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Measurement of whole blood factor Xa-activated clotting time during hemodialysis with low-molecular-weight heparin

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Abstract

Purpose. To determine whole blood factor Xa-activated clotting time (XaACT), a test for monitoring low-molecular-weight heparins (LMWHs).

Methods. Blood was obtained from six healthy volunteers. Dalteparin, a LMWH, was mixed with the blood to concentrations of 0, 0.5, and 1.0 IU·ml^{-1}. XaACT, activated clotting time (ACT), and activated partial thromboplastin time (APTT) were measured at each dalteparin concentration. XaACT of blood from the outflow and inflow sides of the blood circuit in seven hemodialysis patients was measured before and after bolus administration of 1000 IU of dalteparin, followed by continuous infusion at a rate of 500 IU·h^{-1}.

Results. XaACT, ACT, and APTT in dalteparin-containing blood from volunteers were correlated with dalteparin concentration ($y = 312.8x + 86.4$; $r^2 = 0.88$; $P < 0.001$, $y = 41.8x + 113.5$; $r^2 = 0.83$; $P < 0.001$, and $y = 59.5x + 38.8$; $r^2 = 0.80$; $P < 0.001$, respectively). The regression slope of XaACT was steeper than those of ACT and APTT ($P < 0.001$). In hemodialysis patients, dalteparin increased XaACT on the outflow and inflow sides of the circuit ($P < 0.001$, $P < 0.05$, respectively).

Conclusion. The measurement of XaACT can be employed to monitor LMWHs in clinical settings.

Key words: Low-molecular-weight heparin, Fragmin, Hemodialysis

Introduction

Low-molecular-weight heparins (LMWHs) are commonly used to prevent clotting in extracorporeal blood circuits during hemodialysis [1,2]. However, LMWHs cannot be monitored reliably by traditional tests such as activated clotting time (ACT) and activated partial thromboplastin time (APTT), because LMWHs promote the inhibition of thrombin by antithrombin III to a lesser extent than does unfractionated heparin (UFH) [3,4]. The ability of LMWH to prevent fibrin formation is related to its anti-factor Xa activity and not its antithrombin activity [5]. Therefore, the use of LMWH as an alternative to UFH in the intensive care unit requires a convenient and reliable test of anti-factor Xa activity. In the present study, we determined whole blood factor Xa-activated clotting time (XaACT) using the hemochron method, our new test for monitoring LMWHs.

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Materials and methods

Preparation of test tubes for XaACT measurement

Bovine factor Xa (Sigma Chemical, St. Louis, MO, USA) was dissolved in cold distilled water to a concentration of 0.4 U·ml^{-1}. Then, 0.04 U of factor Xa was injected into a plastic test tube (Hemochron P214, International Technidyne, Edison, NJ, USA), from which powdered glass had been previously removed. The factor Xa-containing test tubes were stored frozen at −20°C until they were used for XaACT measurement. Although the insert instruction (Sigma Chemical) recommends that factor Xa should be stored frozen at −20°C after reconstitution, it does not mention the stability of factor Xa. We therefore used the test tubes within a week of freezing.

Measurement of XaACT, ACT, and APTT in LMWH-containing blood

Whole blood was obtained from six healthy male volunteers (age, 33.5 ± 2.3 [SD] years; range, 26 to 35). Immediately after blood sampling, dalteparin (Fragmin,

Kissei Pharmaceutical, Tokyo, Japan), a LMWH, was mixed with the blood to concentrations of 0, 0.5, and 1.0 IU·ml^{-1}. Then, XaACT, ACT (FTCA510, International Technidyne), and APTT were measured at each dalteparin concentration. The volume of the sample injected into the test tube for XaACT measurement was 0.4 ml. XaACT and ACT were measured with a Hemochron 801 (International Technidyne), and APTT was measured with a coagulation analyzer (Coag 1, Wako Pure Chemical Industries, Osaka, Japan). The temperature of the samples was maintained at 37°C with a built-in heater in each apparatus during the measurement of coagulation time.

