



# EX VIVO NEUTRALIZATION OF ENOXAPARIN IN PRIMATES BY A NOVEL HEPARIN ANTAGONIST

W. P. Jeske<sup>1</sup>, E. Litinas<sup>2</sup>, J. Emanuele<sup>1</sup>, D. Hoppensteadt<sup>2</sup>, E. McAllister<sup>3</sup>

<sup>1</sup>Cardiovascular Institute and <sup>2</sup>Pathology, Loyola University Medical Center, Maywood and <sup>3</sup>PolyMedix, Inc., Radnor, United States

## Abstract

In certain clinical situations, it may be necessary to neutralize therapeutic doses of LMWHs to avoid excess bleeding. It is known that protamine does not completely neutralize the anticoagulant activity of LMWHs. In this study, eight non-human primates (*Macaca mulatta*) were anticoagulated with enoxaparin (0.5 mg/kg IV). Blood samples collected at baseline and at 5 minutes post-administration were supplemented *ex vivo* with saline or 10 µg/ml of protamine or a novel salicylamide derivative, PMX 60056. All samples were analyzed by aPTT, thrombin time, anti-Xa and anti-IIa assays. In both clotting and amidolytic assays, antithrombin activity of enoxaparin was completely neutralized by both PMX 60056 and protamine. However, PMX 60056 was more effective at neutralizing enoxaparin's anti-Xa activity (88.2% ± 16.4% inhibition with saline vs. 47.0 ± 30.8% (p=0.003) with PMX 60056 or 76.6 ± 27.6% (p=0.27) with protamine). A similar pattern was observed with the aPTT. Administration of 0.5 mg/kg enoxaparin increased the aPTT nearly 3-fold to 111.9 ± 48.6 sec. Supplementation of PMX 60056 reverted the clotting time back to baseline (43.8 ± 15.0 sec; p<0.05 vs. saline), whereas supplementation with protamine did not affect the clotting time (129.6 ± 77.4 sec). Additionally, when supplemented to baseline samples, protamine produced a mild prolongation of the aPTT [56.2 ± 23.9 sec vs. 29.2 ± 6.9 for saline; p<0.001] that was not observed with PMX 60056 (37.6 ± 16.8 sec; p=0.134 vs. saline). These studies support the idea that salicylamide derivatives are more effective antagonists of LMWHs. Further pharmacodynamic studies are warranted. Because of its molecular size, salicylamide heptagonists may be able to be administered subcutaneously.

## Background

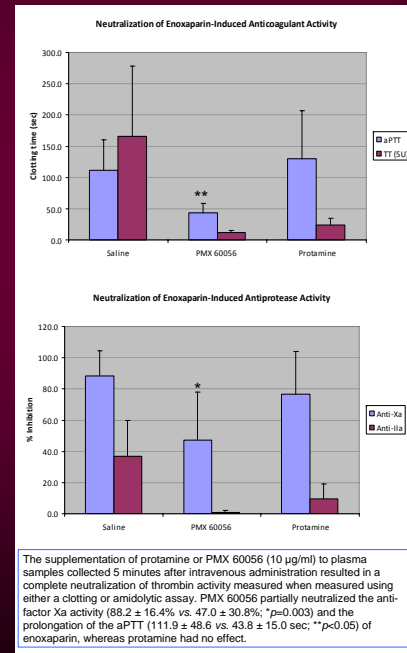
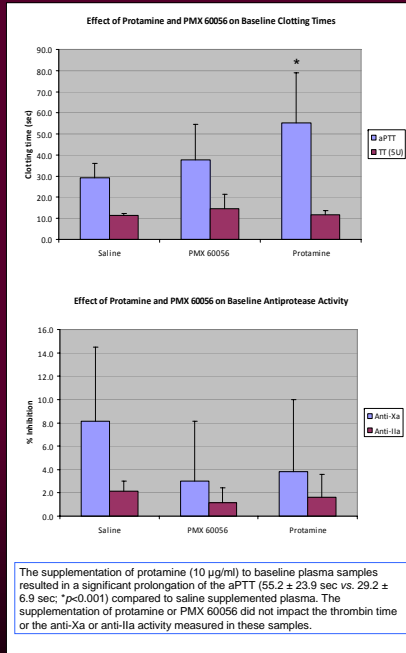
Unfractionated heparin and other heparin-like derivatives such as low molecular weight heparins (LMWHs) and heparin-derived drugs (i.e. fondaparinux) are used in the prophylaxis and treatment of diseases such as deep vein thrombosis and pulmonary embolism and in acute surgical applications including cardiac bypass. Following surgery, heparin's anticoagulation effects must be reversed to prevent excess bleeding. Currently this neutralization is achieved using protamine sulfate, whose cationic properties allow charge-based binding to heparin. However, the use of protamine has potential for serious side effects. Rapid bolus administration can lead to hypotension, bronchoconstriction, or pulmonary hypertension due to the release of histamine and complement activation. Large doses of protamine can also produce a heparin rebound, a reappearance of anticoagulant activity following adequate neutralization by protamine. The use of LMWHs and heparin-derived drugs have increased due to their ease of administration, longer duration and reduced incidence of heparin-induced thrombocytopenia. LMWHs introduce additional problems with neutralization since protamine sulfate is unable to completely reverse the anticoagulant effects. In the past, other polycationic substances such as platelet factor 4 and polybrene (hexadimethrine) and the enzyme heparinase, have been determined to not be clinically useful.

