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—Original Article—

STEROL AND BILE ACID METABOLISM AFTER SHORT-TERM PREDNISOLONE TREATMENT IN PATIENTS WITH CHRONIC ACTIVE HEPATITIS

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Summary

Serum levels of squalene, cholesterol and bile acid were measured before and after short-term prednisolone administration in patients with chronic active hepatitis. Comparison with normal controls indicated that serum bile acid levels were increased significantly ($p<0.01$) in patients with chronic active hepatitis, but serum levels of squalene and cholesterol did not differ significantly between the two groups. After short-term prednisolone treatment, serum levels of squalene and cholesterol were increased significantly ($p<0.01$) as compared with the pretreatment level. On the other hand, while serum fasting bile acid levels were found to be increased significantly ($p<0.01$), serum clearance after oral administration of ursodeoxycholic acid improved significantly ($p<0.05$) after treatment. These results indicated that short-term prednisolone treatment increases sterol metabolism in the liver in patients with chronic active hepatitis, resulting from an increase in hepatic clearance of bile acids.

Key Words: *Squalene, Bile acid, Prednisolone, UDCA tolerance test.*

Introduction

Cholesterol synthesized in the liver is exchangeable with plasma cholesterol, partly as a bile acid precursor[1,2]. A disturbance in the cholesterol turnover rate has been reported in liver disease[3], and glucocorticoid treatment increases the serum cholesterol level. The mechanism involved was considered to be increased cholesterol synthesis in the liver[4-6]. Although glucocorticoid is occasionally administered to patients with chronic active hepatitis, there are few reports concerning its effect on sterol and bile acid metabolism in the same patient.

We reported previously that the serum squalene level may indicate the cholesterol synthesis rate in liver disease[7,8]. In the present study, serum squalene, cholesterol and bile acid levels were determined in patients with chronic active hepatitis before and after prednisolone treatment, and in addition oral ursodeoxycholic acid (UDCA) loading test[9] was carried out.

Materials and Methods

The study was carried out on 10 patients with chronic active hepatitis and 15 healthy

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subjects as controls. The patients with chronic active hepatitis consisted of 7 males and 3 females, aged 17 to 64 (mean, 37 years), and the controls were 10 males and 5 females, aged 25 to 40 (mean 30 years). All cases of chronic active hepatitis were diagnosed by laparoscopy and liver biopsy. HBsAg was measured by redioimmunoassay and detected in 2 of the patients. All patients with chronic active hepatitis were treated with prednisolone 20-30 mg/day orally for a period of 3 weeks or more, for a mean duration of 31 days. Blood samples were collected before the treatment early in the morning following overnight fast and after treatment. During the treatment no abnormality in glucose tolerance test was detected.

Serum cholesterol and routine biochemical tests were measured by a Technicon Autoanalyzer (SMA-60/12). Serum squalene was measured as previously described[7]. Briefly, the serum was saponified at 70°C for 2 hours and the nonsaponified material was extracted using petroleum ether. Next, the extract was transferred to an aluminum column and eluted with petroleum ether. Squalene was measured by a Shimazu GC-5A gas chromatograph with a hydrogen flame ionization detector. Measurement of serum bile acid was performed according to the enzymatic and fluorimetric method[10]. Serum bile acid was extracted on Amberlite XAD-2. Under the presence of 3α-hydroxysteroid dehydrogenase (Nyegaad & Co. Oslo), NADH formed from bile acid and NAD was measured at 460 nm with excitation at 334 nm using A Hitachi model 204 fluorospectrometer.

UDCA (Tokyo Tanabe Pharm. Co., Tokyo, Japan) was more than 98% pure as determined by gas-liquid chromatography. Oral UDCA loading test was carried out following an overnight fast of 12-14 hours[4]. After taking an initial blood sample from the antecubital vein to determine the fasting value, 500 mg of UDCA in 200 ml of water was given to the patient, and blood samples were collected every half hour up to 120 minutes. Sera were separated and stored at 20°C until analysis. The integrated area under the serum concentration curve from 0 to 120 minutes (AUC) was calculated by the trapezoid rule using the formula of Shall et al.[11].

Paired and non-paired Student's t-tests were used in the statistical analysis. The paired Student's t-test was used for analysis before and after prednisolone treatment. Values were considered significant if $p<0.05$.

Results

Responses to the treatment were judged according to the clinical features and levels of GOT and γ-globulin. Improvement was defined as a 50 Karmen unit decrease in GOT (normal 34) and a 2.0 g/dl decrease in γ-globulin (normal 1.70).

