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A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C

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#### Keywords

alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, ursodeoxycholic acid, hepatitis C

#### List of Abbreviations

CH-C, chronic hepatitis C; HCV, hepatitis C virus; UDCA, ursodeoxycholic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase

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## ABSTRACT

**Background:** Combined pegylated interferon and ribavirin has improved chronic hepatitis C (CH-C) therapy, however sustained virologic response is achieved in about half of patients with a 1b genotype infection. We assessed oral ursodeoxycholic acid (UDCA) on serum biomarkers as a possible treatment for interferon nonresponders.

**Methods:** CH-C patients with elevated alanine aminotransferase (ALT) were assigned randomly to 150 (n = 199), 600 (n = 200), or 900 mg/day (n = 197) UDCA intake for 24 weeks. Changes in ALT, aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) were assessed. This study is registered at ClinicalTrial.gov, NCT00200343.

**Results:** ALT, AST, and GGT decreased at week 4 then remained constant during drug administration. The median changes (respectively 150, 600, and 900 mg/day) were: ALT, -15.3, -29.2, and -36.2%; AST, -13.6, -25.0, and -29.8%; GGT, -22.4, -41.0, and -50.0%. These biomarkers decreased significantly less in the 150 mg/day than in the other two groups. Although changes in ALT and AST did not differ between the 600 and 900 mg/day groups, GGT was significantly lower in the 900 mg/day group. In subgroup analysis, ALT decreased significantly in the 900 mg/day group when the baseline GGT exceeded 80 IU/L. Serum HCV-RNA did not change in any group. Adverse effects were reported by 19.1% of the patients, with no differences between groups.

**Conclusions:** A 600 mg/day UDCA dose was optimal to decrease ALT and AST levels in CH-C patients. The 900 mg/day dose decreased GGT levels further, and may be preferable in patients with prevailing biliary injuries.

## INTRODUCTION

Chronic hepatitis C (CH-C) is a common liver disease worldwide. The prevalence of hepatitis C virus (HCV) infection increased recently in several countries,[1] and now results in a growing incidence of HCV-related hepatocellular carcinomas.[2, 3] Following the discovery of HCV, interferon therapy was established as the only treatment to eliminate the viral infection. The introduction of combination therapy with pegylated interferon and ribavirin has substantially enhanced the efficacy of antiviral therapy.[4,5] However, the HCV genotype 1b, the major genotype in Japan, is refractory even to this combination therapy and shows sustained virologic response rates of about 50%. Moreover, interferon therapy is sometimes contraindicated or ended early due to hematological, psychological and other complications.

Ursodeoxycholic acid (UDCA) is a hydrophilic stereo-isomer of chenodeoxycholic acid that was used first to dissolve cholesterol gallstones and recently to treat primary biliary cirrhosis.[6,7] In 1985, Leuschner et al. reported a decrease in serum aminotransferase levels in patients with HBV-negative chronic hepatitis who were given UDCA for concomitant gallstones.[8] Traditional Chinese medicine uses ursine bile for liver diseases. It contains plentiful UDCA and inspired the chemical name. Semi-synthetic UDCA became commercially available in Japan in 1957 and has been used since then for chronic liver diseases. In 1994, Takano et al. reported a randomized, controlled-dose study of UDCA for CH-C: 57 patients were assigned randomly to take 150, 600, or 900 mg/day of UDCA and compared with 17 control patients.[9] They showed that serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) decreased less with 150 mg/day, the dose recommended by Japanese national health insurance policy at that time, than with 600 mg/day or 900 mg/day, while the effects of latter two doses were similar. Although the effects of UDCA on fibrosis progression rates have not been established, the strong association between serum ALT levels and fibrosis progression rates has been well documented.[10,11] and it can be speculated that decreased ALT level is associated with delayed fibrosis progression. Thus, the present study was conducted primarily as a dose-finding trial, using the changes in ALT levels as the primary endpoint.

## PATIENTS AND METHODS

### Patients

Patients with CH-C who were 20 years or older and tested positive for HCV-RNA or HCV core proteins were recruited as candidates for this study. They were observed for 8 weeks prior to administration of the drug, and those who showed ALT of 61 IU/L or higher in week 4 were enrolled. Patients were excluded from the study if they had received antiviral treatment (interferon with or without ribavirin) within 20 weeks before the observation period or were treated with corticosteroids, immunosuppressive

drugs, glycyrrhizic acid, cholestyramine, or other drugs that may affect liver function or interfere with UDCA metabolism. Patients were also excluded if they were 1) suffering from decompensated cirrhosis, viral hepatitis other than hepatitis C, autoimmune liver disease, alcoholic or drug-induced liver injury, malignant tumor, biliary disorder, fulminant hepatitis, or peptic ulcer; 2) required hospitalization for cardiac, renal or pancreatic diseases; 3) pregnant or lactating; 4) alcohol dependent or drinking alcohol more than approximately 22 g/day; 5) participants in another clinical study within 4 weeks before the observation period; or 6) sensitive to UDCA or other bile acid preparations.

