BIOPHYSICS AND BIOCHEMISTRY

Effect of Transplantation of Human Fetal Tissues on Prooxidant-Antioxidant Equilibrium in the Liver and Blood Rats after Partial Hepatectomy in Rats

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We studied the effect of transplantation of fetal liver cells and postnuclear cytoplasmic fraction from human fetal soft tissues on the prooxidant-antioxidant equilibrium in the liver and blood of rats after partial hepatectomy. The preparations increased antioxidant activity and decreased the intensity of lipid peroxidation, which probably contributes to their therapeutic effects.

Key Words: *partial hepatectomy; antioxidant activity; lipid peroxidation; fetal tissue preparations*

The therapeutic effect of transplantation of preparations from fetal tissues is usually attributed to the influence of specific growth factors, cytokines, and other signal molecules potentiating cell regeneration and survival [6]. However, the mechanisms underlying therapeutic properties of preparations from fetal tissues remain unknown. Some cytokines and growth factors modulate expression of genes of antioxidant enzymes in hepatocytes [12]. Lipid peroxidation (LPO) plays an important role in the pathogenesis of various diseases. It can be assumed that the therapeutic effect of preparations from fetal tissues is related to normalization of the prooxidant-antioxidant equilibrium in the body.

Here we studied the effects of fetal liver cells (FLC) and postnuclear cytoplasmic fraction (PCF) from human fetal tissues on the prooxidant-antioxidant equilibrium in the liver and blood of rats after partial hepatectomy (PHE). PHE is followed by initiation of recovery processes [7] and considerable changes in antioxidant activity (AOA) and LPO intensity in liver membranes [1].

MATERIALS AND METHODS

FLC were isolated from human fetuses at 8-12 weeks' gestation [5] and cryopreserved as described previously [13]. PCF was obtained from homogenates of human fetal soft tissues in Hanks solution (1:2 tissue-medium ratio) by centrifugation at 2500*g* for 40 min and frozen at -35°C. Before the experiment FLC and PCF were heated in a water bath at 37°C.

Experiments were performed on male Wistar rats weighing 150-190 g. PHE was performed as described elsewhere [11]. FLC (10^7 cells/0.3 ml) or PCF containing 0.75 mg protein (0.3 ml) were inoculated into the spleen pulp during surgery. Control animals received 0.3 ml cryopreserved medium. The rats were decapitated 24, 48, and 72 h after PHE. The blood from the decapitation wound was collected and the

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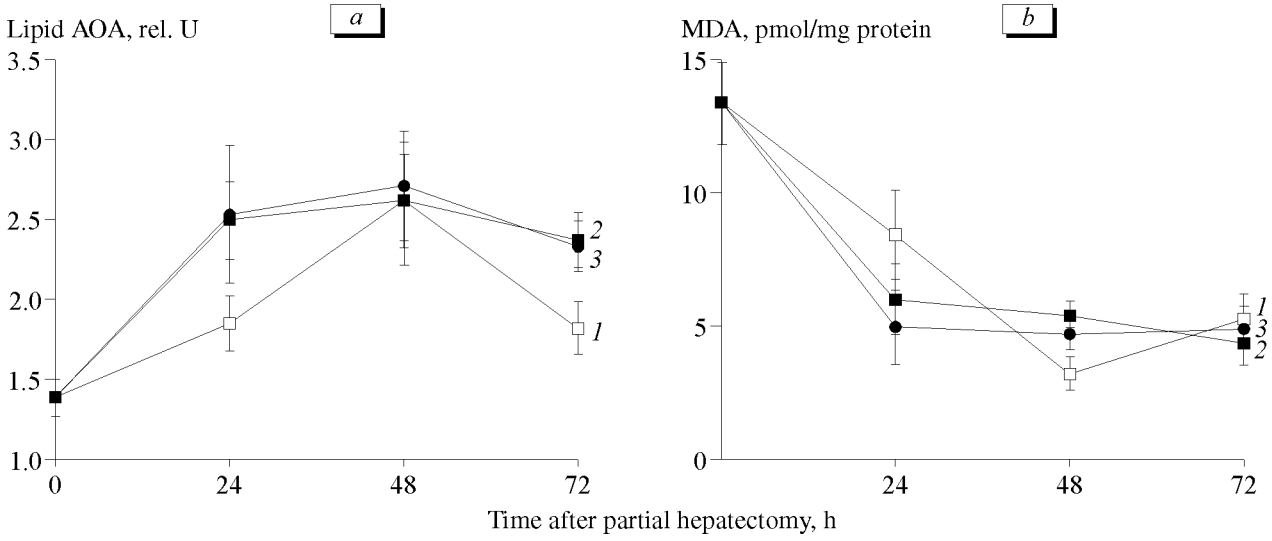

