BIOPHYSICS AND BIOCHEMISTRY

Expression of Prolactin Receptors in Human Liver during Cholestasis of Different Etiology and Secondary Liver Cancer

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Indirect immunoperoxidase assay and computer analysis of photographic images revealed more intensive expression of prolactin receptors in hepatocytes of women compared to men. The intensity of expression was maximum in secondary liver cancer, high in obstructive jaundice of different etiology, and less pronounced in cholelithiasis. The expression of prolactin receptors in cholangiocytes was higher than in hepatocytes and was maximum during obstructive jaundice of different etiology. Cells of secondary tumors were characterized by low expression, while distant hepatocytes most intensively expressed prolactin receptors.

Key Words: *prolactin receptor; cholelithiasis; obstructive jaundice; secondary liver cancer; human*

Previous animal experiments showed that the intensity of prolactin receptor (PrlR) expression increases during proliferation of various liver cells in animals with experimental cholestasis [2,6]. Prolactin stimulates proliferation of liver cells [1,4]. Therefore, the increased expression of prolactin receptors under conditions of abnormal proliferation reflects its stimulating effects on this pathological process.

In humans, cholestasis resulting from cholelithiasis and especially pronounced during obstructive jaundice of different etiology is accompanied by apoptosis and proliferation of various liver cells. Secondary tumor processes in the liver are also associated with intensive proliferation of cells, changes in the ratio between various cells, and structural rearrangement of the hepatic lobule [6,8]. Blood prolactin concentration increases in patients with cholestasis [9]. Here we evaluated the intensity of PrlR expression in various liver cells in patients with liver diseases accompanied by proliferation of liver cells.

MATERIALS AND METHODS

We examined tissue samples from patients with postoperation diagnoses of cholelithiasis not accompanied by jaundice (7 women, 34-71 years), obstructive jaundice of different etiology (5 women, 53-77 years; 3 men, 39-64 years), and secondary liver cancer (5 women, 34-64 years; 4 men, 46-56 years). In patients with secondary liver cancer (metastases of colorectal can-

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cer, *n*=5, cancer of the major duodenal papilla, *n*=2, and common bile duct adenocarcinoma, *n*=1), samples of the central and peripheral region of the secondary tumor and distant liver tissue were studied.

Localization of PrlR in tissue samples from pathologically changed human liver was determined by indirect immunoperoxidase method [5] using monoclonal U5 antibodies against rat PrlR cross-reacting with human PrlR [10]. Two experimental and two control sections of each sample (with and without antibodies against PrlR) were assayed. We examined 50 cells in 5 periportal (PP) zones, 50 cells in 5 pericentral (PC) zones, and all ductal cells on sections. The intensity of PrlR expression was estimated semiquantitatively by the difference between photographic images of the experimental and control sections using a PMIS 2.1 image analysis system (gray scale was proportional to the relative concentration of labeled compounds) [1]. The data were expressed in arbitrary units.

The results were analyzed using Statistica 4.5 software. The significance of differences was estimated by nonparametric Mann—Whitney rank *U* test. The number of measurements in PC and PP zones of the hepatic lobule (50 measurements per sample) or total number of measurements in hepatic lobules (100 measurements per sample) was considered for hepatocytes; for cholangiocytes all measurements (20-80 measurements per sample) were considered.

