Regeneration of the Liver in Mice Treated with a Mixture of Hepatotoxins in Delayed Periods after Bone Marrow Transplantation

T. V. Todriya and T. L. Nikolaeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 4, pp. 462-465, April, 2006
Original article submitted April 28, 2005

Possible effect of bone marrow cells on liver regeneration was studied in mice injected with a mixture of hepatotoxins (allyl alcohol and CCl_4) in a dose equal to LD_{50}. The mixture of hepatotoxins was used to minimize the restitution regeneration of the liver. The dose of allyl alcohol causing (in combination with CCl_4) maximum liver damage was selected beforehand. Increasing the dose of allyl alcohol in the two-component mixture resulted in more severe necrosis of the liver. The maximum dose of alcohol (50 mg/kg) in combination with CCl_4 caused irreversible injury to the liver leading to 100% mortality after 2-4 days. In radiation chimeras reconstituted by bone marrow cell transplantation, in which liver damage was induced by a mixture of hepatotoxins containing the maximum dose of allyl alcohol, we observed normalization of liver tissue structure and function. The mechanism of this effect is not clear.

Key Words: *liver; regeneration; allyl alcohol; carbon tetrachloride; bone marrow*

Exogenous stem cells, *e.g.* hemopoietic stem cells or their derivatives, can serve as the source of regeneration of damaged organs. It has been reported not once that adult bone marrow (BM) contains cells capable of differentiating into mature non-hemopoietic cells of different tissues, including liver cells [6,11], renal cells [9], lung cells [5], skeletal and myocardial cells [2,7], and nervous tissue cells [15]. The capacity of tissue-specific stem cells to differentiate into cells of another histogenetic lineage, so called stem cell plasticity (SCP), was first discovered on the model of regenerating liver in humans, mice, and rats [1,8,11, 12]. After transplantation of genetically labeled cells, donor BM cells in experimental systems were detected in adult animal tissues, including liver tissues [3,11,12]. It was hypothesized that this phenomenon is based on transdifferentiation of cells of BM origin [3], but this hypothesis remained not proven. Spontaneous fusion of donor-recipient cells (but not transdifferentiation) was observed [10]. The therapeutic effect of BM was detected in fumarylacetoacetate hydrolase deficient mice with metabolic disorders [6]. The liver function in fumarylacetoacetate hydrolase (-/-) mice was restored after BM transplantation, presumably due to fusion of donor cells with recipient hepatocytes [13,14].

Markers of extrahepatic stem cells were detected in hepatocytes of patients after transplantation of BM or liver, when the donor and recipient belonged to different sexes [1,11]. The content of these cells varied from 2 to 40%. In model systems the percentage of donor cells in the recipient liver was very low (about 2%). If there was no liver injury (*e.g.*, in parabionts), donor cells had no selective advantage [4,6,8].

On the whole, the data on the kinetics and mechanisms of this phenomenon are scanty. In addition to the possible transdifferentiation and fusion

Hematology Research Center, Moscow. ***Address for correspondence:*** todria@blood.ru. T. V. Todriya

of donor and recipient cells, we cannot rule out activation of recipient stem cells with borderline developmental potential under conditions of stress. These studies on the model of regenerating liver are difficult primarily because of high regeneratory potential of hepatocytes and oval bipotent cells, capable of differentiating into hepatocytes and cholangiocytes under conditions of stress.

The choice of the model of liver injury for the study of SCP is principally important, because donor cells repopulate the damaged organ if its reparative resources are exhausted. In order to evaluate possible effect of donor BM cells on liver regeneration, we used a combination of two models of liver injury: injury caused by different concentrations of allyl alcohol (AlOH) and LD_{50} of CCl_4 administered separately or by a mixture of these two components. AlOH causes periportal and CCl_4 centrolobular necrosis of the liver. The mixture of hepatotoxins was used to minimize the restitutive regeneration of the liver.

MATERIALS AND METHODS

The study was carried out on 8-10-week-old female $(CBA \times C57Bl/6)F_1$ mice. Acute centrolobular hepatic injury was induced by single intraperitoneal injection of CCl_4 in olive oil (1:1) in a dose of 2 ml/kg (1500 mg/kg). Periportal necrosis was induced by intraperitoneal injection of AlOH in doses of 5, 20, 35, and 50 mg/kg (in saline) separately or in a mixture with CCl_4 in a standard dose of 2 ml/kg. The mixture of two hepatotoxins was successively injected into the left and right parts of the peritoneal cavity. Control group of mice received saline intraperitoneally.

