PHARMACOLOGY AND TOXICOLOGY

Dobutamine Prevents Experimental Postintoxication Liver Cirrhosis in Mice

A. P. Vinokurov, A. V. Eremeev*, and N. A. Setkov*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 52-55, July, 2002
Original article submitted March 13, 2002

Various chronic inflammatory and necrotic processes in the liver parenchyma are accompanied by pathological morphofunctional changes, which are associated with hepatocyte death and hyperplasia of the connective tissue. Regeneration of the liver parenchyma should include not only prevention of fibrosis, but also stimulation of hepatocyte proliferation. The adrenoceptor agonist dobutamine stimulated proliferative activity of cultured hepatocytes and prevented the development of postintoxication liver cirrhosis in mice produced by chronic poisoning with CCl_4.

Key Words: *hepatocytes; dobutamine; proliferation; liver cirrhosis*

Chronic diffuse liver diseases, including chronic hepatitis and cirrhosis of the liver, are the major problem of clinical hepatology. The pathogenesis of liver cirrhosis includes necrosis of hepatocytes and development of fibrotic changes. Nonparenchymal liver cells secrete growth factors that stimulate proliferative activity of fibroblasts and formation of the connective tissue [6]. Activated connective tissue cells produce collagen, glycoproteins, fibronectin, and other connective tissue proteins. Uncontrolled proliferation of fibroblasts and increase in their functional activity contribute to the appearance of the connective tissue in Disse's spaces. Fibrotic septa connecting portal and central zones of the hepatic lobule are responsible for liver hypertension. Transforming growth factor-β (TGF-β) stimulates the formation of the connective tissue. This factor acts as a potent mitogen for fibroblasts and as inhibitor for hepatocytes [5,7]. A perspective approach to the therapy of fibroproliferative disease is inhibition of functional activity of fibroblasts and stimulation of hepatocyte proliferation, which can promote functional recovery of the liver. Recent animal experiments showed that treatment with exogenous TGF-β receptors led to elimination of TGF-β from the blood and decreased the severity of cirrhosis [7]. Transplantation of hepatocytes followed by stimulation of their proliferation is another approach to normalization of the cellular composition in the liver [8,13]. We hypothesized that administration of adrenoceptor agonists can reduce functional activity of the connective tissue and stimulate hepatocyte proliferation. Catecholamines decrease the sensitivity of hepatocytes to TGF-β and initiate DNA synthesis *in vivo* and in primary hepatocyte culture (through α_1-adrenoceptors) [4,10].

The use of adrenoceptor agonists in combination with partial hepatectomy (PHE) can activate proliferative processes in damaged liver parenchyma. Here we studied the effects of dobutamine (dobutirex, synthetic catecholamine, α_1-, β_1-, and β_2-receptor agonist) on liver regeneration in mice with chemically induced cirrhosis.

MATERIALS AND METHODS

Experiments were performed on female BALB/c mice weighing 25-27 g and aging 3 months. Experimental

Department of General Surgery, Krasnoyarsk State Medical Academy; *Institute of Biophysics, Siberian Division of the Russian Academy of Sciences, Krasnoyarsk

0007-4888/02/1341-043$27.00

liver cirrhosis was produced by intraperitoneal administration of CCl_4 in olive oil (1.4 ml/kg) 2 times a week for 30-60 days. Some mice were intraperitoneally injected with CCl_4 24 h before isolation of hepatocytes. PHE ($^1/_2$-$^2/_3$) was performed routinely [9].

Liver cells were isolated from adult narcotized mice by non-recirculating two-stage perfusion (non-enzymatic and enzymatic) of the liver by the previously described method [1] with modifications [2]. The experiment was performed under sterile conditions. After isolation and two-step gentle centrifugation (50*g*, 3 min) hepatocytes were suspended in a mixture of DMEM and NCTC-135 media (BDSL, ratio 1:1) containing 0.2% fetal bovine serum (FBS, Vektor) inactivated at 56°C, 0.05% serum albumin for cultured cells (Sigma), 0.1% glucose, 0.5 mg/ml insulin (Sigma), 50 ng/ml dexamethasone, 0.1 mg/ml glucagon (Sigma), 0.02 mM β-mercaptoethanol, 10 mM HEPES buffer, and 100 mg/ml kanamycin sulfate (complete medium).

