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ANTITHROMBOTIC EFFECTS OF INTIMATAN AND HEPARIN

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Introduction: Intimatan is a synthetic Heparin cofactor II agonist that accelerates thrombin inhibition, and has been developed as a therapeutic agent (1). Because heparin has limitations including heparin-induced thrombocytopenia, and the need for antithrombin III, additional methods of anticoagulation are needed. The aim of this experiment was to compare antithrombotic effects of heparin (HEP), Intimatan (IN), and both drugs in combination.

Methods: Intimatan was synthesized by a site-selective 6-O-sulfation of native dermatan sulfate, and was provided by Celsus Laboratories (Cincinnati, Ohio). Blood samples were collected from seven consented volunteers. Platelet poor plasma was obtained by centrifugation (15 min at 2500g). Endogenous thrombin potential (ETP) was measured using a commercially available fluorogenic substrate (Z-GGR-AMC, Bachem Switzerland) with the microplate fluorometer (Fluoroskan Ascent, Labsystems, Finland) set at 390nm (excitation wavelength) and 460 nm (emission wavelength)(2). Briefly, for the thrombin generation experiments, 80 μ l of platelet-poor plasma (PPP), containing either IN (20 μ g/ml), porcine HEP (0.25 u/ml) or combination of IN+HEP (20 μ g/ml+0.25u/ml, respectively) and the thrombin generation trigger (Innovin or Actin) were added to wells of 96-well microtiter plate, followed by 20 μ L of substrate-calcium chloride buffer. PPP without HEP or IN was run as a control. The reaction was monitored by measuring fluorescence for 60 minutes and the acquired data were processed for the thrombin generation parameters: lag time, peak, and ETP.

Results: Both Intimatan and heparin reduced thrombin formation when Innovin (Fig.1) or Actin (Fig.2) was used as a trigger, but the lag time was only affected when Actin was used as a trigger. At the doses used, Intimatan caused less thrombin inhibition than heparin, but the combination of Intimatan and heparin inhibited thrombin generation more than either agent used alone (Tab.1).

Discussion: We found that Intimatan is a potent anticoagulant by itself, and augments heparin anticoagulation, and may be a useful adjunct anticoagulant in cardiac surgical patients to provide better thrombin inhibition. Additional clinical studies are needed to further support these findings.

References:

1. Thrombosis Research 2000;99:603-12,(2) *J of Clin Pharmacol and Therapeutics* 2002;40:135-141

Table 1. Effects of Innovin and Actin on Thrombin generation in PPP.

	Innovin				Actin			
	Control	IN	HEP	IN+HEP	Control	IN	HEP	IN+HEP
Mean ETP	1464.50	588.00	348.25	61.75	1729.33	789.67	540.00	255.67
% Reduction in Thrombin Generation	0.00	59.84	76.22	95.78	0.00	54.33	68.77	85.21

Figure 1.

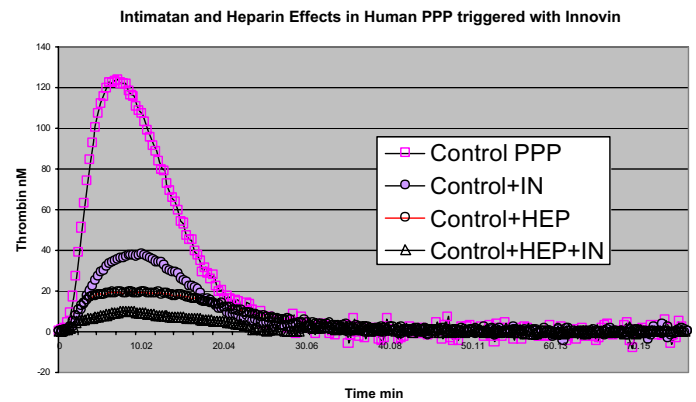


Figure 2.

