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Hepatoprotective Effect of Fetal Tissue Cytosol and Its Thermostable Fraction in Rats with Carbon Tetrachloride-Induced Hepatitis

D. V. Cherkashina and A. Yu. Petrenko

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Pretreatment of rats with cytosol of fetal tissue or its thermostable fraction prevented death of animals from CCl_4 intoxication, decreased serum transaminase activities and level of TBA-reactive products, and normalized the prooxidant/antioxidant balance in the liver. The effect of cytosol was more pronounced than that of its thermostable fraction.

Key Words: CCl_4*-induced hepatitis; liver injury; prooxidant/antioxidant balance; fetal tissue cytosol*

The corrective potential of transplanted stem and progenitor cells can be determined by not only their organ-replacing function, but also by production of bioactive stage-specific compounds regulating metabolic processes, specifically, stimulating regeneration and repair of damaged tissues.

We previously showed that pretreatment of animals with human fetal liver cells or with fetal tissue cytosol (FTC) stimulated liver regeneration after 70% hepatectomy and reduced the severity of CCl_4-induced damage by correction of the prooxidant/antioxidant balance in the organ [3,4]. Cell-free FTC exhibited an effect similar to that of cell transplantation, but short-term and less pronounced, which could be due to degradation of its components. Shortening of the period between its injection and initiation of toxic hepatitis to 4 h prolonged the hepatoprotective effect of pretreatment [4].

In order to detect bioactive fetus-specific factors in FTC responsible for realization of the protective and metabolic effects we selected the method of its thermal denaturing [7], due to which the effect of a wide spectrum of thermolabile proteins could be ruled out. In addition, this method is used for partial purification of the thermostable hepatocyte growth factor, stimulating liver regeneration [1] and present in fetal tissues [6].

We compared the effects of total FTC and its thermostable fraction (TSF) on the dynamics of CCl_4-induced hepatitis and prooxidant/antioxidant balance in damaged organ.

MATERIALS AND METHODS

Experiments were carried out on 28 random-bred male albino rats (150-200 g) kept under standard vivarium conditions. Experiments on animals were carried out in accordance with the regulations of "The European Convention for Protection of Vertebrates Used in Experimental and Other Research Purposes". All manipulations were carried out under light ether narcosis.

Human embryos of 9-12-week gestation were obtained as a result of medical abortions after written consent of donors. FTC was isolated by high-speed staged ultracentrifugation (protein content 1.0±0.2 mg/ml) [5]. TSF was obtained as described

Institute of Cryobiology and Cryomedicine Problems, National Academy of Sciences of Ukraine, Kharkov. ***Address for correspondence:*** danusha@ukr.net. D. V. Cherkashina

previously [7]. All procedures were carried out under sterile conditions on cold, the preparations were stored at -30°C.

Toxic hepatitis was induced by single intraperitoneal injection of 40% CCl_4 in oil (0.3 ml/100 g). The animals were divided into 4 groups: 1) intact; 2) control (intravenous injection of 0.9% NaCl 4 h before intoxication (0.3 ml/100 g CCl_4)); 3 and 4) experimental groups: FTC and TSF (0.3 ml/100 g), respectively.

Serum activities of AST and ALT were measured using Vector-Best kits and the content of TBA-reactive products [2] was measured during the first 3 days after intoxication.

The animals were decapitated 3 days after injection of CCl_4. Basal levels of TBA-reactive products and intensity of Fe^{2+}-ascorbate-induced LPO, activities of catalase (by H_2O_2 utilization, λ=240 nm), glutathione peroxidase (by GSSG accumulation at λ=260 nm), glutathione reductase (by increase in NADPH content) and glucose-6-phosphate dehydrogenase (by decrease in NADPH content, λ=340 nm) were measured in liver homogenates. Protein content in liver homogenates was measured by the biuret method.

The results were processed using nonparametric Mann—Whitney test ($M\pm m$; $p<0.05$).

RESULTS

Pretreatment with FTC (group 3) or its TSF (group 4) 4 h before CCl_4 intoxication completely prevented animal death (40% mortality in the control group).

Serum aminotransferase activities in groups 3 and 4 decreased 24 h after CCl_4 intoxication in comparison with the control (Fig. 1). The effect of pretreatment persisted until the end of the experiment. FTC more effectively prevented transaminase elimination.

Acute CCl_4 intoxication led to an increase in the serum level of TBA-reactive products. Pretreatment with FTC and TSF significantly reduced this parameter on days 1 and 2; on day 3 the effect was observed only in group 3 (Fig. 1).

