BIOCHEMISTRY AND BIOPHYSICS

Expression of an Unusual Estrogen-Binding Protein in the Liver of Androgenized Adult Female Rats During Liver Regeneration after Carbon Tetrachloride Poisoning

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 7, pp. 28-31, July, 1996
Original article submitted April 6, 1995

The expression of an unusual estrogen-binding protein induced in the liver of ovariectomized adult rats by androgen was greatly reduced after their pericentrally located hepatocytes were poisoned with carbon tetrachloride, but was partially restored in the course of subsequent liver regeneration. It is suggested that the androgen program established in the periportal hepatocytes of postpubertal animals is defective.

Key Words: *androgens; imprinting; liver regeneration; unusual estrogen-binding protein; immunohistochemistry*

It has been established that many liver functions are differentiated by sex, which constitutes a basis for the coupling of reproductive and adaptive processes [2]. The endocrine mechanisms by which sex differences are produced in the hepatocyte phenotype are complex and varied, involving direct effects of sex hormones on liver cells; indirect actions of sex hormones mediated by pituitary hormones (growth hormone and prolactin); combined effects of hormones from both groups; and permissive and sensitizing influences of these hormones on the activity of other hormonal factors [2,5,9,10]. The direct and indirect actions of sex hormones on liver cells are manifested in the forms of reversible and irreversible (programming) influences [2,14].

Studies into the mechanisms controlling the formation of an androgenic program for the expression of the unusual estrogen-binding protein (UEBP) specific to the liver in male rats showed that the natural program established in the early postnatal ontogeny under the action of testicular androgens differs by the level of expression of the induced male phenotype from the artificial program elicited by androgens administered to adult females [1,3,13,15].

In the present study we made an attempt to find out whether or not the natural and artificial androgenic programs differ with regard to the distribution, within the hepatic lobule, of hepatocytes carrying the androgenic program. To this end, we utilized the ability of carbon tetrachloride (CCl_4) to selectively destroy hepatocytes located in the pericentral and intermediate zones of the hepatic lobule [8] and expressing UEBP to a much greater extent than do the periportally located hepatocytes [12]. The subsequent proliferation of surviving hepatocytes during liver regeneration in males with the natural androgenic program results in complete restoration of the male phenotype — an indication that the intralobular distribution of program-carrying hepatocytes is uniform [12]. As will be evident from the results of this study, the proliferation of periportally situated

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0007-4888/96/0007-0674$15.00

hepatocytes in androgen-pretreated adult female rats did not lead to complete recovery of the original phenotype of UEBP expression. The defectiveness of the artificial androgenic program in cells of the periportal lobular zone may be due either to insufficient sensitivity of these cells to androgens or to incomplete transfer of the artificial program to their progeny.

MATERIALS AND METHODS

The study was conducted on ovariectomized randombred male rats (body weight 170-200 g), divided into eight groups, as shown in Table 1. Testosterone propionate (TP) was administered once daily in a dose of 3 mg in 0.4 ml of propylene glycol on days 12-14 after ovariectomy. CCl_4 was injected intraperitoneally in a single dose of 5 ml/kg body weight (as a 16% solution in castor oil) on day 17 after ovariectomy.

UEBP concentrations were estimated quantitatively in the cytosol of hepatic lobules by a modified radioligand method [15] in which [2,4,6,7-^{3}H]-estriol with a specific radioactivity of 101 Ci/mmol (Izotop, Russia) is used instead of estradiol as the radioligand. This estriol binds to UEBP with a higher affinity than estradiol (170%) [11] but practically does not bind to α-fetoprotein (4%) [4], which excluded the bias in measurements due to H-ligand binding to the α-fetoprotein expressed in the regenerating liver [7]. Concentrations were measured in picomoles of the ligand-binding UEBP areas per mg cytosolic protein and expressed in percent of their values in the respective control groups taken as 100%, as shown in Table 1. Protein content was measured by Bradford's method [6].

