[P-M-351] THROMBOSTATIN FM19 - A THROMBIN RECEPTOR ACTIVATION ANTAGONIST

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Introduction: The ACE breakdown product of bradykinin, RPPGF, is a thrombin inhibitor and binds to PAR1 and 4. A more potent 5 amino acid peptide form (FM19) has been designed.
Methods: Studies show FM19's ability to inhibit platelet activation and block murine carotid arteries thrombosis.
Results: FM19 is a synthetic peptide consisting of a P1 D-arginine, P2 Oic [((2S,3aS,7aS)-octahydroindol-2-carboxylic acid)] in position 2, P3 proline, P4 D-alanine, and P5 phenylalanine with a para methyl group (rOicPaFpMe). FM19 is a direct inhibitor of alpha- and gamma thrombin with a Ki of 6.6±0.8 and 54±16 micromolar, respectively. FM19 inhibits the thrombin time at 0.39 micromolar. FM19 at 19±7 micromolar blocks threshold gamma thrombin-induced platelet aggregation. Scrambled forms of FM19 don't inhibit platelet aggregation. At 5 micromolar FM19, 69% alpha-thrombin-induced calcium flux is inhibited. On crystal studies by Dr. Enrico Di Cera of Wash U, the D-Arg of FM19 is a retrobinder in thrombin's active site. The P3 Pro interacts with H57 and W60. The P4 D-Ala binds W215. The P5 p-methyl-L-phenylalanine binds D189 and, perhaps, Oic producing a cyclic peptide inhibitor. FM19-biotin binds rPAR1 exodomain on microtiter plates. On single IV bolus, FM19 is cleared with a T1/2 alpha of 27±7 or 33±2 min in rat or dog, respectively. FM19 is stable in intestinal fluids and has 18% intestinal adsorption from rat gut. Adsorbed FM19 prolongs rat and mouse thrombin times and delays carotid artery thrombosis times in mice. In the mouse, there is a significant prolongation of the time to carotid artery thrombosis at > 0.4 mg/kg FM19 IP.
Conclusions: FM19, a synthetic peptide analog derivative of RPPGF, inhibits thrombin-induced platelet activation and murine arterial thrombosis. Since it lacks sequential, naturally occurring L amino acids, it is enzymatically stable in the GI tract and adsorbed in the intestine. FM19 has potential as a single oral agent to inhibit thrombin and PAR1 activation.
References:
Schmaier AH, Burke F, Warnock M, Nieman M, Hilfinger J, Mosberg HI. THROMBOSTATIN FM19 - A THROMBIN RECEPTOR ACTIVATION ANTAGONIST. *J Thromb Haemost 2007*; **5** Supplement 2: P-M-351

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