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Postheparin plasma diamine oxidase activity and the anticoagulant effect of heparin

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Introduction

Diamine oxidase (DAO, EC 1.4.3.6) degrades histamine and other biogenic amines [1]. In man, the highest activities of the enzyme are found in the kidney, intestine, and placenta [2], whereas DAO is usually below the limits of detection in human plasma. DAO activity is detectable in the circulation, however, following an intravenous administration of heparin [3]. Heparin is a sulfated glycosaminoglycan, usually purified from porcine intestinal mucosa, that acts as an anticoagulant via binding to antithrombin and factor Xa [4]. Heparin in unfractionated or size-fractionated form is widely used in anticoagulant/antithrombotic therapy and prophylaxis where it is usually administered subcutaneously. Here we asked whether subcutaneously administered heparin is able to release DAO and whether the anticoagulant effect of intravenously or subcutaneously injected heparin is correlated with postheparin plasma DAO activities.

Materials and methods

A group of healthy male volunteers (n = 6, mean age 29 years, mean weight 77 kg) without gastrointestinal or coagulation disorders received a bolus of 5000 international units (IU) of unfractionated heparin (Heparin Immuno®, MW 8–25 kDa, 5000 IU/ml, Immuno, Vienna, Austria) intravenously. Blood samples were obtained before and 15–120 min after heparin administration. A second group (n = 3, mean age 30 years, mean weight 74 kg) received 5000 IU of the same heparin preparation subcutaneously. In this group blood was obtained before and 30–360 min after heparin administration.

For the determination of DAO activities, blood was collected in tubes containing K-EDTA (final 1.6 mg/ml) and centrifuged immediately for 10 min at 1440 × g at 4 °C. The plasma was stored at –75 °C and assayed for DAO within a week. Plasma DAO activities were determined by a radiometric assay based on the conversion of [^{14}C]putrescine essentially as described previously [5] using 50 µl plasma. DAO activities were calculated in µU/ml where one µU degrades one pmol of putrescine per minute at 37 °C.

For coagulation analyses, blood was collected in tubes containing sodium citrate (10.6 mM), centrifuged as above and the plasma analyzed immediately. Basal coagulation parameters (prothrombin time, thrombin time, fibrinogen) were determined for all subjects. Heparin anticoagulant activity was determined by measuring activated partial thromboplastin time (aPTT) using an electromechanical method [6]. Normal values for aPTT are in the range of 23–40 s.

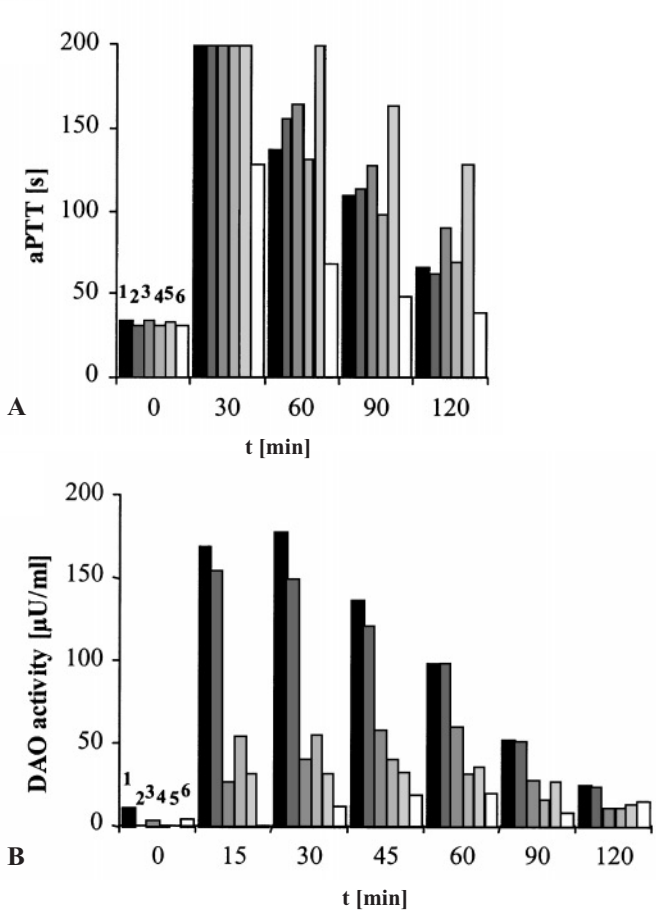

Fig. 1. Effects of intravenous heparin administration. Blood was collected prior to and 15–120 min after an intravenous bolus of 5000 IU of unfractionated heparin for the determination of aPTT values (panel A) and plasma DAO activities (panel B). Values for each individual are represented in the same shade of grey.