In series II we studied incorporation of ^{3}H-thymidine into hepatocytes obtained from normal mouse liver and preincubated with epidermal growth factor (EGF) for 0 (control), 1, 2, and 4 h; otherwise, these cells were preincubated with dobutamine for 2 h. Hepatocytes were washed, and incubation was performed in a medium with 0.2% FBS for 8, 16, and 24 h.

In series III we evaluated proliferative activity of hepatocytes from mice subjected to PHE or treated with CCl_4 and dobutamine. Dobutamine and CCl_4 were injected 24 h before isolation of hepatocytes. When we studied the combined action of PHE and dobutamine or the effects of dobutamine and CCl_4, the preparations were injected 24 h after the corresponding treatment. In a special series dobutamine was injected 2 h after surgery.

The mice received ^{3}H-thymidine (3.7 kBq intraperitoneally) 4 and 2 h before isolation of hepatocytes. After perfusion these cells were placed in flasks (10^5 cells/flask), cultured, washed, and fixed with trichloroacetic acid and ethanol. Radioactivity (incorporated ^{3}H-thymidine) was measured routinely.

The cells obtained from normal liver were cultured in a medium with ^{3}H-thymidine (3.7 kBq/ml) in the presence of various concentrations of dobutamine (0.5-64.0 mg/ml, Lily Pharma) for 8, 16, and 24 h to determine its effects on proliferative activity of mouse hepatocytes *in vitro*.

Proliferative activity of cells was calculated and compared to the control (intact hepatocytes).

RESULTS

Dobutamine stimulated ^{3}H-thymidine incorporation into intact hepatocytes in a monolayer culture. Proliferative activity of hepatocytes was maximum at a dobutamine concentration of 8 mg/ml (Fig. 1). Increasing the concentration of dobutamine by 2-4 times was not followed by the adequate proliferative response. In further experiments we used dobutamine in this subclinical dose.

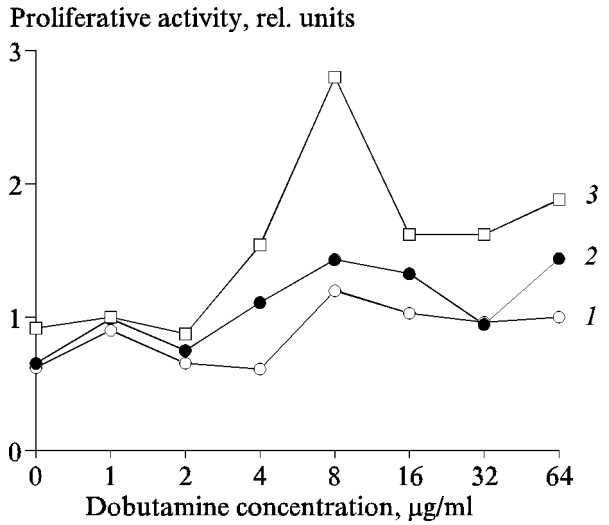

Fig. 1. Proliferative activity of hepatocytes in monolayer culture. Culturing with dobutamine for 8 (*1*), 16 (*2*), and 24 h (*3*).

EGF acts as a physiological regulator of hepatocyte proliferation. We compared proliferative activity of cells induced by EGF and dobutamine.

In physiological solutions dobutamine undergoes degradation within 2 h (as reported by Lily Pharma). We compared the proliferative response of hepatocytes to dobutamine and EGF after 2-h exposure with the growth factor. Dobutamine was more potent than EGF in stimulating proliferative activity of hepatocytes (Fig. 2).

