Effect of Fetal Tissue Transplantation on Reparative Processes in Experimental Liver Cirrhosis

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Reparative regeneration after fetal tissue transplantation and after surgical stimulation was studied in rats with experimental cirrhosis of the liver. Fetal tissue restored the morphology and function of cirrhotic liver and modified functional activity of peritoneal macrophages.

Key Words: *liver cirrhosis; fetal tissue transplantation; reparative regeneration*

Chronic hepatitis and liver cirrhosis are the most prevalent diseases of the liver. They are characterized by decreased count of functioning cells, pronounced fibrosing reaction, restructuring of the parenchyma and vascular network [3,4]. Therapy of these diseases is unsatisfactory because of the absence of effective drugs stimulating liver regeneration. Impaired reparative regeneration (RR) is a specific prerequisite for chronic inflammation in any disease [8], while stimulation of regeneratory activity is an obligatory component of repair. It is now proven that surgical stimulation of regeneration in some forms of chronic hepatitis and cirrhosis of the liver (CL) leads to stabilization of the process. Chronic stimulation of regeneration has not been widely used for certain reasons. An alternative therapeutic approach to the treatment of chronic hepatitis and CL is fetal tissue transplantation. Young cells containing embryospecific growth factors, cytokines, and other signal molecules activate regeneration and improve cell survival in the recipient [2].

We investigated the possibility of stimulating RR of cirrhotic liver by transplantation of allogenic fetal liver tissue.

MATERIALS AND METHODS

Experiments were carried out on 60 adult random-bred male albino rats weighing 200 g kept under standard vivarium conditions at 18-20°C on mixed nutrition with free access to water. Before the experiment the animals were allowed a 2.5-month acclimatization. Activity of reparative processes was assessed with consideration for liver morphology and function and functional activity of peritoneal macrophages (FAPM) as an important parameter of cell-mediated immunity.

Micropreparations for histological analysis were prepared routinely. The severity of degenerative, necrotic, inflammatory, sclerotic, and regeneratory processes in the liver was evaluated by the semiquantitative method and scored by a 4-point scale. The following 10 signs were taken into account: fatty degeneration, glycogen content, hepatocyte necrosis, hypertrophy and hyperplasia of hepatocyte nuclei, proliferation of biliary ducts and stellate reticulocytes, inflammatory infiltration, fibrosis, and the number of false lobules.

In order to evaluate cholestasis and cytolysis, serum bilirubin level and aminotransferase activities were measured routinely.

FAPM was assessed using 1.7-μ latex microspheres; phagocytosis activity and intensity, phagocytic number, and lysosomal activity were evaluated. Production of active oxygen forms by peritoneal macrophages was evaluated in spontaneous and stimulated nitroblue tetrazolium test, as the response to a standard stimulator helps to evaluate the potential cell resources [5].

Baseline values were measured in 10 rats at the beginning of the experiment. In 50 animals CL was induced by subcutaneous injections of 50% oil solu-

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tion of CCl_4 in a dose of 0.4 ml/100 g for 90 days. Eighteen (36%) rats died during this period.

All experimental animals were divided into 5 groups: 1) intact controls; 2) animals with initial CL (examined immediately after the last injection of CCl_4); 3) CL without stimulation of RR (evolution of cirrhotic changes after injections of CCl_4); 4) animals with CL after transplantation of liver tissue from 20-day rat embryos (morphologically corresponding to the liver of 5-month human fetus) for stimulation of RR [1]; and 5) animals with CL subjected to surgical stimulation of RR (resection of 8-10% liver under ether narcosis through laparotomic access, [7]). Allofetotransplant ($3\text{-}5\times10^5$ nuclear cells) was prepared by homogenization of liver fragments and was transplanted by intraperitoneal injection.

Activity of reparative processes in groups 3, 4, and 5 was evaluated 1 month after CCl_4 poisoning and surgical stimulation of RR.

The results were statistically processed using Student's *t* test.

