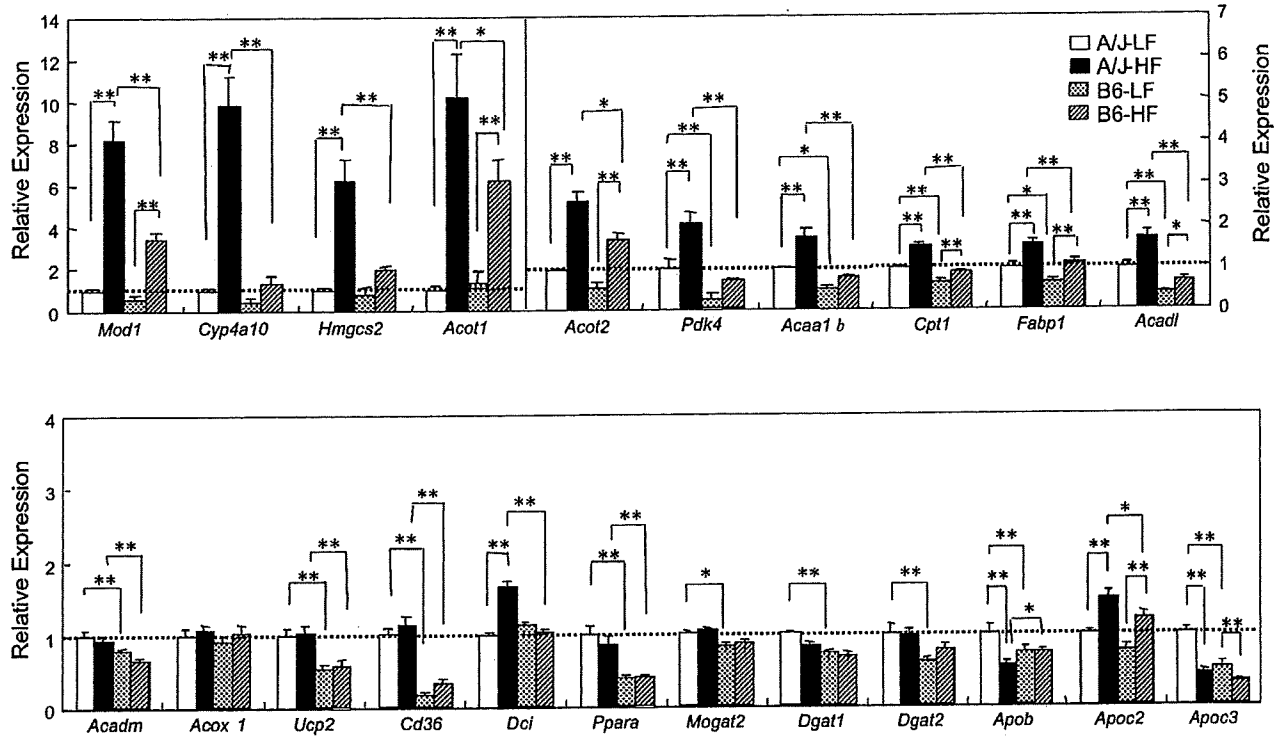

Fig. 1. Quantitative RT-PCR (qRT-PCR) analysis for gene expression in the small intestine. Change in mRNA expression of the lipid metabolism-related genes. qRT-PCR analysis was performed using individual total RNAs prepared from A/J or C57BL/6J (B6) mice fed with a low-fat (LF) or high-fat (HF) diet for 2 wk. Amounts of mRNA were normalized according to the amounts of acidic ribosomal phosphoprotein P0 (*Arbp*) mRNA described in MATERIALS AND METHODS and expressed as a ratio to the corresponding amounts in the A/J mice fed the LF diet. *Mod1*, NADP$^+$-dependent cystolic malic enzyme; *Cyp4a10*, cytochrome P450 4A10; *Hmgcs2*, 3-hydroxy-3-methylglutaryl-CoA; *Acot1*, acyl-CoA thioesterase 1; *Acot2*, acyl-CoA thioesterase 2; *Pdk4*, pyruvate dehydrogenase kinase-4; *Acaa1b*, acetyl-CoA acyltransferase 1B; *Cpt1*, carnitine palmitoyltransferase I; *Fabp1*, fatty acid-binding protein 1; *Acadl*, long-chain acetyl-CoA dehydrogenase; *Acadm*, medium-chain acetyl-CoA dehydrogenase; *Acox1*, acyl-CoA oxidase 1; *Ucp2*, uncoupling protein 2; *Cd36*, fatty acid translocase; *Dci*, dodecenoyl-CoA δ-isomerase; *Ppara*, peroxisome proliferator-activated receptor-α; *Mogat2*, monoacylglycerol-*O*-acyltransferase 2; *Dgat1*, diacylglycerol-*O*-acyltransferase 1; *Dgat2*, diacylglycerol-*O*-acyltransferase 2; *Apob*, apolipoprotein B; *Apoc2*, apolipoprotein C-II; *Apoc3*, apolipoprotein C-III. Values are means ± SE for 6 mice. $*P < 0.05$; $**P < 0.01$.

and *Fabp1* were slightly, but significantly, higher in A/J mice compared with B6 mice under both diet conditions. Gene expressions of *Acox1* and *Ucp2* were increased only in B6 mice by HF feeding.

In the muscle, the response to the HF diet and the strain differences in the lipid metabolism-related genes were negligible or subtle for all of the examined lipid metabolism-related enzymes and fatty acid transporters (Fig. 2*B*).

In the WAT, 2 wk of ingestion of the HF diet did not increase the expression of *Mod1*, *Hmgcs2*, *Acot2*, *Pdk4*, or *Acaa1b* (Fig. 2*C*). *Mod1* expression was rather significantly decreased by HF feeding in A/J mice. In agreement with the previous report (47), *Ucp2* expression in the WAT of obesity-resistant A/J mice was significantly higher compared with obesity-prone B6 mice under the both diet conditions (Fig. 2*C*). Its expression was slightly increased by feeding of the HF diet to A/J mice ($P < 0.01$).

Changes in enzyme activity. To confirm the predominant intestinal lipid metabolism in obesity-resistant A/J mice, suggested by gene expression analysis, lipid metabolism-related enzyme activities were examined.

The cytosolic ME activity in the small intestine was increased in response to the HF feeding, reflecting the expression pattern of the *Mod1* gene. The activity was increased 3.3-fold in A/J mice and 1.5-fold in B6 mice. The activity was 2.5-fold higher in A/J mice compared with B6 mice under the HF diet condition (Fig. 3*A*).

CPT activity was significantly increased by 2 wk of HF feeding in the small intestines of both strains of mice (1.5-fold in A/J mice and 1.7-fold in B6 mice). In parallel with the gene expression pattern, the activities in A/J mice were significantly higher than those in B6 mice under the LF and HF diet conditions (Fig. 3*B*).

The HF feeding also increased the β-oxidation activity in both strains of mice (1.9-fold in A/J mice and 2.9-fold in B6 mice). The activities were not significantly different between the strains (Fig. 3*C*).

DISCUSSION

In this study, we demonstrated that the ingestion of a HF diet induced the expression of several genes related to lipid metabolism and the basal and upregulated expression levels were higher in the obesity-resistant A/J mice compared with the obesity-prone B6 mice. Furthermore, the increased intestinal lipid metabolism in A/J mice was confirmed at the levels of the

