POTENTIAL ACTION OF THE CHIMERIC KALLIKREIN INHIBITOR, rBbKIm, AS A ANTIAGREGANT AGENT OF PLATELET IN VITRO AND EX VIVO.

Medina, A.F. ¹; Brito, M.V. ¹; Salu, B.R. ¹; Ottaiano, T.F. ¹; Maffei, F.H.A. ²; Sampaio. M. U₁; Oliva, M.L.V. ¹

¹Departamento de Bioquímica, UNIFESP-EPM, S.P., ²Departamento de Cirurgia e Ortopedia, UNESP, Botucatu, S.P., Brazil.

INTRODUCTION: Kunitz inhibitors are useful tools in studies of some pathological conditions. rBbKIm is a chimeric protein comprising the sequence RGD and RGE motif found in BrTI inhibitor (1). OBJECTIVE: This study aims to investigate if the inhibitor comprising RGD sequence interferes on the events related to thrombosis and hemostasis, as platelet aggregation and adhesion.

MATERIAL AND METHODS: A synthetic gene codifying the modified BbKI was inserted in the expression vector pET29a, expressed in E.coli BL21(DE3) and purified by chromatographic methods. The inhibition of trypsin, chymotrypsin, plasmin and kallikrein was assayed to ensure its functional characteristics. Human platelet plasma rich (PRP), in vitro, and whole blood of Black 6C57 mice, ex vivo, assays were performed. Protrombin time (PT) and partially activated protrombin time (aPTT) from platelet poor human plasma poor (PPP) were monitored.

RESULTS: rBbKIm showed inhibitory activity on trypsin (K_iapp 1.5 nM), chymotrypsin (K_iapp 7.0 nM), plasmin (K_iapp 12 nM) and kallikrein (K_iapp 4.8 nM). The PRP aggregation, after 5 min pre-incubation with rBbKIm, being fibrinogen (250 µg) present, using a subthreshold-dose of ADP (2 µM) as agonist, decreased by 41 and 80 % with 11 and 22 µM concentrations, respectively. The ex vivo platelet aggregation of mice whole blood, previously treated with 6.0 µM rBbKIm intravenously and induced by ADP (30 µM) was reduced to 51% compared to the control NaCl 0.15 M. rBbKIm did not interfere in PT time, however, aPTT time was prolonged in a dose-dependent manner.

CONCLUSION: Our results showed that the RGD sequence region in rBbKIm provides a potent adhesion to glycoprotein impairing platelet aggregation, in addition to its properties in prolongating clot formation by inhibiting plasma kallikrein. Thus, taken together, our results indicated that rBbKIm may be useful for future studies in a thrombosis model.

Key words: anticoagulant, kallikrein inhibitor, recombinant.

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