

In Vivo Neutralization of Unfractionated Heparin and Low Molecular Weight Heparin by a Novel Salicylamide Derivative

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Abstract

Heparin (UFH) neutralization is critical after coronary bypass surgery to avoid excessive blood loss. The use of protamine can be associated with serious side-effects. This study tests the ability of a salicylamide derivative (PMX 60056; PolyMedix, Radnor, PA) to neutralize hemorrhagic and antithrombotic actions of UFH and enoxaparin (ENOX) in defined animal models. Sprague-Dawley rats were anesthetized and treated with 2 mg/kg UFH or ENOX by IV bolus. 5 minutes later, rats were treated with saline or one of three doses of protamine or PMX 60056. Bleeding time was measured using a tail transection model and antithrombotic activity was assessed using a jugular vein clamping model. UFH and ENOX prolonged the bleeding time (UFH: 29.1±5.1 min, ENOX: 16.7±3.6 min vs. Saline: 7.3±1.4 min; p<0.001). Protamine and PMX 60056 dose-dependently reduced the UFH-induced bleeding with near complete neutralization observed at equigravimetric doses. While protamine and PMX 60056 were able to neutralize the hemorrhagic effects of ENOX, the dose-response relationship was not as clear as with heparin. PMX 60056 was somewhat more effective than protamine (7.4±1.2 vs. 8.7±1.5 min) at neutralizing ENOX-induced bleeding. Residual antithrombotic activity in UFH and ENOX-treated rats (7.2±1.1 and 7.0±0.6 clampings, respectively, 3.4±0.5 in vehicle treated rats) was comparably neutralized by protamine and PMX 60056. A poor correlation between residual anticoagulant/anti-protease activity and neutralization of bleeding time was observed. These results suggest that PMX 60056 is as effective as protamine at neutralizing the antithrombotic and hemorrhagic actions of UFH and may be slightly better at neutralizing ENOX. Further studies to characterize the PK/PD profiles of the salicylamide derivative are warranted.

Hypothesis

The novel synthetic salicylamide-derived heparin antagonist, PMX 60056, is effective in neutralizing anti-coagulant, anti-thrombotic and bleeding effects of heparin and low molecular weight heparin (LMWH).

Background

Unfractionated heparin and the low molecular weight heparins (enoxaparin) are commonly used in the management of heart attacks and to prevent blood clotting during and after surgery^{1, 5, 6}. Unfractionated heparin represents the only anticoagulant for open-heart surgery and interventional cardiovascular procedures. It is sometimes necessary to reverse the effects of heparins to avoid excessive bleeding. Unfractionated heparin and to a lesser extent enoxaparin can be neutralized by protamine sulfate, a protein that binds to heparin and prevents its interaction with the blood clotting system^{5,6,8}. Protamine sulfate use can be associated with serious side-effects such as hypotension, bradycardia, anaphylaxis, varied responses to the drug and heparin rebound. Heparin rebound is a very important consideration with the use of protamine sulfate. Heparin rebound is the re-appearance of anticoagulant activity after adequate neutralization with protamine sulfate and may be due to differential kinetics of heparin and protamine sulfate in the circulation. In addition, large doses of protamine are known to impact the blood clotting system.

Specific Aims

1. Compare ability of Protamine sulfate and PMX 60056 in neutralizing the anti-hemorrhagic (rat-tail transection model^{9,7}) and anti-thrombotic (jugular vein clamping model^{4,7}) effects in animal models.
2. Compare effects of Protamine sulfate and PMX 60056 in routine coagulation and anti-protease tests (aPTT, Heparin, anti-Xa, anti-IIa).

Materials and Methods

In Vivo Methods

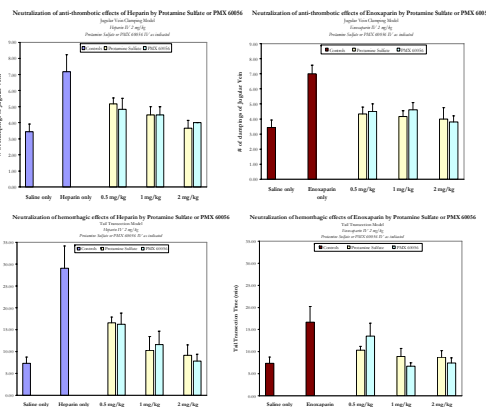
Male, Sprague-Dawley rats (Harlan, Laboratories, Indianapolis, IN) were anesthetized (Ketamine 90 mg/kg and Xylazine 10 mg/kg, Intramuscular bolus) and then treated with an IV bolus of heparin (Institut Choay, Paris France) or enoxaparin (Sanofi-Aventis, Paris, France). Five minutes later, rats were treated with either saline or one of three doses of protamine sulfate (Laboratoire Choay, Paris France) or PMX 60056 (PolyMedix, Radnor, PA). Five minutes later, the distal 2 mm of the rat tail was transected and the time for bleeding to stop was measured. Upon completion of the bleeding model, the jugular veins were isolated and residual antithrombotic activity was measured using a jugular vein clamping model⁴. Flow measurements were made using a Model 806-CB Directional Doppler (Parks Medical Electronics Inc., Aloha, OR). Blood samples were collected via cardiac puncture prior to euthanasia. All procedures carried out with animals were approved by the Institutional Animal Care and Use Committee.

