Changes in Gender-Related Redistribution of Bilirubin Pools in Hyperprolactinemic Rats during Induction and Relieving of Cholestasis

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Gender-related differences in bilirubin concentration in postcholestatic bile, bile excretion from the liver, and bile flow velocity disappeared in hyperprolactinemia in the presence of obstructive cholestasis. Gender-related differences in the alternative routes of bilirubin excretion appeared.

Key Words: *prolactin; gender-related differences; obstructive cholestasis; bilirubin; rat*

Obstructive cholestasis (OC) developing in various liver diseases in human (primarily in woman) blocks bilirubin excretion through the biliary tracts and leads to its accumulation in liver bile, increase in its concentration in the blood, and induces an alternative route of its excretion through the kidneys [12]. We found that gender-related differences in bilirubin concentration and bile-excretory function of the liver became more pronounced in OC [1].

Liver sensitivity to various signal compounds, including prolactin receptors, increases significantly in OC [4,5,8]. Presumably, differences in prolactin concentration in animals of different genders with OC are closely associated with the appearance of gender-related differences in redistribution of bilirubin pools. This hypothesis is supported by the fact revealed in our study: the influence of prolactin on bilirubin level in the bile and the bile excretory function of the liver in female rats increases in OC [2].

We studied the impact of high prolactin level for gender-related changes in bilirubin levels in the bile, blood, and urine in OC and during the initial stage of bile flow recovery.

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MATERIALS AND METHODS

The study was carried out on outbred adult male and female albino rats (190-250 g). The animals were kept under standard vivarium conditions at natural illumination with free access to water and food. Obstructive cholestasis was induced by ligation of the choledochus for 14 days. The initial postcholestatic period (IPP) started 3 h after duct decompression. Persistent hyperprolactinemia was caused by the classical method (transplantation of donor pituitary under the renal capsule of sex-matched recipient [6,10] simultaneously with choledochus ligation). Measurement of prolactin level by enzyme immunoassay using EIA-4493 kit (DRG) showed that transplantation of the pituitary caused a 2-3-fold elevation of serum hormone concentration in comparison with rats without transplantation of the pituitary.

Total bilirubin concentration in the samples, bile flow velocity, and bilirubin excretion were evaluated as described previously [1].

Bile retention in the liver was diagnosed by changed liver to body weight ratio.

Total bilirubin content in blood, bile, and urine samples and of direct serum bilirubin were measured using reagents for serum bilirubin assays (BIL 100S). The percentage of conjugated bilirubin in the blood was evaluated by the proportion of direct to total bilirubin concentrations.

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The data were statistically processed using Statistica 6.0 software. The significance of differences was evaluated by nonparametric Mann–Whitney test. The differences were considered significant at $p<0.05$.

RESULTS

Two-week hyperprolactinemia was inessential for bilirubin concentration in the bile and for bile accumulation in the livers of females and males with normal hepatic function ($p>0.1$; Table 1). Significant gender-related differences ($p<0.05$) in bilirubin concentration in the bile were retained in OC with and without hyperprolactinemia, while liver weights in females and males increased significantly ($p<0.05$) and similarly (2-fold) at the expense of bile retention. Three hours after relieving of cholestasis, the gender-related differences in bilirubin concentration in the bile and in bile accumulation in the liver were present in animals without hyperprolactinemia ($p<0.05$), but disappeared against the background of this condition ($p>0.1$) due to significant (by 1.5 times) elevation of bilirubin concentration in the postcholestatic bile of males and reduced bile accumulation in their livers compared to just slight effects of hyperprolactinemia on these parameters during IPP in females (Table 1; Fig. 1).

Gender-related differences in bile flow velocity ($p<0.05$) during the initial period after relieving of cholestasis also disappear in animals with 2-week hyperprolactinemia ($p>0.1$) due to deceleration of bile excretion, more pronounced in males (Table 2; Fig. 1); hyperprolactinemia is inessential for normal bile flow ($p>0.1$). Importantly that as a result of combined changes in bilirubin concentrations in the bile and in bile flow rate in different groups of animals, the rate of bilirubin excretion in these groups of males and females virtually does not differ (Table 2; $p>0.1$).

