

In vitro characterization of the neutralization of unfractionated heparin and low molecular weight heparin by novel salicylamide derivatives

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Unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) are used to treat acute coronary syndromes, as prophylaxis against venous thromboembolism and to prevent clotting during invasive procedures. UFH neutralization is critical following bypass surgery to avoid excessive blood loss. Protamine use can be associated with hypotension, bronchoconstriction, or pulmonary hypertension. Additionally, protamine less effectively neutralizes LMWHs. This study characterizes the ability of polycationic salicylamide derivatives (PolyMedix, Radnor, PA) to neutralize the anticoagulant actions of UFH and enoxaparin. Plasma was supplemented with 10 µg/ml UFH or enoxaparin (Sanofi-Aventis, Paris, France). Protamine or one of six salicylamide derivatives was added to aliquots of heparinized plasma at final concentrations of 50, 25 and 12.5 µg/ml. The supplemented plasmas were analyzed using clotting (aPTT, Heptest, thrombin time) and amidolytic (anti-Xa, anti-IIa) assays. Protamine concentration-dependently neutralized the actions of UFH in all in vitro assays. Two salicylamide derivatives produced an effect comparable to protamine, while three derivatives exhibited a relatively stronger neutralization of UFH. The extent of neutralization in amidolytic assays was greater with the derivatives. While residual anti-Xa and anti-IIa activities (20% and 10%, respectively) were observed with a 5-fold gravimetric excess of protamine, complete neutralization was observed with the salicylamide derivatives. The anticoagulant activity of enoxaparin was neutralized approximately 50% at a 5:1 protamine to enoxaparin ratio. The derivatives completely neutralized the anticoagulant effects of enoxaparin. A similar pattern was observed with the amidolytic assays where 5 of the 6 salicylamide derivatives concentration-dependently inhibited anti-Xa activity. These studies demonstrate that this series of salicylamide derivatives effectively neutralizes the anticoagulant and anti-protease actions of UFH and enoxaparin. Initial results suggest that such agents are more effective than protamine at neutralizing other LMWHs. Future studies are designed to characterize the compounds PK/PD profiles in animal models of bleeding and thrombosis.

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