Effects of Unfractionated and Low-Molecular-Weight Heparins on Fibrin Polymerization

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In vitro experiments show that therapeutic doses of unfractionated and low-molecular-weight heparins inhibit fibrin polymerization. Sodium and calcium salts of unfractionated heparins exhibit antipolymerization activity. The activity of low-molecular-weight heparins is higher, at 0.75 anti-X_a units/ml fraxiparin produces a stronger inhibiting effect on polymerization of fibrin monomers than clivarin.

Key Words: *blood coagulation; fibrin polymerization; heparins*

Unfractionated heparin sodium salt and its complexes with fibrinogen and other proteins inhibit polymerization of fibrin monomers (FM) [1-3,5]. However, the effects of heparin calcium salt and low-molecular-weight heparins (LMWH) have not been investigated.

In this study we examined the effects of various concentration of unfractionated heparin sodium salt (Biochemie), heparin calcium salt (calciparin, Sanofi), and the LMWHs fraxiparin (Sanofi) and clivarin (Knoll) on FM polymerization. Concentrations of all the preparations were varied within their therapeutic range: 0.25-1.0 units/ml for unfractionated heparins (UFH) and 0.25-1.0 anti-X_a units/ml for LMWH. The rates of polymerization and self-assembly of FM were estimated visually and in a COAG-A-MATE XM optical coagulometer (Organon Teknika).

MATERIALS AND METHODS

The effects of UFH and LMWH on polymerization and self-assembly of FM were investigated *in vitro*. Borate (pH 7.6) and acetate (pH 5.2) buffers with 5 M urea (10% of the total volume) were used.

Self-assembly of FM was studied by two methods.

Method of G. A. Sukhanova and I. G. Peregudova (patent No. 1712873, 15.10.1991). Soluble FM were isolated from platelet-poor plasma of healthy donors [4]. For this purpose 250 mg EDTA, 5 g urea, and 50 mg thrombin dissolved in 5 ml distilled water were consecutively added to 50 ml plasma and heated to 37°C in a water bath for 30 min. Then 90 ml borate buffer and 360 ml normal saline were added. Unstable fibrin clot formed as a result of dilution of urea. It was then dissolved with 35 ml acetate buffer containing 10% urea (5 M). After complete dissolution of the clot, borate buffer was added, and the resultant fibrin clot was dissolved again. This procedure was repeated. The final solution of FM was used for tests.

Protocol. Platelet-poor referent plasma (0.1 ml) was poured into 16 test tubes (4 tubes in line). Heparin sodium salt (0.25-1.0 units/ml) was added to the test tubes of the first line, and the same concentrations of calciparin were added to the test tubes of the second line. Fraxiparin and clivarin (0.25-1.0 anti-X_a units/ml) were added to the test tubes of the third and fourth lines, respectively. Borate buffer (0.2 ml) and FM solution (0.1 ml) were then added, and clotting time was determined visually and in the coagulometer.

Method of A. Sh. Byshevskii et al. [1]. Acetate buffer (0.2 ml) containing 40% urea and 0.2 ml thrombin (activity 10 sec) were consecutively added to 0.2 ml platelet-poor plasma. The mixture was heated to 37°C in a water bath for 10 min and poured (0.1 ml) into 16 test tubes as in the previous test.

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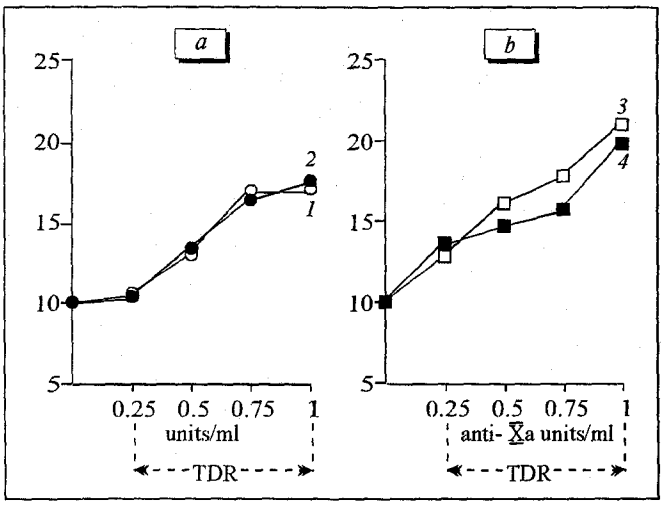