The 10 patients with chronic active hepatitis were classified into Groups A, B and C. Group A (6 patients) showed improvement in levels of both GOT and γ-globulin. Group B (2 patients) demonstrated improvement in either GOT or γ-globulin. Group C (2 patients) failed to show improvement in both GOT and γ-globulin.

Table 1 shows serum levels of squalene, cholesterol and bile acid before and after prednisolone treatment in 10 patients with chronic active hepatitis. Serum squalene levels in normal controls and the patients were 85 ± 22 μg/dl (Mean ± SD) and 68 ± 23 μg/dl respectively, demonstrating no significant difference, and there was also no significant difference between serum cholesterol levels in the two groups. After treatment, the serum levels of squalene and cholesterol were elevated significantly ($p<0.01$) compared with the pretreatment state. The ratio of serum squalene to cholesterol failed to show a significant difference be-

Table 1. Serum levels of squalene, cholesterol and bile acid in patients with chronic active hepatitis

Groups	No. of Cases	Squalene (μg/dl)		Cholesterol (mg/dl)		Sq/Chol ratio		Bile acid (μM)		HBsAg
		Before	After	Before	After	Before	After	Before	After	
Normal controls	15	85±22		179±35		0.45±0.13		5±3		
Chronic active hepatitis	10									
Group A	T.H.	58	62	167	192	0.35	0.32	9	15	(+)
	K.T.	72	105	152	210	0.48	0.50	9	18	(−)
	H.K.	120	138	222	262	0.54	0.53	16	28	(−)
	K.A.	59	152	173	184	0.34	0.83	17	21	(−)
	Y.H.	68	121	185	241	0.37	0.50	17	19	(−)
	I.A.	48	69	150	179	0.32	0.39	6	11	(−)
	M±SD	71±26	108±36*	175±27	211±34*	0.40±0.09	0.51±0.18	12±5††	19±6**	
Group B	T.M.	44	53	106	141	0.42	0.38	6	8	(+)
	A.M.	48	69	150	170	0.32	0.41	ND	ND	(−)
	M±SD	46± 3	61±11	128±31	156±21	0.37±0.07	0.40±0.02	6	8	
Group C	K.N.	88	94	197	218	0.45	0.43	0	22	(−)
	H.A.	70	77	147	183	0.48	0.42	15	15	(−)
	M±SD	79±13	86±12	172±35	201±25	0.47±0.02	0.43±0.001	12±4	19±5	
Total	M±SD	68±23	91±37**	165±32	199±35**	0.41±0.08	0.47±0.14	12±5††	17±6**	

Group A; B; C, See text. ND=not done

†$p<0.05$ ††$p<0.01$ as compared chronic active hepatitis with normal controls.

*$p<0.05$ **$p<0.01$ as compared before treatment with after treatment.

tween controls and patients and was unchanged by prednisolone treatment. Fasting serum bile acid levels were 5 ± 3 μM in 15 normal controls, while the mean value in patients was significantly elevated to 12 ± 5 μM ($p<0.01$), and after treatment a significant increase was noted ($p<0.01$).

To clarify the mechanism of bile acid elevation after prednisolone treatment, oral UDCA loading test was carried out in 8 patients with chronic active hepatitis. In normal controls serum bile acid levels peaked at 60 min after UDCA administration and then returned to nearly normal at 120 min (**Fig. 1**). The fasting serum bile acid level in normal controls was 5 ± 1 μM (mean ± SEM), which increased to 18 ± 3, 15 ± 1, 12 ± 1 and 9 ± 1 μM at 30, 60, 90 and 120 min after UDCA administration, respectively. Patients with chronic active hepatitis showed moderate bile acid intolerance with peak levels occurring after 60 min, and their levels were significantly higher than the controls, being 12 ± 2 ($p<0.01$), 38 ± 7 ($p<0.01$),

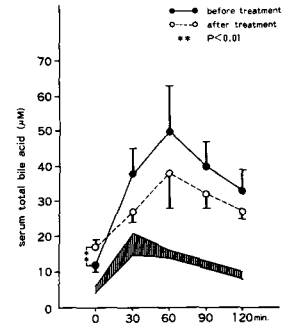

Fig. 1. UDCA loading test in patients with chronic active hepatitis before and after prednisolone treatment. Results are expressed as means ± SEM. Hatched area presents the normal range (Mean ± SEM).

