Heparin binding copolymer reverses the anticoagulant activity of low molecular weight heparins: safety and efficacy data in rats.

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Background: Protamine, the only registered antidote of unfractionated heparin (UFH), may cause unacceptable toxicity. We developed heparin binding copolymer (HBC), a new synthetic agent directly binding UFH, enoxaparin and fondaparinux, and neutralizing their anticoagulant effect in animal models. However, it is necessary to explore a possible application of HBC to reverse the effects of other low molecular weight heparins (LMWHs), and exclude the potential toxicity before first use in humans.

Purpose: Our aim was to evaluate the safety profile of HBC and its efficacy against tinzaparin, dalteparin and nadroparin in rats.

Methods: The in vitro neutralization of tinzaparin, dalteparin and nadroparin was evaluated by measuring anti-factor Xa activity (anti-Xa). The in vivo neutralization was evaluated by measuring the time of bleeding from male Wistar rats tail (N=70). The tinzaparin (10 mg/kg), dalteparin (800 U/kg) and nadroparin (800 U/kg) were injected alone or followed by intravenous infusion of HBC (20 mg/kg). Blood samples were taken from the heart for anti-Xa activity estimation after measuring of bleeding time. HBC was incubated for 72 hours with human umbilical vein endothelial cell lines (HUVEC) to investigate potential in vitro vascular cytotoxicity. The maximum tolerated dose (MTD) of HBC studies were performed in Wistar rats (N=20), by 4-days postdose observation for clinical signs of toxicity and mortality/morbidity. HBC was administered intravenously in doses: 5, 10, 20, 40 and 80 mg/kg until MTD was determined. On the last day of MTD experiment rats were sacrificed and gross necropsy was performed. Additionally, the possible acute toxicity of HBC (6, 20, 40 mg/kg) was assessed by one-hour monitoring of blood pressure, heart rate, body temperature, oxygen saturation, perfusion and respiratory rate in male Wistar rats (N=32). All experiments involving animals were approved by Local Ethical Committees.

Results: HBC completely neutralized the anticoagulant activity of tinzaparin, dalteparin and nadroparin at in vitro conditions. Anticoagulants prolonged bleeding time, but infusion of HBC restored this parameter to baseline level, as is shown in the Figure 1 (*P < 0.05, **P < 0.01, ***P < 0.001 vs vehicle, ###P < 0.001 vs appropriate LMWH). HBC did not show cytotoxic effects on HUVEC (IC50=7386 nM). The MTD was estimated to be 40 mg/kg. The therapeutic doses of HBC did not influence cardiovascular and respiratory parameters of the rats.

Conclusions: HBC successfully neutralized tinzaparin, dalteparin and nadroparin at in vitro and in vivo conditions. The safety data indicates that HBC could be a novel antidote for all parenteral anticoagulants in patients who suffer a major bleeding or require emergency surgery.
Abstract:

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