

SCA 46

DOSE ESCALATING EFFECTS OF INTIMATAN, A HEPARIN COFACTOR II AGONIST, ON THROMBIN GENERATION

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Introduction: Thrombin plays a central role in coagulation; therefore its inhibition is of paramount importance during cardiopulmonary bypass (CPB). Intimatan is a novel synthetic anticoagulant that accelerates Heparin Cofactor II agonist (HCII)-mediated thrombin inhibition, and is currently being evaluated as an alternative to heparin anticoagulation (1). Because there is a paucity of data available on this new agent, we characterized the in vitro dose response inhibitory effects of Intimatan on thrombin generation using human platelet poor plasma (PPP) and an automated system able to measure endogenous thrombin potential (ETP).

Methods: Intimatan was synthesized by a site-selective 6-O-sulfation of native dermatan sulfate, and was provided by Celsus Laboratories (Cincinnati, Ohio). The drug was dissolved in saline at concentrations of 2 and 20 mg/ml and added to PPP in the smallest volumes possible. Blood samples were collected from seven consented volunteers and PPP was obtained by centrifugation (15 min at 2500g). 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 IN (0, 5, 10, 20, 50, and 100 g/ml) and the thrombin generation trigger (Actin 1:20 dilution, or Innovin, Dade-Behring 1:750 dilution) was added to wells of 96-well microtiter plate, followed by 20 μ L of substrate-calcium chloride buffer. The reaction was monitored at 37C, and the fluorescence was measured from the top at 20 sec intervals for 60-90 min. Four identical experiments were run in parallel. Acquired data were processed for the thrombin generation parameters: lag time, peak, and endogenous thrombin potential.

Results: Addition of increasing concentrations of Intimatan to PPP caused dose dependent reduction in thrombin generation when the trigger was either Actin (37.4-85.7 %) or Innovin (46.5-88.5 %) (Fig 1 and 2). Intimatan delayed Actin but not Innovin induced thrombin generation (Fig 1 and 2) in human PPP.

Discussion: Intimatan effectively suppressed thrombin generation in plasma, and may provide an alternative anticoagulation strategy in cardiac surgical patients especially when heparin is contraindicated because of heparin induced thrombocytopenia.

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

This abstract has 2 Additional Files -- Converted Files are included below:

Figure 1.

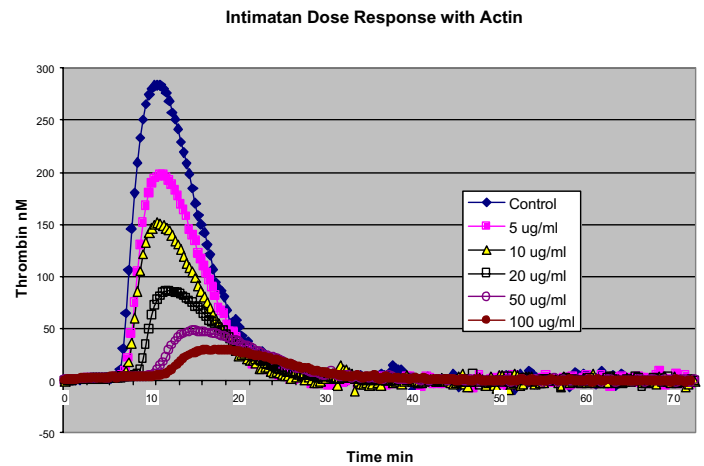


Figure 2.