Bilirubin concentration in the blood of normal animals was below the method resolution capacity; in animals with normal hepatic function with hyperprolactinemia, low concentration of bilirubin in the blood was detected in just few rats. No gender-related differences in blood concentrations of bilirubin and percentage of its conjugated form were detected in animals with OC ($p>0.1$; Table 3). Hyperprolactinemia against the background of OC led to the appearance of significant gender-specific differences in the levels of total and conjugated bilirubin at the expense of significant increase of its blood concentration in females ($p<0.05$) and reduced percent of conjugation in males. During IPP, the level of total bilirubin in the blood decreased significantly ($p<0.05$) in both males and females; moreover, the percentage of conjugated bilirubin decreased in males ($p<0.05$; Table 3), still in the absence of gender-related differences in this parameter ($p>0.1$). Relieving of cholestasis leads to a drop (by 4.3 times) of total bilirubin concentration in the blood of hyperprolactinemic females and causes virtually

TABLE 1. Hyperprolactinemia (HPRL) Effects on Bilirubin Concentration in the Bile and Liver Weight in Female and Male Rats (*M*±*SEM*)

Group	Bilirubin concentration in bile, μmol/liter/kg		Liver weight/body weight, %	
	females	males	females	males
Intact	470.6±26.8 (*n*=44)	479.5±20.1 (*n*=36)	2.7±0.1 (*n*=7)	2.8±0.1 (*n*=5)
Intact+HPRL	505.9±32.7 (*n*=10)	474.3±41.0 (*n*=12)	3.2±0.1 (*n*=4)	2.9±0.1 (*n*=3)
OC	467.9±98.1 (*n*=16)	217.9±37.0* (*n*=26)	6.2±0.3 (*n*=13)	6.6±0.2 (*n*=19)
OC+HPRL	617.7±70.2 (*n*=12)	271.8±70.4* (*n*=10)	6.6±0.5 (*n*=8)	6.3±0.3 (*n*=6)
IPP	2241.5±237.5 (*n*=12)	1385.6±248.2* (*n*=8)	5.4±0.4 (*n*=10)	6.4±0.3* (*n*=8)
IPP+HPRL	1861.2±477.2 (*n*=4)	2022.9±220.6 (*n*=4)	5.1±0.2 (*n*=4)	5.6±0.4 (*n*=4)

Note. Here and in Tables 2, 3: *$p<0.05$ compared to the corresponding group of females.

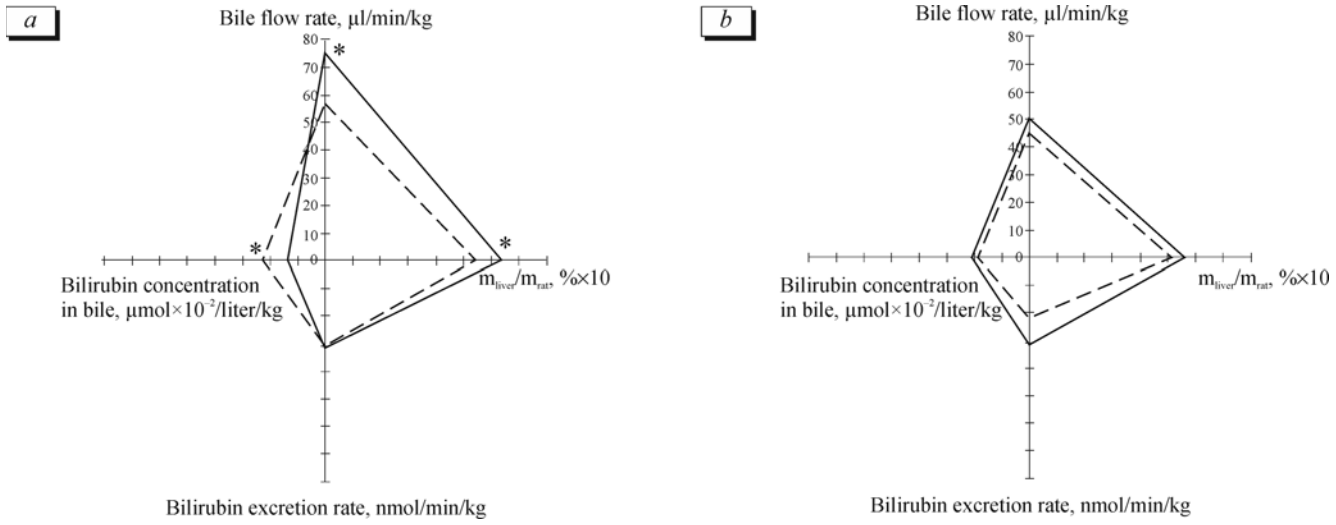

Fig. 1. Gender-related characteristics of recovery of the excretory function of the liver in rats during IPP under conditions of normal (*a*) and high (*b*) prolactin levels. Here and in Fig. 2: dotted line: females; solid line: males. *$p<0.05$ compared to females.