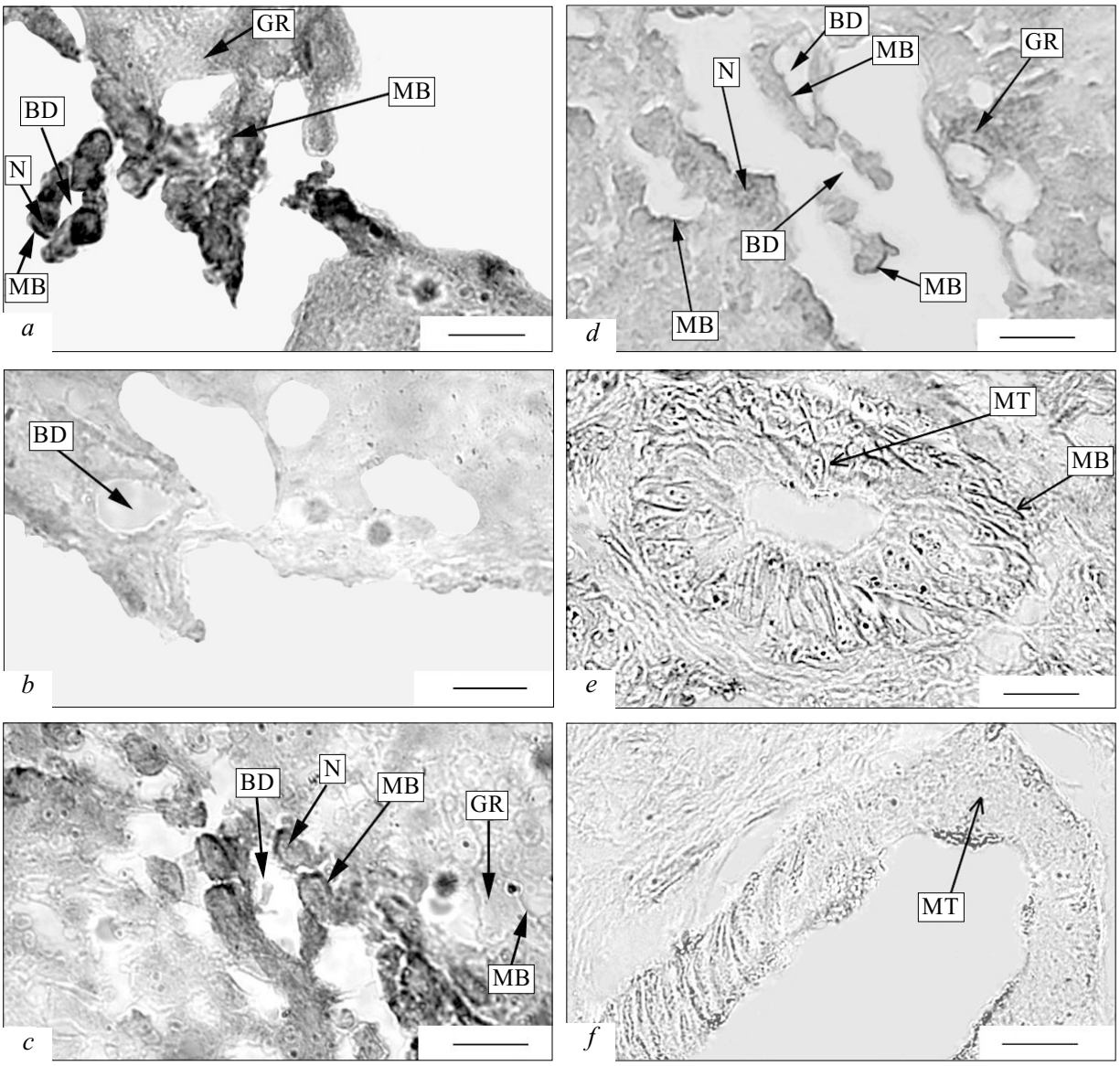

Fig. 1. Immunoperoxidase identification of prolactin receptors in cells of pathologically changed human liver. Cholelithiasis (*a*, *b*), obstructive jaundice (*c*), liver cells in a patient with metastases of colorectal cancer (*d*), and colorectal cancer cells metastasizing into the liver (*e*, *f*). PrlR-positive staining with monoclonal U5 antibodies (*a*, *c*, *d*, *e*); without primary antibodies (*b*, *f*). Arrows: bile duct (BD), metastasis (MT), and specific immunoperoxidase staining of membranes (MB), cytoplasmic granules (GR), and nuclei (N). Scale: 25 μ.

TABLE 1. Expression of Prolactin Receptor in PC and PP Zones of Hepatic Lobule in Patients with Liver Diseases of Different Etiology (*M*±*SEM*)

Disease		Expression, arb. units	
		PC	PP
Cholelithiasis (without jaundice)	women	102.5±4.5	110.6±4.0
Obstructive jaundice	women	246.0±12.4	216.0±7.0
	men	201.9±9.8	186.0±10.1
Metastases into the liver, liver tissue distant from tumor	women	307.0±9.0	300.0±18.0
	men	245.0±13.0	263.0±11.0

RESULTS

In patients with various liver diseases PrlR-positive staining was seen in plasma membranes and cytoplasmic granules of hepatocytes and cholangiocytes and sometimes in their nuclei. Most cells of secondary tumors were characterized by membrane staining (Fig. 1). The subcellular distribution of PrlR was previously revealed in patients with other liver diseases and animals with experimental cholestasis [2,5].

The intensity of PrlR expression in hepatocytes in PC and PP zones of the hepatic lobule was similar in women and men with liver diseases of different etiology (Table 1). This suggests that the sensitivity of hepatocytes to prolactin and other hormones does not depend on their localization in the hepatic lobule [1]. This fact allowed us to combine the results for these zones.

The intensity of PrlR expression in hepatocytes in women with obstructive jaundice of different etiology and secondary liver cancer was higher than in men with these diseases (Table 2). Cholelithiasis is more prevalent in women than in men. Therefore, we examined liver samples from women with cholelithiasis. More intensive expression of PrlR in hepatocytes in women with cholelithiasis is consistent with the results of experiments on female animals. These differences are probably related to the positive effect of estrogens on expression of PrlR [5].

The intensity of PrlR expression in hepatocytes was low in women with cholelithiasis, high during obstructive jaundice of different etiology, and maximum in secondary liver cancer (Fig. 2).

In cholangiocytes the intensity of PrlR expression in women and men with various liver diseases was higher than in hepatocytes (Fig. 2). PrlR expression in ductal cells was most intensive in patients with obstructive jaundice of different etiology (Fig. 2). It should be emphasized that the number of bile ducts per sections was maximum in these patients. This is an indirect evidence of most intensive proliferation of ductal cells.

