, R. J. S.³, Tanaka, A. S.³, Tanaka-Azevedo, A. M.¹,²

¹Laboratório de Herpetologia, Instituto Butantan, São Paulo, Brasil; ²Interunidades em Biotecnologia, Instituto de Ciências Biomédicas-Instituto de Pesquisas Tecnológicas-Instituto Butantan, Universidade de São Paulo, São Paulo, Brasil; ³Departamento de Bioquímica, Universidade Federal de São Paulo, São Paulo, Brasil.

Introduction: Antithrombin is known as the most important natural coagulation inhibitor and has been shown to present anti-inflammatory properties. These properties are mediated through the interaction of AT with Syndecan-4, a cell-surface heparan sulfate proteoglycan, which initiates intracellular signaling responses in leukocytes and endothelial cells, thereby producing an increase in prostacyclin production. Objectives: The present study aimed to compare the anti-inflammatory properties of human and Bothrops jararaca (B. jararaca) snake antithrombin.

Material and methods: Antithrombin was purified by affinity chromatography using HiTrap Heparin HP column. To evaluate edema formation, antithrombin (20 µg) was endovenously administered 1 hour after subplantar injection of carrageenan (15 mg kg⁻¹) into the mouse hind paw. The binding of AT with heparin experiments were performed using a Biacore T200 instrument equipped with a SA sensor chip. Biotinylated heparin was immobilized onto the chip at a density of ~310 resonance units (RU).

Results and discussion: Treatment with B. jararaca AT significantly reduced the paw edema formation induced by carrageenan in all time intervals analyzed. This reduction was about 35, 28.1, 39.8, 58.2, 45.5, 43 and 60.6% in 2, 4, 6, 24, 48, 72 and 96 hours, respectively. On the other hand, treatment with human antithrombin significantly reduced the paw edema formation in two out of seven time intervals analyzed, 4 ad 6 hours after carrageenan administration. This reduction was about 47.2 and 56.5, respectively. Biacore measurements revealed that B. jararaca antithrombin was bound to heparin with a dissociation constant of 6.3x10⁻⁸ M while human antithrombin was bound to heparin with a dissociation constant of 2.1x10⁻⁷ M. These results suggest that B. jararaca antithrombin presents higher inhibitory activity on edema formation induced by carrageenan when compared to human antithrombin. The better results obtained with B. jararaca antithrombin treatment may be due to its higher affinity to glycosaminoglycans.

Keywords: inflammation, antithrombin, B. jararaca, paw edema, Biacore T200.

Supported by FAPESP (2009/50199-2, 2009/03484-3)