FIBRINOLYTIC COMPLEXES OF LOW MOLECULAR WEIGHT HEPARIN WITH ACETYLSALICYLIC ACID

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It was shown previously that acetylsalicylic acid (ASA) forms complex compounds with high-molecular-weight heparin with a ratio by weight between the reacting components of 10:1 [2, 4]. The resulting complex possessed not only anticoagulant, but also nonenzymic fibrinolytic activity, both in vitro and on intravenous injection into animals [3]. However, besides high-molecular-weight heparin (HMH), its separate fragments may also be present in man and animals [6]. Researchers have recently paid great attention to low-molecular-weight heparin (LMH), which possesses considerable activity as a neutralizer of factor X_a but has a weaker action on thrombin than HMH. LMH has been shown to be a powerful antithrombotic agent, and when taken into the body it does not give rise to a bleeding tendency in the case of an overdose [7]. LMH can interact with the vascular endothelium and thereby exert a prolonged and moderate anticoagulant effect in vivo [5]. Considering previous data on the properties of a complex of HMH with ASA [3, 4], as well as information on the high antithrombotic action of LMH, the need has arisen for a study of the ability of LMH to form a complex with ASA.

The aim of the present investigation was to obtain an LMH–ASA complex, to study its anticoagulant and fibrinolytic properties in vitro, and also to examine the thrombolytic effect of the complex on a model of thrombosis in animals.

EXPERIMENTAL METHOD

A commercial preparation of LMH, from "Celsus" (USA), with antithrombin activity of 82 IU/mg and activity against factor X_a of 30 IU/mg, and with a molecular weight of 4.4 kD, was used. The ASA used in the experiments was of German or USSR origin. The process of complex formation between LMH and ASA was monitored by crossed electrophoresis [9]. Electrophoresis was carried out in phosphate buffer, pH 7.0, for 1 h in a density gradient of 15 V/cm. The gels were stained for heparin with a 0.1% solution of azure A. The background was washed out with 2% acetic acid. Preparations of LMH–ASA complexes were obtained, on the basis of the method in [8], by mixing 0.1% solutions of heparin and acid in the ratio by weight of 1:1, 1:5, and 5:1 respectively, followed by precipitation of the complex by changing the pH of the medium. During analysis of the complexes LMH and ASA were used separately in concentrations equivalent to those in the complex, as the control; a 0.85% solution of sodium chloride also was used for this purpose. The anticoagulant properties of the resulting complexes were determined in vitro by the recalcification time and thrombin time tests in the usual way. The thromboplastin-induced plasma clotting time was determined by the method in [10]. The total and nonenzymic fibrinolytic activity of the complexes was determined by the method in [1]. The thrombolytic action of the preparations of LMH–ASA complexes was determined by the method in [11] in vivo on male albino rats weighing 170-200 g. A 0.1% solution of the complex was injected intravenously into the animals in a volume of 0.5 ml/200 g body weight, 10 min after provocation and thrombus formation

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TABLE 1. Anticoagulant and Fibrinolytic Activity of 0.01% Solution of LMH–ASA Complex

Specimen	Ratio of components in complex	Anticoagulant activity			Fibrinolytic activity	
		RT (sec)	TT (sec)	PTT (sec)	TFA (mm^2)	NFA (mm^2)
LMH–ASA complex	1:1	183±8,1*,**	21,5±1,5*	195±1,1*,**	19±3,2*,**	13±6,1*,**
	1:5	162±5,7**	14,1±1,0*	120±2,8	21±1,7*,**	21±1,7*,**
	5:1	412±27*,**	28±2,3*	245±2,3*,**	30±2,9*,**	30±2,9*,**
Control LMH solution equivalent to content in complex	1:1	>5 min	20,9±1,2	145,6±2,4	0	0
	1:5	>5 min	11,8±0,9	115±5,7	0	0
	5:1	>5 min	32,5±4,5	203±25	0	0
Control ASA solution equivalent to content in complex	1:1	141±5,1	10,0±0,05	112±13	0	0
	1:5	155±6,3	10,2±0,3	105±6,4	0	0
	5:1	141±4,3	10,1±0,1	111±12	0	0
Control –0.85% sodium chloride solution		145±7,1	10,7±0,5	119±5,8	0	0

Legend. Significance of differences (*p) calculated by comparison with control – sodium chloride solution; (**p) compared with control LMH solution. RT) recalcification time; TT) thrombin time; PTT) partial thromboplastin time; TFA) total fibrinolytic activity; NFA) nonenzymic fibrinolytic activity.

TABLE 2. Thrombolytic Action in Vivo of 0.1% solution of LMH–ASA Complex (ratio 5:1 by weight)

Group of animals	Mass of thrombus, mg	% of thrombolysis
1. Control (0.85% NaCl solution)	5,5±0,3	0
2. Experiment (LMH–ASA complex)	3,0±0,27	54,5
3. Control 1 (LMH in does equivalent to its content in complex)	3,5±0,25	37
4. Control 2 (ASA in does equivalent to its content in complex)	5,0±0,33	9,9

in the jugular vein by intravenous injection of thrombin. The segment of the vein with the thrombus was excised after 5 h and the thrombus was dried at 37°C for 24 h and weighed. Animals of the same weight and age, but receiving the component parts (heparin or ASA) instead of the LMH–ASA complex, in doses equivalent to their content in the complex. The results obtained on 48 rats were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The fact that complex formation took place by the use of 0.1% solutions of LMH and ASA was first proved by crossed electrophoresis. Staining for heparin showed curving of the band on interaction between heparin and acid, evidence of strong interaction between them.

It was found (Table 1) that the LMH–ASA complexes obtained in ratios by mass of 1:1 and 5:1 possessed anticoagulant activity in the partial thromboplastin and thrombin time tests, but the complex obtained with the components in the ratio of 1:5 had a weakened anticoagulant action than control specimens of LMH alone. In the plasma recalcification time test also the complexes exhibited higher anticoagulant activity than the control samples of ASA and of 0.85% NaCl solution, but it was significantly lower than the anticoagulant activity of the control dose of LMH.

Table 1 shows that solutions of the LMH–ASA complexes exhibit nonenzymic fibrinolytic properties. Highest fibrinolytic activity was identified with the complex obtained with the components in the ratio of 5:1. Control samples of heparin and ASA in concentrations equivalent to their content in the complex produced no zones of lysis.

Complexes of LMH and ASA thus possess both anticoagulant and nonenzymic fibrinolytic activity. The anticoagulant activity of the complexes according to some tests did not exceed that of the control samples of LMH, which eliminates any possible risk of development of a bleeding tendency after their administration to animals.

In the next experiments on rats the thrombolytic action of 0.1% solutions of the LMH–ASA complex obtained with components in the ratio of 5:1 was studied (Table 2).

According to the data in Table 2, the highest thrombolytic activity (50%) was observed in the experimental animals (group 2) which had received an injection of the complex. The mass of the thrombus in this case was only half of that in the control animals, receiving an injection of physiological saline. The results are evidence that a solution of LMH alone, equivalent to its content in the complex, also has a thrombolytic action, but it is manifested to a lesser degree (40%) than the action of the complex. An even weaker thrombolytic action is exerted by administration of ASA alone to the animals.

The experimental results can be summed up as follows. A complex of low-molecular weight heparin with acetylsalicylic acid in the ratio of 5:1 has not only an anticoagulant and a nonenzymic fibrinolytic action, but also has a considerable thrombolytic effect. This combination of nonenzymic fibrinolytic activity and a considerable thrombolytic effect makes preparations of the LMH–ASA complex promising candidates for the role of fibrinolytic agents.

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