Acceleration of Liver Regeneration by Malotilate in Partially Hepatectomized Rats

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Abstract—The effect of malotilate (diisopropyl 1,3-dithiol-2-ylidenemalonate) on liver regeneration was studied by using partially hepatectomized rats. Malotilate administration (100 mg/kg/day, p.o.) facilitated the weight gain of the liver after partial hepatectomy. Protein, RNA and DNA contents of the regenerating liver correlated well with the weight gain. The weight gain, RNA and DNA contents, and mitotic index were significantly suppressed in the alloxan-diabetic rats 24 hr after partial hepatectomy. However, malotilate administration significantly improved the delayed recovery of RNA content. Other parameters were not significantly improved by malotilate, but tended to increase to a level comparable to those of partially hepatectomized control rats. These results show that malotilate accelerates cell proliferation, resulting in facilitated liver regeneration in rats (as well as in alloxan-diabetic rats).

Malotilate (Diisopropyl 1,3-dithiol-2-ylidenemalonate) is effective against liver cirrhosis and chronic hepatitis (1). The primary action is considered to be related with the enhanced synthesis of protein in the liver (2). Malotilate characteristically induces liver microsomal cytochrome b_5 rather than P-450, which are attributable to the enhancement of protein synthesis in the liver (3, 4). Intraperitoneal administration of malotilate (100 mg/kg) can facilitate the liver regeneration in cirrhotic rats, but not in normal rats after partial hepatectomy (5). In the current paper, malotilate was orally given to partially hepatectomized rats and its effect on post-hepatectomized regenerating liver was evaluated, using such parameters as liver weight, protein and nucleic acid contents, and mitotic index.

Portal factors such as insulin and glucagon accelerate liver regeneration (6–9). Damage of pancreatic B cells by alloxan leads to a lowered insulin level and retards liver regeneration (10–13). Therefore, the effect of malotilate on liver regeneration after partial hepatectomy is also studied in alloxan-diabetic rats.

Materials and Methods

Materials: Malotilate (Diisopropyl 1,3-dithiol-2-ylidenemalonate) used in this experiment was synthesized in the Chemical Research Center of Nihon Nohyaku Co., Ltd. Its purity was more than 99.5%. Other chemicals used were of analytical reagent grade.

Animals: Five-week old male Sprague-Dawley rats were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan) and used after acclimatization for 1 week under free access to food pellets (F-2, Funabashi Nojo, Funabashi, Japan) and tap water. Malotilate suspended in 2% gum arabic solution was administered orally to rats at a dose of 100 mg/kg/day. The control rats received an equivalent volume of 2% gum arabic solution.

Partial hepatectomy and liver regeneration: Partial hepatectomy was performed under ether anesthesia according to the methods of Higgins and Anderson (14). The sham

hepatectomy was carried out in the same manner except that the liver was not excised. The rats were first administered with malotilate and/or its vehicle 2 hr after the operation and then daily around 10 a.m. They were killed by cervical dislocation 1, 3, 5 or 10 days after sham or partial hepatectomy. Each sacrifice was carried out 24 hr after the last dose of malotilate and/or its vehicle. The liver was then removed and weighed. The contents of protein, RNA and DNA in the liver were determined by homogenization and separation according to the methods devised by Schmidt and Thannhauser (15), which was followed by quantification according to the reported methods (16–18). Small portions of the liver were obtained from the middle part of the *lobus quadratus*. The obtained samples were then fixed in 10% neutral formalin or Bouin solution, embedded in paraffin, sectioned and stained with hematoxylin-eosin for microscopic observation. The mitotic activity was determined by counting the number of parenchymal cells undergoing mitosis in 50 randomly-selected fields under magnification ×400. The results were expressed as the mitotic index (the average number of mitoses per 100 parenchymal cells).

Rats were intravenously injected with 40 mg/kg of alloxan monohydrate dissolved in physiological saline. After 3 days, they were fasted for 16 hr and orally loaded with 30% glucose solution at a dose of 1.5 g/kg. After 1 hr, their blood was collected from the femoral vein, and the plasma glucose concentration was determined by the O-TB method (19). The rats were partially hepatectomized 5 days after alloxan treatment. They were given malotilate 2 hr after the hepatectomy and sacrificed for the examination of liver regeneration 24 hr after the hepatectomy. The parameters used were liver weight, protein and nucleic acid contents, and mitotic index.