The intensity of PrlR expression in tumor cells of secondary liver cancer varied, but was lower than in hepatocytes in the same patients. Hepatocytes of patients with secondary liver cancer were characterized by intensive expression of PrlR, which increased from the center of the secondary tumor node toward the periphery (Table 3).

Our results show that in cholelithiasis not accompanied by jaundice (i.e. in mild cholestasis little affecting proliferative activity of liver cells) PrlR expression was relatively low in both hepatocytes and cholangiocytes. In contrast, obstructive cholestasis accompanied by jaundice and inducing proliferation of ductal cells and hepatocytes is characterized by enhanced expression of PrlR in hepatocytes and cholangiocytes. Intensive expression of PrlR in patients with obstructive cholestasis of different etiology indicates that these receptors can serve as a target for pharmacological preparations. Since changes in the

TABLE 2. Sex Differences in Expression of Prolactin Receptors in Hepatocytes from Patients with Liver Diseases of Different Etiology (*M*±*SEM*)

Disease	Expression, arb. units	
	women	men
Obstructive jaundice	227.0±6.4	193.0±7.1*
Metastases into the liver, liver tissue distant from tumor	305.0±8.0	258.0±10.0*

Note. *p<0.001 compared to women.

TABLE 3. Changes in Expression of Prolactin Receptors in Liver Tissue and Tumor Node in Women (*M*±*SEM*)

Liver zone		Expression, arb. units	
		tumor cells	hepatocytes
Metastasis	center	190.2±39.5	222.5±25.0
	periphery	256.0±12.0*	256.0±9.0
Liver tissue distant from tumor		—	305.0±8.0**

Note. *p<0.01 and **p<0.001 compared to the central tumor region.

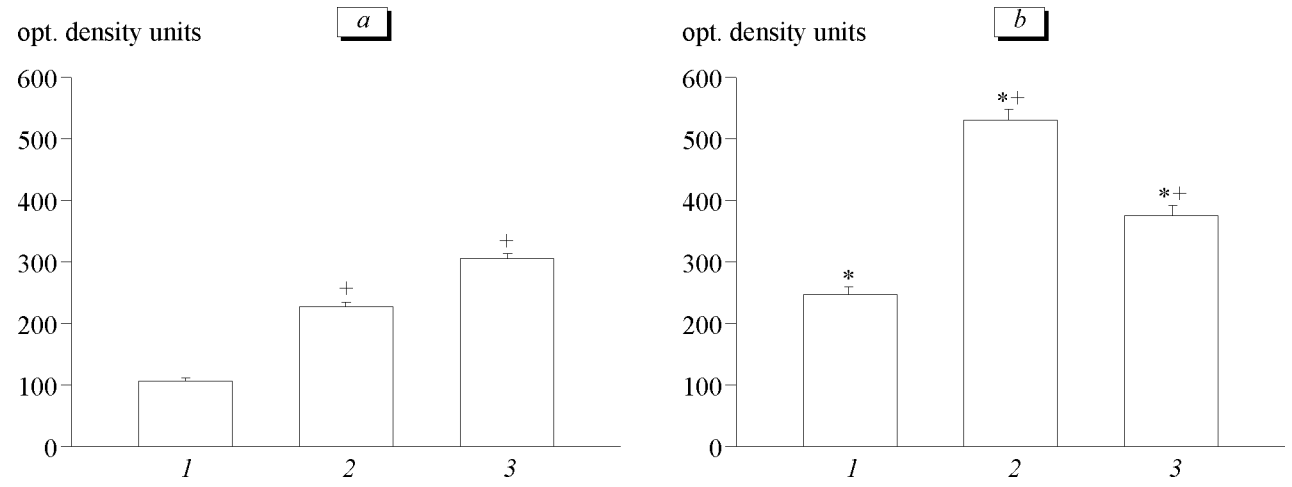

Fig. 2. Prolactin receptor expression in hepatocytes (*a*) and cholangiocytes (*b*) in women with liver diseases of different etiology: cholelithiasis (without jaundice, *1*); obstructive jaundice (*2*); metastases into the liver, liver tissue distant from tumor (*3*). **p*<0.001 compared to expression in hepatocytes. +*p*<0.001 compared to cholelithiasis.

intensity of PrlR expression in liver cells are similar in patients with obstructive cholestasis and animals after common bile duct ligation, this animal model can be used for studying pharmacological effect of drugs modulating secretion and action of prolactin on various liver cells during this pathology.

The intensity of PrlR expression was relatively low in secondary tumor cells, which indicates that prolactin plays little role in progression of liver metastases. However, it should be emphasized that the presence of secondary liver tumors considerably stimulates PrlR expression in hepatocytes not contacting with tumor cells and, to a lesser extent, in cholangiocytes. This can be explained by increased plasma level of estrogens acting as positive regulators of PrlR [8]. Our results indicate that the effects of tamoxifen, a competitive estrogen receptor antagonist used for chemotherapy of liver tumors [9], are related not only to blockade of these receptors, but also to inhibition of estrogen-induced increase in PrlR expression in hepatocytes.

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