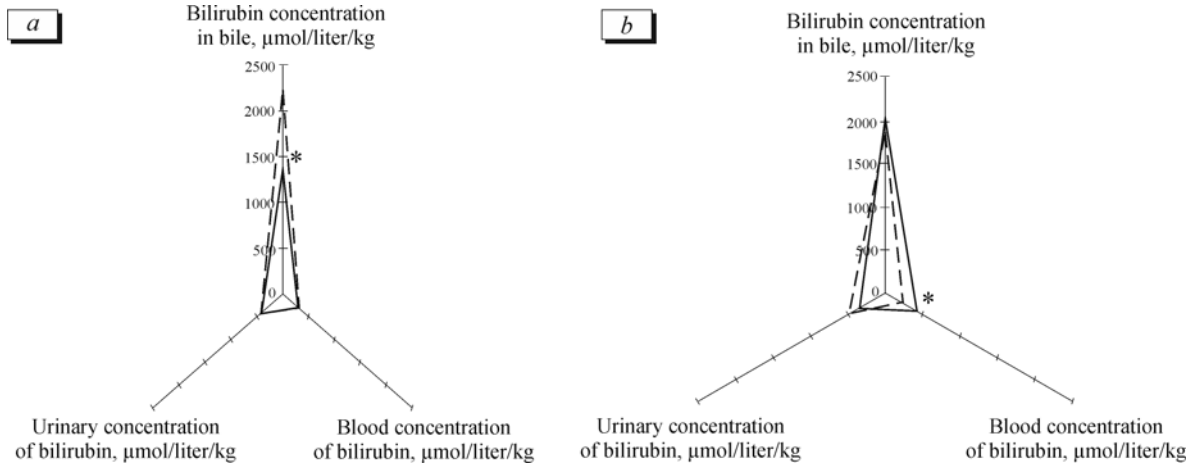

Fig. 2. Gender-related redistribution of bilirubin pools during IPP under conditions of normal (*a*) and high (*b*) prolactin levels.

TABLE 2. Impact of HPRL for Bile Flow Velocity and Bilirubin Excretion with Bile in Female and Male Rats (*M±SEM*)

Group	Bile flow rate, µl/min/kg		Bilirubin excretion rate, nmol/min/kg	
	females	males	females	males
Intact	36.0±1.4 (*n*=43)	35.5±1.2 (*n*=36)	4.0±0.2 (*n*=43)	4.2±0.2 (*n*=36)
Intact+HPRL	31.3±2.6 (*n*=10)	34.6±1.5 (*n*=12)	3.9±0.4 (*n*=10)	4.8±0.4 (*n*=12)
IPP	56.7±3.7 (*n*=12)	75.9±7.8* (*n*=8)	30.5±3.5 (*n*=12)	32.1±5.9 (*n*=8)
IPP+HPRL	44.7±3.4 (*n*=4)	50.1±11.6 (*n*=4)	21.4±5.8 (*n*=4)	30.8±7.2 (*n*=4)

TABLE 3. Impact of HPRL for Blood and Urinary Bilirubin Concentrations in Female and Male Rats (*M*±*SEM*)

Group	Total bilirubin concentration in blood, μmol/liter/kg		% of conjugated bilirubin in blood		Total bilirubin concentration in urine, μmol/liter/kg	
	females	males	females	males	females	males
OC	519.4±58.6 (*n*=13)	455.6±40.2 (*n*=16)	76.4±6.6 (*n*=10)	83.6±4.7 (*n*=12)	603.0±82.0 (*n*=17)	845.2±104.6 (*n*=21)
OC+HPRL	1029.0±140.8 (*n*=6)	515.7±37.4* (*n*=5)	77.1±1.5 (*n*=6)	72.2±2.7* (*n*=5)	738.3±39.5 (*n*=12)	536.2±71.2 (*n*=10)
IPP	296.6±33.7 (*n*=10)	318.1±54.3 (*n*=8)	75.6±5.4 (*n*=8)	64.1±5.1 (*n*=7)	406.4±71.4 (*n*=12)	409.8±81.1 (*n*=7)
IPP+HPRL	240.6±48.7 (*n*=4)	418.4±43.0* (*n*=4)	81.2±8.1 (*n*=4)	67.3±4.8 (*n*=4)	487.4±194.9 (*n*=4)	334.4±77.2 (*n*=4)

no changes in this parameter in males, as a result of which the concentration of this pigment starts to predominate significantly in the blood of males ($p<0.05$; Table 3).