Measurement of XaACT in hemodialysis patients

The study protocol was approved by our hospital ethics committee, and informed consent was obtained from seven maintenance hemodialysis patients (two men and five women; age 63.0 ± 5.7 years, range 60 to 75; weight 45.6 ± 4.7 kg, range 37 to 49). Blood samples (0.4 ml) were taken from both the outflow and the inflow sides of the extracorporeal blood circuit immediately before, and 5 min and 1, 2, 3, and 4 h after bolus injection of dalteparin 1000 IU. The infusion rate of dalteparin during hemodialysis was 500 IU·h^{-1}.

Statistical analysis

Relationships between blood dalteparin concentration and XaACT, ACT, and APTT were analyzed by simple linear regression and comparison of two regression slopes. Changes in XaACT in hemodialysis patients were analyzed by analysis of variance for repeated measurements and Scheffé's test, and differences between the inflow and outflow sides of the circuits were analyzed by the paired t-test. Values are means ± SD. P values less than 0.05 were considered significant.

Results

Relationships between LMWH concentration and XaACT, ACT, and APTT

XaACT, ACT, and APTT in dalteparin-containing whole blood obtained from healthy volunteers were each positively correlated with dalteparin concentration (regression lines: $y = 312.8x + 86.4$; $r^2 = 0.88$; $P < 0.001$, $y = 41.8x + 113.5$; $r^2 = 0.83$; $P < 0.001$, and $y = 59.5x + 38.8$; $r^2 = 0.80$; $P < 0.001$, respectively) (Fig. 1). However, the regression slope of XaACT was significantly steeper than those of ACT and APTT ($P < 0.001$).

XaACT in hemodialysis patients

After bolus injection of dalteparin 1000 IU, XaACT of blood taken from the outflow and inflow sides of the circuit increased significantly from 68 ± 10 s at baseline to 327 ± 56 s ($P < 0.001$) and 472 ± 254 s ($P < 0.05$), respectively (Fig. 2). Then, XaACT levels on both sides of the circuit decreased but remained significantly

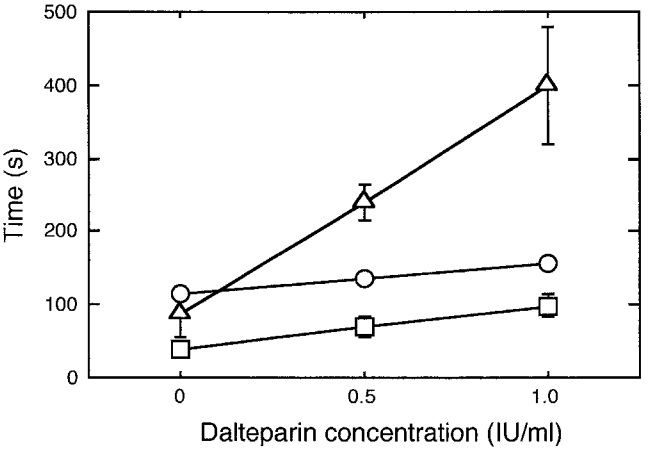

Fig. 1. Relationships between the concentration of dalteparin, a low-molecular-weight heparin and factor Xa-activated clotting time (XaACT) (*triangles*), activated clotting time (ACT) (*circles*), and activated partial thromboplastin time (APTT) (*squares*) in whole blood obtained from six healthy volunteers ($r^2 = 0.88$; $P < 0.001$, $r^2 = 0.83$; $P < 0.001$, and $r^2 = 0.80$; $P < 0.001$, respectively). Values are means ± SD

