Gastroenterologia Japonica
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Vol. 23, No. 6
Printed in Japan

—Original Article—

Effects of malotilate treatment on the serum markers of hepatic fibrogenesis in liver cirrhosis

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Summary: Malotilate, a hepatotropic agent, was given to 39 cirrhotic patients for more than 32 weeks. The serial changes in the serum levels of hepatic fibrogenesis markers, such as procollagen type III N-terminal peptides (P-III-N-P) and immunoreactive prolyl hydroxylase beta-subunit (IR-BPH) were analyzed. Serum albumin levels, transaminase and choline esterase activities and the Normotest values were found to be significantly improved by malotilate treatment. The levels of both serum markers of hepatic fibrogenesis were also significantly reduced by malotilate. The prognoses of the decompensated liver cirrhosis patients treated with malotilate were significantly better than those who did not receive malotilate. These results indicate that the effects of malotilate on chronic liver diseases are not simply biocosmetic, but rather are related to an improvement in the basal changes of the liver, including a decrease in the fibrogenetic stimulus. These effects of malotilate improved the prognosis of liver cirrhosis. *Gastroenterol Jpn 1988;23:639–645*

Key words: *Hepatic fibrogenesis, Liver cirrhosis, Malotilate, Procollagen type III N-terminal peptides (P-III-N-P), Prolyl hydroxylase beta-subunit (IR-BPH)*

Introduction

It has been reported that malotilate (diisopropyl 1, 3-dithiol-2-ylidenemalonate), a new hepatotropic drug, improves protein metabolism in the liver[1,2]. The treatment of chronic liver diseases with malotilate resulted in improvements in serum transaminase activity and serum markers of hepatic protein synthesis[3,4]. However, the possibility that these effects of malotilate were merely cosmetic improvements and not genuine improvements in the basal changes of the diseases cannot be ruled out. Although animal studies have shown that malotilate inhibits hepatic fibrosis[5,6], virtually nothing is known about its effect on fibrogenesis in chronic liver diseases in human subjects. It has been pointed out that serum procollagen type III N-terminal peptides (P-III-N-P) and serum prolyl hydroxylase beta-subunit are good markers of hepatic fibrogenesis[7-12]. We have recently reported that malotilate treatment tended to improve the values of these markers in chronic liver disease in a study on a small number of patients[13]. In the present study, these serum markers were measured and the effects of long-term malotilate administration on hepatic fibrogenesis and the prognosis in liver cirrhosis were analyzed in order to confirm the results of the previous study[13] in a larger number of patients.

Materials and Methods

Thirty nine patients (15 males and 24 females) with liver cirrhosis were given malotilate

Received February 24, 1988. Accepted June 13, 1988.
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Table 1 Backgrounds of the 39 patients

Sex	male	15 cases
	female	24 cases
Age	40-49 years old	7 cases
	50-59 years old	14 cases
	over 60 years old	18 cases
Etiology	alcohol	7 cases
	hepatitis B	9 cases
	cryptogenic	23 cases
Severity	compensated	22 cases
	decompensated	16 cases
	unclassified	1 case

(600mg/day) orally for more than 32 weeks. Five of these patients received malotilate for 32 weeks, and the remaining 34 for more than 48 weeks. Cirrhosis was confirmed by liver biopsy or peritoneoscopy in most patients. In patients with severely decompensated cirrhosis, diagnosis was made from the clinical features and laboratory findings, including computed tomography of the liver and endoscopic examination of esophageal varices. Sixteen cases were determined to be in the decompensated stage, while 22 were in the compensated stage. The remaining case had aortic stenosis and ascites complications which were considered to perhaps be caused by chronic heart failure. The backgrounds of the patients, including their etiologies, are presented in **Table 1**.

Various hepatic tests, serum P-III-N-P levels and serum immunoreactive prolyl hydroxylase beta-subunit (IR-BPH) levels were measured before and after malotilate treatment at 8 week intervals until 32 weeks in most cases, and until 48 weeks in 34 cases. The values before and after treatment were compared. In 30 out of 39 cases, malotilate treatment was started after a 24-week control basal level observation period. The hepatic test values and hepatic fibrogenesis serum marker levels during the 24 week of the basal observation period were compared with those during the 24 week malotilate treatment period (cross-over study).