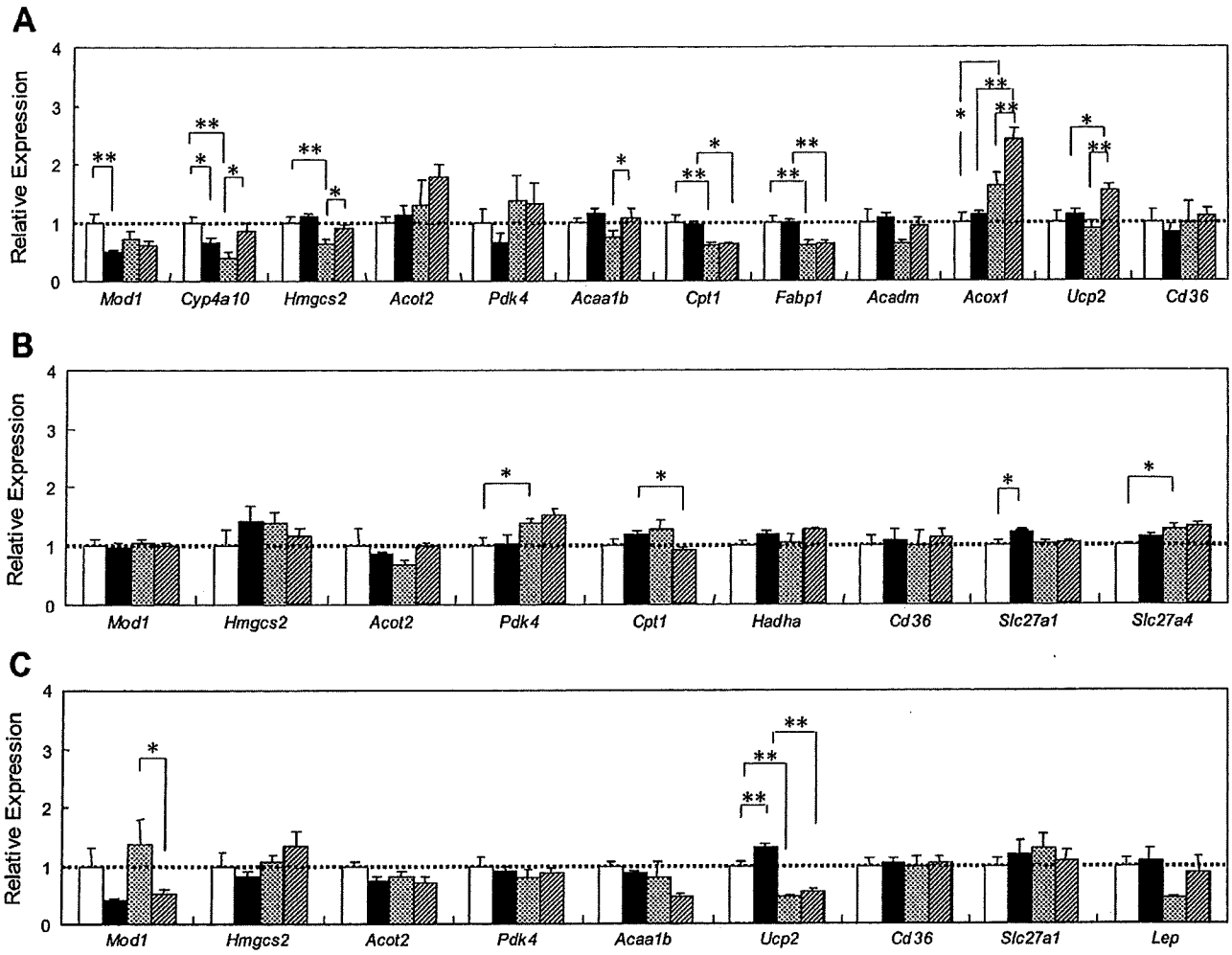

Fig. 2. Gene expression in the liver, skeletal muscle, and white adipose tissue (WAT). Total RNA isolated from the liver (*A*), gastrocnemius muscle (*B*), and epididymal WAT (*C*) was subjected to qRT-PCR analysis as described in MATERIALS AND METHODS. *Hadha*, hydroxyacyl-CoA dehydrogenase; *Slc27a1*, solute carrier family 27, member 1; *Slc27a4*, solute carrier family 27, member 4; *Lep*, leptin. Values are means ± SE of 6 mice. $*P < 0.05$; $**P < 0.01$.

enzyme activities; ME activity, CPT activity, and β-oxidation activity were increased in response to the ingestion of the HF diet, and the basal enzyme activities and/or the activities upregulated by the HF feeding were higher in the A/J mice compared with those in the B6 mice.

A notable change in gene expression by HF feeding was observed in *Mod1*, *Cyp4a10*, *Hmgcs2*, *Acot1*, *Acot2*, *Pdk4*, and *Acaa1b*. Expression of these genes was significantly higher in obesity-resistant A/J mice than in obesity-prone B6 mice. There is little information about the functions of the genes in the small intestine, but previous studies have shown their physiological roles in liver and muscle. ME encoded by *Mod1* is known as a representative lipogenic enzyme in the liver. The enzyme catalyzes the synthesis of pyruvate and NADPH from malate and $NADP^+$, and the NADPH generated by the reaction promotes fatty acid synthesis (5). *Cyp4a10* belongs to the CYP4A subfamily encoding several cytochrome P450 enzymes that catalyze the ω-oxidation of fatty acids (3). Because CYP4A subfamily enzymes require NADPH as a coenzyme, the increase of ME activity in the small intestine could result in the stimulation of the ω-oxidation of fatty acid by generating NADPH. *Hmgcs2* is highly expressed in the liver and plays a role as a rate-limiting enzyme in the synthesis of ketone bodies from the acetyl-CoA generated by fatty acid β-oxidation (12, 49). *Acot1* and *Acot2* encode acyl-CoA thioesterases that catalyze the hydrolysis of acyl-CoAs of various chain lengths to free fatty acids and CoA. It has been proposed (26, 41, 46) that these enzymes promote β-oxidation by modulation of the cellular concentrations of acyl-CoA and CoA or by acting in concert with uncoupling protein 3 in the liver and skeletal muscle. Thus all of the genes mentioned above are possibly associated with the β- or ω-oxidation in the small intestine. *Pdk4* encodes an isozyme of the enzyme that catalyzes pyruvate dehydrogenase complex phosphorylation. It has been reported (43) that the upregulation of *Pdk4* expression in the kidney facilitated the entry of acetyl-CoA derived from β-oxidation into the TCA cycle via the increased carboxylation of pyruvate to oxaloacetate. *Acaa1b* has been identified as a gene for an enzyme involved in the peroxisomal β-oxidation (10). In the present study, it has been demonstrated that the intestinal

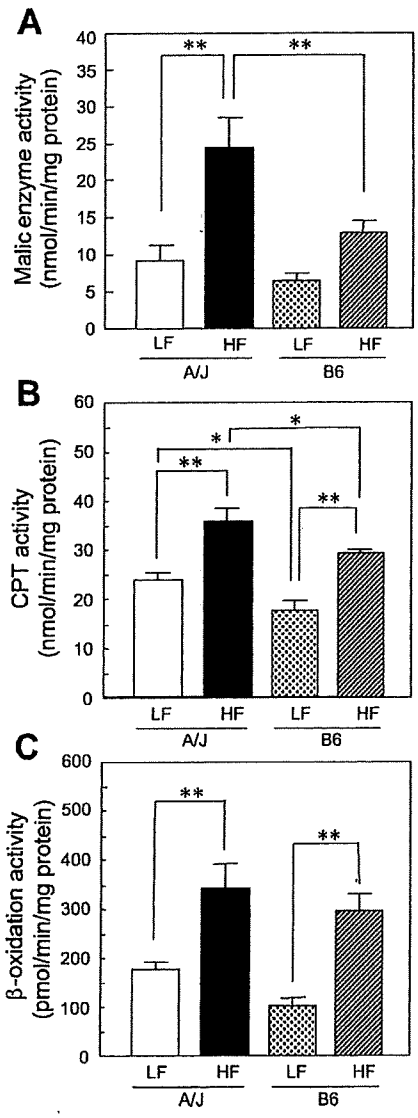

Fig. 3. Malic enzyme (*A*), carnitine palmitoyltransferase (CPT; *B*), and β-oxidation activity (*C*) in the small intestine. Small intestines of A/J or B6 mice fed the LF or HF diet for 2 wk were collected, and the cytoplasmic malic enzyme, CPT, and β-oxidation activities were measured as described in MATERIALS AND METHODS. Values are means ± SE of 6 mice. $*P < 0.05$; $**P < 0.01$.

lipid metabolism-related genes were expressed at higher levels in obesity-resistant A/J mice than in obesity-prone B6 mice. Previous studies have reported that the upregulation of fatty acid catabolism induced by PPARα-specific activators in liver and/or brown adipose tissue was associated with a reduction in body weight gain and fat accumulation in rodent models of HF diet-induced or genetic insulin resistance (2, 16). Taken together, it is likely that the capacity for fatty acid catabolism in the small intestine also relates to body weight gain and fat accumulation; i.e., low levels of intestinal fatty acid catabolism may result in a reduced energy expenditure and, therefore, the development of obesity on a long-term basis.

HF feeding also affected the gene expression of *Apoc2* and *Apoc3*. It has been reported (7, 19) that the lipid-binding domain of apoC-II is essential for the activation of lipoprotein lipase (LPL) (19, 32) and that apoC-III inhibits LPL activity. Because LPL catalyzes the hydrolysis of the TG circulating as chylomicrons or very low-density lipoproteins, the putative activation of LPL may promote utilization by peripheral tissues of free fatty acids and 2-monoacylglycerol. The observed changes in the gene expression of apoCs may also be an adaptive response to the excess intake of lipid. However, the physiological importance needs to be clarified by further study.