Statistical analysis: The significance of the difference between two groups was assessed by the *t*-test.

Results

Effect of malotilate on liver regeneration: The partial hepatectomy was performed according to the methods of Higgins and Anderson (14), where about 70% of the liver was resected. The ratio of the resected portion was 3.42±0.05 (mean±S.E.) g/100 g body weight. The changes in liver weight, protein and nucleic acid contents, and mitotic index are summarized in Table 1. The liver weight of the partially hepatectomized rats was 3.32, 5.58, 6.52 or 8.70 g after 1, 3, 5 or 10 days, respectively. That of the malotilate-administered partially hepatectomized rats was 3.40, 6.51, 8.24 or 9.88 g, respectively. Daily administration of malotilate made liver regeneration rapid. No significant difference in the liver weights was observed between the malotilate- administered rats and the sham hepatectomized rats after 5 days, although liver weights were still significantly lighter in the hepatectomized control rats than in the sham hepatectomized rats after 10 days. The protein, RNA and DNA contents were also significantly lower in the partially hepatectomized control rats than in the sham hepatectomized rats after 10 days. However, no significant differences of the parameters between the sham-operated and the malotilate-administered groups were observed after 10 days. As shown in Table 1, the mitotic index of regenerating liver tended to increase in the malotilate-administered rats after 1 or 3 days. After 5 days, the mitotic stage was through completion, and the indices were as low as 0.6 in the partially hepatectomized rats. Therefore, the effect of malotilate on mitoses of liver parenchymal cells could no longer be observed after 5 days. Few mitoses were observed in the liver of sham-operated rats throughout the experimental period.

Effect of malotilate on the liver regeneration in alloxan-treated rats: Rats were given glucose (1.5 g/kg, p.o.) 1 hr prior to the determination of their plasma glucose. Plasma glucose concentration in the alloxan-treated rats (474.2±27.5 mg/dl) was about 3 times as high as that in the untreated rats (165.7± 4.2 mg/dl). This could indicate that alloxan damaged pancreatic B cells, causing a decrease of insulin secretion (10). As already shown in Table 1, the mitotic index can be an indicator for liver regeneration within a couple of days after the partial hepatectomy. In the alloxan-treated rats, the mitotic index after

Table 1 Effect of malotilate on liver weight, protein and nucleic acid (RNA and DNA) contents and mitotic index of remaining liver after the partial hepatectomy in rats

Days after hepatectomy	Hepatectomy	Malotialte administration	No. of animals	Liver weight (g)	Protein (g/liver)	RNA (mg/liver)	DNA (mg/liver)	Mitotic index (mitosis/100 cells)
1	Sham	−	5	7.67±0.35	1.27±0.07	113.7±2.4	11.6±0.4	0.00±0.00
	Partial	−	5	3.32±0.21***	0.55±0.04***	55.9±3.7***	5.4±0.4***	2.04±0.83*
	Partial	+	5	3.40±0.23***	0.60±0.03***	59.0±3.7***	5.6±0.4***	3.47±1.28*
3	Sham	−	5	8.48±0.08	1.58±0.05	124.3±4.4	13.3±0.4	0.00±0.00
	Partial	−	5	5.58±0.05***	0.86±0.04***	101.5±5.3*	9.8±0.6**	1.38±0.23***
	Partial	+	5	6.51±0.14***+	0.96±0.04***	121.0±5.7+	11.5±0.3**	1.98±0.52**
5	Sham	−	5	8.75±0.13	1.61±0.06	127.1±7.4	13.5±0.4	0.02±0.01
	Partial	−	5	6.52±0.16***	1.15±0.07**	121.0±2.5	11.2±0.3	0.54±0.21*
	Partial	+	5	8.24±0.42++	1.43±0.09*	161.5±8.9*++	14.1±0.7++	0.11±0.01
10	Sham	−	5	9.89±0.26	1.83±0.07	148.6±4.3	15.9±0.6	0.05±0.02
	Partial	−	4	8.70±0.18**	1.54±0.07*	129.0±6.5*	13.9±0.3*	0.33±0.08**
	Partial	+	5	9.88±0.50	1.77±0.69	145.7±13.8	15.7±1.0	0.56±0.19*

Malotilate was administered orally at a dose of 100 mg/kg/day after the partial hepatectomy. (First administration was carried out 2 hr after the partial hepatectomy, and following daily administrations were carried out around 10 a.m.) Rats were killed 1, 3, 5 or 10 days post-operatively. Each value is expressed as a mean±S.E. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ vs. sham hepatectomized rats; +$P<0.05$ and ++$P<0.01$ between partially hepatectomized control rats and malotilate-administered rats.