Serum P-III-P-N levels were determined with a radioimmunoassay kit (Hoechst AG Radiochemishes Lab., West Germany) based on the method of Rohde et al[7]. Serum IR-BPH levels were measured with an enzyme immunoassay kit (Fuji Chemical Industries Ltd., Japan) using monoclonal anti-human prolyl hydroxylase beta-subunit mouse antibodies, based on the method of Yoshida et al[14]. Normal serum P-III-N-P and IR-BPH values were less than 10 ng/ml and 70 ng/ml, respectively.

All results are expressed as the mean ± standard error (SE). The paired Student's t-test and the Wilcoxon test were applied to the mean of the individual differences.

For the cirrhotic patients in the decompensated stage, the indocyanine green maximum removal rate (ICG-Rmax) and hepatic volumes were measured according to the description of Nakaya[15], a modified method of Paumgartner et al.[16], and computed tomography[17], respectively. Using the values of these 2 examinations as well as serum choline esterase activity, the total functioning hepatic cell masses were evaluated. The 24 month prognoses in these patients (malotilate group) were compared with those in 30 other cirrhotic patients in the decompensated stage who had not received malotilate treatment and who possessed virtually the same total functioning hepatic cell mass capacities (control group) as determined by the life table method[18]. Significant differences in the cumulative survival rates were evaluated according to the description of Greenwood[19].

Results

Forty eight week observation

During the 48-week malotilate treatment period, serum transaminase activity tendend to decrease. The decreases in serum GOT activity in the malotilate treatment period were statistically significant, except for the 32nd week. On the other hand, serum bilirubin levels and alkaline phosphatase activity did not change due to malotilate treatment. Serum gamma-glutamyl transpeptidase (GGT) activity was increased by malotilate treatment (**Table 2**). Hepatic tests reflecting hepatic protein synthe-

Table 2 Changes brought about by malotilate administration in various hepatic tests (I) in liver cirrhosis patients

(n=39, mean±SE)

Hepatic tests	Malotilate treatment period (weeks)					
	0	8	16	24[(a)]	32	48[(b)]
T-bil. (mg/100ml)	1.21±0.11	1.20±0.10	1.18±0.13	1.28±0.14	1.36±0.14	1.42±0.25
S-GOT (INTL unit/l)	104.4±10.7	84.7±7.45*	84.1±7.57*	92.9±16.4#	86.4±8.51	76.4±7.64#
S-GPT (INTL unit/l)	110.3±14.7	96.5±10.1	93.5±9.29	104.0±18.2	94.0±10.2	87.2±7.64
S-Al P-ase (INTL unit/l)	127.1±14.7	119.5±11.3#	123.7±13.1	127.7±11.7	126.3±10.5	120.6±11.1
S-GGT (INTL unit/l)	66.0±13.7	76.9±14.7#	72.0±13.0#	85.5±18.3	97.6±25.4#	86.8±19.7##

Normal values are less than 1.2 for T-bil (serum total bilirubin), less than 41 for S-GOT (serum glutamic oxaloacetic transaminase), less than 45 for S-GPT (serum glutamic pyruvic transaminase), less than 105 for S-Al P-ase (serum alkaline phosphatase), and than 60 for S-GGT (serum γ-glutamyl transpeptidase), respectively.

*: $p<0.05$, **: $p<0.01$ vs the corresponding 0 week period by Student's t-test.

#: $p<0.05$, ##: $p<0.01$ vs the corresponding 0 week period by the Wilcoxon test.

