Thrombolytic and Antithrombotic Effects of the Low-Molecular-Weight Heparin—Serotonin Complex

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Thrombolytic activity of the low-molecular-weight heparin—serotonin complex is tested in rats. Thrombi are formed in the left jugular vein. The complex, its components, or normal saline is injected in the right jugular vein 5 min after thrombosis. The complex exhibits high thrombolytic activity in comparison with the control (normal saline) and similar to that of heparin. Antithrombotic activity of the complex is higher than that of heparin, normal saline, and serotonin. Thrombolytic and antithrombotic activities of the complex may be associated with its non-enzyme fibrinolytic activity.

Key Words: *thrombolytic activity; heparin; serotonin; complex*

Low-molecular-weight heparin (LMWH) exhibits antithrombotic activity in provoked thrombosis at the organism level [6]. There is evidence that the antithrombotic effect of therapeutic doses of LMWH is longer than that of high-molecular-weight heparin [7]. The high-molecular-weight heparin—serotonin complex produces a thrombolytic effect, while none of its components possesses thrombolytic activity [3]. Serotonin released into the circulation due to degranulation of mast cells [5] promotes blood coagulation [4]. Complexation with high-molecular-weight heparin abolishes the coagulating effect of serotonin, the resulting complex displaying both anticoagulant and fibrinolytic properties [1].

The aim of the present study was to synthesize the LMWH—serotonin complex and evaluate its thrombolytic and antithrombotic activities in experimentally provoked thrombosis.

MATERIALS AND METHODS

The complex was prepared from LMWH (Celsus) and serotonin (Reanal). Heparin and serotonin (3:1, w/w) were incubated at 37°C for 30 min. The complex was precipitated by adjusting pH to 5.2 with cold acetic acid (1%) and adding 4 volumes of cold acetone. The precipitate was centrifuged (20 min, 8000 rpm), dried, and dissolved in 0.85% NaCl immediately before studies. Complexation between LMWH and serotonin was controlled by cross electrophoresis [9].

Male albino rats (n=80) weighing 180-200 g were used. The animals were injected with 0.06 ml 2.5% aminazine. Two series of experiments were performed. In series I, the thrombolytic effect of LMWH—serotonin complex was assessed. Thrombosis was induced in the jugular vein as described elsewhere [8]. Five min after thrombosis, 0.5 ml 0.1% LMWH—serotonin complex in normal saline (group 1), 0.5 ml heparin (group 2), 0.5 ml serotonin (group 3), or 0.5 ml normal saline (group 4) was injected into the contralateral jugular vein.

In series II, rats (n=40) were also divided into 4 groups. Prior to thrombosis, they were injected with 0.5 ml 0.1% LMWH—serotonin complex (group 1), 0.5 ml LMWH (group 2), 0.5 ml serotonin (group 3), or 0.5 ml normal saline (group 4). Low-molecular-weight heparin and serotonin were administered in doses equivalent to their contents in the studied complex. Blood was collected 10 min after injection, and

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TABLE 1. Plasma TFA and NFA After Injection of the LMWH—Serotonin Complex

Parameter	Control	Serotonin	LMWH	LMWH—serotonin complex
TFA, mm^2 5.7±0.8	6.7±1.3	12.8±0.5*	14.2±1.1*	
NFA, mm^2 3.3±0.6	3.1±0.6	8.0±0.9*	10.5±0.5*	

Note. *$p<0.001$ compared with the control.

thrombosis was induced. After 15 min, thrombi were excised, dried and weighed.

Sodium citrate (3.8%, 1:9) was used as anticoagulant. Plasma total fibrinolytic activity (TFA) and non-enzymatic fibrinolytic activity (NFA) were assayed as described previously [2] and expressed in mm^2.

Data were analyzed using the Fisher—Student test.

RESULTS

The LMWH—serotonin complex exhibited pronounced thrombolytic activity. As early as 15 min after injection, the weight of thrombi decreased by 79.4% in comparison with the control (normal saline). Thrombolytic activity of the complex was similar to that of LMWH: the weight of thrombi in group 1 (0.29±0.03 g) did not differ significantly from that in group 2 (0.27±0.06 g). Serotonin administered in an equivalent dose slightly increased the weight of thrombi, the difference being significant at $p<0.05$. It can be concluded that after complexation with LMWH serotonin lost its coagulant activity due to formation of ionic bonds with LMWH.

The antithrombotic effect of the LMWH—serotonin complex is of particular interest. After injection of the complex, the weight of thrombi was lower than that after injection of LMWH alone (0.035± 0.006 *vs.* 0.043±0.004 g, $p<0.05$). Serotonin had no antithrombotic effect: the weight of the thrombi after its injection did not differ from that in the control (1.412±0.02 *vs.* 1.482±0.09 g).

It should be noted that 10 min after injection of the LMWH—serotonin complex, plasma NFA was 3.1-fold higher compared with that in the control (Table 1), being slightly higher than after injection of LMWH alone ($p<0.05$). As expected, serotonin alone did not enhance NFA. Similar regularities were revealed for plasma TFA. From these observations it can be concluded that LMWH alone and the LMWH—serotonin complex increase plasma TFA due to activation of non-enzymatic fibrinolysis.

Thus, in our experiments the LMWH—serotonin complex not only prevented thrombosis in mammals (the antithrombotic effect) but also lysed fresh thrombi. These properties are related to the presence of LMWH. The antithrombotic effect of the complex is higher than that of LMWH. Presumably, both thrombolytic and antithrombotic properties of the complex result from its high non-enzyme activity. It should be noted that after complexation serotonin completely lost its coagulating activity. The thrombolytic effect of the LMWH—serotonin complex develops within a 15-min period, while the effect of the high-molecular-weight heparin—serotonin complex (15:1, w/w) develops within an hour [3].

REFERENCES

1. B. A. Kudryashov, T. M. Kalishevskaya, and L. A. Lyapina, *Vopr. Med. Khimii*, **19**, No. 3, 269-274 (1973).
2. B. A. Kudryashov, L. A. Lyapina, and I. P. Baskova, *Vestn. Mosk. Univ. Ser. Biologiya*, No. 5, 40-43 (1974).
3. B. A. Kudryashov and L. A. Lyapina, *Biochimiya Zhivotnykh i Cheloveka*, No. 6, 64-67 (1982).
4. M. D. Mashkovskii, in: *Drugs* [in Russian], Moscow (1993), pp. 366-367.
5. V. M. Uspenskii and V. B. Grinevich, *Arkh. Pat.*, No. 6, 14-19 (1980).
6. J. Hirsh, *Thromb. Haemost.*, **70**, No. 1, 104-207 (1993).
7. J. Hirsh and M. N. Levine, *Blood*, **79**, No. 1, 1-17 (1992).
8. D. Hoppesteadt, J. H. Valenga, and J. Fareed, *Thromb. Res.*, **60**, No. 3, 191-200 (1980).
9. S. Nakamura, R. Takeo, K. Tanaka, *et al.*, *Hoppe-Seylers Z. Physiol. Chem.*, **318**, 115-118 (1960).