



Novel Antagonists for Low Molecular Weight Heparin and Heparin-Like Drugs

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Abstract

Unfractionated heparin and other heparin derivatives such as low molecular weight heparins (LMWHs) are used for treatment of coagulation diseases and in surgical applications. Currently neutralization is achieved using protamine sulfate, whose cationic properties allow charge-based binding to heparin. However, protamine is unable to completely reverse LMWH anticoagulant effects and has potential for side-effects. Our objective was to characterize the heparin activity of a series of salicylamide derivatives (PolyMedix, Inc., Radnor, PA) to neutralize the activities of a LMWH (enoxaparin) and a heparin-derived drug containing the antithrombin binding sequence (fondaparinux) to better understand the structural features required for effective neutralization. Human plasma (n=4) was supplemented with enoxaparin (0.9 – 15 µg/ml) or fondaparinux (0.5 – 10 µg/ml) to develop concentration-response curves. Each of 18 salicylamide derivatives (i.e. PMX 60056, etc.) or protamine sulfate was added to the enoxaparin- and fondaparinux-supplemented plasma samples at concentrations of 2.5 or 5.0 µg/ml. Anticoagulant activity was measured by coagulation (activated partial thromboplastin time and Heparin) and anti-protease (anti-factor Xa and anti-thrombin) assays using an automated coagulation analyzer. Data was analyzed in terms of percent neutralization of therapeutic (7.5 and 1.25 µg/mL) and prophylactic concentrations (3.75 and 0.625 µg/mL) of enoxaparin and fondaparinux, respectively. For the LMWH, the ratios of neutralizing agent to enoxaparin ranged from 0.33 to 1.33. As the ratio increased, more effective neutralization was observed. Consistent with previous results, protamine was only capable of partially neutralizing the *in vitro* activities of LMWH. While approximately 60% of the anti-thrombin activity was neutralized, much less neutralization was observed in terms of anti-Xa and anticoagulant activity. The first salicylamide advanced into clinical development (PMX 60056) more effectively neutralized the anti-thrombin and anti-Xa activities of enoxaparin. Of the 17 new derivatives, approximately half were as effective as PMX 60056 at neutralizing anti-thrombin activity, and several were notably better at neutralizing anti-Xa activity. It was also noted that several compounds were not effective at neutralizing anticoagulant or anti-protease activities. The ratios of neutralizing agent to fondaparinux ranged from 2.0 to 8.0. At any of the ratios tested, protamine was ineffective at neutralizing anti-Xa or Heparin activity. Although all salicylamide derivatives exhibited a reduced capacity to neutralize fondaparinux in comparison to enoxaparin, at higher ratios, several of the derivatives were more potent than PMX 60056. This study shows that salicylamide derivatives can neutralize the anticoagulant and anti-protease actions of LMWH and fondaparinux. Manipulation of chemical structure may allow for identification of even more potent and/or selective agents.

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 in and also 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, Inc., 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 anti-protease activities of low molecular weight heparin and heparin-derived drugs using *in vitro* assays.

Test Agents

Lovenox – Sanofi-Aventis, Paris, France
Fondaparinux – GlaxoSmithKline, Research Triangle Park, NC
Protamine sulfate – Institute Choay, Paris, France
Salicylamide derivatives (PMX 60056, PMX 60098, PMX 60102, PMX 0000487, PMX 10215, PMX 10156, PMX 60137, PMX 60114, PMX 60106, PMX 60124, PMX 60126, PMX 60138, PMX 60139, PMX 0000415, PMX 0000418, PMX 60100, PMX 60121, PMX 0000414) – PolyMedix, Inc., Radnor, PA

Methods

Anticoagulant and Antiprotease activity assays:
Anticoagulant activity was measured in terms of prolongation of Heparin and aPTT time, and antiprotease activities were measured using anti-factor Xa and anti-thrombin assays. Enoxaparin or Fondaparinux and each of the salicylamide derivatives (or protamine sulfate) were supplemented to normal human plasma (n=4) and all assays were performed according to the manufacturer's recommended procedures using an automated coagulation analyzer. Data was then analyzed in terms of percent neutralization of therapeutic (7.5 µg/mL and 1.25 µg/mL) and prophylactic concentrations (3.75 µg/mL and 0.625 µg/mL) of enoxaparin and fondaparinux, respectively.