Relieving of OC significantly reduced urinary level of bilirubin in females and males ($p<0.05$). Despite the absence of significant differences in urinary bilirubin concentrations in females and males, gender-specific trends to its changes in hyperprolactinemia were noted in the presence of OC and after its relieving (Table 3; Fig. 2).

Hence, hyperprolactinemia, particularly during the initial period after relieving of cholestasis, is essential for the parameters of the bile excretory function of the liver, leading to disappearance of their gender-related differences (Fig. 1). The gender-specific differences in hyperprolactinemia disappear because of increasing concentration of bilirubin in the postcholestatic bile and more intense bile excretion from the liver in males and because of reduced bile flow rate in females and males. However, hyperprolactinemia leveling the gender-related differences in the bile excretory function of the liver induced the appearance of gender-related alternative pathways of bilirubin excretion in OC during IPP (Fig. 2). The concentration of total bilirubin and percentage of its conjugated form in OC against the background of hyperprolactinemia were significantly higher in the blood of females compared to those in males. During IPP, hyperprolactinemia stimulating bilirubin elimination from the blood in females and not changing this parameter in males leads to inversion of the gender-related differences in the blood bilirubin level. In many cases, the more pronounced effect of persistent hyperprolactinemia on males can be explained by artificial elevation of the concentration of this hormone (simulating stable little fluctuating female pattern of prolactin secretion) in the males, as a result of which the increase of the total hormone level in the males is paralleled by replacement of the pattern of its secretion by the female pattern [3,9].

The detected gender-related effect of prolactin on redistribution of bilirubin pools and bile excretion function of the liver can be related to the involvement of this hormone into the regulation of the expression and exposure on the membrane of transporters of bilirubin and bile acids, such as multiple drug resistance proteins 2 and 3 (MRP2, MRP3), sodium taurocholate cotransporter (NTCP), and some others, whose expression is changed in OC and, according to some findings, is gender-specific [7,11,12].

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REFERENCES

1. N. S. Kushnareva and O. V. Smirnova, *Byull. Eksp. Biol. Med.*, **146**, No. 11, 495-498 (2008).
2. N. S. Kushnareva and O. V. Smirnova, *Ibid.*, **148**, No. 11, 511-514 (2009).
3. V. B. Rozen, G. D. Mataradze, O. V. Smirnova, and A. N. Smirnov, *Gender-Specific Differentiation of Liver Functions* [in Russian], Moscow (1991).
4. D. Alvaro, G. Alpini, P. Onori, *et al.*, *Gastroenterology*, **119**, No. 6, 1681-1691 (2000).
5. R. L. Bogorad, T. Yu. Ostroukhova, A. N. Orlova, *et al.*, *J. Endocrinol.*, **188**, No. 2, 345-354 (2006).
6. R. S. Bridges and P. T. Dunckel, *Biol. Reprod.*, **37**, No. 3, 518-526 (1987).
7. X. Cheng, D. Buckley, and C. D. Klaassen, *Biochem. Pharmacol.*, **74**, No. 11, 1665-1676 (2007).
8. S. Glaser, D. Alvaro, T. Roskams, *et al.*, *Am. J. Physiol. Gastrointest. Liver Physiol.*, **284**, No. 4, G683-G694 (2003).
9. A. Mode and J. A. Gustafsson, *Drug. Metab. Rev.*, **38**, Nos. 1-2, 197-207 (2006).

10. F. Sato, H. Aoki, K. Nakamura, *et al.*, *J. Androl.*, **18**, No. 1, 21-25 (1997).
11. F. R. Simon, M. Iwahashi, L. J. Hu, *et al.*, *Am. J. Physiol. Gastrointest. Liver Physiol.*, **290**, No. 4, G595-G608 (2006).
12. G. Zollner, H. Marschall, M. Wagner, and M. Trauner, *Mol. Pharm.*, **3**, No. 3, 231-251 (2006).