Pretreatment with FTC and TSF normalized the basal level and velocity of accumulation of TBA-reactive products in the liver of animals with CCl_4 intoxication (Fig. 2). Decreased intensity of LPO processes after FTC pretreatment was paralleled by normalization of catalase, glutathione reductase and peroxidase activities, and a significant increase in glucose-6-phosphate dehydrogenase activity (Fig. 3). On the other hand, injection of TSF led to a significant, though incomplete elevation of catalase

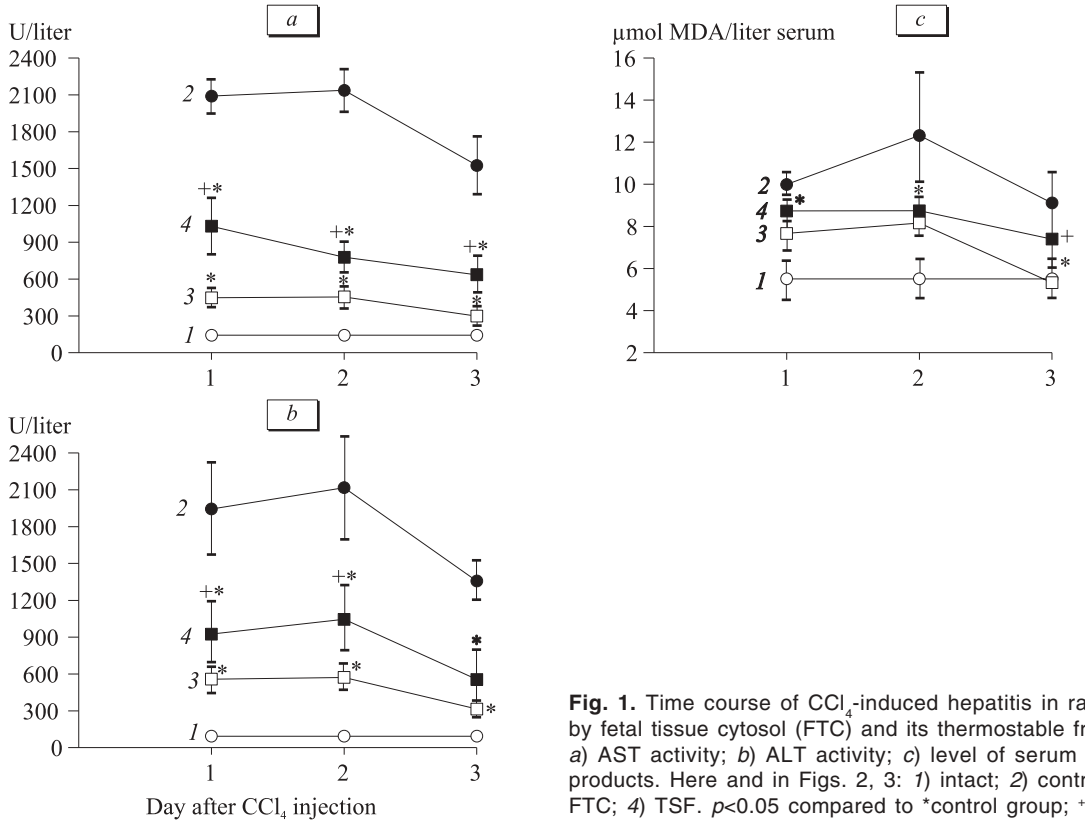

Fig. 1. Time course of CCl_4-induced hepatitis in rats pretreated by fetal tissue cytosol (FTC) and its thermostable fraction (TSF). *a*) AST activity; *b*) ALT activity; *c*) level of serum TBA-reactive products. Here and in Figs. 2, 3: *1*) intact; *2*) control groups; *3*) FTC; *4*) TSF. $p<0.05$ compared to *control group; +group 3.

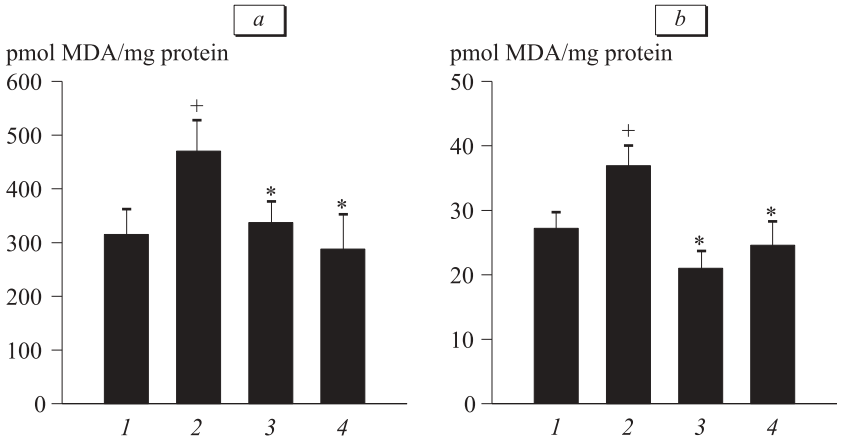

Fig. 2. Effects of FTC and TSF on basal level (*a*) and velocity of TBA-reactive products accumulation (*b*) in the liver of rats with CCl_4 intoxication. $p<0.05$ compared to: *control group; +intact group.

and glutathione peroxidase activities against the background of normalization of glutathione reductase and glucose-6-phosphate dehydrogenase activities. These differences between experimental groups can indicate a switch-over of TSF action to nonenzymatic components of antioxidant systems, because NADPH generated in glucose-6-phosphate dehydrogenase reaction is essential for replenishment of the pool of low-molecular-weight free radical traps (*e.g.* glutathione) [8].