(a): 38 cases except for T-bil, (b): 34 cases

Table 3 Changes brought about by malotilate administration in various hepatic tests (II) in liver cirrhosis patients

(n=39, mean±SE)

Hepatic tests	Malotilate treatment period (weeks)					
	0	8	16	24[(a)]	32[(b)]	48[(c)]
T-protein (g/100ml)	6.96±0.10	6.99±0.12	7.00±0.09	6.87±0.11	6.75±0.11	6.84±0.10
Albumin (g/100ml)	3.36±0.10	3.40±0.11	3.62±0.15#	3.50±0.10*	3.38±0.10	3.49±0.11
Ch-E (ΔpH)	0.44±0.03	0.48±0.03*,##	0.49±0.03*,#	0.50±0.04*,#	0.47±0.04	0.50±0.04
Normotest (%)	63.5±2.96	70.8±3.96**,##	72.8±3.44***,##	70.9±3.85*,#	71.9±4.28**,#	73.2±4.49*,#
T-cholest (mg/100ml)	153.0±7.35	167.1±7.69***,##	164.7±6.16*,#	165.5±6.14*,#	162.0±6.95	156.0±7.53

Normal values are 6.0-8.0 for T-protein (total protein), 3.5-5.5 for albumin, 0.7-1.2 for Ch-E (choline esterase), and 124-207 for T-cholest (total cholesterol), respectively.

*: $p<0.05$, **: $p<0.01$ ***: $p<0.001$ vs the corresponding 0 week period by Student's t-test.

#: $p<0.05$, ##: $p<0.01$ vs the corresponding 0 week period by the Wilcoxon test.

(a): 38 cases except for 36 cases in Ch-E, (b): 38 cases in T-protein, (c): 34 cases.

Table 4 Changes in hepatic fibrogenesis serum markers in liver cirrhosis patients brought about by malotilate treatment

(n=39, mean±SE)

Serum markers	Malotilate treatment period (weeks)					
	0	8[(a)]	16[(b)]	24[(c)]	32	48[(d)]
P-III-N-P (ng/ml)	21.1±1.26	19.3±1.05	19.8±1.23	19.1±1.19#	18.6±0.93*,#	16.6±1.20**,##
IR-BPH (ng/ml)	112.4±10.7	99.9±8.35##	91.2±7.28##	87.9±4.56*,##	95.8±5.70	84.4±5.45#

Normal values are less than 10.0 for P-III-N-P (procollagen type III N-terminal aminopeptide), and less than 70 for IR-BPH (immunoreactive prolyl hydroxylase beta-subunit), respectively.

*: $p<0.05$, **: $p<0.01$ vs the corresponding 0 week period by Student's t-test.

#: $p<0.05$, ##: $p<0.01$ vs the corresponding 0 week period by the Wilcoxon test.

(a): 37 cases, (b): 36 cases, (c): 37 cases, (d): 34 cases.

sis, such as serum albumin levels, choline esterase activity and Normotest values, but not serum total protein levels, increased significantly after malotilate treatment, particularly during the initial 24 weeks of treatment. Serum cholesterol levels also increased significantly during malotilate treatment (**Table 3**). In all patients, serum P-III-N-P levels were considerably higher than the normal range before malotilate treatment, and the levels decreased significantly after the 24th week of malotilate treatment. Serum IR-BPH levels, which were

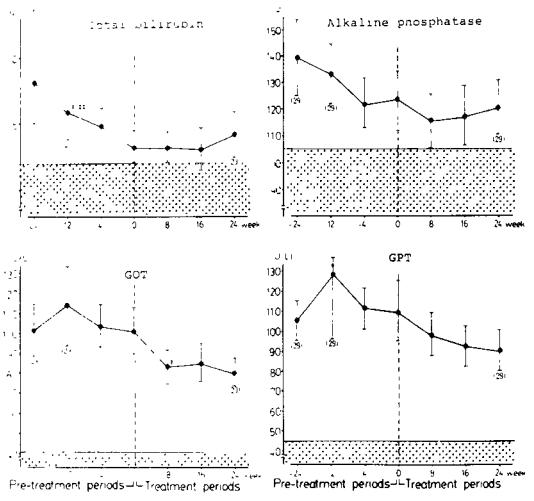

Fig. 1 Serial changes in various hepatic tests (I) in liver cirrhosis during pre- and post-treatment periods with malotilate.

