Availability of Danaparoid, an Anticoagulant, for Routine Hematological Tests

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ABSTRACT

In the Multiphasic Health Check-up System, medical laboratory testing has been changing lately to utilize a small and compact type of testing systems. The system uses heparinized blood for chemistry tests to reduce laboratory’s workload and sample volume. The danaparoid, a mixture of glycosaminoglycans (Heparan sulfate), has been used clinically for treatment of patients with disseminated intravascular coagulopathy instead of heparin sulfate. Heparin has been used for routine laboratory tests, although it has a strong activity of platelet aggregation. This study aimed to examine availability of Danaparoid for routine hematology tests as a substitute of heparin. Although Danaparoid induced platelet aggregation directly, the reaction was weaker than that of heparin. The platelet count of Danaparoid blood was decreased after five minutes of mixing. Addition of MgSO4 or of kanamycin sulfate to Danaparoid blood was able to prevent this decrease. When Danaparoid blood was applied to chemistry tests, major chemistry test data showed acceptable results, although level differences were observed slightly. It was concluded that Danaparoid would be a good candidate for a universal anticoagulant for both chemical and hematological laboratory tests.

Key Words: Anticoagulant; Heparan Sulfate; Heparin; Platelet Aggregation; Laboratory Testing.

INTRODUCTION

In the Multiphasic Health Check-up System, medical laboratory testing has been changing lately to utilize a small and compact type of testing systems. The system uses heparinized blood for chemistry tests to reduce laboratory’s workload and sample volume.

On the other hand, Danaparoid is a mixture of glycosaminoglycans from the intestinal mucosa of swine, which consist of heparan sulfate (84%), dermatan sulfate (12%) and chondroitin sulfate (4%).[1] It is clinically used for patients with deep vein thrombosis[2] and disseminated intravascular coagulation (DIC).[3] Danaparoid is also known to have potent anticoagulant activity similar to that of heparin,[4,5] although platelet aggregation was in some cases observed in blood with heparin[6]-[8] due to unclarified mechanism. Fortunately, it was reported that Danaparoid also enhanced platelet aggregation, but that its extent was lesser than that of heparin.[9] Danaparoid would be then a promising anticoagulant for use in the clinical laboratory. We, therefore, performed this study to establish the use of Danaparoid as a new anticoagulant for routine hematology tests.

MATERIALS AND METHODS

Subjects

Twenty healthy volunteers between the ages of 25 and 55 years were studied.

Anticoagulants

In this study, we used Orgaran® Injection (Organon, Holland) as danaparoid sodium. It consists of heparan sulfate, dermatan sulfatel and chondroitin sulfate (Fig. 1). A 22.5 unit portion of danaparoid sodium (danaparoid) or heparin sodium (heparin) (Novoheparin, Novo Nordisk A/S, Denmark) for 1 mL whole blood was added to plastic tubes. EDTA-3K vacuum tubes (Terumo, Tokyo Japan) were used as a control.

Inhibitors of platelet aggregation

MgSO4 solution (30 mg/ml) was made from MgSO4-7H2O (Wako Pure Chemical Industries, Osaka, Japan), and Kanamycin sulfate was obtained from Meiji Seika Co., Ltd (Tokyo, Japan).

Measurement of platelet aggregation

Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from sodium-citrate anticoagulated blood by centrifugation, then platelet aggregation was measured by an aggregometer (NBS HEMATRACER 801, MC Medical, Tokyo, Japan). To detect mild platelet aggregation, the recorder scale was expanded by using a 1:1 mixture of PRP and PPP instead of alone PPP as a control.

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Fig. 1 Repeating units of constituents of danaparoid.
control. PRP was pre-incubated for one minute in the incubating hole of the aggregometer.

### Automated blood counting

Heparin (22.5 U) or Danaparoid (22.5 U) was placed in plastic test tubes as an anticoagulant, and 1mL of whole blood was added to the tubes in duplicate. The blood was analyzed with a NE-8000 automatic blood counter (Sysmex Co. Ltd., Kobe, Japan) immediately after blood collection (0 hours). One of the duplicate tubes was kept still for two hours and analyzed, while the other was mixed for five minutes (inverted 20 times/minute) and then analyzed. Blood anti-coagulated with EDTA-3K was used as a control. As an inhibitor of platelet aggregation, 10 mg MgSO₄ or 20 mg kanamycin sulfate (Meiji Pharmaceutical Co., Tokyo) was added in anticoagulated tubes with heparin or Danaparoid.

### Biochemical analysis

Biochemical parameters were measured with a SPOTCHEM SP-4420 automated clinical chemistry analyzer (ARKRAY, Inc., Kyoto, Japan).

### Statistics

Statistical analysis for comparison of mean values was conducted with Wilcoxon signed-rank test. Correlation coefficients were determined by Pearson’s correlation coefficient test.

### RESULTS

#### Platelet aggregation

When Danaparoid or heparin was added to citrated PRP, mild platelet aggregation was observed (Fig. 2), which increased in a dose-dependent manner. However, this effect of Danaparoid was weaker than that of heparin, suggesting that physiological polysaccharides might have stimulatory effects on platelets.

