

HEPARIN AND MAST CELLS IN THE COURSE OF EXPERIMENTAL EXTRAHEPATIC CHOLESTASIS

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The blood heparin concentration and the number of mast cells in the tissues were studied in rats at various times after ligation of the common bile duct. The blood heparin level rose during cholestasis. The total number of mast cells was slightly reduced on the third day, increased on the seventh day, and back to normal on the 14th day. The ratio between granulated and degranulated forms of cells was sharply altered in favor of the latter. Changes in the number of mast cells and the increase in the number of degranulated forms are regarded as the result of irritation of mast cells by bile acids and pigments which accumulate in the body.

KEY WORDS: heparin; mast cells; extrahepatic cholestasis.

In obstructive jaundice the coagulability of the blood decreases and in some cases hemorrhagic complications develop [2, 8, 11]. Increased vascular permeability [6], a deficiency of vitamin-K-dependent factors [7, 19], a disturbance of the formation of active thromboplastin [4], and hyperheparinemia [1, 13] combine to play an important role in the mechanism of hypocoagulation and the bleeding tendency. In obstructive jaundice the blood heparin concentration is increased [1, 10, 11] and correlates with the increase in the direct bilirubin concentration in the blood [12].

Since the main producers of heparin are mast cells, it was decided to study the blood heparin concentration and the number of mast cells at various times during cholestasis; no corresponding data could be found in the accessible literature.

EXPERIMENTAL METHOD

The common bile duct was ligated in albino rats of both sexes weighing 170-200 g. Cholestasis was manifested as a change in the color of the skin, feces, and urine, and also as an increase in the serum total bilirubin concentration. Determination of the blood heparin concentration [5] and histological examination of the tissues were carried out on the third, seventh, and 14th days after the operation. Later, recanalization of the common bile duct can occur [20]. In control rats laparotomy was performed but the bile duct was not ligated.

Mast cells were counted in serial sections through the skin and subcutaneous areolar tissue in the supramuscular layer in 50 fields of vision under a magnification of 400, the result being expressed as the number of cells in ten fields. The total number of mast cells was determined by staining with alcian blue-heparin-containing mast cells were identified by staining with methylene blue at pH 3.65-3.85 [17]. Since degranulation of the mast cells is regarded as the morphological expression of their secretory activity [14, 18], the mast cells were divided into three groups and their relative percentages calculated. Group 1 included oval cells with a round nucleus, surrounded by dark bluish-green cytoplasm densely packed with granules. Degranulation was absent. Group 2 consisted of oval cells with an eccentric nucleus and with large granules in their cytoplasm; in some areas the integrity of the cell was disturbed and granules had

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TABLE 1. Number of Mast Cells (in 10 fields of vision) at Different Periods of Cholestasis ($M \pm m$)

Group and number of animals	Staining with alcian blue			
	total number of cells	number of cells depending on degree of degranulation (in %)		
		group 1	group 2	group 3
Intact (20)	56,3 \pm 2,04	80,8 \pm 0,98	18,3 \pm 0,95	0,95 \pm 0,198
Control (10)	50,9 \pm 2,06	79,3 \pm 1,93	19,5 \pm 1,72	1,2 \pm 0,2
Experimental:				
3rd day of cholestasis (9)	44,5 \pm 2,05*	46,0 \pm 3,35*	44,3 \pm 1,88*	9,7 \pm 2,22*
7th day of cholestasis (11)	69,0 \pm 3,21*	58,9 \pm 1,38*	32,2 \pm 1,1*	8,9 \pm 0,77*
14th day of cholestasis (9)	49,6 \pm 3,23	47,6 \pm 2,22*	43,7 \pm 1,62*	8,7 \pm 1,33*

TABLE 1 (continued)

Group and number of animals	Staining with methylene blue			
	total number of cells	number of cells depending on degree of degranulation (in %)		
		group 1	group 2	group 3
Intact (20)	44,4 \pm 1,55	70,3 \pm 1,18	28,2 \pm 1,21	1,5 \pm 0,85
Control (10)	51,9 \pm 2,76	64,3 \pm 1,84	33,3 \pm 1,93	2,4 \pm 0,29
Experimental:				
3rd day of cholestasis (9)	43,6 \pm 2,5	45,0 \pm 2,71*	40,4 \pm 1,72*	14,6 \pm 1,75*
7th day of cholestasis (11)	54,2 \pm 5,1	53,4 \pm 2,99*	38,5 \pm 1,63*	8,1 \pm 1,63*
14th day of cholestasis (9)	41,4 \pm 1,62	48,2 \pm 2,76*	40,7 \pm 1,13*	11,1 \pm 2,69*

* $P < 0.05$.

escaped into the surrounding tissues (initial stage of degranulation), whereas in other cases the cytoplasm contained pale areas devoid of granules, reflecting the stage of most active of degranulation. Group 3 contained completely destroyed cells, the presence of which could be judged from an accumulation of granules with specific granular structure (the final stage of degranulation).

EXPERIMENTAL RESULTS AND DISCUSSION

The serum bilirubin concentration, which was normally 0.15 mg %, rose on the third day of cholestasis to 3.93 \pm 0.196 mg %, on the seventh day it was 3.28 \pm 0.26 mg %, and on the 14th day 3.1 \pm 0.1 mg %. The blood heparin level of the intact rats was 5.83 \pm 0.42 units, and in rats undergoing the mock operation it was 5.05 \pm 0.41 units ($P > 0.05$). On the third day after the operation the heparin level was 7.25 \pm 0.34 units, on the seventh day 9.14 \pm 0.63 units ($P < 0.05$), and on the 14th day 9.97 \pm 1.25 units ($P < 0.05$). Correlation between the increase in the blood levels of bilirubin and heparin was particularly strong on the seventh day of cholestasis ($r = 0.71$). The total number of mast cells was a little reduced on the third day, but increased on the seventh day; on the 14th day it was back to normal. On the third day after the beginning of cholestasis, when the total number of mast cells was reduced, the number of completely degranulated forms of these cells was increased almost tenfold. All the cells contained heparin. On the seventh and 14th days of cholestasis the number of degranulated mast cells also was high (Table 1).

There is evidence in the literature of correlation between the increase in the heparin concentration and the increase in the degree of degranulation of the mast cells [3]. However, the correlation between the heparin concentration in the tissues and the number of mast cells is not direct [15]. The increase in the number of degranulated mast cells during cholestasis is evidently the result of their irritation by bile acids and pigments which have accumulated in the body. Similar responses of the mast cells have been described to hypoxia [9] and to acoustic, photic, and emotional stimulation [16]. Against the background of intensive degranulation of the mast cells, in cholestasis there is an increase in the free heparin concentration in the blood. These two processes are possibly directly connected. However, this is a matter for further study.

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