Fig. 1. Effect of transplantation of preparations from human fetal tissues on lipid antioxidant activity (AOA, *a*) and content of TBARS in rat liver (*b*) after partial hepatectomy. Here and in Fig. 2: control (*1*), fetal liver cells (*2*), and postnuclear cytoplasmic fraction (*3*). Zero point on abscissa: normal.

plasma was isolated. The liver was rapidly removed, cooled, mixed with 100 mM Tris-HCl (pH 7.4, 1:3 tissue-buffer ratio), and homogenized.

AOA of the plasma was estimated by the inhibition of accumulation of thiobarbituric acid-reactive substance (TBARS) in yolk lipoprotein suspension [3]. AOA of liver lipids isolated from homogenates by the method of Folch was studied in a model system with linetol [4] and calculated by the ratio of optical densities of standard and test samples (5 mg total lipids per 1 ml linetol). The content of TBARS in the plasma and liver homogenates was measured as described elsewhere [8.9]. The intensity of spontaneous LPO in rat liver homogenates was estimated by the rate of malonic dialdehyde (MDA) accumulation [2]. Total lipid content was measured by the method of Gribanov. Protein content was estimated by the method of Lowry with modifications proposed by Miller. The results were analyzed by Student's *t* test.

RESULTS

AOA of liver lipids increased after PHE, this increase was most pronounced 48 h after surgery (Fig. 1). Seventy-two hours after surgery this parameter decreased but remained above the control. The content of TBARS underwent opposite changes: the content of LPO products 4.2-fold decreased 48 h after PHE, but increased 72 h after surgery (Fig. 1). The intensity of spontaneous LPO changed similarly (Table 1). These changes in test parameters are consistent with published data [1,10,14]. Plasma AOA increased 24 h after PHE (Fig. 2). Further changes in AOA in rat plasma differed from those in the liver. The content of LPO products markedly decreased 24-48 h after PHE, but then returned to normal (Fig. 2). Thus, in hepatectomized rats AOA and the content of LPO products in the plasma changed earlier than in the liver. Suppression of LPO was probably related not only to activation of the antioxidant systems, but also to exhaustion of oxidation substrates. However, in our experiments the concentration of total lipids did not decrease, but even increased (Table 1). Previous studied showed that the content of total lipids and their individual fractions in the liver and plasma increases in animals subjected to PHE [1].

In animals receiving FLC the increase in liver lipid AOA was more pronounced 24 h after PHE. This parameter remained unchanged throughout the observation period (Fig. 1). PCF produced similar changes in AOA. The content of LPO products in animals

TABLE 1. Effects of PHE and Preparations from Fetal Tissues on the Intensity of Spontaneous LPO (nmol MDA/mg Protein, 30-min Incubation) and Content of Total Lipids (mg/g Tissue) in Rat Liver (*M*±*m*, *n*=5-8)

Time after PHE, h		Control	FLC	PCF
24	LPO	1.31±0.14	1.44±0.26	1.59±0.15
	total lipids	45.5±5.0^{+}	86.8±16.5^{*+}	96.9±19.0^{*+}
48	LPO	0.97±0.23^{+}	1.10±0.18^{+}	1.08±0.16^{+}
	total lipids	69.0±4.8^{+}	59.7±9.8^{+}	52.3±6.7^{+}
72	LPO	1.30±0.11^{+}	0.82±0.17^{*+}	1.12±0.11*
	total lipids	48.0±3.5^{+}	48.4±5.0^{+}	46.8±3.6^{+}

Note. $p<0.05$: *compared to the control; $^{+}$compared to normal (1.75±0.15 nmol/MDA/mg protein and 34.1±2.7 mg lipids/g tissue).