Fig. 1. Effects of unfractionated (*a*) and low-molecular-weight (*b*) heparins on polymerization of fibrin monomers (method of G. A. Sukhanova and I. G. Peregudova). Here and in Fig. 2: *1*) heparin sodium salt; *2*) heparin calcium salt; *3*) fraxiparin; *4*) clivarin. *TDR*) therapeutic dose range. Ordinate: clotting time, sec.

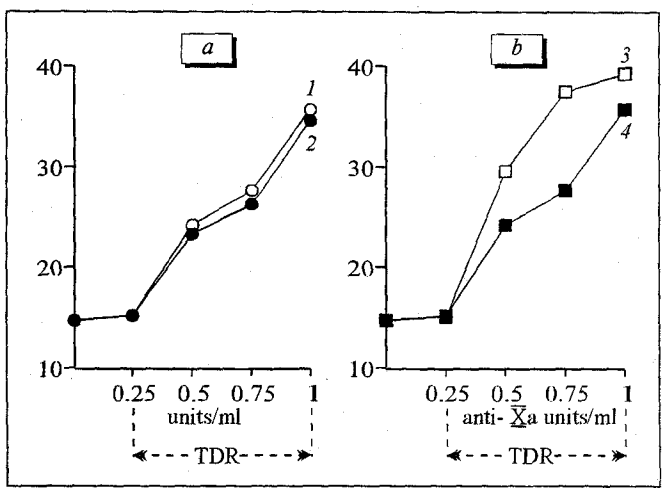

Fig. 2. Effects of unfractionated (*a*) and low-molecular-weight (*b*) heparins on spontaneous polymerization of fibrin monomers [1].

Polymerization of FM was triggered by the addition of 0.2 ml borate buffer. Clotting time was determined visually and in the coagulometer.

Thirty-four samples of normal platelet-poor plasma were studied. Two UFH, each in 4 therapeutic concentrations (0.25, 0.5, 0.75, and 1.0 units/ml) and two LMWH in 4 concentration (0.25, 0.5, 0.75, and 1.0 anti-X_a units/ml) were used. A total of 2312 determinations were performed.

RESULTS

As Figs. 1 and 2 show, all studied heparins inhibited polymerization and self-assembly of FM. However, the effect of LMWH was significantly higher ($p< 0.01$) than those of heparin sodium and calcium salts. The effects of UFH on FM self-assembly did not differ considerably from each other. The antipolymerization activity of fraxiparin was significantly higher than that of clivarin ($p<0.05$) starting from the dose 0.75 anti-X_a units/ml.

Thus, our results show that all studied heparins, including LMWH, not only block the proteases involved in blood coagulation but also inhibit self-assembly of fibrin, which should be taken into consideration when the antithrombotic potential of these preparations is determined. Low-molecular-weight heparins possess a higher anticoagulating activity than that of UFH, and fraxiparin is a more potent anticoagulant that clivarin.

REFERENCES

1. A. Sh. Byshevskii, S. L. Galyan, P. I. Leven, *et al.*, *Regulation of Coagulation Transformations of Fibrinogen* [in Russian], Sverdlovsk (1987).
2. D. M. Zubairov, *Kazan. Med. Zh.*, No. 1, 62-72 (1976).
3. B. A. Kudryashov, L. A. Lyapina, and E. A. Grigor'eva, *Vopr. Med. Khimii*, No. 3, 318-321 (1980).
4. I. M. Radzevich and E. L. Khodorova, *Ukr. Biokhim. Zh.*, **41**, No. 4, 367-370 (1984).
5. S. I. Tazhudinova, in: *Mechanisms of Hemostatic Regulation at the Level of Molecular Interactions* [in Russian], Sverdlovsk (1988), pp. 13-18.