UEBP distribution in the cells of liver sections was analyzed histochemically by means of an indirect immunoperoxidase reaction [12] using rabbit polyclonal antibodies isolated from antiserum by immunoadsorption on an immobilized highly purified UEBP in a concentration of 7.2 μg/ml. As second-stage antibodies, donkey antibodies to rabbit immunoglobulins (from the Gamaleya Institute of Epidemiology and Microbiology, Moscow) were used, after which the sections were treated with a rabbit PAP complex (Arnel). The color reaction was developed by treatment with a 0.05% diaminobenzidine solution in the presence of 0.06% H_2O_2 and the staining intensity was enhanced by OsO_4. To verify specificity of the reaction, the anti-UEBP antibodies were replaced by the immunoglobulin fraction of nonimmune rabbit serum.

RESULTS

Extensive zones of necrosis located around central hepatic lobular veins and covering 40% to 80% of the total parenchymal area were observed 24 h after CCl_4 poisoning. As shown in Table 1, UEBP concentrations in the TP-untreated and TP-treated groups had fallen by that time to 15% (group 2) and 17% (group 4) of their control values (groups 1 and 3). On day 22 after CCl_4 poisoning, the lobular structure was completely restored owing to the proliferation of periportal hepatocytes and their migration to the central lobular region. UEBP levels in the group given no TP were also restored by that time (group 6 in the Table 1) and did not differ significantly from the control values (group 5 in the Table 1) because of the return to normal of hepatocyte numbers and functions, in particular as regards the basal program of UEBP expression. The immunohistochemical analysis of UEBP distributions in the latter two groups showed a weak specific staining only in hepatocyte layers adjacent to the central veins (Fig. 1, *a* and *b*), which corresponded to a very low UEBP level (0.2 pmol/mg cytosolic protein).

In contrast, UEBP levels in the TP-treated group were on day 22 significantly lower (group 8 in the Table 1) than the respective control values (group 7). The specific staining in liver sections from both groups has a typical graded pattern, being the most intensive around the central veins and extending in

TABLE 1. Effect of CCl_4 Poisoning on the Liver Content of Unusual Estrogen-Binding Protein (UEBP) in Ovariectomized Adult Rats (*M±m*)

Group	TP dosage	Days after CCl_4 injection	UEBP, % of control value		
1	-	0	100±36	(5)	$p<0.02$
2	-	1	15±6	(7)	
3	3 doses of 3 mg	0	100±39	(10)	$p<0.05$
4	-"-	1	17±8	(14)	
5	-	0	100±23	(28)	$p>0.1$
6	-	21	263±147	(20)	
7	3 doses of 3 mg	0	100±19	(24)	$p<0.02$
8	-"-	21	48±8	(24)	

Note. Figures in parentheses are the number of rats.