Serum transaminase activity tended to decrease after malotilate treatment.

In this figure and Figs. 2 and 3, the vertical bars represent standard error of the mean and the numbers of examined cases are shown in parentheses, except for 30 cases. The same symbols such as *: $p<0.05$, **: $p<0.01$ in Student's t-test and #: $p<0.05$, ##: $p<0.01$ in the Wilcoxon test, are used. The shaded areas in this figure and Figs. 2 and 3 represent the normal ranges of the values.

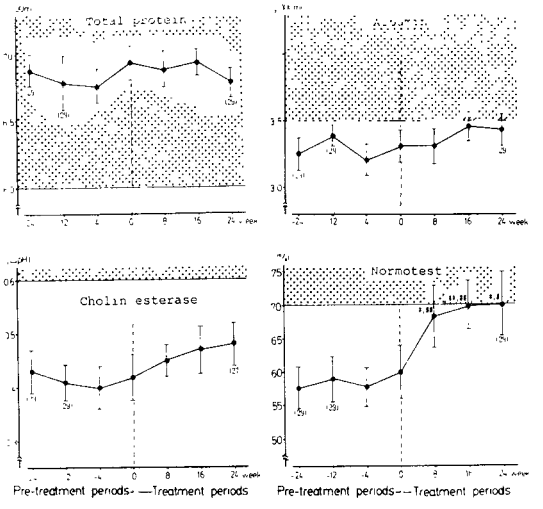

Fig. 2 Serial changes in various hepatic tests (II) in liver cirrhosis during pre- and post-treatment periods with malotilate.

Serum choline esterase activity tended to increase after malotilate treatment. The Normotest values significantly increased after malotilate treatment.

also considerably high before treatment in 29 cases, decreased significantly after the 8th week of malotilate treatment, except for the 32nd week (**Table 4**).

Cross-over study

No hepatic test values, serum P-III-N-P or serum IR-BPH levels, except serum total bilirubin levels and GGT activity, changed significantly during the 24 week basal level pre-treatment observation period. Serum transaminase activity tended to decrease after malotilate treatment, with the change in GOT during the 8th week being statistically significant. Serum total bilirubin levels clearly decreased during the basal pre-treatment period, however, the levels did not change after malotilate treatment. Serum alakaline phosphatase activity did not change significantly during the observation periods, although the activity tended to decrease (**Fig. 1**). Serum total protein and albumin levels did not change significantly during the observation periods. Serum choline esterase activity tended to increase after malotilate treatment, however, the changes were statistically insignificant. The Normotest values were found to be significantly increased after malotilate treatment (**Fig. 2**). Serum total cholesterol levels increased significantly after malotilate treatment. Serum GGT activity decreased during the basal pre-treatment periods and then increased after malotilate administration. Serum P-III-N-P levels were significantly decreased after malotilate treatment and serum IR-BPH levels tended to decrease after malotilate administration, however, the changes were not statistically significant because of the large individual variations (**Fig. 3**). When serum IR-BPH levels were analyzed in 22 cases who showed higher levels than the normal range before malotilate treatment, the change after the 24th week of malotilate treatment was statistically significant (129.3 ± 16.3 vs 97.9 ± 5.6, $p<0.05$).

Survival rates of decompensated cirrhosis

Sixteen patients with liver cirrhosis in the

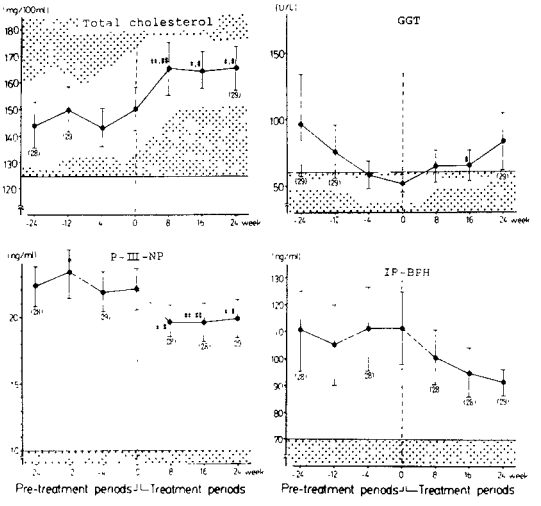

Fig. 3 Serial changes in serum GGT, total cholesterol and hepatic fibrogenesis markers in liver cirrhosis during pre- and post-treatment periods with malotilate.