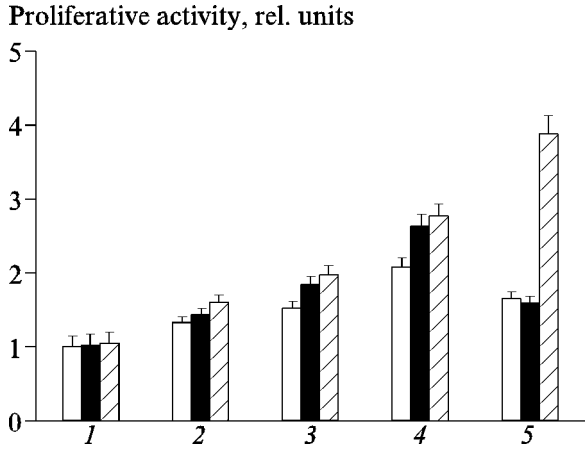

Fig. 2. Proliferative activity of hepatocytes after preincubation with epidermal growth factor or dobutamine and incubation in a medium with 0.2% FBS for 8 (light bars), 16 (dark bars), and 24 h (shaded bars). Control (*1*); preincubation with epidermal growth factor for 1 (*2*), 2, (*3*), and 24 h (*4*); and preincubation with dobutamine for 2 h (*5*).

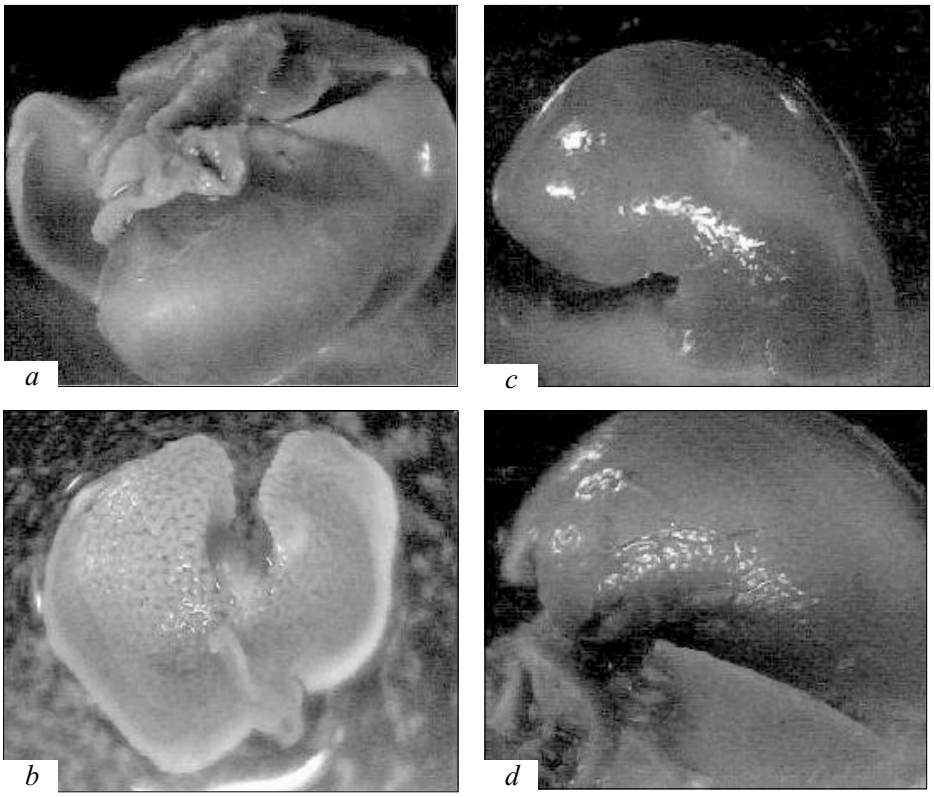

Fig. 3. Liver samples from mice exposed to CCl_4 for 1 (*a*, *c*) and 2 months (*b*, *d*). Fixation in ethanol. No therapy (*a*, *b*) and treatment with dobutamine after (*c*) or during intoxication (*d*).