Results

Neutralization of Heparin by Protamine and PMX 60056

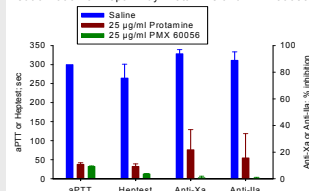


Figure 1: Anticoagulant effects of unfractionated heparin alone and subsequent neutralization by protamine sulfate or the clinical lead salicylamide derivative (PMX 60056).

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 even more potent and/or selective agents. These results warrant further studies on the neutralization profile of the series of salicylamide derivatives in animal models of bleeding and thrombosis.

Anticoagulant Activity of Heparin-like Drugs

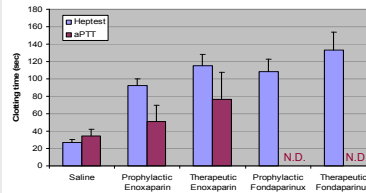


Figure 2: Anticoagulation effects of enoxaparin and fondaparinux.

Anti-Protease Activity of Heparin-like Drugs

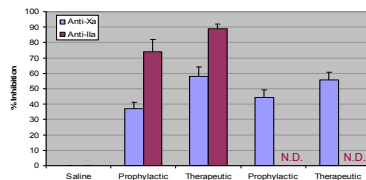
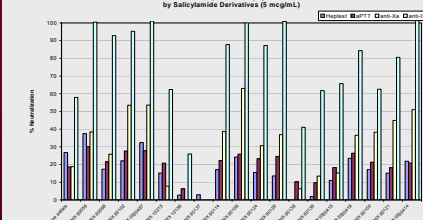


Figure 3: Anti-protease effects of enoxaparin and fondaparinux as measured by anti-Xa and anti-thrombin assays.

% Neutralization of Enoxaparin at Prophylactic Concentrations by Salicylamide Derivatives (5 mcg/mL)



% Neutralization of Enoxaparin at Therapeutic Concentrations by Salicylamide Derivatives (5 mcg/mL)

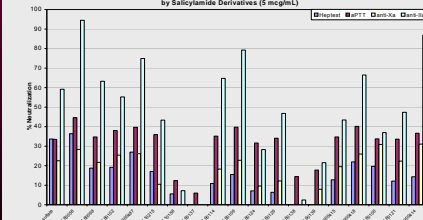
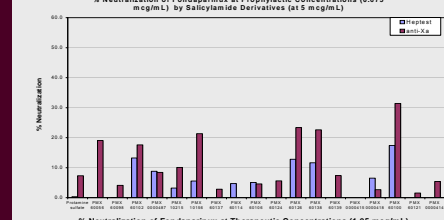


Figure 4: Demonstration of the neutralization of therapeutic and prophylactic levels of enoxaparin by 18 salicylamide derivatives and protamine sulfate using coagulation tests (Heparin and aPTT) and anti-protease (anti-Xa and anti-thrombin) assays. Approximately half of the derivatives were as effective as PMX 60056 at neutralizing anti-thrombin activity, and several were notably better at neutralizing anti-Xa activity.

% Neutralization of Fondaparinux at Prophylactic Concentrations (0.625 mcg/mL) by Salicylamide Derivatives (15 mcg/mL)



% Neutralization of Fondaparinux at Therapeutic Concentrations (1.25 mcg/mL) by Salicylamide Derivatives (15 mcg/mL)

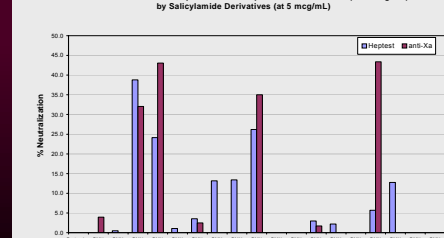


Figure 5: Heparin and anti-Xa assays confirmed that protamine was ineffective at neutralizing fondaparinux anticoagulation activity at therapeutic and prophylactic concentrations. Although all salicylamide derivatives exhibited a reduced capacity to neutralize fondaparinux in comparison to enoxaparin, at higher ratios, several of the derivatives were more potent than PMX 60056.