In Vitro Methods

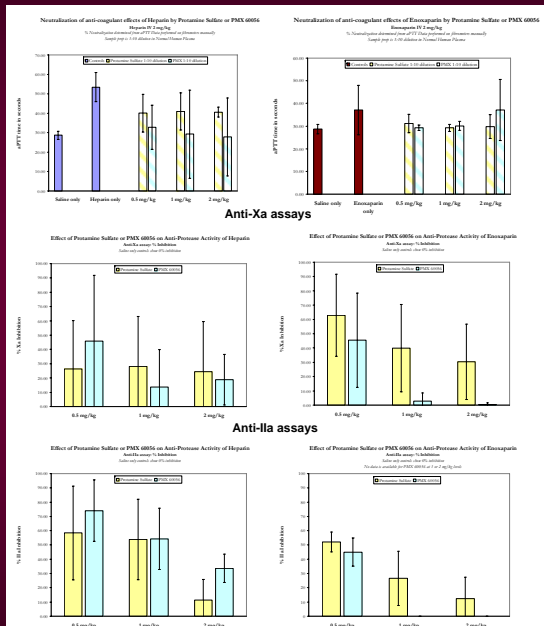
Anticoagulant activity assays: Anticoagulant activity was measured in terms of prolongation of the aPTT (bioMérieux, Durham, NC), and Heparin (Haemachem, St. Louis, MO), Heparin (or LMWH) and protamine sulfate (or PMX 60056) were supplemented with normal human plasma or were measured whole in 1:10 citrate. aPTT and Heparin clotting times were determined using fibrometers (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).

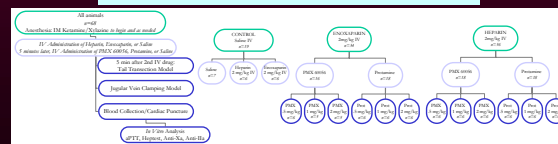
In Vivo Results



In Vitro Results



Study Design



Clinical Relevance

Unfractionated heparin and the low molecular weight heparins (LMWHs) are commonly used in the treatment of acute coronary syndromes, as prophylaxis against deep vein thrombosis and pulmonary embolism and to prevent clotting during interventional and surgical procedures. The neutralization of unfractionated heparin is critical following the completion of coronary bypass surgery to avoid excessive blood loss. The development of an improved heparin antagonist will not only minimize observed adverse reactions² but will also fill an unmet need in providing an effective neutralizing agent for LMWHs. Moreover, PMX 60056 is synthetic agent of non-biological origin which may pose an improved safety profile in regards to anaphylaxis and allergic side-effects.

Summary

At a dose of 2 mg/kg, both UFH and enoxaparin significantly prolonged the bleeding time compared to vehicle-treated control (UFH: 29.1±5.1 min vs. 7.3±1.4 min, p<0.001; enoxaparin: 16.7±3.6 min vs. 7.3±1.4 min; p<0.001). Over a concentration range of 0.5 to 2.0 mg/kg, both protamine and PMX 60056 dose-dependently reduced the heparin-induced increase in bleeding time; at equigravimetric doses, both protamine and PMX 60056 produced near complete neutralization of the heparin-induced bleeding. Both protamine and PMX 60056 were also able to neutralize the hemorrhagic effects of enoxaparin, and PMX 60056 appeared to be somewhat more effective than protamine (7.4±1.2 vs. 8.7±1.5 min) at equigravimetric concentrations of enoxaparin and neutralizing agent. Residual antithrombotic activity was comparable in the heparin and enoxaparin-treated rats (7.2±1.1 and 7.0±0.6 clampings, respectively, vs. 3.4±0.5 in vehicle treated rats). Comparable neutralization of antithrombotic activity for both protamine and PMX 60056 was observed. Blood samples collected prior to euthanasia were analyzed for anticoagulant (aPTT and Heparin) and anti-protease (anti-Xa and anti-IIa) activities. While variable degrees of neutralization could be observed in these assays, overall there was a poor correlation between residual anticoagulant/anti-protease activity and neutralization of bleeding time.

Conclusions

These studies demonstrate that novel salicylamide derivatives can effectively neutralize the antithrombotic and hemorrhagic actions of unfractionated heparin and LMWHs such as enoxaparin. Initial results suggest that PMX 60056 is equally effective as protamine at neutralizing UFH and may be slightly better at neutralizing enoxaparin. Further studies to characterize the PK/PD profiles of the salicylamide derivative are warranted.

References

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