It has been demonstrated (3, 8, 17, 18, 22, 36, 50) that the expression of *Mod1*, *Cyp4a10*, *Hmgcs2*, *Acot1*, *Acot2*, *Pdk4*, and *Acaa1b* was upregulated by hypolipidemic fibrate, and most of the genes have a PPAR response element in their promoter region. Therefore, the increase in the expression of these lipid metabolism-related genes in response to the HF feeding might be explained by the increased cellular concentration of free fatty acids, an endogenous ligand for PPAR, in the intestinal mucosa. The different expression levels of PPARα observed in this study (Fig. 1) may be a factor responsible for the strain difference in the expression of the lipid metabolism-related genes. Indeed, previous studies (23, 25) have shown that the infusion or oral administration of PPARα ligands caused an increase in the mRNA levels of these PPAR-dependent genes such as *Acox1* and *Fabp1*. Poirier et al. (34) have shown that the PPARδ isoform plays a role as a transcription factor for *Fabp1* in the small intestine of PPARα-null mice, suggesting its contribution to the metabolic adaptation of the small intestine to changes in the lipid content of the diet. However, we could not find any strain difference in the expression of PPARδ in the small intestines of A/J and B6 mice by qRT-PCR analysis (data not shown).

Consistent with previous reports (35, 47), we confirmed a significant increase of *Ucp2* expression due to the HF diet in the WAT of A/J mice. Furthermore, *Ucp2* expression in WAT was higher in A/J mice compared with that in B6 mice. Therefore, the thermogenic capacity of the adipose tissue may contribute to the development of obesity. Previous studies (6, 9, 30) using other rodent models have shown that HF feeding induced the upregulation of the β-oxidation activity and the gene expression of the related enzymes in the liver and muscle. Brady et al. (6) have demonstrated that 4 wk of HF feeding increased the enzyme activity and mRNA expression of mitochondrial and peroxisomal CPT. Cheng et al. (9) have shown that Long-Evans rats fed a HF diet exhibited a higher CPT activity in muscle compared with rats fed laboratory chow diets. However, in the present study, changes in the expression of lipid metabolism-related genes in the small intestine were more prominent than those in the liver, muscle, and adipose tissue after 2 wk of HF feeding, suggesting that the small intestine is one of the organ's most sensitive to dietary lipids. Longer term HF feeding may be required for the upregulation of lipid metabolism in the liver and muscle in A/J and B6 mice under our experimental condition.

Because the small intestine is directly exposed to dietary fat, the activation of intestinal fatty acid catabolism by ingestion of large amounts of fat may result in a substantial reduction in the amount of lipid entering the bloodstream. Because the small intestine is also exposed to other orally ingested substances and the surface area of the villus mucosa is quite large, the regulation of the intestinal lipid metabolism by food ingredients or chemicals may become an efficient measure for pre-

venting the development of obesity. Indeed, it has been reported (27, 28) that dietary diacylglycerol reduced the HF-induced body weight gain in B6 mice and genetic body weight gain in C57BL/KsJ *db/db* mice accompanied by the stimulation of intestinal β-oxidation.

In the present study, we demonstrated that the intestine is highly responsive to fat ingestion and that the activation by HF feeding of the lipid metabolism-related genes in the intestine was more pronounced in obesity-resistant A/J mice than in obesity-prone B6 mice. These findings suggest that the capability for fatty acid catabolism in the small intestine is associated with the development of obesity.

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Selective protection of curcumin against carbon tetrachloride-induced inactivation of hepatic cytochrome P450 isozymes in rats

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Abstract

We investigated the effects of curcumin, a major antioxidant constituent of turmeric, on hepatic cytochrome P450 (CYP) activity in rats. Wistar rats received curcumin-containing diets (0.05, 0.5 and 5 g/kg diet) with or without injection of carbon tetrachloride (CCl_4). The hepatic CYP content and activities of six CYP isozymes remained unchanged by curcumin treatment, except for the group treated with the extremely high dose (5 g/kg). This suggested that daily dose of curcumin does not cause CYP-mediated interaction with co-administered drugs. Chronic CCl_4 injection drastically decreased CYP activity, especially CYP2E1 activity, which is involved in the bioactivation of CCl_4, thereby producing reactive free radicals. Treatment with curcumin at 0.5 g/kg alleviated the CCl_4-induced inactivation of CYPs 1A, 2B, 2C and 3A isozymes, except for CYP2E1. The lack of effect of curcumin on CYP2E1 damage might be related to suicidal radical production by CYP2E1 on the same enzyme. It is speculated that curcumin inhibited CCl_4-induced secondary hepatic CYPs damage through its antioxidant properties. Our results demonstrated that CYP isozyme inactivation in rat liver caused by CCl_4 was inhibited by curcumin. Dietary intake of curcumin may protect against CCl_4-induced hepatic CYP inactivation via its antioxidant properties, without inducing hepatic CYPs.

Keywords: Curcumin; Carbon tetrachloride; Free radicals; CYP2E1; Hepatotoxicity

Introduction

Recently, interest in complementary and alternative medicine has grown rapidly in industrialized countries, and the demand for herbal remedies has currently increased (De Smet, 2002; Ammon and Wahl, 1991). Turmeric, the rhizome of *Curcuma longa* L., has traditionally been used for treatment of gastrointestinal colic, flatulence, hemorrhage, hematuria, menstrual difficulties and jaundice. The anti-inflammatory and hepatoprotective characteristics of turmeric and its constituents have been widely investigated (Govindarajan, 1980; Luper, 1999; Miquel et al., 2002). The most well-researched component of turmeric is curcumin (diferuloylmethane, Fig. 1). Curcumin is the major yellow pigment comprising 3–6% of turmeric, and has been widely used in curry, mustard, cosmetics and drugs (Govindarajan, 1980; Miquel et al., 2002). Curcumin is well known for its pharmacological properties including antioxidant, anti-inflammatory, antimutagenic and anticancer activity (Miquel et al., 2002; Okada et al., 2001; Asai and Miyazawa, 2001; Ramirez-Tortosa et al., 1999; Sharma et al., 2004). The preventive and improved effects of curcumin on symptoms of liver diseases are shown to stem from its antioxidant effects (Rukkumani et al., 2004; Park et al., 2000; Nanji et al., 2003).

Many alternative remedies including turmeric may be taken with medicine; hence, pharmacological interactions are a concern in clinical therapy (Ernst, 2002; Williamson, 2001). Changes in the pharmacokinetics and pharmacodynamics of co-administered drugs affects clinical efficacy, and occasion-

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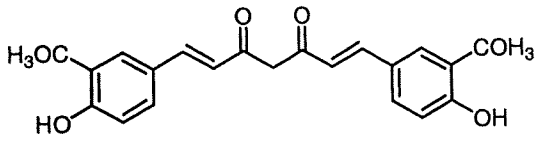

Fig. 1. Chemical structure of curcumin.

ally severe adverse reactions occur. We have examined cytochrome P450 (CYP)-mediated interactions with herbal remedies and medication (Umegaki et al., 2002; Kubota et al., 2004; Sugiyama et al., 2004). Regarding the metabolic functions of the liver, the effects of curcumin on metabolic enzymes, especially hepatic CYP activity, have not been completely elucidated. In addition, many liver diseases are related to lipid peroxidation in liver tissue, and some antioxidant components have a protective effect against liver damage. Curcumin has antioxidative properties and prevents some oxidative stress, and the action of curcumin has been shown to be beneficial for inhibition of tissue injury (Luper, 1999; Miquel et al., 2002; Okada et al., 2001; Khopde et al., 2000). Carbon tetrachloride (CCl_4), a well-known model compound for producing chemical hepatic injury, requires biotransformation by hepatic microsomal CYP to produce toxic metabolites, namely trichloromethyl free radicals (Recknagel et al., 1989; Brattin et al., 1985; Brautbar and Williams, 2002). CYP2E1 is the major isozyme involved in bioactivation of CCl_4 and subsequent production of free radicals (Recknagel et al., 1989). It has been proposed that the antioxidative action of curcumin plays an important role in its hepatoprotective effects against CCl_4-induced liver injury (Park et al., 2000). However, the mechanism by which curcumin protects the liver against CCl_4-induced toxicity is unclear, particularly in association with CYP activity.

This study was undertaken to evaluate the effect of repeated curcumin ingestion on hepatic CYP enzymes and to examine the protective effect of curcumin on CCl_4-induced hepatic CYP damage in rats.