Table 2. Effect of malotilate on liver weight, protein and nucleic acid (RNA and DNA) contents and mitotic index of remaining liver in normal or alloxan-treated partially hepatectomized rats 24 hr after the operation

Alloxan treatment	Malotilate administration	No. of animals	Liver weight (g)	Protein (g/liver)	RNA (mg/liver)	DNA (mg/liver)	Mitotic index (mitotsis/100 cells)
−	−	5	3.86±0.10	0.66±0.02	72.5±1.8	5.9±0.3	2.27±0.34
−	+	5	4.15±0.16	0.75±0.04	84.4±2.7**	7.2±0.3	3.91±1.11
+	−	6	3.23±0.13**	0.62±0.03	54.9±1.7***	4.6±0.4*	0.53±0.26**
+	+	6	3.67±0.13+	0.66±0.03	69.3±2.5++	5.3±0.2	2.45±1.05

Alloxan was intravenously injected at a dose of 40 mg/kg 5 days before the partial hepatectomy. Rats were administered orally with malotilate (100 mg/kg) 2 hr after the partial hepatectomy. Each value is expressed as a mean±S.E. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ vs. normal partially hepatectomized rats without malotilate administration; +$P<0.05$ and ++$P<0.01$ between alloxan-treated partially hepatectomized rats without and with malotilate administration.

the partial hepatectomy was as low as 0.53±0.26 (Table 2). Even in the alloxan-treated rats, the mitoses of liver parenchymal cells 24 hr after the partial hepatectomy tended to be increased by malotilate administration to the value of 2.43±1.05 per 100 parenchymal cells, which was very close to that (2.27±0.34) in the partially hepatectomized untreated rats. DNA content also showed an increasing tendency by malotilate, and RNA content showed a significant increase (Table 2).

Discussion

Acceleration of liver regeneration after partial hepatectomy by malotilate was observed in the rat. The enhancement of liver regeneration by malotilate well-reflected the changes in protein, RNA and DNA contents. It was considered that the nucleic acid contents increased by malotilate meant an acceleration of cell proliferation. Indeed, malotilate tended to increase the mitotic index 1–3 days after partial hepatectomy in the rats (Table 1) as well as in the rats treated with alloxan (Table 2). The alloxan-diabetic rats seemed to be suffering from deficiency in insulin (10), which is one of the stimulating factors of liver regeneration (11–13). In the partially hepatectomized alloxan-diabetic rats, indeed, the mitotic index was as low as 0.53 instead of 2.27 as found in the untreated (partially hepatectomized) rats. The effect of malotilate on the liver regeneration in the alloxan-diabetic rats may suggest that malotilate acts directly on the hepatocytes in regenerating liver, but not through the hormonal action of insulin. Although no synthetic drug has been known to accelerate liver regeneration, malotilate was shown to accelerate liver regeneration. Malotilate tended to increase mitoses of hepatocytes and the following weight gain of the liver during liver regeneration.

Igarashi et al. (5) have reported that the intraperitoneal administration of malotilate for 7 days also accelerates the regeneration rate of the partially hepatectomized cirrhotic rat liver, but not that of the partially hepatectomized non-cirrhotic rat liver. However, the incorporation of ^{14}C-leucine into regenerating liver protein was enhanced in both groups of rats. Therefore, the acceleration of liver regeneration by malotilate seems to be due to enhanced protein synthesis and cell proliferation in a few days after partial hepatectomy. Under the conditions of Igarashi et al. (5), liver regeneration in the partially hepatectomized non-cirrhotic rat might be completed 7 days after the operation, but not in the partially hepatectomized cirrhotic rat.

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