Sex-Related Differences in Redistribution of Total Bilirubin Pool after Induction and Removal of Cholestasis in Rats

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Experiments on rats revealed sex-related differences in bilirubin concentration in the bile and bile flow rate against the background of obstructive cholestasis. These differences disappeared after gonadectomy. There were no sex-related differences in the rate of bilirubin excretion with the bile after removal of cholestasis, which was attained through different mechanisms in males and females.

Key Words: *obstructive cholestasis; total bilirubin; bile flow rate; sex differences; rat*

Efficiency of liver excretory function is determined by the intensity of exo- and endotoxin (including bilirubin) transport into the bile and by activity of systems determining bile flow rate. Obstructive cholestasis, a widespread pathological condition accompanying various liver diseases in humans, disturbs excretory function of the liver, increases blood bilirubin level, and induces an alternative (renal) pathway of its removal. Liver diseases accompanied with obstructive cholestasis are sex-dependent [1].

Here we evaluated sex-dependence of compensatory changes in the blood, bile, and urine bilirubin levels in obstructive cholestasis and their normalization in the initial period of bile flow resumption.

MATERIALS AND METHODS

Outbred albino mature male and female rats weighing 190-250 g were used (intact and 4 weeks after gonadectomy, 4-18 animals per group).

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To investigate the causes of the appearance of sex-related differences in bile bilirubin concentration against the background of obstructive cholestasis and after its removal we studied bile flow rate in intact animals and during the initial post-cholestatic period.

Obstructive cholestasis was induced by ligation of the common bile duct for 14 days. In some of rats, the common bile duct was decompressed 14 days after surgery. One day before bile duct surgery, the rats were deprived of food, but had free access to water; the surgery was conducted under ether anesthesia. The initial post-cholestatic period was considered to start 3 hours after decompression.

The bile was collected as follws: in rats without obstructive cholestasis, 1 hour after the beginning of common bile duct drainage for 120 min with 10-min intervals; in animals with obstructive cholestastasis, the bile accumulated over 2 weeks in the dilated duct; in rats after decompression, the bile was collected 150-180 min after the beginning of duct drainage. The blood and urine were collected from animals without obstructive cholestasis, against the background of obstructive cholestasis and in the initial post-cholestatic period after termination of duct drainage (on minute 180).

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Total bilirubin concentration in the bile, blood serum, and urine was estimated using BIL 100S kits (expressed in μmol/liter/kg body weight).

Bile flow rate (μl/min/kg body weight) in rats without obstructive cholestasis was measured over 120 min of common bile duct drainage and in animals in the initial post-cholestatic period during the final 30 min of drainage (minutes 150-180).

The rate of bilirubin excretion with the bile (in μmol//min/kg body weight) was calculated using the data on bilirubin concentration (μmol/liter) in the bile, volume (liter) of the bile sample, and the time of secretion of this volume (min).

Statistical analysis of the data was performed using nonparametric Mann—Whitney test (Statistica 6.0). The differences were significant at $p<0.05$.

RESULTS

In animals with normal liver function, no sex-related differences in total bile bilirubin concentration were revealed in rats with intact gonads and after gonadectomy (Fig. 1).

Obstructive cholestasis induced opposite changes in total bilirubin concentration in the bile in male and female rats with intact gonads: in males, the concentration of this bile pigment significantly decreased ($p<0.005$), while in females it tended to increase (Fig. 1). This led to the appearance of significant sex-related differences in total bilirubin concentration in cholestatic bile. Induction of obstructive cholestasis against the background of gonadectomy in males and females neutralized these sex-related differences (Fig. 1).

In the initial post-cholestatic period, the total bilirubin concentration in the bile significantly increased in all groups ($p<0.05$). Sex-related differences in this index were preserved in rats with intact gonads, but disappeared in gonadectomized animals due to significant decrease in bilirubin level in the bile of ovariectomized females ($p<0.05$; Fig. 1).

In animals with normal liver function (both with intact gonads and gonadectomized), bilirubin concentration in the serum and urine was below the sensitivity limit of the method. Obstructive cholestasis led to elevation of serum and urine bilirubin concentrations, which did not depend on sex both against the background of obstruction and after decompression (Table 1). The percent of conjugated bilirubin in the blood was also similar in males and females under these pathological conditions.