Materials and methods

Materials

Curcumin was purchased from Wako Pure Chemical Ltd. (Osaka, Japan). Resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, testosterone, 6β-hydroxytestosterone, corticosterone, *p*-nitrophenol, 4-nitrocatechol and 7-ethoxycoumarin were purchased from Sigma (St. Louis, MO, USA). (*S*)-Warfarin and 7-hydroxywarfarin were obtained from Ultrafine (Manchester, England). NADPH was obtained from Oriental Yeast (Tokyo, Japan). Other reagents were obtained from Wako Pure Chemical Ltd. (Osaka, Japan).

Animal experiments

Male Wistar rats (5 weeks old) obtained from Japan SLC (Shizuoka, Japan) were housed individually in stainless steel, wire-bottomed cages at a constant temperature (23±1 °C) under a 12 h light–dark cycle. Rats were given AIN-93G based diets (containing 53.2% (w/w) α-corn starch, 20% milk casein, 10% sucrose, 7% corn oil, 5% cellulose, 3.5% mineral mix (AIN-93G-MX), 1.0% vitamin mix (AIN-93G-VX), 0.3% L-cysteine and 0.0014% *tert*-butylhydroquinone) (Reeves et al., 1993) with or without curcumin (0.05, 0.5 and 5 g/kg diet) for 4 consecutive weeks. In order to examine the protective effects of curcumin on the liver damage, rats were subcutaneously injected with CCl_4 (50% (v/v in olive oil) for 0.2 ml/100 g body weight) twice a week during the 7 weeks of curcumin ingestion. After these treatments, rats were anesthetized with pentobarbital and sacrificed, the blood was collected, and the livers were immediately removed and weighed. The glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in plasma were determined using an assay kit, transaminase CII-Test Wako (Wako Pure Chemical Ltd., Osaka, Japan).

All procedures were in accordance with the National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals.

Preparation of microsome and cytosolic fractions from the liver

The liver was rinsed with 0.9% (w/v) NaCl solution and homogenized in 50 mmol/L Tris–HCl buffer (pH 7.4) containing 0.25 mol/L sucrose. The homogenate was centrifuged at 10,000×*g* at 4 °C for 30 min. The supernatant was further centrifuged at 105,000×*g* at 4 °C for 60 min. The supernatant was used as the cytosolic fraction for the assay of glutathione *S*-transferase, the activity of which was determined using 1-chloro-2,4-dinitrobenzene as a substrate (Habig and Jakoby, 1981). The pellet was washed once with 50 mmol/L Tris–HCl buffer (pH 7.4) containing 0.25 mol/L sucrose by centrifugation at 105,000×*g* at 4 °C for 60 min, and the concentration and activities of CYP were analyzed.

Protein concentrations of microsomal and cytosolic fractions were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Analysis of CYP enzyme activities

The CYP content was quantified by the method of Omura and Sato (1964). The activities of various CYP enzymes were determined by HPLC methods as reported previously (Umegaki et al., 2002). The subtypes of CYP enzymes examined and the corresponding CYPs were ethoxyresorufin *O*-deethylase, CYP1A1; methoxyresorufin *O*-demethylase, CYP1A2; pentoxyresorufin *O*-dealkylase, CYP2B; (*S*)-warfarin 7-hydroxylase, CYP2C9; *p*-nitrophenol hydroxylase, CYP2E1; and testosterone 6β-hydroxylase, CYP3A (Hanioka et al., 2000; Mishin et al., 1996; Lang and Bocker, 1995).

Statistical analysis

The data are presented as means with standard deviation (S.D.) for the individual groups. Statistical analysis of the data was carried out using ANOVA followed by a post hoc test of Fisher's

Table 1
Effects of curcumin on the weights of body and liver, and hepatic drug metabolizing enzymes in rats

	Untreated control	Curcumin		
		(0.05 g/kg)	(0.5 g/kg)	(5 g/kg)
Body weight (g)	211.8±9.9	216.1±6.7	215.1±5.5	219.9±9.6
Liver weight (%/body weight)	2.90±0.08	2.93±0.13	3.02±0.11	3.04±0.09
Hepatic metabolizing enzymes				
Cytochrome P450 content (nmol/mg protein)	0.615±0.077	0.584±0.088	0.686±0.044	0.675±0.055
Glutathione *S*-transferase (μmol/mg protein/min)	0.346±0.075	0.522±0.088*	0.416±0.030	0.476±0.068*

Wistar rats were given diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 4 weeks. Each value is the mean±S.D. for five rats. Significant difference from the untreated control group is indicated by $*P<0.01$.

PLSD. A P-value <0.05 was considered to be significant. These statistical analyses were performed using a computer program (Stat View 5.0, ASA Institute Inc., Cary, NC, USA).

Results

Dose-dependent effects of curcumin on the hepatic CYP activity

During the curcumin treatment, there was no difference in the dietary intake or the body weight gain between each group. The average intake dose of curcumin was calculated based on the intake amount of diet and the body weight of the group, at a dose of 0.05 g/kg diet for about 6.2 mg/kg body weight per day. The effects of curcumin on the weights of body and liver, and hepatic metabolizing enzymes of rats are shown in Table 1. Curcumin had no influence on the liver weight and hepatic CYP content in rats (Table 1). Glutathione *S*-transferase activity increase correlated with the ingestion of curcumin (Table 1). The effects of curcumin on the various CYP activities of rats are shown in Table 2. Treatment with curcumin (0.05 and 0.5 g/kg diet) did not change the activity of the six types of CYP, while the extremely high dose (5 g/kg diet) of curcumin tended to increase the activity of pentoxyresorufin *O*-dealkylase as corresponding to CYP2B and (*S*)-warfarin 7-hydroxylase as CYP2C9 (Table 2).

Effects of curcumin on the changes of hepatic CYP activities induced by chronic CCl_4 injection in rats

Liver weight was increased with CCl_4 treatment, by 1.2-fold (4.32±0.12%/body weight, $P<0.05$) compared with the untreated control group (3.69±0.13%), and slight inductions were observed by co-administration of curcumin. The liver weights were 4.60±0.22% in 0.05 g/kg diet group, 4.59±0.17% in 0.5 g/kg diet group and 4.64±0.21% in 5 g/kg diet group, respectively. The GOT and GPT activities in plasma were significantly increased by chronic CCl_4 treatment: 40.3±4.8 IU/L and 17.6±2.2 IU/L in the untreated control group, 120±7.4 IU/L and 78.9±19.3 IU/L in the CCl_4-treated group. Repeated administrations of curcumin did not influence the increases in GOT and GPT activities, even high dose 0.5 g/kg diet: 105±11.1 IU/L and 87.7±10.9 IU/L, respectively. Effects of curcumin on the changes in the content of CYP and the activities of the CYPs and glutathione *S*-transferase in CCl_4-treated rats are shown in Fig. 2 and Table 3. Chronic CCl_4 treatment markedly decreased hepatic total CYP content to 29%, compared to the level of the untreated control group (Fig. 2A). In contrast, the ingestion of higher doses of curcumin (0.5 and 5 g/kg diet) significantly moderated the reduction of CYP content to 55% of the level of the untreated control group. Similarly, the activities of the six types of CYPs were drastically decreased by CCl_4 treatment, while higher doses of curcumin (0.5 and 5 g/kg diet) inhibited the decreases of CYP activity, except for *p*-nitrophenol hydroxylase corresponding to CYP2E1 (Table 3). Glutathione *S*-transferase activity was reduced by CCl_4 treatment, while the effects of co-administered curcumin were not significant (Fig. 2B).

Discussion

The objectives of the present study were two-fold: firstly, to examine the effects of curcumin on hepatic CYP activity in order to analyze hepatic drug-metabolizing function and CYP-

Table 2
Effects of curcumin on the activity of various hepatic CYPs in rats

	Untreated control	Curcumin		
		(0.05 g/kg)	(0.5 g/kg)	(5 g/kg)
	Activity (pmol/mg protein/min)			
Ethoxyresorufin *O*-deethylase (CYP1A1)	10.1±2.80	10.1±1.22	9.94±1.17	12.7±2.02
Methoxyresorufin *O*-demethylase (CYP1A2)	6.19±1.34	6.41±0.93	5.95±0.92	6.68±1.19
Pentoxyresorufin *O*-dealkylase (CYP2B)	2.71±0.76	2.71±0.36	2.69±0.23	3.58±0.70*
(*S*)-Warfarin 7-hydroxylase (CYP2C9)	2.09±0.61	2.05±0.25	2.13±0.24	3.02±0.52*
p-Nitrophenol hydroxylase (CYP2E1)	7330±1235	7162±674	6531±527	7331±872
Testosterone 6β-hydroxylase (CYP3A)	1641±506	1425±157	1505±115	1693±157

Wistar rats were fed diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 4 weeks. Each value is the mean±S.D. for five rats. Significant difference from the untreated control group is indicated by $*P<0.05$.

