EFFECT OF HEPARIN ON ACTIVITY OF OXIDATIVE ENZYME SYSTEMS OF LIVER MICROSOMES IN HEALTHY RATS AND RATS WITH EXPERIMENTAL GLOMERULONEPHRITIS

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To study the effect of heparin on activity of the oxidative enzyme systems of the liver microsomes of healthy rats and of rats with glomerulonephritis the hexobarbital test was carried out, the content of cytochrome P_{450} in the liver and the relative weight of the liver were determined, and a histological and histochemical investigation of the liver was undertaken. Heparin was found to stimulate the detoxicating function of the liver, disturbed in experimental glomerulonephritis.

KEY WORDS: glomerulonephritis; heparin; liver microsomes.

During the last decade heparin has been given with good results for the treatment of glomerulonephritis [1, 2, 7-9, 18]. Heparin has also been shown to prevent the development of experiment glomerulonephritis [12, 16, 17]. In the pathogenesis of the glomerulonephritis, the hepatorenal syndrome accompanied by depression of the antitoxic function of the liver plays an important role. [5]. It is interesting to study the possibility of enhancing the antitoxic function of the liver in glomerulonephritis by administration of heparin. According to data in the literature, heparin has a detoxicating effect in various forms of poisoning due to cyanides [11, 13], curare [10], digitalis glycosides [14], bacterial toxins [11], etc.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 110-200 g, in two series: I) on 160 healthy rats and II) on 40 rats with nephrotoxic glomerulonephritis [15]. Heparin (5000 units/ml) was given as single and repeated daily subcutaneous injections in doses of 2.5, 5, 10, 20, and 60 mg/kg.

To judge the effect of heparin on the state of the oxidative enzyme system of the endoplasmic reticulum of the liver the pharmacological method of the hexobarbital test (50 and 70 mg/kg) was used. During repeated injections of heparin the hexobarbital test was carried out at 11 a.m. Other determinations included the cytochrome P_{450} content in the liver [4] and the relative weight of the liver; the liver was also studied histologically in sections stained with hematoxylin–eosin. The nicotinamideadenine dinucleotide (NAD) content was determined histochemically and succinate dehydrogenase (SD) activity was estimated by Nachlas's method.

EXPERIMENTAL RESULTS

As Table 1 shows, a single injection of heparin led to a decrease in the duration of hexobarbital sleep, within the dose range of heparin from 2.5 to 10 mg/kg; with a dose of 20 mg/kg the duration of sleep increased.

The dynamics of the effect of heparin on the hexobarbital test is shown in Fig. 1: With a dose of 5 mg/kg a maximum appeared after 2 h, but with a dose of 10 mg/kg after 3 h. After 24 h the duration of sleep was reduced by one-third. During repeated injections of heparin the duration of hexobarbital sleep decreased over a period of 2-3 days without any further reduction despite continuing administration of heparin.

As a result of administration of heparin the cytochrome P_{450} content in the liver of the rats increased. After heparin in a dose of 5 mg/kg a significant difference ($P < 0.05$) between the control and the experimental series was observed only in the case of a single injection of heparin, and after two injections the difference

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TABLE 1. Decrease in Duration of Hexobarbital Sleep (0.75 mg/kg) in Rats 1 h after a Single Injection of Heparin in Different Doses

Preparation	Dose of preparation, mg/kg	Number of animals	Duration of sleep, % of control
Hexobarbital (control)	75	10	100
Hexobarbital	75		
Heparin	2,5	10	84,3
Hexobarbital	75		
Heparin	5	10	82,0
Hexobarbital	75		
Heparin	10	10	78,0
Hexobarbital	75		
Heparin	20	10	106,9

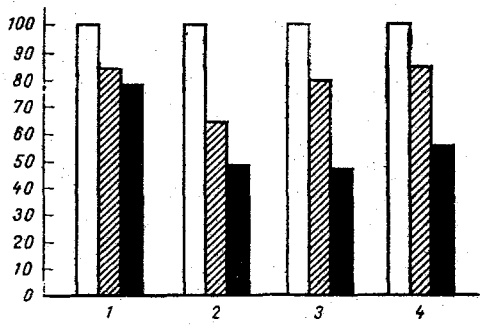

Fig. 1. Decrease in duration of hexobarbital sleep compared with control at different times after injection of heparin. Unshaded columns show duration of hexobarbital sleep in group of control rats; obliquely shaded columns – in group of rats receiving heparin subcutaneously in dose of 5 mg/kg; black columns – in group of rats receiving heparin in dose of 10 mg/kg. Ordinate, degree of shortening of sleep (in % of control). Abscissa, time after injection of heparin (in h).

was smaller. With more frequent (3-6) injections of heparin the cytochrome P_{450} level was virtually the same as in the control (Table 2).

The relative weight of the liver increased after therapeutic doses of heparin by between 16 and 34%, whereas after higher than therapeutic doses it decreased by 10-15%. Histological changes appeared in the liver under the influence of heparin only when given repeatedly in doses of over 10 mg/kg.

An increase in the NAD content was detected histochemically after four injections of heparin in a dose of 5 mg/kg. Changes in SD activity after a single injection of heparin (5 mg/kg) were ill-defined, although the number of formazan granules showed some increase over the control. The granules were situated not only in the center, but also at the periphery of the lobule. Four daily injections of heparin caused a decrease in SD activity.

The hexobarbital test, carried out on the 10th and 30th days of development of nephrotoxic glomerulonephritis, showed a marked increase in the duration of hexobarbital sleep compared with healthy rats: On the 10th day of glomerulonephritis the duration of sleep was increased by 63%, and on the 30th day by 80%.

TABLE 2. Content of Cytochrome P_{450} in Endoplasmic Reticulum of Rat Hepatocytes 24 h After Injections of Heparin in Dose of 5 mg/kg (M ± m; n = number of experiments)

Experimental conditions	Number of injections					
	1	2	3	4	5	6
Control (n = 10)	2,49±0,78	0,87±0,12	1,62±0,55	1,48±1,01	1,49±0,97	1,49±0,97
Heparin (n = 10)	9,36±0,62	1,19±0,25	0,74±0,13	2,65±0,50	0,71±0,13	0,62±0,15
P	<0,05	<0,05	≥0,05	>0,05	>0,05	>0,05

The lengthening of hexobarbital sleep was proportional to the severity of the glomerulonephritis. Heparin considerably shortened the duration of hexobarbital sleep in the rats with glomerulonephritis; the effect of heparin under these conditions was much more clearly manifested than in healthy rats. For instance, 1 h after injection of heparin in a dose of 10 μg/kg into rats with glomerulonephritis the duration of hexobarbital sleep was reduced by 59% compared with rats not receiving heparin, whereas in healthy rats under the same conditions it was reduced by only 24%.

The results of this investigation thus show that heparin has a positive effect on the activity of oxidative enzyme systems of the liver microsomes. In glomerulonephritis, when the antitoxic function of the liver is considerably depressed, heparin can activate it.

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