Serum total cholesterol levels increased and P-III-N-P levels decreased significantly after malotilate treatment. Serum IR-BPH levels tended to decrease after malotilate treatment.

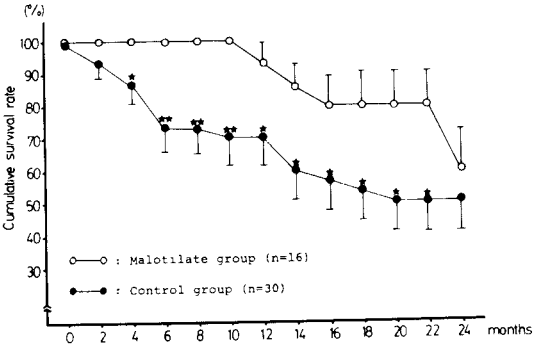

Fig. 4 Cumulative survival rates in decompensated liver cirrhosis.

The rates were significantly better in the malotilate treated group than in the control group. Vertical bars represent the standard error of the mean. *:$p<0.05$, **:$p<0.01$ based on the analysis of Greenwood[19].

decompensated stage were treated with malotilate for 12 to 24 months (malotilate group). Among these patients, one patient died of hepatocellular cancer and 5 of hepatic encephalopathy. No patients died due to gastrointestinal bleeding. Therefore, 30 patients with liver cirrhosis in the decompensated stage, but without hepatocellular cancer and who neither died of gastrointestinal bleeding nor received malotilate, were selected retrospectively as the control group. The cumulative survival rates for 24 months of both groups were compared. The backgrounds, results of the various hepatic tests and the total functioning

Table 5 Backgrounds of the patients with decompensated cirrhosis treated with or without malotilate

Groups	Malotilate (n=16)	Control (n=30)	P-values
Sex (male/female)	4/12	16/14	n.s.
Age: Mean	57.9± 8.2	57.7±10.6	n.s.
(range)	(41-72)	(33-72)	
Etiology			
Alcohol	1 (6.3%)	7 (23.3%)	n.s.
HBV	6 (37.5%)	9 (30.0%)	n.s.
Lupoid type	0	1 (3.3%)	n.s.
Cryptogenic	9 (56.2%)	13 (43.4%)	n.s.
Hepatic tests values			
Albumin (g/100ml)	2.8±0.1	2.6±0.07	n.s.
Bilirubin (mg/100ml)	2.2±0.25	2.6±0.18	n.s.
GOT (unit/l)	115.1±12.6	98.2±11.1	n.s.
GPT (unit/l)	103.0±13.3	77.7±15.1	n.s.
Normotest (%)	41.3±4.3	40.8±2.41	n.s.
Ch-E (delta pH)	0.25±0.015	0.24±0.015	n.s.
BSP R-45 (%)	33.8±2.0	36.2±1.82	n.s.
ICG Rmax (mg/kg/min)	0.28±0.03	0.27±0.027	n.s.
Hepatic volume	439±20.7	458 ±17.7	n.s.
(cm^3/m^2 body surface	n=15	n=20)	

Ch-E: choline esterase, BSP R-15: bromosulfalein retention test for 45 minutes, ICG Rmax: indocyanine green maximum removal rate.

hepatic cell mass values in the patients from both groups are presented in **Table 5**. There were no significant differences in these values between two groups before observation was started. The cumulative 24 month survival rates for both groups are shown in **Figure 4**. The survival rate was significantly better in the malotilate group than in the control group after the 4th month until the 22nd month. By the 24th month, the differences were not statistically significant, because of the small number of cases (only 3) who were followed up to this period in the malotilate group.