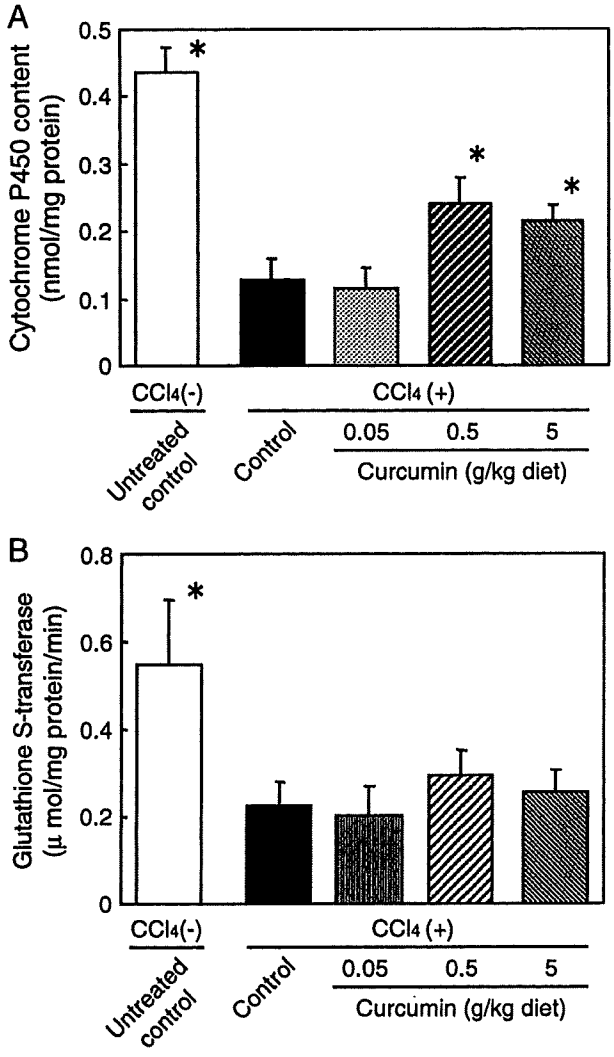

Fig. 2. Effects of curcumin on the reduction of hepatic cytochrome P450 content and glutathione *S*-transferase activity induced by CCl_4 injection. Wistar rats were fed diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 7 weeks and intraperitoneally injected with CCl_4 twice a week. (A) Cytochrome P450 content, (B) glutathione *S*-transferase activity. Each column is the mean ± S.D. for five rats. Significant difference from the CCl_4-treated control group is indicated by $*P<0.01$.

mediated drug interaction, and secondly, to determine the protective effect of curcumin on hepatic CYP damage induced by chronic CCl_4 injection.

As shown in the results, rats were given curcumin-containing diets for 4 consecutive weeks. The dose of curcumin was calculated to be about 5 mg/kg body weight per day based on the recommended dose of curcumin (500 mg/day or more) in human therapy (Sharma et al., 2004; Cheng et al., 2001), and the 10- and 100-fold doses (50 and 500 mg/kg body weight) were also tested. Even in the highest dose group (5 g/kg in diet; about 500 mg/kg body weight), the repeated ingestion of curcumin had no effect on body weight gain, liver weight or the total content of hepatic CYP enzyme of rats (Table 1). Likewise, the activities of six CYP isozymes remained unchanged after curcumin treatment at doses of 0.05 g/kg diet, i.e. 5 mg/kg body weight (Table 2). These results indicate that daily doses of curcumin have no influence on hepatic CYP activities, namely phase I drug-metabolizing enzymes. On the other hand, curcumin increased the activity of glutathione *S*-transferase, one of phase II drug-metabolizing enzymes (Table 1), as previous reports (Iqbal et al., 2003; Okada et al., 2001). Glutathione *S*-transferase is a soluble protein located in the cytosol, and plays an important role in the detoxification and excretion of xenobiotics (Mannervik, 1985; Mannervik et al., 1985). Compounds that increase the glutathione *S*-transferase activity and convert toxic substances to nontoxic substances are known to protect the liver. Some reports have indicated that curcumin increases intracellular glutathione levels and activities of glutathione *S*-transferase and some antioxidative enzymes (Okada et al., 2001; Rinaldi et al., 2002; Piper et al., 1998; Iqbal et al., 2003). These results indicate that curcumin might be beneficial for glutathione-mediated detoxification of electrophilic products of lipid peroxidation.

Recently, herb–drug interactions have become a concern in clinical therapy. Alternative remedies containing curcumin or turmeric are consumed by many patients receiving medical therapy for liver disease (Luper, 1999; Miquel et al., 2002). The fact that repeated intake of curcumin has no influence on hepatic CYP activity suggests that curcumin does not change the efficacy or pharmacokinetics of co-administered medicines. Moreover, because CYPs mediate the biosynthesis and metabolisms of various hormones, it is unlikely that a daily dose of curcumin cause adverse reactions involved in changes of CYP activity.

The free radical scavenging activity of curcumin is beneficial to liver injury caused by a variety of hepatotoxic substances, including CCl_4, ethanol, pentobarbital and acetaminophen (Luper, 1999; Miquel et al., 2002; Park et al., 2000). However, the changes of various CYP activities are not clear in simultaneous injection of CCl_4 and curcumin in rats. Thus, we focused on the changes in CYP activity in CCl_4-induced hepatopathy-modeled rats. Chronic CCl_4 injection increased the liver weight, while drastically reducing the content and activity of CYP enzymes, especially CYP2E1, as shown by the *p*-nitrophenol hydroxylase activity (Fig. 2A, Table 3). These results support recent reports of the reductions of mRNA expression and activity of some CYP enzymes in the liver of rats given various doses of CCl_4 (Lee et al., 2004). CYP2E1 is the major isozyme involved in CCl_4 bioactivation and generated cytotoxic trichloromethyl radicals are thought to cause hepatotoxicity (Recknagel et al., 1989; Wong et al., 1998; Williams and Burk, 1990). Furthermore, alterations in CYP2E1 activity can affect susceptibility to hepatic injury from CCl_4 (Wong et al., 1998; Takahashi et al., 2002). Moreover, the reactive free radicals inactivate CYP enzymes and subsequent depletion of CYP2E1 (Guengerich et al., 1991; Jeong, 1999; Zhou et al., 2004). In this way, CCl_4 injection decreased the CYPs 1A, 2B, 2C and 3A isozymes activities, similar to CYP2E1 (Table 3). In contrast, repeated curcumin ingestion in higher doses (0.5 and 5 g/kg diet) significantly relieved the CCl_4-caused reductions of total CYP content (Fig. 2A) and the activities, except for CYP2E1

Table 3
Effects of curcumin on the activity of various hepatic CYPs in rats treated with and without CCl_4

	Untreated control	CCl_4-treated			
		Control	Curcumin		
			(0.05 g/kg)	(0.5 g/kg)	(5 g/kg)
	Activity (pmol/mg protein/min)				
Ethoxyresorufin *O*-deethylase (CYP1A1)	16.92±1.78*	3.70±1.15	3.16±0.51	7.67±3.42*	6.67±2.67
Methoxyresorufin *O*-demethylase (CYP1A2)	10.31±0.49*	1.97±0.27	1.65±0.14	3.43±0.49*	3.22±0.47
Pentoxyresorufin *O*-dealkylase (CYP2B)	3.25±0.37*	1.28±0.18	1.24±0.13	1.82±0.36*	1.77±0.49*
(*S*)-Warfarin 7-hydroxylase (CYP2C9)	1.00±0.25*	0.146±0.044	0.192±0.042	0.416±0.142*	0.419±0.276
p-Nitrophenol hydroxylase (CYP2E1)	6444±1043*	462±147	419±127	458±148	462±67
Testosterone 6β-hydroxylase (CYP3A)	917±171*	340±63	224±63	579±263*	502±237

Wistar rats were fed diets containing curcumin (0.05, 0.5 and 5 g/kg diet) for 7 weeks and intraperitoneally injected with CCl_4 twice a week. Each value is the mean±S.D. for five rats. Significant difference from the CCl_4-treated control group is indicated by $*P<0.05$.