TABLE 1. Parameters of Excretory Function of the Liver under Normal Conditions, during Obstructive Cholestasis (OCh), and in the Initial Postcholestatic Period (IPP) in Intact and Gonadectomized Male and Female Rats

Group	Bilirubin concentration, μmol/liter/kg			Bile flow rate, μl/min/kg	V_{ex} for bilirubin nmol/min/kg
	bile	blood	urine		
Intact					
males	469.2±45.7 (9)	N.d.	N.d.	39.7±3.1 (9)	4.10±0.51 (9)
females	387.4±32.2 (19)	N.d.	N.d.	35.5±1.8 (18)	3.20±0.28 (18)
Against the background of Och					
males	196.4±29.1 (12)	531.8±57.2 (8)	807.8±193.0 (7)	No	No
females	643.3±147.3 (9)	670.0±53.7 (7)	550.3±82.4 (11)	No	No
In the IPP					
males	1272.0±364.4 (5)	256.5±60.6 (5)	297.4±108.1 (4)	92.4±7.7 (5)	30.9±10.4 (5)
females	2423.2±337.0 (8)	290.7±40.6 (8)	377.2±104.3 (8)	57.8±5.2 (8)	31.9±4.9 (8)
Gonadectomized					
males	378.0±34 (11)	N.d.	N.d.	33.3±1.3 (11)	3.70±0.33 (11)
females	344.2±45.2 (9)	N.d.	N.d.	27.2±1.9 (9)	2.70±0.25 (9)
Gonadectomized upon Och					
males	243.3±57.2 (10)	390.1±131.2 (4)	273.7±86.3 (6)	No	No
females	500.1±132.8 (9)	706.9±125.9 (3)	399.1±108.6 (8)	No	No
Gonadectomized in the IPP					
males	1372.8±323.0 (5)	313.9±82.2 (5)	312.0±137.1 (4)	70.9±3.5 (5)	30.40±6.54 (5)
females	1270.2±254.2 (4)	247.1±99.4 (4)	194.2±107.9 (4)	56.1±9.0 (4)	19.9±1.6 (4)

Note. N.d. — not detected; in parentheses — number of animals.

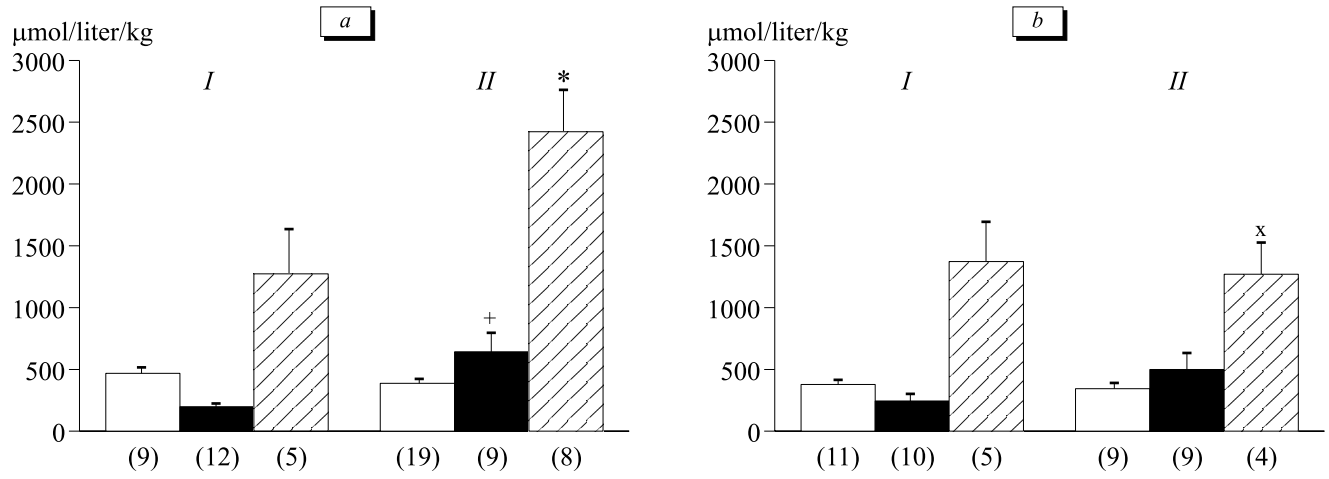

Fig. 1. Sex differences in total bile bilirubin concentration upon OCh and in the IPP. *a*) animals without gonadectomy; *b*) animals with gonadectomy. Light bars: intact animals; black bars: Och; dashed bars: in the IPP. *I*: males, *II*: females. $^{+}p<0.01$ compared to males upon OCh; $^{*}p<0.05$ compared to males in the IPP; $^{x}p<0.05$ compared to females in the IPP. Here and on Fig. 2: number of animals is shown in brackets.

Removal of cholestasis led to bilirubin pool redistribution as soon as after 3 hours at the expense of significant increase of its bile level in males and females and simultaneous significant decrease of its serum level ($p<0.05$) without significant changes in total urine bilirubin concentration (Table 1). These findings suggest that the sex-dependent differences in bile bilirubin concentration during obstructive cholestasis and after its removal are primarily determined by sex-dependent disturbances in the liver function.