RESULTS

Treatment with CCl_4 induced toxic CL with characteristic manifestations: decreased motor activity and body weight, partial loss of hair, *etc.* Autopsy showed ascites in the abdominal cavity (2-5 ml light opalescent exudation), enlarged liver with small tubercles (regeneration nodes) and rounded edges. Plethoric foci were seen under the capsule. Small (0.1-0.5 mm) lobules, yellowish-brown tissue with small plethoric foci were seen on the surface of the section. Histological examination showed a typical picture of cirrhotic transformation of the liver, histological structure was impaired by fibrosis and nodular rearrangement. Hepatocytes were in a state of pronounced small- and large-droplet fatty degeneration. Glycogen content markedly decreased. Hepatocyte necrosis manifested by clusters of 2-3 anuclear cells with degenerative homogenous cytoplasm. Inflammatory infiltration of the portal tracts and parenchyma consisted of lymphocytes and solitary plasma cells and polymorphonuclear neutrophils. Sclerotic changes (fibrosis) manifested by newly formed collagen fibers along the portal tracts and the formation of porto-portal and portocentral septae with false lobules. This morphological picture corresponded to the initial stage of monobular CL.

Cirrhotic changes in the liver were accompanied by shifts in blood biochemical parameters: hyperbilirubinemia and hypertransferasemia (high alanine aminotransferase activity), which was due to the involvement of parenchymatous cells and hepatocytes (Table 1).

FAPM was changed in rats with CL (Table 2): phagocytic intensity and activity and NBT test decreased, while the phagocytic number, functional reserve, and lysosomal activity slightly increased. This resulted from hepatocyte necrosis caused by direct damaging effect of the hepatotoxic agent and cell autoimmune reaction (secondary immune reaction) directed at the neutralization of the toxic agent located inside hepatocytes or on their membrane [3,5].

Hence, 3-month poisoning with CCl_4 induced monobular CL associated with hyperbilirubinemia and impaired macrophagal activity.

The intensity of morphological manifestations of regression of cirrhotic transformation of the liver was different in groups 3 and 5 (Table 3). Comparative histological analysis in groups 4 and 5 (RR stimulation) showed more pronounced intracellular and cellular hepatocyte regeneration in comparison with group 3 (control). There were many hepatocytes with 2-3-fold enlarged nuclei containing coarsely dispersed chromatin and 1-3 nucleoli. This was paralleled by pronounced proliferation of stellate reticulocytes. Solitary cells and groups of 3-5, sometimes 8-10 cells were seen. Regeneration of hepatic tissue was most pronounced in group 4: degenerative, inflammatory, necrotic, and sclerotic changes were less pronounced, glycogen content in hepatocyte cytoplasm was increased, and marked nuclear hyperplasia was seen in this group. Bi- and polynuclear hepatocyte clusters containing 5-8 cells were more often seen in this group.

TABLE 1. Biochemical Parameters of Liver Function ($M\pm m$, n=7-10)

Parameter	Healthy	CL			
		initial	after 1 month (control)	+transplantation	+resection
Bilirubin, μmol/liter	11.83±0.65	27.14±2.08+	15.19±1.90*	13.70±1.35*	13.58±4.94*
ALT, μmol/liter/h	1.10±0.22	4.32±1.17+	3.05±0.67	2.40±0.46*	1.85±0.29*
AST, μmol/liter/h	0.73±0.15	1.80±0.98+	1.49±0.30*	1.14±0.22*	0.99±0.06*

Note. Here and in Tables 2 and 3 $p<0.05$: *vs.* initial values, +*vs.* healthy animals.

TABLE 2. Parameters of FAPM in Animals with Experimental CL after RR Stimulation (*M±m*)

Parameter		Healthy	CL			
			initial	after 1 month (control)	+transplantation	+resection
Number of macrophages		76.25±3.79	66.00±6.77	77.75±9.37	74.20±8.81	72.67±13.38
Lysosomal activity, %		43.5±3.3	44.50±7.76	67±1*	33.20±7.47	27.33±9.96
Phagocytosis activity, %		42.83±2.90	21.00±3.00*	48.25±7.71*	36.2±9.2	33.33±3.53*
Phagocytosis intensity		0.92±0.11	0.76±0.29	1.86±0.45*	0.85±0.20	0.99±0.41
Phagocytic number		2.12±0.13	2.36±0.16	3.70±0.42*	2.61±0.61	2.82±0.87
NBT test						
spontaneous	%	56.17±2.74	30.50±6.40*	55.5±8.5*	65.33±10.91*	65.33±10.72*
	C	0.79±0.03	0.38±0.09*	0.73±0.12*	1.01±0.23*	1.12±0.27*
induced	%	76.00±3.86	67.50±7.93	78.00±8.00	71.40±8.58	76.67±8.51
	C	1.08±0.05	0.97±0.12	1.07±0.23	1.15±0.20	1.06±0.08
Functional reserve						
	%	1.35±0.07	2.15±0.15*	1.29±0.13*	1.40±0.28*	1.22±0.21*
	C	1.39±0.07	1.83±0.23	1.45±0.24	1.32±0.23	1.09±0.32

Note. C: macrophage activity coefficient.