The protocol was approved by the ethics committee of each clinical study institution. Patients were informed of details of the clinical study and agreed to participate. We conducted this clinical study in accordance with the Declaration of Helsinki and Good Clinical Practice.

### **Study design**

After the 8-week observation period patients were treated with oral (prandial) UDCA (Urso, Mitsubishi Pharma. Co., Ltd, Osaka, Japan) for 24 weeks at 150, 600 or 900 mg/day, divided into 3 doses, under double-blind conditions. Double blinding used placebo, 50 and 100 mg tablets identical in appearance as the test drug. The UDCA doses were established from a previous clinical study of UDCA in patients with CH-C.[9] Concomitant use of drugs and therapies included in the exclusion criteria were prohibited throughout the observation and treatment periods.

Changes in serum ALT levels were previously reported to be -26 and -25.5% with 600 and 900 mg/day of UDCA, respectively, compared to untreated control and no significant changes were observed with 150mg/day.[9] Based on these data, we assumed a standard deviation of 30% for percent changes in ALT, and the necessary sample size was calculated to be 200 in each group to detect any superiority of the 600 and 900 mg/day doses over 150 mg/day at a significance level of 0.05 and a power of 0.9.

We enrolled patients who met all criteria and gave written informed consent between July 2002 and May 2004 in 62 institutions with liver clinics throughout Japan. Each patient was assigned randomly to one of the three dose groups by using numbered containers provided based on a permuted block method (block size: 6).

When treatment or evaluation was discontinued because of a patients' request, aggravation of symptoms, adverse events or other reasons, prior data were included in the evaluation as final observation data.

To investigate long-term effects of UDCA, the protocol included an option for additional UDCA administration for 28 weeks at minimum and 80 weeks at maximum (total 52 - 104 weeks including the initial 24 weeks) if ALT level decreased by at least 15% at Week 20 compared to the baseline. In the additional period, the double-blind

setting was discontinued and the dose of 600 mg/day was adopted, which could be increased to 900 mg/day by the decision of each patient and responsible physician. Patients who entered the additional phase could discontinue UDCA administration anytime after Week 52.

### **Laboratory tests**

Blood was collected every 4 weeks from the start of observation period to the end of drug administration. Serum ALT was measured as a primary endpoint of liver function, and AST and GGT as secondary endpoints, using conventional methods. Blood samples taken at the start of observation, at 0, 4 and 12 weeks of treatment, and at the final observation were analyzed to determine leukocyte and erythrocyte counts, hemoglobin, hematocrit, thrombocyte count, and the levels of ALT, AST, GGT, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, cholinesterase, total bilirubin, direct bilirubin, total cholesterol, urea nitrogen, creatinine, Na, K, and Cl.

For bile acid composition analysis, blood was collected at the start of treatment and the final observation in a fasted condition. Serum total bile acid was measured by the 3 $\alpha$ -hydroxysteroid dehydrogenase method. Bile acid fractions were determined by a specific liquid chromatography-electrospray mass spectrometry, using an HPLC system (Agilent 1100 series, Agilent Technologies, CA, USA) equipped with a C18 cartridge (CAPCELL PAK C18 UG120A, Shiseido, Tokyo, Japan) and a mass spectrometer (Quattro Ultima, Micromass Technologies, Manchester, UK).

Serum HCV-RNA level was measured prior to treatment and at the final observation by a reverse transcriptional polymerase-chain-reaction method.

All analyses and measurements were performed in a single contract laboratory (SRL, Inc., Tokyo, Japan).

### **Statistical analysis**

Patients' backgrounds were compared among the three dose groups by chi-square and ANOVA. Changes in serum ALT, AST, and GGT levels due to UDCA administration were compared among the groups by repeated-measure ANOVA. Differences between groups were tested by using linear contrasts. Subgroup analyses of median changes in serum ALT at the final observation, relative to the pre-treatment levels, were performed according to gender, body weight, and pre-treatment serum GGT level with Wilcoxon signed-ranks tests. Changes in bile acid and serum HCV-RNA levels were analyzed by paired Student's *t*-test. Fischer's exact probability test was applied to the incidences of adverse reactions. P value < 0.05 in a two-tailed test was considered significant. Analyses were done on full analysis set. This study is registered at ClinicalTrial.gov, number NCT00200343, and compliant with the published CONSORT guidelines for performance and publication of clinical trials.[12]