Our objective was to characterize the ability of various salicylamide derivatives (PolyMedix Corp, Radnor, PA) to neutralize the activities of LMWHs (enoxaparin) and other heparin-derived drugs containing the same antithrombin-binding pentasaccharide sequence (fondaparinux). We hypothesized that the salicylamide derivatives will be as effective, if not more effective, than the current heparin antagonist, protamine sulfate, at neutralizing the anticoagulant and antiprotease actions of these heparin-like drugs.

## Purpose

To characterize the ability of salicylamide derivatives to neutralize the anticoagulant and antiprotease activities of low molecular weight heparin and heparin-derived drugs using *in vitro* assays.

## Results



## Test Agents

Enoxaparin (Lovenox) – Sanofi-Aventis, Paris, France  
Protamine sulfate – Institute Choay, Paris, France  
PMX 60056 – PolyMedix, Radnor, PA

## Methods

### Sample Collection

Non-human primates (*Macaca mulatta*, n=8) were anesthetized by an intramuscular injection of ketamine (10 mg/kg). Baseline blood samples were collected via venipuncture of the saphenous vein. Enoxaparin was administered as an intravenous bolus at a dose of 0.5 mg/kg. Five minutes post-injection, a second blood sample was collected.

All blood samples were collected into sodium citrate (3.2%) at a ratio of 9 parts whole blood to 1 part citrate. Samples were centrifuged within 30 minutes of collection to make platelet poor plasma. Aliquots of plasma were frozen for future analysis.

Baseline and 5 minute post-enoxaparin plasma samples were supplemented with saline, 10 µg/ml PMX 60056 or 10 µg/ml protamine sulfate. Samples were gently mixed and then analyzed in terms of anticoagulant activity (aPTT and thrombin time) and amidolytic antiprotease activity (anti-Xa and anti-IIa).

### Anticoagulant and Antiprotease activity assays:

**Anticoagulant activity assays:** Anticoagulant activity was measured in terms of prolongation of the aPTT (bioMérieux, Durham, NC), and thrombin time (Enzyme Research Laboratories (South Bend, IN) using fibrinometers (BBL, Cockeysville, MD).

**Antiprotease assays:** Human factor Xa and Human Thrombin were obtained from Enzyme Research Laboratories (South Bend, IN). Amidolytic Substrates were obtained from American Diagnostica (Stamford, CT). Anti-factor Xa and anti-factor IIa assays were performed on an ACL 300 Plus fast kinetics coagulation analyzer (Beckman-Coulter, Hialeah, FL).

## Conclusion

These studies demonstrate that the PolyMedix series of salicylamide derivatives can neutralize the anticoagulant and anti-protease actions of LMWH and fondaparinux. Manipulation of chemical structure may allow for the identification of agents that are more effective than currently available antagonists. These results warrant further studies on the neutralization profile of the series of salicylamide derivatives in animal models of bleeding and thrombosis.