In series II the mice were irradiated in a dose of 10 Gy (2×5 Gy, with 3-h interval) on a ^{127}Cs gamma device specially designed for Institute of Blood Transfusion. Dose power was 0.165 Gy/min. Hemopoiesis in irradiated recipients was restored by injection of 10×10^6 syngeneic donor BM cells. After 3 months radiation chimeras were injected with AlOH in a maximum dose (50 mg/kg) solely or in combination with the standard dose of CCl_4. Liver regeneration was evaluated 2, 4, 7, and 10 days after hepatotoxin injection (3-5 mice per term were analyzed). The blood was collected from the orbital sinus and total bilirubin and serum AST and ALT activities were measured photometrically using DiaSis kits. The degree of necrosis and subsequent regeneration of the liver were evaluated by the structure of the parenchyma on routine histological sections stained with hematoxylin and eosin. The sections (5 μ) were prepared from the major left and median lobes of the liver. Thirty visual fields per section were analyzed by selectively evaluating hepatocyte count in zone 1 (zone of portal triad of the hepatic lobule), 2 (central vein zone), and 3 (interzonal space).

Statistical analysis was carried out using Student's *t* test.

RESULTS

For correct selection of hepatotoxin dose causing irreversible damage to the liver, standard LD_{50} of CCl_4 (2 ml/kg) and different concentrations of AlOH (5, 10, 35, and 50 mg/kg) were used separately in experimental series I. CCl_4 caused centrolobular necrosis of the liver. Restitutive regeneration in necrotic zones started from day 2 after injury and eventuated in proliferation of intact hepatocytes after 1-2 weeks. After AlOH treatment the location of necrotic zones was confined to the portal triad of the hepatic lobule. Light microscopy showed that recovery of the liver structure took 5-7 days, with the following successive stages: necrosis with hepatocyte apoptosis in non-necrotic zones, focal periportal edema and minimum inflammation, proliferation and differentiation of minor periportal cells. The degree of liver destruction depended on the concentration of chemical agents. Despite vast necrosis caused by hepatotoxins in LD_{50} (2 ml/kg CCl_4 and 50 mg/kg AlOH), all mice survived for 10-15 days, which attests to high restitution capacity of hepatic tissue. The combination of two hepatotoxins augmented liver destruction and reduced hepatocyte capacity to regeneration. Individual intolerance depending on AlOH dose led to mortality of 8-30% (20 and 35 mg/kg AlOH, respectively) for 10-15 days. The maximum concentration of AlOH (50 mg/kg) in the two-component mixture caused 100% mortality of mice on days 2-4 after injection of hepatotoxins.

Necrosis of the liver caused by CCl_4 and AlOH was associated with changes in activities of serum AST and ALT and in total blood bilirubin level, indicating liver dysfunction. The maximum cell destruction with the release of AST and ALT into the blood was observed on days 1-3 after injection of hepatotoxins. The start of regeneration (day 4) was associated with gradual normalization of transaminase and bilirubin activities. Histograms reflecting changes in ALT activity and bilirubin concentration during the period of maximum injury to the liver (day 2) are presented (Fig. 1). No strict correlation between the dose of AlOH and blood bilirubin content was observed. Destructive effect of the two-component mixture manifested in increased biliru-