## RESULTS

### Patients

We enrolled 596 patients, and 199 received UDCA at 150 mg/day, 200 at 600 mg/day, and 197 at 900 mg/day. Safety was evaluated in all patients as adverse events based on signs and symptoms and abnormal laboratory test results. Efficacy was evaluated in 586 patients (195, 150 mg/day; 198, 600 mg/day; and 193 at 900 mg/day), excluding 10 who lacked sufficient data. At the end of 24 weeks administration, 392 patients were eligible for additional long-term administration. Of these patients, 280 chose to participate in the study and others refused mainly because of lack of time. 23 patients discontinued before Week 52, one of them for biochemical relapse, and other 10 patients violated protocol. The effects of long-term administration were evaluated among the remaining 247 patients (**Figure 1**).

Patients' backgrounds are summarized in **Table 1**. Differences observed in gender, body weight, treatment history with interferon between the three groups are indicated ( $P < 0.15$ ).

**Table 1.** Characteristics of patients with chronic hepatitis C treated with UDCA (full analysis set)

	150 mg/day (n=195)	600 mg/day (n=198)	900 mg/day (n=193)	P-value
Gender				
Male	97 (49.7%)	117 (59.1%)	123 (63.7%)	0.018
Female	98 (50.3%)	81 (40.9%)	70 (36.3%)	
Age (years)	58.0 ± 12.2	57.7 ± 12.0	59.8 ± 10.1	0.152
Height (cm)	160.1 ± 9.5	161.9 ± 9.2	160.8 ± 8.7	0.163
Weight (kg)	58.8 ± 11.4	61.8 ± 11.2	61.6 ± 11.9	0.017
ALT (IU/L)	109.2 ± 49.7	106.3 ± 59.4	110.6 ± 57.3	0.745
AST (IU/L)	84.0 ± 39.1	82.4 ± 41.8	85.2 ± 45.0	0.796
GGT (IU/L)	87.5 ± 73.0	82.4 ± 62.2	85.9 ± 66.3	0.744
Previous interferon treatment				
Absent	119 (61.0%)	100 (50.5%)	96 (49.7%)	0.044
Present	76 (39.0%)	98 (49.5%)	97 (50.3%)	

Data represent the number of patients or mean ± SD.

### Changes in ALT, AST and GGT

Serum ALT, AST and GGT levels before and during treatment are shown in **Figures 2 to 4**. The responses of ALT, AST and GGT over time were greater for 600 and 900 mg/day administration compared to 150 mg/day (ALT,  $P < 0.001$  and  $P = 0.021$ ; AST,  $P < 0.001$  and  $P < 0.001$ ; GGT,  $P < 0.001$  and  $P < 0.001$ , respectively). No difference was observed between the 600 and 900 mg/day groups in ALT ( $P = 0.926$ ) and AST ( $P = 0.429$ ), but GGT differed significantly ( $P < 0.001$ ). Serum ALT, AST and GGT levels decreased by 4 weeks into treatment and remained constant. Serum ALT, AST and GGT levels at the final observation, together with median changes relative to 0 week (baseline), are shown in **Table 2**. The mean decreases in serum ALT levels from the baseline value were 13.4, 30.6 and 29.3 IU/L in the 150, 600 and 900 mg/day groups, respectively. The median changes in ALT at the final observation were -15.3%, -29.2% and -36.2% in the corresponding groups (**Table 2**).

**Table 2.** Serum ALT, AST and GGT levels in patients with chronic hepatitis C after treatment with UDCA

	Dose (mg/day)	Pre-treatment	Post-treatment	Change (%)
		Mean $\pm$ SD	Mean $\pm$ SD	Median (Range)
ALT (IU/L)	150	109.2 $\pm$ 49.7	95.8 $\pm$ 60.2	-15.3 (-80.7 – 375.9)
	600	106.3 $\pm$ 59.4	75.7 $\pm$ 41.9	-29.2 (-88.3 – 95.2)
	900	110.6 $\pm$ 57.3	81.3 $\pm$ 90.5	-36.2 (-81.4 – 1696.9)
AST (IU/L)	150	84.0 $\pm$ 39.1	75.5 $\pm$ 43.6	-13.6 (-74.2 – 347.2)
	600	82.4 $\pm$ 41.8	63.1 $\pm$ 32.9	-25.0 (-82.7 – 72.5)
	900	85.2 $\pm$ 45.0	65.5 $\pm$ 49.6	-29.8 (-79.0 – 1026.1)
GGT (IU/L)	150	87.5 $\pm$ 73.0	70.4 $\pm$ 58.3	-22.4 (-74.6 – 145.9)
	600	82.4 $\pm$ 62.2	49.7 $\pm$ 43.0	-41.0 (-81.1 – 153.1)
	900	85.9 $\pm$ 66.3	43.8 $\pm$ 44.8	-50.0 (-80.1 – 213.9)

The mean decreases in serum AST levels from the baseline value were 8.5, 19.3 and 19.7 IU/L in the 150, 600 and 900 mg/day groups, respectively. The mean decreases in serum GGT levels from the baseline value were 17.1, 32.7 and 42.1 IU/L in the 150, 600 and 900 mg/day groups, respectively.