(Table 3). Glutathione *S*-transferase activity was also decreased by CCl_4 treatment and co-administered curcumin (0.5 g/kg diet) tended to recover the decreased activity, but there was no significance. The activities of GOT and GPT, well-known biomarkers, were markedly elevated by CCl_4 injection, indicating severe tissue damage. Co-administered curcumin, even in high dose, did not inhibit the increase in these activities. These results suggested that curcumin did not significantly relieve tissue damage by CCl_4 as indicated by the transaminase activities, but relieved the decreased hepatic CYPs activity in the present experimental condition. Interestingly, among the six CYP enzymes examined, CYP2E1 was degraded the most by CCl_4 injection and no amelioration was observed with curcumin ingestion (Table 3). CYP2E1-mediated metabolism of CCl_4 generated reactive free radicals, and CYP2E1 protein might be more susceptible to CCl_4 toxicity than other CYP isozymes. Curcumin could not moderate the decrease of CYP2E1 activity. In other words, curcumin was unavailable to additionally precipitate the bioactivation of CCl_4 and exacerbated liver damage. Curcumin did not change the hepatic CYP activity in normal rats (Tables 1 and 2), indicating that curcumin indirectly improved the inactivation of CYPs induced by severe CCl_4 toxicity.

Many previous investigations regarding CCl_4-induced liver injury have focused only on CYP2E1 activity, but not on other CYP isoforms (Yokogawa et al., 2004; Jeong et al., 2002; Jeon et al., 2003). In this study, different susceptibilities to CCl_4 were observed between in CYP2E1 and other isozymes, i.e. CYPs 1A, 2B, 2C and 3A, and the effects of curcumin were also different. The mechanism underlying the CCl_4-induced degradation of CYP activity may be different between CYP2E1 and other isoforms. CYP2E1 mediated CCl_4 bioactivation and produced reactive free radicals, and accordingly, the most suicidal damaged among the CYP isozymes. It is speculated that the antioxidant properties of curcumin inhibit the secondary inactivation of CYPs caused by reactive free radicals.

In conclusion, curcumin ingestion has no influence on hepatic CYP activity in rats, indicating no pharmacokinetic interaction with co-administered drugs. Curcumin does not prevent the decrease of CYP2E1 activity related to the first step of metabolic activation of CCl_4. However, curcumin is beneficial for ameliorating the subsequent inactivation of other CYP isozymes caused by CCl_4. The antioxidant properties of curcumin may contribute to the inhibition of the reactive free radicals produced from CCl_4 bioactivation. Further detail study will be needed to clarify the mechanism of curcumin against CCl_4-induced liver injury.

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—Notes—

経口投与したコンドロシンのラット軟骨への ^{35}S 硫酸取り込み効果

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Effect of Orally Administered Chondrosine on Uptake of ^{35}S Sulfate into Mice Cartilage

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Chondroitin sulfate is widely distributed in animal tissues and possibly plays an important role in different types of metabolic reactions as well as protecting joints, the internal wall of blood vessels, skin, bone, etc. In cartilage, glycosaminoglycans have a protective function; in particular, chondroitin sulfate stabilizes fibrous and cellular elements of the connective tissue and, at the same time, lubricates and protects the membranes in joints. Recently, chondroitin sulfate has been used as a nutraceutical for the treatment of joint diseases such as osteoarthritis, although acidic and large molecules such as chondroitin sulfate might not be able to be absorbed through digestive apparatus such as the intestine. In this study, we investigated the effects of orally administered chondrosine derived from shark chondroitin sulfate on the uptake of inorganic ^{35}S sulfate into rat cartilage and found that chondrosine stimulates the incorporation of ^{35}S sulfate into cartilage compared with intact chondroitin sulfate.

Key words——chondrosine; ^{35}S sulfate; uptake; rat cartilage

緒　　言

軟骨組織に含まれるコンドロイチン硫酸プロテオグリカンの1つであるアグリカンは，他の機能性分子，例えばヒアルロン酸と複合体を形成し，細胞の接着，増殖，分化等の重要な細胞活動の調節に関与していることが知られている.[1,2] 様々な原因により，この軟骨組織からのプロテオグリカンの分解・遊離が促進され，かつ組織におけるプロテオグリカンの生合成が低下すると，軟骨組織の破壊が進み，変形性関節症等の疾患が惹起するものと考えられている.[3] これまでにも各種軟骨疾患の予防あるいは治療を目的とするプロテオグリカン生合成促進物質についての報告があり，例えばベンゾチエピン誘導体,[4] 肝細胞増殖因子（HGF）,[5] ヒアルロン酸[6]等が報告されている．しかしながら，安全かつ有効性に優れた軟骨細胞プロテオグリカン生成促進のための栄養補助剤（サプリメント）あるいは医薬品については，いまだその開発途上といっても過言ではない.

一方，グルクロン酸と *N*-アセチルガラクトサミンを構成糖とするコンドロイチンやコンドロイチン硫酸は，変形性関節症の治療等に現在用いられており,[7,8] その効果についてもアメリカ合衆国国立保健局が中心となり，結合組織疾病の予防や治療のための調査が進行中である.[9] しかし，高分子量でかつ硫酸基，カルボキシル基を持つコンドロイチン硫酸などは，経口投与しても吸収される割合が低いため，1回に大量投与が行われている.[10] さらに作用機序も明確でない上に一般的な硫酸化多糖の消化管障害等の副作用を惹起することも懸念されている.[11]

今回，コンドロイチン硫酸から単離精製した構成2糖であるコンドロシンに着目し，コンドロイチン硫酸プロテオグリカン生合成の指標として，^{35}S 標識硫酸の取り込みについて，ラット血漿，膝軟骨，剣状軟骨を対象に検討を行い，若干の知見を得たので報告する.

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実 験 の 部

1. 実験動物 Wistar 系オスのラット（7 週齢，体重 161.6—188.4 g，日本エスエルシー㈱）を用いた．11 日間 SPF 動物舎（室温 23±3℃，湿度 55±20%，照明サイクル 12 時間）で予備飼育し，実験に用いた．飼料（オリエンタル酵母工業㈱）及び水道水は自由に摂取させた．投与当日に各ラットの体重を測定し，群内の体重範囲が平均値の±10%以内となるように 1 群 4 匹で選択した．

2. 試薬 ^{35}S 標識硫酸ナトリウムは市販品（比放射能，21.53 GBq/mmol, PERKINELMER）を使用した．投与及びコンドロシン調製用のコンドロイチン硫酸は市販品（サメ軟骨由来，分子量 50000，三栄源㈱）を用いた．核磁気共鳴スペクトル測定用重水はメルク㈱製（重水素純度 99.9%）を用いた．

その他の試薬は市販品試薬特級をそのまま使用した．水はラットの飼育以外は精製水を用いた．

3. 投与実験 入手した^{35}S 標識硫酸ナトリウム全量を生理食塩液で希釈して最終 12 ml とし，92.5 MBq (2.5 mCi) /ml の投与液を用時調製した．コンドロイチン硫酸 2 g に水を加えて 10 ml としたのち，超音波処理を行い懸濁し，0.2 g/ml の投与液を用時調製した．コンドロシン 2 g に水を加えて 10 ml としたのち，超音波処理を行い懸濁し，0.2 g/ml の投与液を用時調製した．^{35}S 標識硫酸ナトリウム単独投与群では，^{35}S 標識硫酸ナトリウム投与液を 2 ml/kg の用量で 25 G 注射付シリンジを用いてラットの尾静脈内に単回投与した．

コンドロイチン硫酸又はコンドロシンと^{35}S 標識硫酸ナトリウムを併用投与する群では，コンドロイチン硫酸投与液は 1 g/5 ml/kg，コンドロシン投与液は 1 g/5 ml/kg の用量で，各群の組織内濃度の測定を実施する当日まで 1 日 1 回，一定時刻に経口ゾンデを装着したシリンジを用いて胃内に強制投与し，^{35}S 標識硫酸ナトリウム投与液は併用するコンドロイチン硫酸及びコンドロシンの初回投与 1 時間後に

