TILIPO 33, A POTENT INHIBITOR OF COLLAGEN-INDUCED PLATELET AGGREGATION THAT BINDS TO a2ß1 INTEGRIN

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The platelet activation is a redundant process which can be initiated by several agonists like ADP, thrombin, collagen and PAF (platelet activating factor). To control this process, Triatomine insects produce a protein family named Lipocalin. The present work shows the characterization of a potent inhibitor of collagen-induced platelet aggregation, named Tilipo 33, from *Triatoma infestans* salivary glands. We performed the expression and purification of Tilipo33, a lipocalin from a cDNA library of *T. infestans* salivary glands. High level of Tilipo 33 was expressed by *E. coli* Roseta-Gami strain (protein level of 3-4 mg/L). It was purified by affinity chromatography on Ni-Agarose column and size exclusion chromatography on Superdex75 column. Purified Tilipo 33 (10 nM) markedly inhibited platelet aggregation induced by collagen (10 µg), but not by convulxin, and slightly affected platelet aggregation induced by ristocetin. By flow cytometry assay Tilipo 33 bound to washed platelet and interfered with the binding of a a2ß1 (an important collagen receptor on platelet surface) integrin monoclonal antibody to platelets, These data were confirmed by confocal microscopy, so that Tilipo 33-FITC co-localized with anti-a2ß1 on platelet surface. Our perspectives are to immune precipitate Tilipo 33 with its receptor and resolve its tridimensional structure. **Financial Supported by: FAPESP and CNPq.**