#### Counting of hematological items

Platelet counts of blood treated with Danaparoid or heparin were lower than those of blood treated with EDTA-3K (Fig. 3). Platelet count decreased significantly after five minutes of mixing in Danaparoid-treated-blood. The same phenomenon was observed in heparin blood. The extent of platelet aggregation was lower with Danaparoid than with heparin. No further decrease in platelet count was observed with additional standing for 2 hours without mixing. The heparin- or Danaparoid-induced platelet aggregation could be partially prevented by addition of MgSO₄ or kanamycin sulfate. White blood cell count tended to increase after five minutes of mixing in association with the decrease in platelet count, but no significant differences were observed between mixed

![Fig. 2](image-url) Platelet aggregation induced by danaparoid or heparin. In PRP, a final concentration of danaparoid 2.25 U/ml (a), danaparoid 22.5 U/ml (b), heparin 0.225 U/ml (c), or heparin 22.5 U/ml (d) was added. Physiological saline solution was used as a control (e).

![Fig. 3](image-url) Platelet counts of danaparoid or heparin blood at 0 hours (open bars), after 2 hours (filled bars) and with mixing for five minutes (hashed bars). Thin bars indicate S.D. and significant differences from 0 hours are shown by *: P<0.05 or **: P<0.01.

![Fig. 4](image-url) White blood cell counts of danaparoid or heparin blood at 0 hours (open bars), after 2 hours (filled bars) and with mixing for five minutes (hashed bars). Thin bars indicate S.D. and significant differences from 0 hours are shown by *: P<0.05 or **: P<0.01.

![Fig. 5](image-url) Red blood cell counts of danaparoid or heparin blood at 0 hours (open bars), after 2 hours (filled bars) and with mixing for five minutes (hashed bars). Thin bars indicate S.D. and significant differences from 0 hours are shown by *: P<0.05 or **: P<0.01.
and non-mixed specimens in this count (Fig. 4). No difference change in red blood cell count was observed with or without mixing (Fig. 5).

**Correlation of platelet counts**

The correlation coefficient for blood platelet count was over 0.9 between EDTA-3K blood and heparin or Danaparoid blood without mixing (Fig. 6). After five minutes of mixing, the degree of correlation was decreased because of decrease in platelet count due to partial aggregation of platelets. Addition of MgSO₄ or kanamycin sulfate prior to mixing was enable to prevent the platelet aggregation resulting in keeping the high correlation coefficient over 0.9.

**Analysis of biochemical test results**

Compared with heparin, Danaparoid blood showed slight decreases in total protein and albumin concentration (Table 1). Addition of MgSO₄ cannot prevent the decreases in total protein and albumin concentrations. Furthermore, the addition made LDH activity strongly inhibited, although the addition was able to prevent the decrease in platelet count. When kanamycin was added to Danaparoid blood albumin and ALT levels increased, and AST level lowered, compared with the result of heparin blood.

**DISCUSSION**

Danaparoid is clinically used for deep vein thrombosis and DIC as a substitute for heparin, since its anticoagulant effect is controllable more easily than that of heparin.⁹ Although Danaparoid enhances platelet aggregation by other stimulants, such as ADP, adrenaline and collagen, the effect of Danaparoid is not so strong than that of heparin.⁹ We demonstrated previously that the blood anticoagulated with heparin exhibited decrease in platelet counts after strong mixing.¹⁹ The study proved that heparin caused platelet aggregation directly in the presence of shear stress. As Danaparoid-induced platelet aggregation was known to be weak, it would be more advantageous than heparin to apply Danaparoid to routine hematology tests.

The platelet count of Danaparoid blood to be measured was lower than that of EDTA blood, but the decrease was apparently lower than the count decrease induced by heparin. Addition of MgSO₄ or kanamycin sulfate could partially prevent the heparin- or Danaparoid-induced count decrease due to platelet aggregation, although the preventive mechanism towards platelet aggregation has not been clarified. MgSO₄ and kanamycin sulfate obviously inhibited the count decrease due to aggregation. A common molecular residue of such substances are SO₄²⁻ ions with strong negative electric charge, the SO₄²⁻ ions may play some roles to prevent the platelet aggregation. In the Multiphasic Health Check-up System, the platelet counting is not essential, and both Danaparoid and heparin blood would be applied to chemistry and hematology tests. If platelet count is needed, Danaparoid blood mixed with MgSO₄ or kanamycin would be preferable to get routine hematology data.

On analysis of biochemical parameters, Danaparoid without aggregation inhibitor yielded the same levels of total protein, albumin and total bilirubin as the results obtained by heparin blood. However, the addition of MgSO₄ resulted in a slightly low value of albumin. On the other hand, addition of kanamycin resulted in a slightly higher value of albumin and ALT.

The Multi-phasic Health Check-up System is now widely utilized by Japan workers for prevention of life-style-related disorders. The system employs wet-type chemistry tests since the test-
ing promises stable and reliable tests. One of disadvantageous points of the wet chemistry is requires much volume of blood for laboratory testing, and many of laboratories then have been changing their testing system from the wet one to dry-chemistry one using heparinized blood. Recently the use of Danaparoid has been popular even in the routine laboratory. For simultaneous determination of both chemistry and hematology with a minimum amount of blood, Danaparoid would be more desirable than heparin.

We confirmed that another kind of dry-chemistry-based chemistrip tests, Ektachem (Kodak), demonstrated good data to Danaparoid blood close to those of heparin blood, although the number to be tested was only 2 cases. Thus, the results showed in Table 1 might be due to the specific matrix effects on the chemistrip we used.

In conclusion, Danaparoid would be applicable to automated hematological tests as a substitute of heparin blood, if platelet count is not needed. Regarding chemistry tests, re-calibration or value assignment measures, and further studies would be required.

REFERENCES