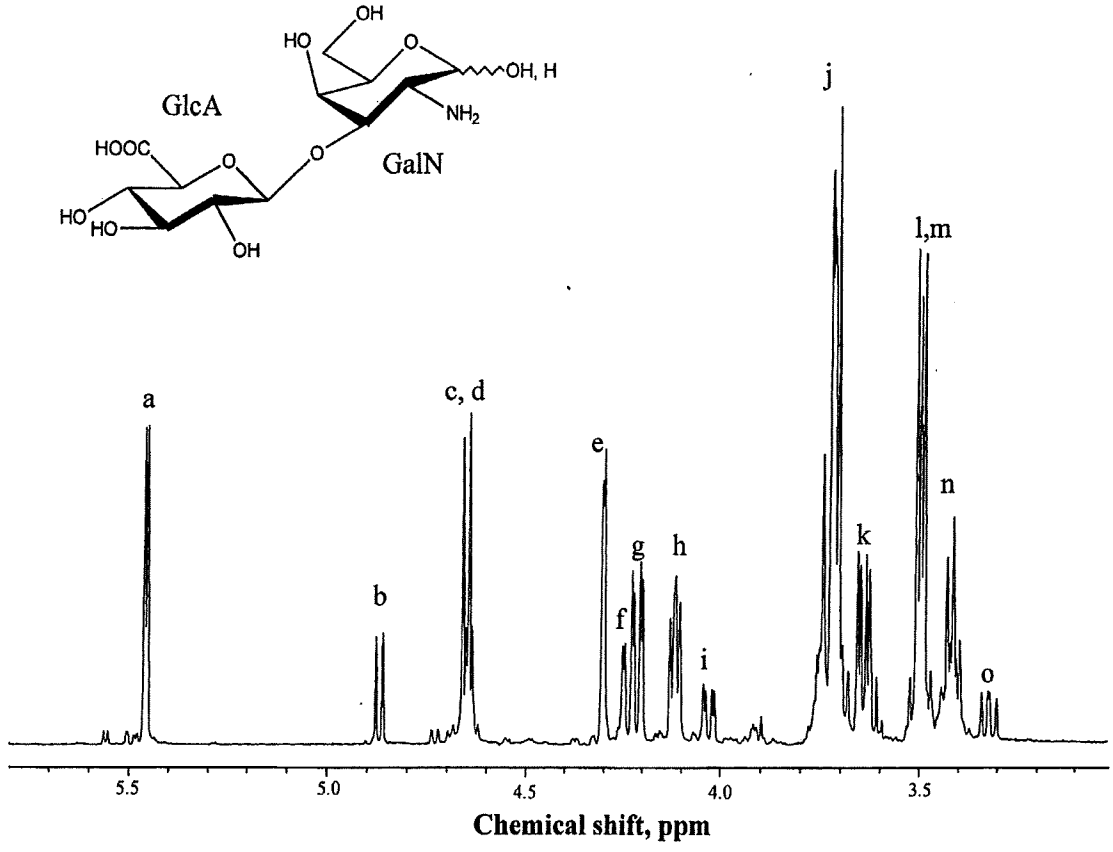

Fig. 1. ^{1}H-NMR Spectrum of Chondrosine Prepared from Chondroitin Sulfate

GlcA: glucuronic acid, GalN: galactosamine. a: GalNαH-1, b: GalNβH-1, c, d: GlcAβH-1. e: GalNaH-4, f: GalNβH-4, g: GalNαH-3, h: GalNαH-5; I: GalNβH-3, j: GalN H-6, k: GalNαH-2, l, m: GlcAH-3, -4, n, o: GlcAH-2.

2 ml/kg の用量で 25 G 注射付シリンジを用いてラットの尾静脈内に投与した．すなわち“投与後 24 時間”群に対しては試料採取 1 時間前までに計 2 回，また“投与後 72 時間”群に対しては計 4 回，コンドロイチン硫酸及びコンドロシンを投与した．^{35}S 標識硫酸投与後 24 時間後，72 時間後に血液及び組織を採取した．各群については 4 匹ずつのラットを用いて検討した．

取り込まれた ^{35}S 硫酸については血漿，剣状軟骨，膝関節軟骨を採取し，液体シンチレーションカウンターを用いて分析した．すなわち，血漿は腹大動脈よりヘパリンナトリウム入り真空採血管を用いて血液を採取した．遠心分離（1800×g, 4℃, 15 min）して得られた血漿は，その 100 μl を放射能測定用試料としてバイアルに採取し，組織溶解剤 SOLUENE-350 を 2 ml 加えて溶解した．溶解後，シンチレーター（HIONIC-FLUOR）を 10 ml 加えた．剣状軟骨，膝関節軟骨については全量を放射能測定用試料として秤量後，バイアルに採取し，組織溶解剤 SOLUENE-350 を 2 ml 加えて加温・溶解した．溶解後，シンチレーター HIONIC-FLUOR を 10 ml 加えた．得られた結果は，コンドロイチン硫酸及びコンドロシン非投与群に対して，小標本法による有意差検定（スチューデントの t 検定）を行った．

4. コンドロシンの調製　コンドロシンは既報の方法[12]に従いサメ軟骨由来コンドロイチン硫酸を加水分解し，電気透析装置により脱塩後，最終濃度が 90％になるようにエタノールを加えて遠心分離を繰り返し，沈殿を乾燥して調製した．コンドロシンの構造は日本電子㈱製 ECP600 を用いて ^{1}H-NMR スペクトルを測定し確認した．

結果と考察

Figure 1 に調製したコンドロシンの ^{1}H-NMR スペクトル及び各シグナルの帰属を示す．還元末端のガラクトサミンのアノメリックプロトンが α あるいは β 配置の違いにより，グルクロン酸に由来するシグナルも化学シフトが変化することが分かる．また，それぞれのシグナルの結合定数から，重水中でコンドロシンのガラクトサミン，グルクロン酸は椅子型コンフォーメーションをとることが推察できる．また 5.5 ppm, 4.7 ppm 付近に若干単糖に由来するシグナルが混在するものの顕著な不純物に由来するシグナルは観察されず，98％以上の純度のコンドロシンが調製できたことを示している．また，原料のコンドロイチン硫酸からのコンドロシンの回収率は 60％以上であった．

Figure 2 (a) にラット血漿中の ^{35}S 硫酸放射能を調べた結果を示す．72 時間後における血漿中放射能についてコンドロシン投与群で高い傾向があるが，24 時間後における有意差は観察されなかった．一方，Fig. 2 (b) に示すようにラット膝関節における ^{35}S 硫酸放射能は 24 時間後におけるコンドロイチン硫酸同時投与群で ^{35}S 硫酸の取り込み低下が観察さ

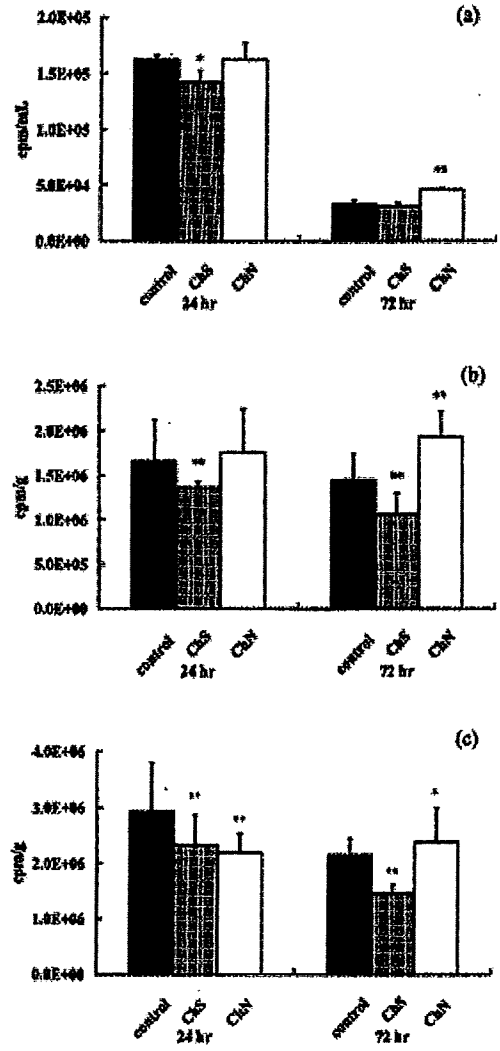

Fig. 2. $Na_2{}^{35}SO_4$ Concentrations in Plasma and Uptake in Cartilage Tissues of Rats after *i.v.* Administration with/without Chondrosine

a: plasma, b: articulatio genus cartilage, c: xiphoid cartilage. ChN: chondrosine, ChS: chondroitin sulfate. Each point represents the mean± S.E. obtained from 4 experiments. * $p<0.05$; ** $p<0.01$ against control.