### Long-term effects

The decreases in ALT, AST, GGT levels from the baseline value were maintained during long-term administration of UDCA, as shown in **Table 3**.

**Table 3.** Serum ALT, AST and GGT levels in patients with chronic hepatitis C during long-term administration of UDCA

	Pre-treatment	Treatment period		
	Week 0	Week 24	Week 48	Week 104
No. of patients	247	242*	243**	149***
ALT (IU/L)	114.8 ± 54.1	70.7 ± 37.4	67.9 ± 36.3	63.5 ± 31.9
AST (IU/L)	86.6 ± 41.7	59.0 ± 31.5	56.6 ± 27.4	54.1 ± 23.7
GGT (IU/L)	87.3 ± 67.6	49.5 ± 42.6	47.3 ± 40.5	41.8 ± 30.1

Data are expressed as mean ± SD.

Corresponding data missing in 5 patients\* and in 4 patients\*\*

Administration between Week 52 and Week 104 was optional and 149 patients opted for the maximum term.\*\*\*

### Subgroup analyses

The decrease in serum ALT was significantly larger in the 600 and 900 mg/day groups than in the 150 mg/day group for most subgroups by gender, body weight, or baseline serum GGT levels (**Table 4**). Although the difference between the 600 and 900 mg/day groups as a whole was not significant, the subgroup of baseline GGT ≥ 80 IU/L showed significantly lower level of GGT with 900 mg/day administration (P = 0.004).

**Table 4.** Subgroup analyses of change in serum ALT in patients with chronic hepatitis C after treatment with UDCA

	Dose (mg/day)	No. of patients	Change (%)	P-value		
			Median (Range)	vs 150 mg	vs 600mg	
Gender	Male	150	97	-14.9 (-80.7 – 375.9)		
		600	117	-33.1 (-88.3 – 93.1)	< 0.001	
		900	123	-36.4 (-79.1 – 1696.9)	< 0.001	0.430
	Female	150	98	-18.0 (-79.0 – 175)		
		600	81	-25.0 (-74.7 – 95.2)	0.058	
		900	70	-35.8 (-81.4 – 315.3)	0.002	0.076
Body weight (kg)	< 60	150	115	-14.9 (-80.7 – 375.9)		
		600	82	-28.6 (-74.7 – 95.2)	0.002	
		900	91	-35.2 (-81.4 – 315.3)	0.001	0.356

	≥ 60	150	80	-16.7 (-73.4 – 166.1)		
		600	116	-30.3 (-88.3 – 93.1)	0.003	
		900	102	-36.6 (-77.1 – 1696.9)	< 0.001	0.096
GGT (IU/L)	≤ 39	150	45	-14.5 (-73.4 – 71.4)		
		600	39	-32.7 (-62.9 – 93.1)	0.049	
		900	45	-26.6 (-81.4 – 1696.9)	0.112	0.616
	40 – 79	150	79	-15.2 (-69.1 – 175)		
		600	90	-30.3 (-74.7 – 95.2)	0.001	
		900	70	-36.3 (-77.7 – 200)	< 0.001	0.633
	≥ 80	150	71	-18.2 (-80.7 – 375.9)		
		600	69	-28.6 (-88.3 – 53.8)	0.057	
		900	78	-41.2 (-79.1 – 119.3)	< 0.001	0.004

P-values refer to Wilcoxon signed-ranks tests.

### Bile acid in serum

Total bile acid concentration in serum increased dose-dependently from the start of drug administration to the final observation, as shown in **Table 5**. The ratio of UDCA to total bile acid was increased significantly in all groups at the final observation compared to baseline. The ratio of UDCA at the final observation was similar in the 600 and 900 mg/day groups. The proportion of less hydrophilic bile acids was decreased accordingly. The proportion of chenodeoxycholic acid at the final observation decreased significantly in all groups, and was similar in the 600 and 900 mg/day groups. The proportions of cholic acid and deoxycholic acid were also decreased significantly compared to the baseline.