TABLE 3. Degree of Histological Changes (in Scored) (*M±m*, *n*=8-9)

Histological sign	LC			
	initial	after 1 month (control)	+transplantation	+resection
Fatty degeneration	3.4±0.3	1.00±0.67*	2.2±0.7*	1.67±0.33*
Glycogen content	1.2±0.2	1.50±0.07	2.4±0.8*	1.33±0.33
Hepatocyte necrosis	1.8±0.2	0.75±0.12*	1.2±0.2*	1.33±0.33
Hepatocyte hypertrophy	1.8±0.2	2.00±0.67*	3.2±0.7*	3.33±0.33*
Hepatocyte hyperplasia	1.6±0.3	1.50±0.33*	2.4±0.3*	2.00±0.07*
Proliferation of biliary ducts	2.4±0.8	1.75±0.58	1.6±0.3	2.00±0.15
Proliferation of stellate reticulocytes	1.8±0.2	1.75±0.25*	2.6±0.3*	3.00±0.06*
Cell infiltration	1.8±0.7	1.5±0.6	1.6±0.3	2.00±0.12*
Fibrosis	3.0±0.5	3.00±0.67	2.2±0.2*	3.0±0.2*
Number of false lobules	3.00±0.67	2.50±0.91	1.8±0.7	2.67±0.33

Serum biochemical parameters more rapidly returned to normal after RR stimulation (groups 4 and 5), surgical stimulation being more effective (Table 1).

Macrophage activity disordered in CL more rapidly normalized in group 4 (Table 2). Increased lysosomal activity and phagocytosis in the control group in comparison with group 1 (intact healthy animals) indicated autoimmune process (delayed hypersensitization reaction).

Therefore, transplantation of allogenic fetal liver tissue to animals with experimental CL stimulated RR and promoted recovery of liver morphology and function. The intensity of morphological manifestations of hepatic tissue after fetal tissue transplantation was higher than after surgical stimulation of RR, which indirectly indicated its more potent stimulatory effect on RR of the liver. The efficiency of RR stimulation was confirmed by normalization of serum biochemical parameters in animals with CL. FAPM disorders more rapidly regressed after RR stimulation, which attested to a direct relationship between the processes in the liver with this basic parameter of cell-mediated immunity.

Positive results of our experiments prompted us to use fetal tissue transplantation in the treatment of 21 patients with chronic hepatitis and initial forms of

CL. The patients were observed for 1.5 years, and the results proved the efficiency of this method.

Our findings suggest that transplantation of allogenic fetal liver tissues in experimental CL promoted activation of RR processes (restoration of liver parenchyma and function, normalization of FAPM). This opens new vistas in the search for new methods of the treatment for chronic hepatitis and LC.

REFERENCES

1. V. K. Verin, *Histogenesis and Reactivity of the Liver in the Ontogeny*, Abstract of Doct. Med. Sci. Dissertation, Leningrad (1973).
2. V. I. Kulakov, G. T. Sukhikh, and E. M. Molnar, *Transplantation of Human Fetal Tissues and Cells* [in Russian], Moscow (1996), pp. 5-9.
3. A. S. Loginov and Yu. S. Blok, *Chronic Hepatitis and Cirrhosis of the Liver* [in Russian], Moscow (1987).
4. D. K. L'vov, *Ros. Zhurn. Gastroenterol. Gepatol.*, **5,** No. 1, 4-6 (1995).
5. A. N. Mayanskii and D. N. Mayanskii, *Essays on the Neutrophil and Macrophage* [in Russian], Novosibirsk (1983).
6. V. G. Perederii, I. L. Klyarinskaya. S. M. Tkach, *et al., Gastroenterologiya,* No. 22, 82-85 (1990).
7. S. A. Pyshkin, *Possibility of Surgical Treatment of Chronic Hepatitis and Cirrhosis of the Liver,* Abstract of Doct. Med. Sci. Dissertation, Chelyabinsk (1988).
8. V. V. Serov and A. B. Shekhter, *Connective Tissue* [in Russian], Moscow (1981).