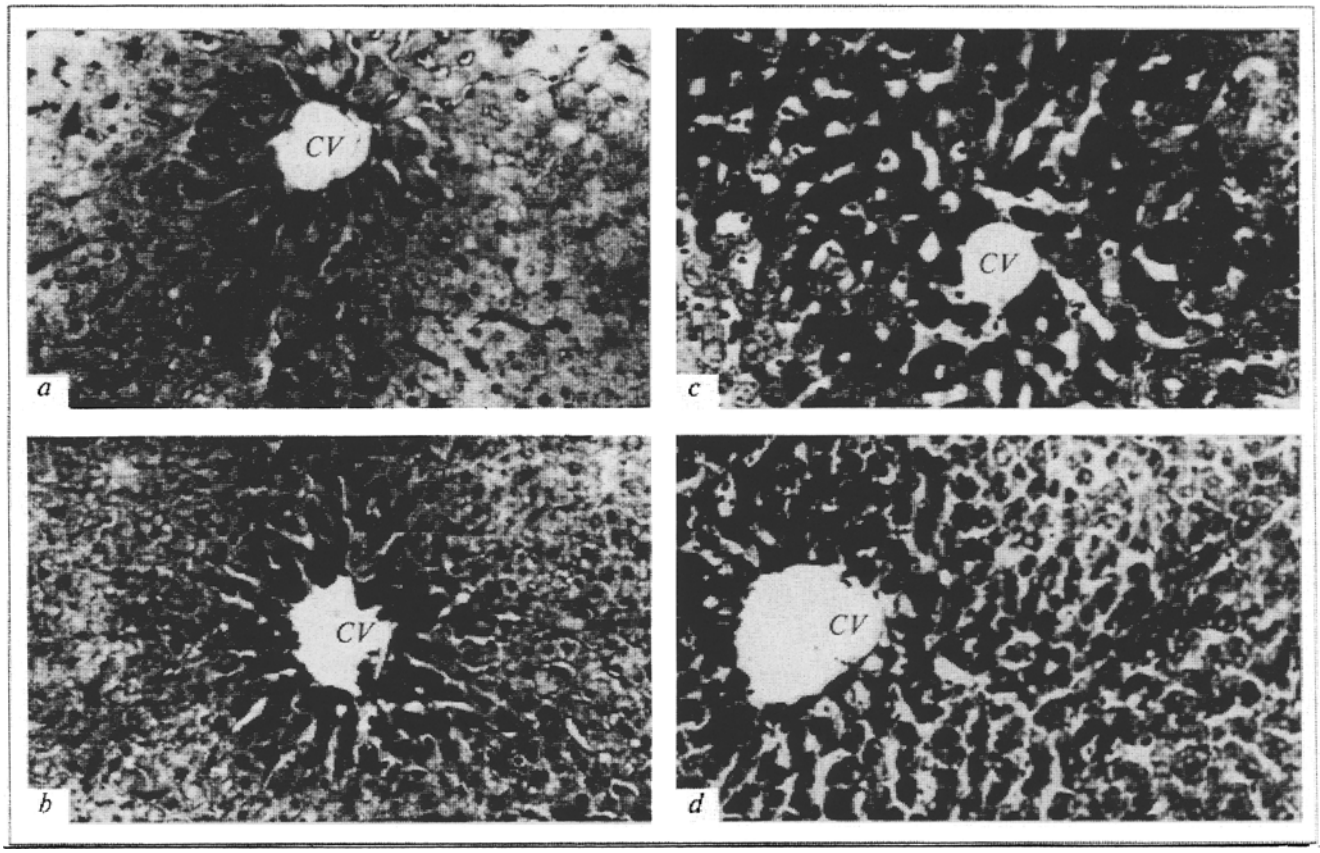

Fig. 1. Immunoperoxidase staining of UEBP in hepatocytes from regenerating livers of ovariectomized rats on day 22 after CCl_4 poisoning. *a*) rats before CCl_4 poisoning; *b*) CCl_4-poisoned rats; *c*) testosteone propionate(TP)-treated rats; *d*) TP-treated rats poisoned with CCl_4. × 160. *CV* = central vein.

some cases into the interlobular region (Fig 1, *c* and *d*). Such staining reflected fairly high UEBP concentrations in the androgenized females — 2.9 pmol/mg protein in the control group (group 7). On day 22 after ovariectomy, UEBP concentrations in the androgenized females of group 8 were much higher in absolute terms than in the females not given TP (group 6). This implies that the periportal hepatocytes of females carry an androgenic program and pass it on to the new cell progeny. However, as UEBP synthesis failed to reach the control values, this program should be regarded as defective in that it either fails to be firmly established in periportal hepatocytes or is inadequately reproduced by daughter cells. Such a defect in the androgenic program appears to be peculiar to periportal hepatocyes since partially hepatectomized androgenized females were found to show a completele restoration of UEBP expression during liver regeneration when all lobular hepatocytes proliferated [12].

The postpuberal artificial androgenic program of UEBP expression thus differs from the neonatal natural program not only in the level of male phenotype expression but also in the intralobular distribution of cells carrying the program.

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