**Table 5.** Composition of serum bile acid in patients with chronic hepatitis C treated with UDCA

	Dose (mg/day)	Before Treatment	After Treatment	P-value
Total bile acid	150	8.63 ± 9.76	13.69 ± 19.28	< 0.001
concentration	600	9.42 ± 12.04	21.89 ± 24.20	< 0.001
(µmol/L)	900	9.17 ± 9.30	28.74 ± 39.78	< 0.001
Cholic acid	150	17.69 ± 10.33	11.35 ± 7.08	< 0.001
(%)	600	17.75 ± 10.35	5.93 ± 4.53	< 0.001

	900	18.15 ± 9.54	5.14 ± 4.19	< 0.001
Deoxycholic acid	150	21.62 ± 16.24	13.84 ± 11.39	< 0.001
(%)	600	19.86 ± 16.84	6.50 ± 7.06	< 0.001
	900	18.74 ± 15.29	5.68 ± 6.58	< 0.001
Chenodeoxycholic acid	150	54.46 ± 14.12	39.93 ± 11.61	< 0.001
(%)	600	55.37 ± 13.95	24.66 ± 10.01	< 0.001
	900	55.95 ± 13.65	23.31 ± 12.72	< 0.001
Ursodeoxycholic acid	150	5.93 ± 8.72	34.25 ± 13.75	< 0.001
(%)	600	6.70 ± 9.72	62.26 ± 13.69	< 0.001
	900	6.83 ± 10.6	65.12 ± 16.84	< 0.001
Lithocholic acid	150	0.30 ± 0.99	0.62 ± 1.66	0.010
(%)	600	0.33 ± 1.23	0.66 ± 1.35	0.010
	900	0.33 ± 1.12	0.75 ± 1.49	0.001

Data are expressed as mean ± SD. P-values refer to paired t-test (before vs. after treatment).

### Virus load

HCV-RNA levels (mean ± SD) changed from the baseline of 1477 ± 1280 to 1366 ± 1224 kIU/mL in the 150 mg/day group, from 1463 ± 1299 to 1358 ± 1233 kIU/mL in the 600 mg/day group, and from 1553 ± 1318 to 1552 ± 1398 kIU/mL in the 900 mg/day group. None of those changes were significant.

### Safety

The observed adverse reactions with possible association to UDCA administration are collected in **Table 6**. The overall incidences of adverse reactions were 18.1%, 21.5% and 17.8% in the 150, 600 and 900 mg/day groups, respectively, with no significant difference between the groups. Diarrhea was reported most often. No severe adverse reactions were seen.

**Table 6.** Summary of adverse reactions

	150 mg/day	600 mg/day	900 mg/day
Overall incidence	18.1% (36/199)	21.5% (43/200)	17.8% (35/197)
Total number of adverse reactions	44	62	45
Common adverse reactions*	Number of patients (%)		
Abdominal distension	2 (1.0)	2 (1.0)	2 (1.0)
Upper abdominal pain	2 (1.0)	4 (2.0)	2 (1.0)
Constipation	3 (1.5)	4 (2.0)	2 (1.0)
Diarrhea	7 (3.5)	8 (4.0)	8 (4.1)
Dyspepsia	3 (1.5)	2 (1.0)	2 (1.0)
Loose stool	1 (0.5)	6 (3.0)	5 (2.5)
Stomach discomfort	2 (1.0)	2 (1.0)	3 (1.5)
Pruritus	3 (1.5)	3 (1.5)	2 (1.0)

\* The adverse reactions which were observed in 1% or more of the patients.

## DISCUSSION

UDCA is frequently used for cholestatic liver diseases, primary biliary cirrhosis in particular. UDCA improves biochemical indices such as serum GGT, ALT, and bilirubin. Histopathologic improvements have been shown[13] and prolonged survival reported.[14,15] Although its effect on survival remains controversial,[16,17] UDCA is the only approved medication for primary biliary cirrhosis. Suggested mechanisms for UDCA include reducing the cytotoxicity of hydrophobic bile acids, stimulation of hepatobiliary secretion, and anti-apoptosis.[18]

UDCA was used to decrease serum aminotransferases levels for so-called non-A non-B chronic hepatitis before the discovery of HCV.[8,19,20] Takano *et al.*, restricted their study to CH-C patients and found 600 mg/day as an optimal dose of UDCA.[9] The reduction was greater in GGT (40.5%) than in ALT (26.0%), as observed also in the current study. The reported effect of UDCA was stronger among CH-C patients with morphologic bile duct injury,[21] and UDCA administration was accompanied by histologic improvement of biliary lesions, but not of hepatitis.[22] These data suggest that UDCA may act on the biliary system in CH-C through enhanced bile formation and/or modification of bile acid compositions. In fact, bile duct injury is characteristic of CH-C, though not specific.[23] In this study, the changes in bile acid compositions were similar in the 600 and 900 mg/day groups but smaller in the 150 mg/day group, and this may have been associated with the changes in serum biomarkers.