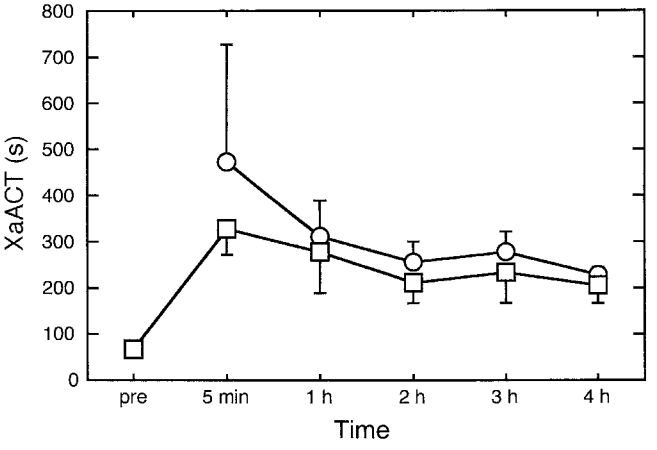

Fig. 2. Changes in factor Xa-activated clotting time (XaACT) of whole blood taken from the outflow (*squares*) and inflow (*circles*) sides of the extracorporeal blood circuit in seven hemodialysis patients before and 5 min and 1, 2, 3, and 4 h after bolus administration of 1000 IU of dalteparin, followed by continuous infusion at a rate of 500 IU·h^{-1}. Values are means ± SD

increased until 4h, and were steady during 2 to 4h of hemodialysis (Fig. 2). The mean values of XaACT were higher on the inflow side than on the outflow side, but the differences were not statistically significant (Fig. 2).

Discussion

We demonstrated that XaACT reflected LMWH concentration more sensitively than did ACT and APTT, and that the measurement of XaACT may be useful for monitoring LMWH concentration during hemodialysis. These findings suggest that measurement of XaACT will enable LMWH to be used for treatment with extracorporeal circulation, which requires more time than hemodialysis. Another finding of the study was that continuous infusion of LMWH into the blood circuit did not produce a significant difference in XaACT values between the outflow and inflow sides of the circuit, indicating that either side of the circuit is available for blood sampling.

LMWHs induce less prolongation of the ACT and APTT than does UFH, since LMWHs have high anti-factor Xa to anti-thrombin activity ratios [3]. Therefore, chromogenic substrate-based assays measuring the anti-factor Xa activity of LMWH have been recommended for quantification of LMWH concentration [6]. However, assays of this type must be performed in the laboratory and are time-consuming, since the procedures required are based on the photometric determination of inactivation of factor Xa after incubation with sample plasma in the presence of antithrombin III. The Heptest (Haemachem, St. Louis, MO, USA) may be available for monitoring the anti-factor Xa activity of LMWH. With this kit, measurement of clotting time is performed by mixing factor Xa and sample, followed by the addition of calcium chloride, rabbit brain cephalin, factor V, and fibrinogen to complete prothrombinase and the substrate. A more convenient method was reported by Naganuma et al. [7], who measured celite and factor Xa-activated coagulation time by the hemochron method using glass test tubes containing celite, factor Xa, and calcium chloride. In their method, since coagulation is initiated by the activation of contact factors as well as exogenous factor Xa following the injection of whole blood diluted with sodium citrate into the test tube, endogenous factor Xa promotes coagulation and shortens clotting time. In contrast, the fundamental principle of our method is the ability of LMWH to catalyze the inhibition of exogenous factor Xa by plasma antithrombin III. It is therefore likely that the clotting time of blood determined by our method accurately reflects the level of LMWH present. Furthermore, the absorption of factor Xa to celite or glass surfaces possibly causes inactivation of factor Xa. Our plastic test tubes and preparation method seem to minimize this reaction and contribute to preservation of the stability of factor Xa. Additionally, our method offers the following advantages: easy preparation of test tubes, a rapid one-stage procedure, and use of nondiluted whole blood as test sample. However, further study is needed to determine the stability of factor Xa in test tubes stored frozen and the therapeutic range of XaACT required for anticoagulation in extracorporeal blood circuits.

In summary, we compared the measurement of XaACT with conventional tests measuring ACT and APTT in LMWH-containing blood, and observed changes in XaACT in hemodialysis patients. We found that XaACT reflected LMWH concentration more sensitively than did ACT and APTT and could be used to monitor LMWH in hemodialysis patients, suggesting that the measurement of XaACT can be employed to monitor LMWHs in clinical settings.

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