50 ± 13 ($p<0.01$), 40 ± 7 ($p<0.01$) and 33 ± 6 μM ($p<0.01$) at 0, 30, 60, 90 and 120 min. After prednisolone treatment the fasting serum bile acid level was significantly increased to an average of 17 ± 2 μM, as compared to the pretreatment level ($p<0.01$). The levels at 30, 60, 90 and 120 min were 27 ± 3, 38 ± 5, 32 ± 4 and 27 ± 2 μM, respectively (**Fig. 1**), but were not significantly different from those before treat-

Table 2. Area under curve (AUC) after UDCA loading test in patients with chronic active hepatitis

Cases			AUC (μM•min) Before	AUC (μM•min) After
Normal controls	(15)		688±82	
Chronic active hepatitis	(10)			
Group A		T.H.	3670	1740
		K.T.	2100	570
		H.K.	2820	2190
		K.A.	—	—
		Y.H.	2040	1950
		I.A.	1650	1500
Group B		T.M.	760	960
		A.M.	—	—
Group C		K.N.	1890	420
		H.A.	1140	930
Total		M±SEM	2009±324††	1283±234*

AUC is the integrated area under the serum concentration curve from 0 to 120 minutes, estimated by the trapezoid rule.
††$p<0.01$ as compared chronic active hepatitis with normal controls
*$p<0.05$ as compared before treatment with after treatment.

ment at any time. **Table 2** shows the integrated area under the serum concentration curve (AUC) after UDCA loading test in normal controls and patients with chronic active hepatitis. AUC was 688 ± 82 (SEM) μM. min in controls and 2009 ± 324 μM. min in patients, showing a significant increase in the latter group ($p<0.01$). After prednisolone treatment AUC decreased significantly in 7 of 8 patients ($p<0.05$).

Discussion

In some patients with chronic active hepatitis, clinical, biochemical and histological remissions have been obtained with corticosteroid and/or azathioprine treatment[12-14]. Although steroid seems to improve liver function in general, the patients with HBs Ag and/or those manifesting non A non B hepatitis respond less well. It has been reported that the cholesterol synthesis rate was decreased in patients with chronic active hepatitis[3], but there have been few reports on changes in sterol and bile acid metabolism during administration of corticosteroid.

Most serum squalene early in the morning after an overnight fast is derived from endogenous squalene. In plasma of healthy subjects, half of the squalene appears in the very low density lipoprotein (VLDL) fraction, while the remaining half appears in the high density lipoprotein (HDL) and low density lipoprotein (LDL) fractions[15]. As plasma VLDL and HDL were reduced markedly in parenchymal liver disease[16], serum squalene and cholesterol levels may be due, in part at least, to changes of these carrier proteins. The present study showed that serum levels of squalene and cholesterol were lower in patients, especially in severe chronic active hepatitis.

Glucocorticoid is known to induce hyperlipemia (Type II and/or IV)[6]. Though the details are unknown, its mechanism seems to be related to increased sterol metabolism in the liver and overproduction of VLDL apoprotein[17]. This our study showed that short-term prednisolone treatment caused a significant increase in serum squalene and cholesterol levels in patients with chronic active hepatitis, but no change in the serum squalene to cholesterol ratio. Although the decrease in GOT and γ-globulin and the changes of serum squalene and cholesterol levels are not always in parallel in Groups B and C, it has been suggested that prednisolone may improve the sterol metabolism in patients with chronic active hepatitis. Therefore, serum squalene and cholesterol levels seem to be a sensitive parameter of liver response in glucocorticoid treatment.

In the present study, serum fasting bile acid levels were elevated in all patients with chronic active hepatitis after prednisolone treatment. This finding suggests either a disturbed bile acid metabolism or an increased pool size of bile acid. As a previous study by the authors revealed, oral UDCA loading test is a useful liver test for chronic liver disease[9], and AUC following UDCA gives a quantitative estimate of the hepatic UDCA clearance which is determined mainly by hepatic first-pass clearance[18,19]. In our present study, patients with chronic active hepatitis were found to have significantly larger AUC values than controls, suggesting that first-pass clearance of UDCA was impaired in patients. After prednisolone treatment, oral UDCA loading test levels improved in 7 of 8 patients and AUC decreased significantly ($p<0.05$) as compared with pretreatment values. Therefore, the elevation of serum fasting bile acids after treatment may not be due to diminished hepatic clearance, but to an increased pool size of bile acid.

It is generally accept that glucocorticoid induces activity of β-hydroxy-β-methylglutaryl Coenzyme A (HMG-CoA) reductase and Cholesterol 7α-hydroxylase in the liver of ad-

renalectomized rats[20], and further the activity of cholesterol 7α-hydroxylase even in the liver of infant rats[21]. Therefore, glucocorticoid may increase pool sizes of these substances. Although we did not examine pool sizes of cholesterol and bile acid, available data indicate that glucocorticoid improves sterol and bile acid metabolism in patients with chronic active hepatitis. At present, however, the use of prednisolone in patients with chronic active hepatitis is controversial, because this agent is known to increase viremia and to cause severe side effects in patients with HBsAg[22,23].

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