Nakamura *et al.* reported that UDCA had a greater effect among CH-C patients with autoimmune characteristics, i.e., high immunoglobulin G concentration or positive anti-nuclear or anti-smooth muscle antibodies,[24] which suggests involvement of immunomodulative mechanisms. Indeed, studies *in vitro* have shown

that UDCA suppresses NF-kappaB-dependent transcription by binding to the glucocorticoid receptor[25] and decreases proinflammatory cytokine-induced transcription of phospholipase A2.[26] These mechanisms may act cytoprotectively *in vivo*. The choleric and cytoprotective mechanisms are not necessarily mutually exclusive.

We examined the effect of UDCA on CH-C in terms of serum biochemical markers in a large-scale, double-blind investigation. We confirmed that a dose of 600 mg/day, i.e., 10 mg/kg body weight on average, was more effective than 150 mg/day while adverse effects remained similar and minimal. The doses of 600 and 900 mg/day induced similar decreases in serum ALT and AST. Consequently, it appears that 600 mg/day is the preferred dose of UDCA, assuming that serum transaminases levels reflect the degree of hepatocellular damage.

The decrease in serum GGT did differ significantly between 600 and 900 mg/day administration of UDCA. In contrast to the decrease in ALT or AST, that of serum GGT may represent improved cholestasis from biliary injury in CH-C. Although the importance of biliary injury in CH-C is unclear, it is possible that a 900 mg/day dose has additional benefits compared to 600 mg/day, as the incidence of adverse effects did not differ between the two doses. It is of interest that the decrease in ALT was significantly different between the two doses in patients with high baseline GGT levels (**Table 4**).

The long-term effects of UDCA therapy in CH-C patients are yet to be elucidated. Changes in liver histology by UDCA administration are not evident in short-term observations. However, it is possible that delayed progression of fibrosis by UDCA can be revealed only by much longer-term observation, because the fibrosis progression in the natural course of CH-C is usually slow, taking decades to establish cirrhosis.[27,28] The effect of UDCA lasted for at least 104 weeks without attenuation (**Table 3**).

In the natural course of CH-C those patients with normal serum aminotransferase levels show slow fibrosis progression[29] and a low incidence of hepatocellular carcinoma.[30,31] By multivariate analysis, the risk of hepatocellular carcinoma after interferon treatment without virologic response was shown to be 0.26, 0.36, and 0.91 in patients whose ALT levels were normal, moderately elevated (less than twice the upper normal limit), and highly elevated, respectively, compared to untreated patients. It may be that when UDCA lowers serum ALT levels the risk of hepatocellular carcinoma is decreased. A retrospective study showed that hepatocellular carcinoma developed within 5 years in 10 of 56 patients (18%) from onset of HCV-related early cirrhosis who took UDCA and 18 of 46 patients (39%) who did not.[32] Interestingly, ALT levels similar in the two groups, possibly because UDCA was likely to be prescribed to those patients with high baseline ALT levels. Although these data were obtained from a non-randomized, retrospective study, it suggests that UDCA may

provide cancer protective effects independent of decreasing ALT.

In summary, we confirmed, in a large-scale, double-blind study, that a UDCA dose of 600 mg/day was optimal to decrease serum ALT and AST levels in CH-C patients without serious adverse effects. A dose of 900 mg/day resulted in additional decreases in serum GGT levels, and may be preferred in cases with prevailing biliary injuries. Long-term effects of UDCA administration on prognosis, hepatocarcinogenesis in particular, remain to be investigated in future studies.

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**Statement of competing interests:** none to declare

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## Figure legends

Figure 1. Trial profile.

Figure 2. Changes in serum ALT levels in patients with chronic hepatitis C before and during the treatment period.

Data are expressed as mean  $\pm$  SD. Open circles, 150 mg/day; filled circles, 600 mg/day; open triangles, 900 mg/day; \*,  $P < 0.01$ , paired t-test (vs. week 0). P-values refer to repeated measures ANOVA.

Figure 3. Changes in serum AST levels in patients with chronic hepatitis C before and during the treatment period.

Data are expressed as mean  $\pm$  SD. Open circles, 150 mg/day; filled circles, 600 mg/day; open triangles, 900 mg/day; \*,  $P < 0.01$ , paired t-test (vs. week 0). P-values refer to repeated measures ANOVA.

Figure 4. Changes in serum GGT levels in patients with chronic hepatitis C before and during the treatment period.

Data are expressed as mean  $\pm$  SD. Open circles, 150 mg/day; filled circles, 600 mg/day; open triangles, 900 mg/day; \*,  $P < 0.01$ , paired t-test (vs. week 0). P-values refer to repeated measures ANOVA.

Figure 1.