Discussion

It was reported that malotilate treatment improved serum albumin and cholesterol levels, serum choline esterase activity and the Normotest values in patients with chronic liver diseases in a large scale double blind control 12-week study[3]. In the present study on liver cirrhosis, during the initial 24 weeks these parameters and serum GOT activity were improved significantly by malotilate administration. However, the changes in albumin, total cholesterol and chline esterase after 32 weeks were not statistically significant, indicating the possibility that metabolic adaptation to protein and cholesterol synthesis in the liver may occur with long-term malotilate treatment. An improvement in the Normotest values, which reflect the total activity of blood coagulation factors II, VII and IX[20], by malotilate treatment was the most prominent finding of the present study, although the changes reported in previous studies[3] were not as prominent. In the cross-over control analysis, virtually the same effects of malotilate, except for albumin levels, were observed, although the changes were somewhat slight. These results indicate that the changes in various hepatic tests observed with long-term malotilate administration are attributed to the direct effects of malotilate. The results of the previous report[3] were confirmed by the present study.

Since P-III-N-P is cleaved by an endopeptidase from procollagen when it is polymerized into collagen fiber and then appears in the blood, it has been suggested that the measurement of this peptide in serum serves as an indicator of fibrogenesis[7,8]. Many reports have found a correlation between serum P-III-N-P values and hepatic fibrosis[7-10]. The formation of hydroxyproline from proline by prolyl hydroxylase is a key step in the biosynthesis of collagen due to the critical role of hydroxyproline in maintaining the stability of the triple helix in the collagen molecule[21,22] and the activity of prolyl hydroxylase correlates well with the rate of collagen synthesis in many tissues and cells[23,24]. However, serum activity can not function as a marker of hepatic fibrogenesis because prolyl hydroxylase is cleaved into its subunits which subsequently lose their activity in the serum very quickly[12,14]. Recently, an enzyme immunoassay method for detecting serum prolyl hydroxylase beta-subunit, which is stable in serum, using monoclonal antibodies was developed by Yoshida et al[14]. It has been reported that serum IR-BPH levels correlate well with hepatic fibrogenesis[12,25]. In the present study, both serum P-III-N-P and IR-BPH levels were decreased significantly by malotilate treatment. Decreases in the fibrogenesis serum markers were also confirmed by cross-over control analysis. These results indicate that long-term malotilate administration decreases collagen neosynthesis in the cirrhotic liver. Although, at the present time, it is unclear whether malotilate inhibits collagen neosynthesis directly or indirectly, the changes in the serum markers of fibrogenesis were gradual and the improvements in the various hepatic tests preceded the changes in the hepatic fibrogenesis serum markers. These results strongly imply that malotilate may decrease the fibrogenetic stimulus by reducing chronic inflammation in the liver. Serum P-III-N-P levels are not only related to collagen neosynthesis, but also to the degradation of collagen fibers[8]. Serum IR-BPH levels are more selectively related to collagen neosynthesis[25]. However, both markers were clearly decreased

by malotilate treatment.

The present study has shown that long-term malotilate treatment improved not only various hepatic test values, but also the levels of the serum markers of hepatic fibrogenesis, indicating that the effects of malotilate are not simply biocosmetic, but rather that they may be related to an improvement in the basal hepatic changes of cirrhosis. When the survival rates of cirrhotic patients whose hepatic functions were severely impaired were compared according to whether or not they had received malotilate, patients in the malotilate-treated group had significantly better survival rates than the control patients. These results indicate that malotilate also inhibits the inflammatory processes of chronic liver disease as well as improving their prognosis. In the present study, the number of female patients with decompensated liver cirrhosis was greater in the malotilate group than in the control group; therefore, the final conclusion on the effects of malotilate on the prognosis of liver cirrhosis may wait until sex difference becomes minimum. However, the difference of sex between both groups was statistically insignificant in the present study.

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