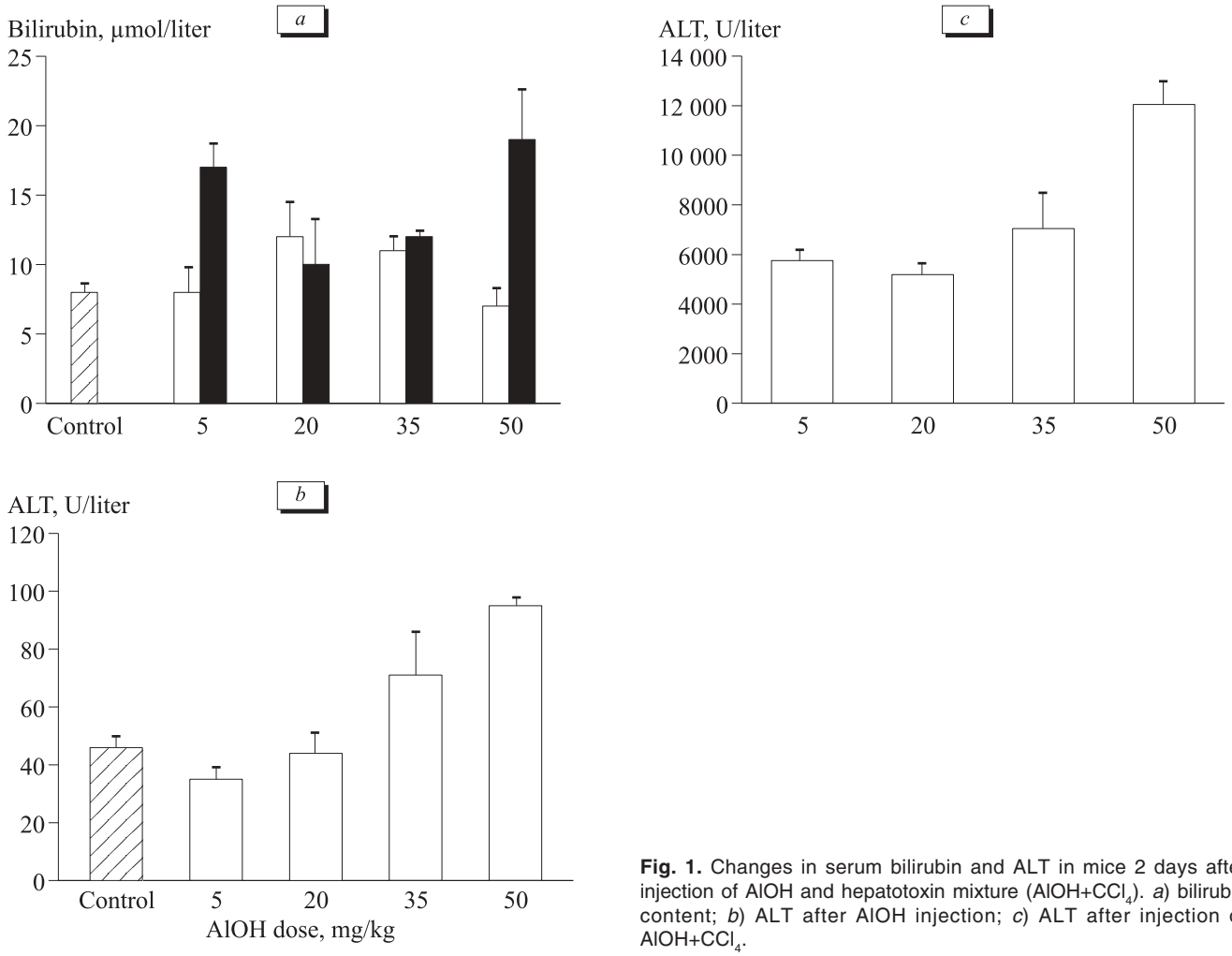

Fig. 1. Changes in serum bilirubin and ALT in mice 2 days after injection of AlOH and hepatotoxin mixture (AlOH+CCl_4). *a*) bilirubin content; *b*) ALT after AlOH injection; *c*) ALT after injection of AlOH+CCl_4.

bin level after injections of the minimum and maximum concentrations of AlOH (Fig. 1, *a*). Dose-dependent increase of ALT indicated moderate cytolysis of hepatocytes (Fig. 1, *b*), whose degree reached a critical level after the maximum dose of AlOH in the mixture (Fig. 1, *c*). A sharp release of ALT was provoked by the presence of CCl_4 in the mixture. Activity of ALT after single injection of CCl_4 in LD_{50} was equal to 4200±250 U/liter. AlOH in the maximum concentrations potentiated the to-

TABLE 1. Main Biochemical and Histological Characteristics of Regenerating Liver 2 Days after Acute Injury Caused by Injection of Hepatotoxin Mixture CCl_4 (2 ml/kg) and AlOH (50 mg/kg)