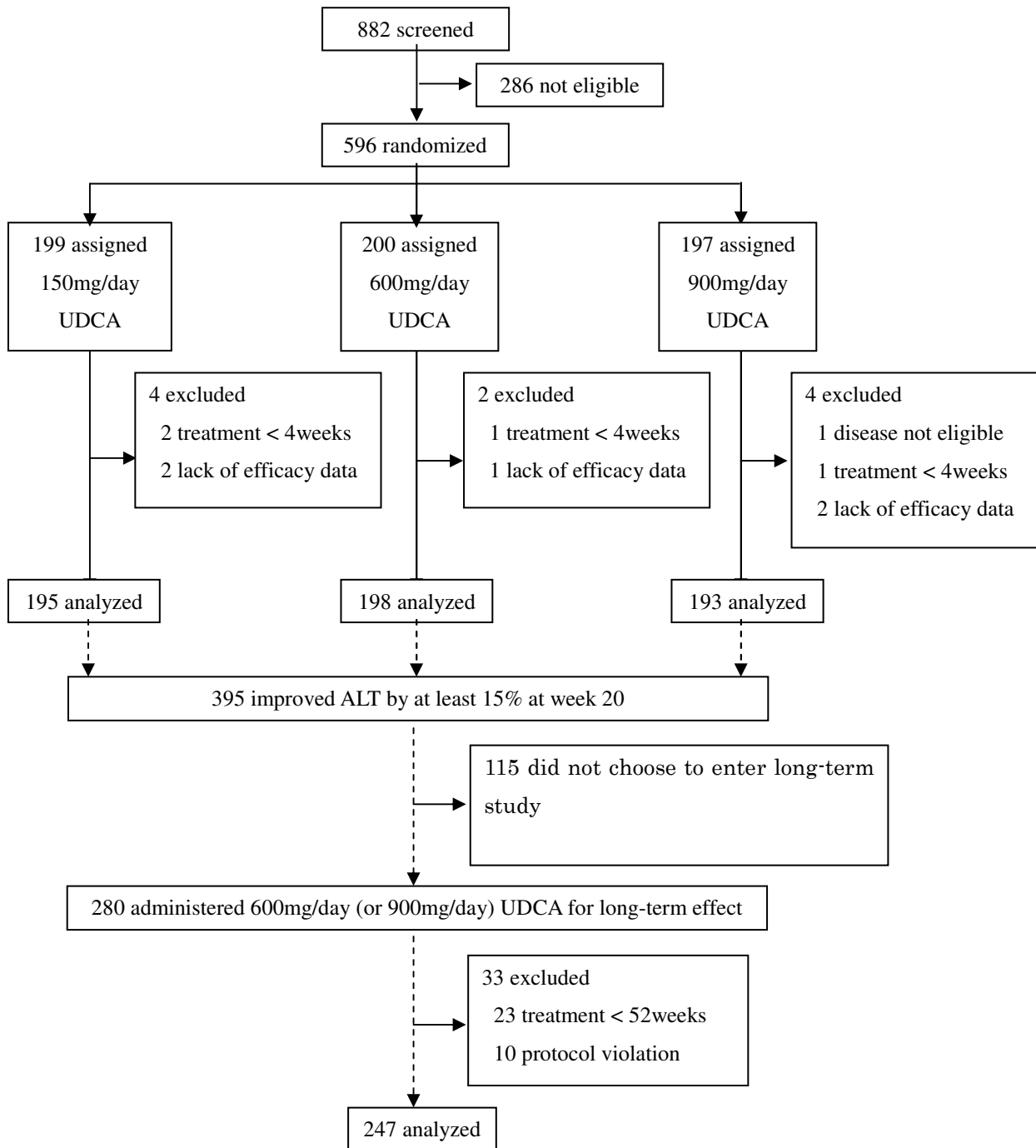


Figure 2.