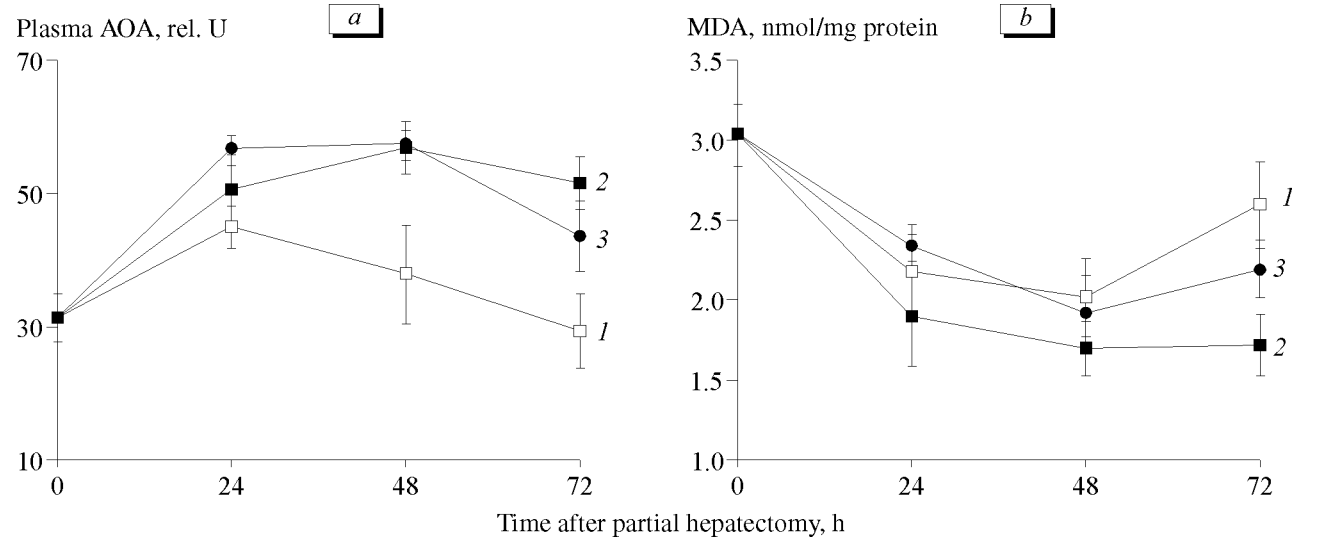

Fig. 2. Effect of transplantation of preparations from human fetal tissues on antioxidant activity (AOA, *a*) and content of TBARS in rat plasma (*b*) after partial hepatectomy.

treated with FLC and PCF decreased by 1.4 and 1.7 times, respectively, 24 h after PHE and remained low 48 and 72 h after surgery. The preparations had no effect on the intensity of spontaneous LPO in liver homogenates 24 and 48 h after PHE, while 72 h after surgery LPO intensity in liver homogenates from animals receiving FLC was much lower than in the control. Twenty-four hours after PHE plasma AOA in rats receiving FLC and PCF increased more significantly than in the control (Fig. 2). Plasma AOA in control animals decreased 48 h after PHE, while in rats receiving FLC and PCF this parameter remained high 48 and 72 h after the surgery. In all rats the content of LPO products in the plasma decreased 48 h after PHE. Seventy-two hours after surgery the content of TBARS returned to normal in control animals, but was 1.8- and 1.4-fold lower in rats receiving FLC and PCF, respectively. FLC and PCF produced similar effects on test parameters. However, FLC was more potent in modifying the intensity of spontaneous LPO in liver homogenates and the content of LPO products in the plasma.

Previous studies showed that lipid antioxidants play an important role in cell proliferation. The increase in lipid content after hepatectomy reflects the formation of high-energy materials and intensification of anabolic processes [1]. In our experiments FLC and PCF increased the concentration of total lipids by 1.9 and 2.1 times, respectively, 24 h after PHE (Table 1).

The rate of cell division during regeneration positively correlates with AOA and is inversely related to the content of free radicals [1]. Our findings suggest that FLC and PCF increase AOA and inhibit LPO after PHE, which probably contributes to their therapeutic effects. Further studies of the effects of these preparations on antioxidant systems, and in particular, on antioxidant enzymes, are required for elucidation of the molecular mechanisms of changes induced by fetal tissue preparations.

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