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REGULAR ARTICLE

The *in vitro* anticoagulant effects of Danaparoid, Fondaparinux, and Lepirudin in children compared to adults

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Abstract

Introduction: Major physiological differences in the coagulation system of children compared to that of adults are well documented. We have previously investigated the age-related differences in response to Unfractionated Heparin (UFH). However, the impact of developmental haemostasis on more recent anticoagulant drugs is unknown. A number of these drugs are approved for use in specific indications in adults and none are approved for use in children. This study aimed to determine whether age-related differences in effect and impact on monitoring tests exist *in vitro* for danaparoid, fondaparinux and lepirudin.
Materials and Methods: Plasma samples were obtained from healthy children and pooled into age-specific pools, in order to obtain sufficient quantity of plasma required for the analysis of the three drugs. Each age-specific pool was spiked with different concentrations of danaparoid, fondaparinux and lepirudin and response was measured using standard techniques. All experiments were repeated using three separate plasma pools. The effect of each drug in children's plasma was compared to the effect in the respective adult plasma pool.
Results: Age-related differences in effect on thrombin potential and monitoring tests were observed only with the drug lepirudin. Specifically, APTT for children up to 5 years of age was increased compared to adults; all children had lower ECT results

Abbreviations: APTT, Activated Partial Thromboplastin Time; ECT, Ecarin Clotting Time; ETP, Endogenous Thrombin Potential; UFH, Unfractionated Heparin.

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compared to adults; children up to 10 years of age had increased inhibition of ETP as compared to adults.
Conclusions: This study confirms age-related differences in response to anticoagulants with predominant anti-IIa effect and highlights the need for further research into this area.

Introduction

Thromboembolic disease remains a major health problem in our society. Historically, therapies for thromboembolic disease, including heparin and warfarin, are commonly complicated by bleeding. Since bleeding and clotting are at opposite ends of the physiological spectrum, the search for an ideal anticoagulant that inhibits clotting, while not increasing clinical bleeding is ongoing. The general increase in the understanding of the coagulation system has led to a rapid increase in the development of new anticoagulants. Many of these new drugs have specific target proteins within the coagulation system and a number of these drugs, such as danaparoid, fondaparinux and lepirudin are approved for use in specific indications in adults. However, these anticoagulants are not approved for use in children. Danaparoid is a low molecular weight heparinoid which acts as a Factor Xa (FXa) and Factor IIa (FIIa) inhibitor in a ratio of 28:1 [1]. Lepirudin is a recombinant, desulfated form of hirudin; a direct thrombin inhibitor that binds to both fibrin-bound, as well as fluid-phase thrombin [2]. Fondaparinux is synthetic analogue of the pentasaccharide sequence required for the binding of Heparin to Antithrombin (AT) and as such is an AT-dependent specific FXa inhibitor.

Danaparoid and lepirudin have been used for the treatment of Heparin Induced Thrombocytopenia (HIT) in children [3–8]. However, there are no studies comparing the age-related effect of these drugs. Hirudin has been studied in cord plasma, with outcomes suggesting that dosing strategies for neonates should not be derived from studies performed in adults [9]. This is due to the fact that higher doses of the drug were required to achieve the same level of anticoagulation as compared to adults. To date there is no published data on fondaparinux in the paediatric setting.

Paralleling the development of new anticoagulants is the expansion in the understanding of Developmental Haemostasis. This concept was established by Andrew et al. and confirms fundamental differences in the haemostatic system of neonates and children compared to adults [10–12]. Differences in weight-adjusted dosing for Unfractionated Heparin (UFH), warfarin and Low Molecular Weight Heparin (LMWH), have been well documented. More importantly, we have recently shown, first *in vitro* and then *ex vivo*, the impact of Developmental Haemostasis on age-related effect and monitoring of UFH therapy in children [13,14].

This study was designed to determine whether age-related differences in effect and impact on monitoring tests exist *in vitro* for danaparoid, lepirudin and fondaparinux, as an initial step prior to studying these drugs *in vivo*.

Materials and Methods

Plasma samples were obtained from healthy children and adults, without previous thromboembolic events and not receiving any form of anticoagulant therapy. Paediatric samples were collected from healthy children about to undergo minor day surgery, while the adults were healthy volunteers. Informed consent was obtained from the parents of children and from the adult participants themselves. This study was approved by the Royal Children's Hospital Ethics in Human Research Committee (EHRC #24124B).

Blood samples were collected in tubes containing 0.105 mol/L (i.e., 3.2%) trisodium citrate anticoagulant in a ratio of 9 volumes of whole blood to 1 volume anticoagulant. Citrated samples were centrifuged at 3000 rpm for 10 minutes at 10°C (Megafuge 1.0R, Heraeus) and platelet-poor plasma was frozen at -70°C for batch-testing.

Plasma samples were pooled into the following age-related pools: <1 years old, 1-5 years old, 6-10 years old, 11-16 years old and Adults (>18 years old). Each age-specific plasma pool consisted of a minimum of fifteen individual donors and was not significantly different between the different pools. Three separate groups of donors were used to create three separate plasma pools for each age-group.

Each age-specific plasma pool was divided into sub-pools and spiked with different concentrations of: danaparoid (0.3, 0.5, 0.7, 1.0 and 1.5 anti-FXa U/ml plasma), lepirudin (0.375, 0.75, 1.5, 2.25 and 3.0 μg/ml plasma) and fondaparinux (0.5, 1.0, 1.5, 2.0 and 4.0 μg/ml plasma). Unspiked, age-specific plasma pools were used as controls.

Danaparoid (Orgaran), lepirudin (Refludan) and fondaparinux (Arixtra) were obtained from Organon, Australia; Pharmion, Australia and Sanofi-Synthelabo, Australia, respectively.

The *Activated Partial Thromboplastin Time (APTT)* was measured using a commercially available STA® PTT-A reagent. The *Ecarin Clotting Time (ECT)* was determined using a commercially available Ecarin reagent, in combination with the STA® analyser (Diagnostica STAGO, France). The maximum measurement times for the APTT and ECT assays was modified allowing for measurement of results up to 600 sec. This is the upper limit of time measurement possible on the STA® analyser.