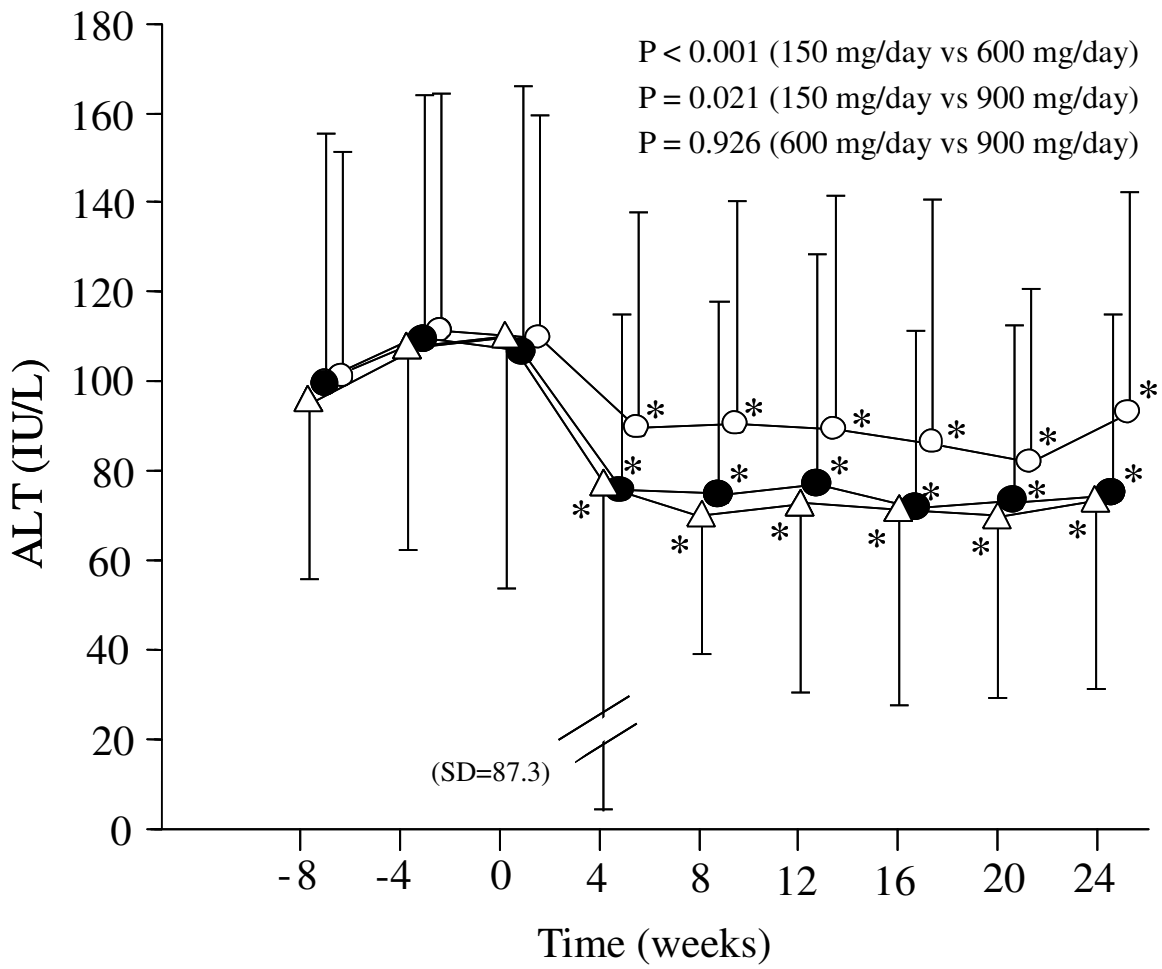


Figure 3.

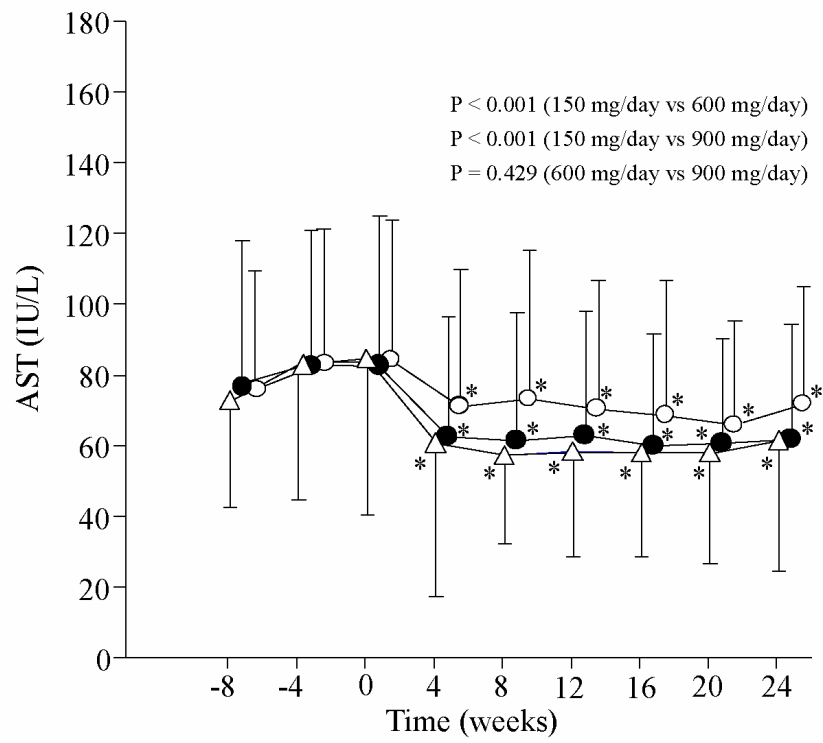


Figure 4.