Parameter	Control (saline)	Experimental groups	
		not exposed	radiation chimeras transplanted BM
Bilirubin, μmol/liter	9.5±0.6	19.0±3.6	8.0±2.8*
AST, U/liter	55.00±3.25	10544±816	5620±1627*
ALT, U/liter	49.5±9.9	12044±903	7362±1043*
Hepatocytes in visual field (×1000)	29.80±0.27	17.90±2.15	26.6±2.6 *
Hepatocytes in zone 1[x]	29.03±0.32	15.60±1.15	19.0±1.5
Hepatocytes in zone 3[+]	28.29±1.21	13.1±2.5	22.9±2.7*
Mitoses/100 hepatocytes	0.16±0.03	6.7±0.14	12.7±2.38*

Note. *$p<0.05$ compared to non-irradiated mice. [x]Zone of portal triad of hepatic lobule. [+]Central vein zone. Control: mean values for 12 mice.

xic effect of CCl_4 more than 2.5 times. Hence, the summary toxicity significantly surpassed the maximum toxicity of each component alone. A similar regularity was observed for AST.

Combination of two LD_{50} caused overall mortality, and therefore in series II of experiments AlOH (50 mg/kg) with the standard dose of CCl_4 or without it was injected to radiation chimeras 3 months after BM transplantation. In this case the maximum necrosis was observed on day 2 after injection of the hepatotoxin mixture (Table 1). Despite variations in individual values, the content of bilirubin, AST, and ALT in chimeras was virtually 2-fold lower than in mice receiving no BM transplantation. Rapid regeneration was also observed at the histological level. The state of the liver parenchyma was evaluated by the retained structure of the liver beams, degree of vacuolation and fatty degeneration of hepatocytes, by their number in the visual field and in zones 1 and 2, and by mitotic index; according to these parameters, it approached the normal.

On the whole, regeneration of the liver depends on the dose of the destructive agent, the natural restitutive response was observed after treatment with hepatotoxins in a wide concentration range. However, regeneration of the liver tissue is exhausted after exposure to a combination of two LD_{50} of hepatotoxins in a binary mixture. BM injected before hepatotoxic treatment presumably activates regeneration processes in the liver tissue or protects it from acute injury. The mechanism of this effect is not clear. It remains to clear out whether donor BM cells produce a therapeutic effect or they are directly involved in regeneration of the liver tissue, *e.g.*, by fusion with the recipient hepatocytes, and what, specifically, hierarchical category of BM cells is responsible for liver regeneration after massive necrosis.

REFERENCES

1. M. R. Alison, R. Poulsom, R. Jeffery, *et al.*, *Nature* (London), **406**, 257 (2000).
2. G. Ferrari, G. Gusella-De Angelis, M. Coletta, *et al.*, *Science*, **279**, 1528-1530 (1998).
3. M. A. Goodell, *Curr. Opin. Hematol.*, **10**, No. 3, 208-213 (2003).
4. Y. Kanazawa and I. M. Verma, *Proc. Natl. Acad. Sci. USA*, **100**, Suppl. 1, 11,850-11,853 (2003).
5. D. N. Kotton, B. Y. Ma, W. V. Cardoso, *et al.*, *Development*, **128**, No. 24, 5181-5188 (2001).
6. E. Lagasse, H. Connors, M. Al-Dhalimy, *et al.*, *Nat. Med.* (NY), **6**, No. 1, 1229-1234 (2000).
7. D. Orlik, J. Kajstura, S. Chimenti, *et al.*, *Ann. NY Acad. Sci.*, **938**, 221-230 (2001).
8. B. E. Petersen, W. C. Bowen, K. D. Patrene, *et al.*, *Science*, **284**, 1168-1170 (1999).
9. R. Poulsom, S. J. Forbes, K. Hodivala-Dilke, *et al.*, *J. Pathol.*, **195**, No. 2, 229-235 (2001).
10. N. Terada, T. Hamazaki, M. Oka, *et al.*, *Nature*, **416**, 542-545 (2002).
11. N. D. Theise, S. Badve, R. Saxena, *et al.*, *Hepatology*, **31**, No. 1, 234-240 (2000).
12. N. Theise, M. Nimmakalaylu, R. Gardner, *et al.*, *Ibid.*, **32**, No. 1, 11-16 (2000).
13. G. Vassilopoulos, P. R. Wang, and D. W. Russel, *Nature*, **422**, 901-904 (2003).
14. X. Wang, S. Ge, G. MacNamara, *et al.*, *Blood*, **101**, No. 10, 4201-4208 (2003).
15. L. R. Zhao, W. M. Duan, M. Reyes, *et al.*, *Exp. Neurol.*, **174**, No. 1, 11-20 (2002).