It was found that bile flow rate was similar in intact rats, but increased significantly after removal of cholestasis in both females and males ($p<0.5$; Fig. 2). At the same time, this parameter became dependent on sex after cholestasis removal due to more pronounced increase in bile flow rate in the initial post-cholestatic period in males ($p<0.05$). In gonadectomized animals, no sex-related differences in bile flow rate were revealed in the initial post-cholestatic period (Fig. 2) due to significant decrease of this parameter in castrated males.

Sex differences in the rate of bilirubin biliary excretion were absent under normal conditions and in the initial post-cholestatic period (Table 1). After cholestasis removal this was achieved via different mechanisms: due to high bile flow rate and low bilirubin concentration in the bile in males and due to high bilirubin concentration and relatively low bile flow rate in females. It can be assumed that sex differences in bilirubin concentration in the bile in obstructive cholestasis are also associated with different bile flow rate in males and females.

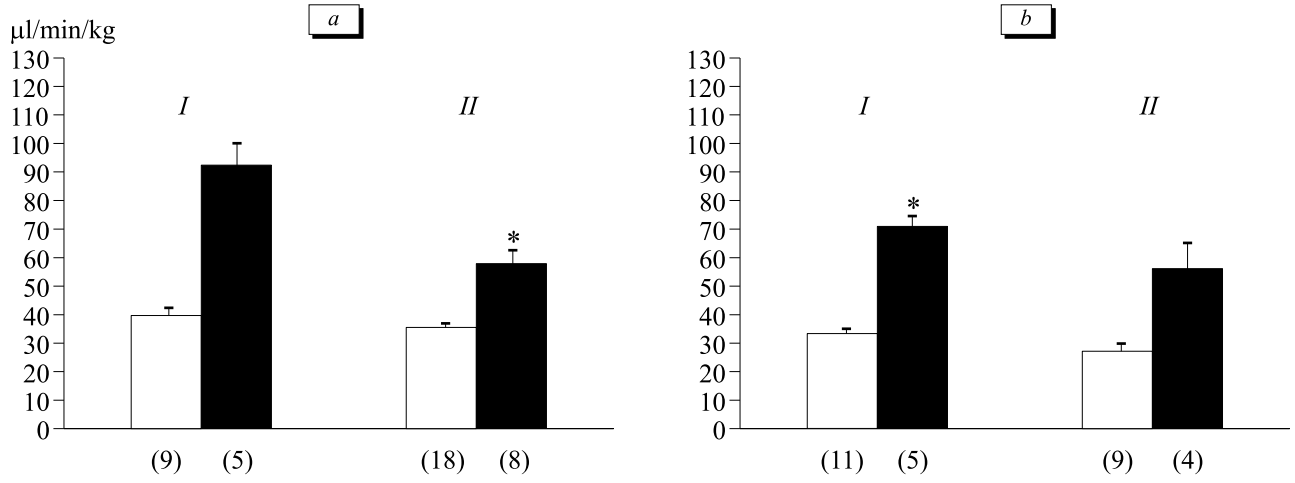

Fig. 2. Sex differences in bile flow rate in the IPP. *a*) animals without gonadectomy; *b*) animals with gonadectomy. Light bars: intact animals, black bars: in the IPP. *I*: males, *II*: females. $^{*}p<0.05$ compared to males in the IPP.

Sex differences in bile flow rate and bilirubin excretion were studied in different animal species, but the obtained data are contradictory [2,3,6,7]. We revealed sex differences in bile flow rate and bile bilirubin concentration against the background of obstructive cholestasis only. However, the probability of this scenario appears from published reports. It was demonstrated that expression of multiple drug resistance protein 2 (MRP2) mRNA and the corresponding protein involved in bilirubin transport into the bile in the liver of intact animals in higher in females. However, these differences normally did not manifest at physiological level, because the density of MRP2 protein on apical hepatocyte membrane (parameter determining the efficiency of functioning of this transporter) was equal in males and females [5]. In cholestasis, expression of MRP2 mRNA and protein decreased, cellular localization of MRP2 was also altered as a result of its retrieval from the canalicular membrane to pericanalicular vesicles which was accompanied by bile flow reduction [4]. Obstructive cholestasis is probably a condition when the latent sex-related differentiation in MRP2 expression appears and affects the rate of protein retrieval from the membrane compartment and the rate of its re-incorporation after removal of cholestasis, which can be implemented as a sex-dependent influence on bile flow rate after decompression.

The revealed sex-related features of the excretory function of the liver under conditions of obstructive cholestasis and its removal can serve as the index, which would enable gender differentiation of the stage of disease and severity of the pathological process in the liver and the search for sex-dependent ways to treat the diseases associated with obstructive cholestasis in the future.

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