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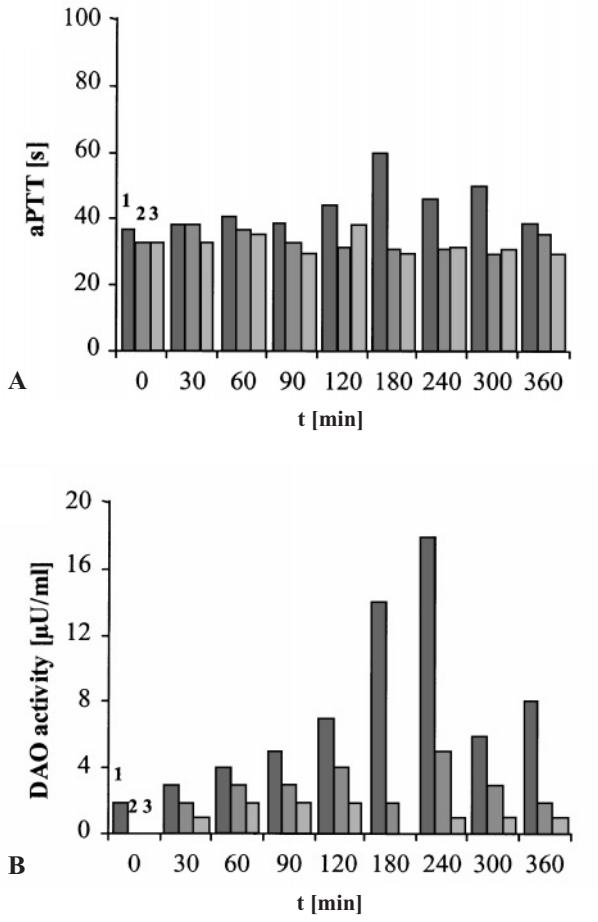

Fig. 2. Effects of subcutaneous heparin administration. Blood was collected prior to and 30–360 min after a subcutaneous bolus of 5000 lU of unfractionated heparin for the determination of aPTT values (panel A) and plasma DAO activities (panel B). Values for each individual are represented in the same shade of grey.

Results and discussion

Intravenous administration of 5000 IU unfractionated heparin caused significant increases in aPTT values within 30 min in all subjects and values were still elevated 120 min after heparin injection (Fig. 1 A). Plasma DAO activities increased from undetectable levels to a maximum median value of 50 µU/ml within 30 min of intravenous heparin administration (Fig. 1 B). Whereas a relatively uniform response was observed in the anticoagulant effect of heparin reflected by aPTT values, DAO activities showed considerable inter-individual variations regarding both absolute values and the time course of release. The anticoagulant activity of heparin and DAO release appear not to be correlated.

Following subcutaneous administration of 5000 IU unfractionated heparin, aPTT values and plasma DAO activities were significantly increased in only one out of three subjects (Fig. 2). As expected, the response was much weaker and significantly delayed compared to intravenous heparinization. Nevertheless, this is the first demonstration that subcutaneously injected heparin can also release DAO.

Since the intestine is the organ containing most of the DAO activity present in man [2] and is presumably the major source of releasable DAO, postheparin plasma DAO activity was investigated as a possible marker to assess mucosal integrity in various intestinal diseases [7, 8]. Our results showing considerable inter-individual variations of heparin-induced DAO release in a very homogenous population of healthy individuals challenge the applicability of postheparin plasma DAO activities as a diagnostic test. Differences in postheparin plasma DAO activities might be the consequence of a variable pool of releasable DAO in each individual. Future studies will have to clarify how releasable DAO is related to total DAO activities available for the degradation of histamine and other diamines.

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