Hence, pretreatment with FTC and its TSF prevents the death of rats after CCl_4 intoxication due to reduction of the severity of liver injury and normalization of the prooxidant/antioxidant balance.

Comparative analysis of the effects of FTC and TSF showed that preventive effect of FTC is not confined to its thermostable low-molecular-weight components, but is due to complex oppositely directed effects on metabolic processes in the liver, *e.g.* regulation of the antioxidant enzyme activities.

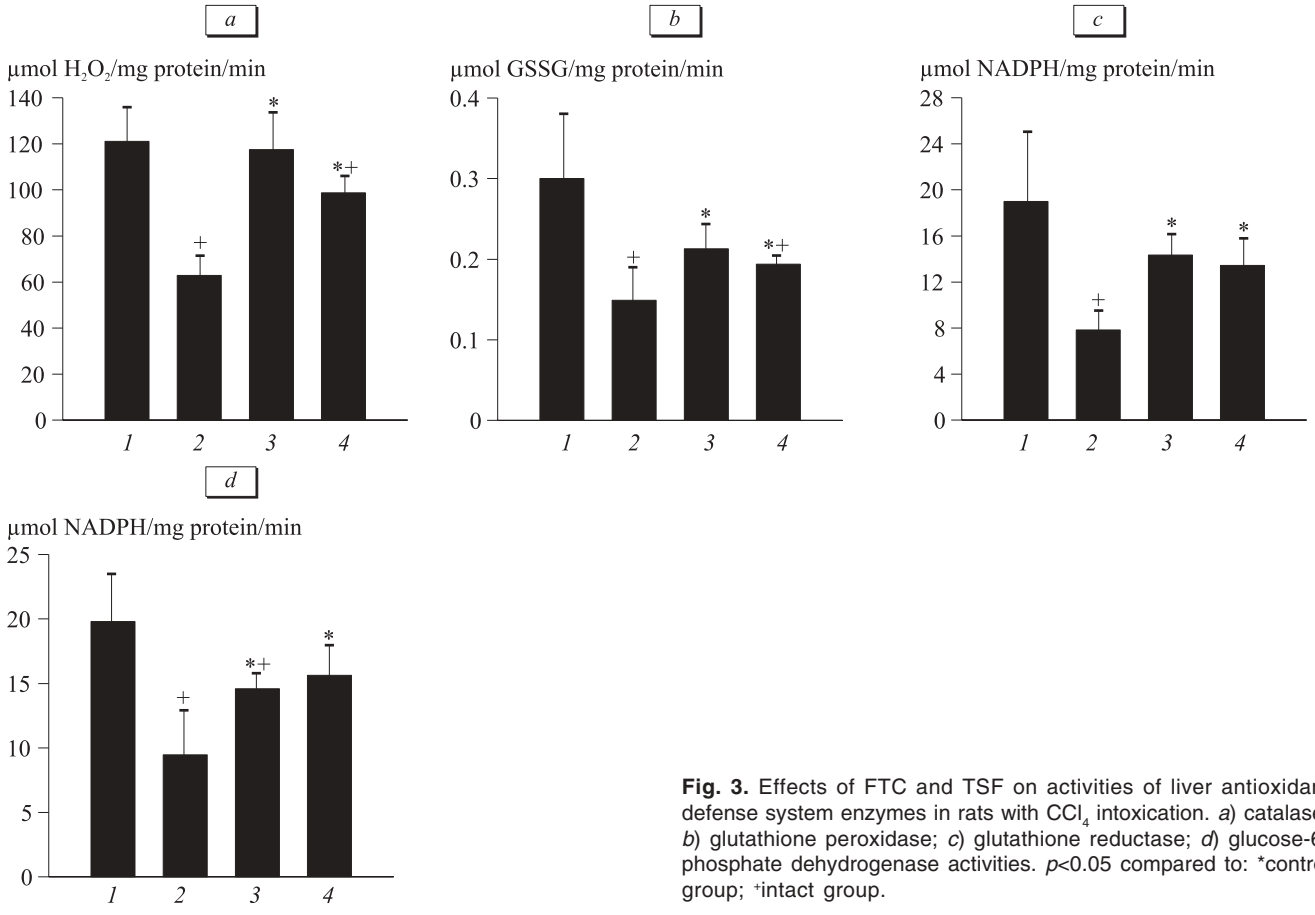

Fig. 3. Effects of FTC and TSF on activities of liver antioxidant defense system enzymes in rats with CCl_4 intoxication. *a*) catalase; *b*) glutathione peroxidase; *c*) glutathione reductase; *d*) glucose-6-phosphate dehydrogenase activities. $p<0.05$ compared to: *control group; +intact group.

Pretreatment with FTC proved to be more effective for the realization of the early defense mechanisms providing cell integrity. The results necessitate more detailed study of cell-free FTC composition and of possible approaches to realization of its hepato-protective effect.

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