Hepatocytes from the intact liver exhibit low proliferative activity [3] and insensitivity to growth factors [11]. In the next series we evaluated whether dobutamine *in vivo* stimulates proliferative activity of hepatocytes. Dobutamine *in vivo* stimulated proliferative activity of hepatocytes. Treatment with dobutamine 24 h after PHE enhanced the proliferative response of hepatocytes to surgery (Table 1). By contrast, administration of dobutamine 2 h after PHE suppressed proliferative activity of hepatocytes stimulated by surgery. Dobutamine infusion after CCl_4 intoxication produced a less pronounced stimulatory effect (Table 1).

These results show that dobutamine *in vitro* and *in vivo* increases proliferation-inducing activity of mouse hepatocytes.

TABLE 1. Proliferative Activity of Hepatocytes after PHE or Treatment with CCl_4 in Combination with Dobutamine ($M \pm m$)

Experimental conditions		^{3}H-thymidine, rel. units
Control		1.00±0.11
Intact hepatocytes and dobutamine		1.62±0.08
PHE		2.66±0.06
+dobutamine	after 24 h	4.82±0.18
	after 2 h	0.71±0.04
CCl_4+dobutamine	after 24 h	1.40±0.09

Published data show that hepatocytes more rapidly react to proliferative stimuli than nonparenchymal liver cells. Previous studies showed that 3 h after surgery hepatocytes lose their sensitivity to TGF-β, which acts as a potent inhibitor of their proliferation [11,14]. Since catecholamines decrease the sensitivity of hepatocytes to TGF-β and increase their proliferative activity, we hypothesized that dobutamine stimulates regeneration of the liver and prevents the development of fibrosis after poisoning with CCl_4. In the next series we studied whether dobutamine prevents the development of experimental postintoxication liver cirrhosis in mice. The animals received CCl_4 for 1-2 months. Then some mice were intraperitoneally injected with dobutamine in physiological saline 2 times a week (8 mg), while others received dobutamine 2 days after treatment with CCl_4. Dobutamine not only prevented the development of postintoxication cirrhosis, but also normalized morphofunctional state of the liver (Fig. 3).

REFERENCES

1. A. Ya. Dudina-Barkovskaya and L. A. Mittel'man, *Tsitologiya*, **23**, No. 8, 944-946 (1981).
2. N. A. Setkov, V. N. Kazakov, and T. V. Andreeva, *Ibid*., **33**, No. 6, 76-83 (1991).
3. I. V. Uryvaeva and V. M. Faktor, *Ontogenez*, **6**, 438-445 (1975).
4. J. L. Cruise and K. A. Houck, *Science*, **227**, 749-751 (1985).

5. M. Date, K. Matsuzaki, M. Matsushita, *et al*., *Gut*, **46**, 719-724 (2000).
6. S. L. Friedman, *J. Biol. Chem*., **275**, 2247-2250 (2000).
7. J. George, D. Roulot, V. E. Koteliansky, and D. M. Bissel, *Proc. Natl. Acad. Sci. USA*, **96**, No. 22, 12,719-12,724 (1999).
8. S. Gupta and C. E. Rogler, *Gastrointestinal Liver Physiol*., **277**, 1097-1102 (1999).
9. G. Higgins and R. Anderson, *Arch. Pathol*., **12**, 186-202 (1931).
10. K. A. Houck and G. K. Michalopoulos, *J. Cell. Physiol*., **141**, No. 3, 503-509 (1989).
11. A. C. Huggett, H. C. Krutzsch, and S. S. Thorgeirsson, *J. Cell. Biochem*., **35**, 305-314 (1987).
12. P. Loyer, G. Ilyin, S. Cariou, *et al*., *Prog. Cell Cycle Res*., **2**, 37-47 (1996).
13. J. A. Rhim, E. P. Sanoqren, J. L. Degen, *et al*., *Science*, **263**, 1149-1152 (1994).
14. A. J. Strain, *Scand. J. Gastroenterol. Suppl*., **151**, 37-45 (1988).