れたが，コンドロシン投与群と対照群で差は見られなかった．これに対し，72時間後におけるコンドロシン投与群では明らかに^{35}S硫酸の取り込みが上昇していた．この傾向はFig. 2(c)に示すようにラット剣状軟骨においても観察され，コンドロシンは^{35}S硫酸の取り込みを上昇させる傾向が観察された．

またコンドロイチン硫酸を経口投与すると，関節における軟骨組織の分解酵素を阻害し，炎症の進行を抑制するとの報告がある[13,14]が，今回正常なラットを用いた場合，明確な^{35}S硫酸の取り込み低下が観察された．現在実験的炎症性関節炎モデルを用いた検討を行っている．一方，コンドロシンを経口投与した場合，顕著なラット軟骨への^{35}S硫酸の取り込みが観察された．コンドロシンの場合その分子量が小さいため，消化管からの吸収もコンドロイチン硫酸に比べて非常に高いことが予想できる．また，生体内に存在するコンドロイチン硫酸の分解系で働くグルクロニダーゼは，コンドロシンに対して作用しない可能性[15]があり，さらには阻害作用も考えられることから，今回の結果は，コンドロシンの^{35}S硫酸取り込み促進効果というよりもむしろ，生合成されたコンドロイチン硫酸の分解抑制効果と考えられる．今後さらにコンドロシンの定量法を確立することにより，その体内動態及び生物活性について詳細に検討する予定である．

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Immunological Activity of Chondroitin Sulfate

I. Chapter Overview

The use of chondroitin sulfate (CS) for the symptomatic treatment of osteoarthritis (OA) has become very popular; however, it has also been the subject of controversy for several reasons. First, the nutraceutical industry is less regulated than the pharmaceutical industry and thus, the nutraceutical CS often suffers from poor quality control. Second, the bioavailability of orally administered CS is not generally accepted. Third, the mechanism of the effect of CS for treatment of OA remains unclear. There is abundant *in vitro* and *in vivo* evidence from animal and human clinical studies demonstrating the efficacy and safety of CS. This chapter focuses on the immunological activity of structurally regulated CSs. The mechanism of this immunological activity appears to be through CS binding to receptors related to cytokine production in lymphocytes such as splenocytes.

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II. Introduction

Most important pharmaceuticals have their origin in natural products, such as herbs and antibiotics, however, many physicians are deeply skeptical about the use of natural remedies. This skepticism is based on the concerns about the lack of scientific evidences of their efficacy. A new class has emerged called nutraceuticals, which are nutritional supplements with presumed pharmaceutical properties and efficacy. Because these substances are relatively unregulated, there is no requirement for rigorous scientific evidences before marketing. This lack of regulation also poses severe problems with purity and quality control. Glucosamine and CS sales alone in Japan are estimated at several billion JPY (several hundred million US dollars) in retail sales. Furthermore, the combination of glucosamine and CS is a very popular nutraceutical in the USA. While there is no scientific evidence on the efficacy of glucosamine and CS in the treatment of joint disease, the market of this nutraceutical product continues to grow. Self-medicating patients represent the driving force making nutraceutical products bestsellers throughout the world. Glucosamine and CS have been widely studied in tissue culture, animal models of arthritis, veterinary clinical trials, and human comparative or placebo controlled trials. All published studies suggest a positive effect, and no trial has shown significant side effects. Based on the absence of conclusive data, the National Institute of Health has started "*NIH Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT)*" (http://www.niams.nih.gov/ne/press/2000/gait_qa.htm#what) to obtain definitive scientific evidence for the efficacy of glucosamine and CS in the treatment of arthritis.

Glucosamine and CS are integral components of articular cartilage and are important to the physiologic and mechanical properties of this tissue. Glucosamine is involved in cartilage formation by acting as the precursor of the disaccharide unit in glycosaminoglycans (GAGs) (Baker and Ferguson, 2005; De los Reyes *et al.*, 2000; Scott *et al.*, 2005). Chondroitin sulfate is a GAG that is a component of the aggrecan structure that makes up articular cartilage (Freeman, 1979). It binds collagen fibrils and limits water content by cooperating with hyaluronan, which is also a GAG. Chondroitin sulfate plays a role in allowing the cartilage to resist tensile stresses during various loading conditions by giving the cartilage resistance and elasticity (Muir, 1986). Exogenously administered glucosamine and CS have been shown *in vitro* to have other physiological effects. Glucosamine stimulates chondrocytes to increase secretion of GAGs and proteoglycans (PGs) *in vitro* (Jimenez, 1996). There is also evidence of CS-based anti-inflammatory activity not related to prostaglandin metabolism, probably through a free radical scavenging effect (Raiss, 1985). Osteoarthritis is clinically characterized as the decomposition of cartilage by degradative enzymes. These enzymes are competitively inhibited by CS *in vitro* (Bartolucci *et al.*, 1991;

Bassleer *et al.*, 1992). Moreover, laboratory studies have demonstrated a synergistic effect when glucosamine and CS are administered together. Lippiello *et al.* (2000) noted that the coadministration of CS and glucosamine resulted in a greater increase of $^{35}SO_4$ incorporation into GAGs (97%) than demonstrated by either agent alone (glucosamine, 32%; CS, 32%). This synergistic effect was also observed in experiments on CS's antiprotease activity *in vitro* (Arner, 2002). However, the orally administered CS has to be absorbed through gastric/intestinal system into blood flow to show these effects in its intact form.

There are many arguments regarding whether or not orally administered CS is absorbed through gastric/intestinal system (Owens, 2004). We have found only very small amounts of relatively low-molecular weight CS chains (average molecular weight 15,000) in the blood over 24 h following oral administration to mice. The failure to observe significant bioavailability suggests a novel concept that CS might act in the absence of absorption, on the humoral immunosystem by stimulating the intestinal intraepithelial lymphocytes (IEL) through cytokine production (Akiyama *et al.*, 2004; Sakai *et al.*, 2002a). This chapter describes the effects of CS on immunosystem *in vivo* and *in vitro*.

III. Clinical Experience (David and Lynne, 2003)

In an artificially induced cartilage injury model, Uebelhart *et al.* (1998) noted that treatment with CS resulted in a marked reduction in the loss of PSs as compared with controls. Lippiello *et al.* (1999) reported that the effect of CS given to normal dogs was an increase in the serum GAG levels. Using indirect assessments of cartilage metabolism, they found that serum from treated dogs increased biosynthetic activity (incorporation of radioactively labeled glucosamine) and decreased proteolytic degradation (release of ^{35}S) from prelabeled normal calf cartilage segments. Using a rabbit instability model created by transecting the anterior cruciate, Lippiello *et al.* (2000) found that the articular matrix was severely degraded in the untreated group while remaining essentially intact in the treated group. In a canine model of unilateral carpal synovitis, although no effect was observed if the treatment was started after the synovitis occurred, dogs pretreated with the combination of glucosamine and CS have shown less evidence of bone remodeling and lower lameness scores (Canapp *et al.*, 1999).

Glucosamine and CS are often used either separately or in combination for the treatment of arthritic ailments (Dechant *et al.*, 2005). The safety profile of these nutraceuticals has been reviewed (Hungerford and Valaik, 2003). When recommending a supplement to patients, the physicians should take into account the purity of the ingredients, reputation of the manufacturer, and the molecular weight of chondroitin supplied. An analysis of

marketed products indicated that the amounts of glucosamine and CS present in the products sold often fell short of the declared values on the label (Adebowale *et al.*, 2000). Most of the commercially available supplements sold in Japan analyzed in our laboratory contained less CS than indicated on their label, and significant amounts of carrageenan was found in many of these products (data not published). These discrepancies may introduce the confusion underlying the potential benefits of these nutraceuticals in treating arthritic disease.

Several clinical trials exploring the efficacy of both glucosamine and CS in the treatment of OA have been performed over the past 30 years as indicated in an earlier section; the outcomes of these studies have also been reviewed (Leeb *et al.*, 2000; McAlindon *et al.*, 2000; Richy *et al.*, 2003). The goal of these reviews was to assess both the potential symptom-modifying (e.g., pain and functional efficacy) and structure-modifying (e.g., changes in joint space narrowing) activities of glucosamine and CS in alleviating symptoms of OA of the knee using outcome-oriented metaanalysis of these randomized clinical trials. The general conclusion from these reviews is that glucosamine ingestion shows efficacy in both narrowing joint space and some symptom-modifying parameters. However, although CS ingestion showed similar symptom-modifying effects, the structure-modifying benefits still need to be confirmed. Given this clinical evidence, there is clearly a need for more basic research aimed at elucidating the cellular and molecular mechanisms involved with these two interesting nutraceuticals.

IV. Metabolic Fate of Orally Administered Chondroitin Sulfates

The metabolic fate of orally administered CS is ambiguous (Ronca and Conte, 1993). Baici *et al.* (1993) investigated the ability of an oral dose of CS to impact the concentration of GAGs in humans. In this study, CS samples were administered to six healthy volunteers, six patients with rheumatoid arthritis, and six patients with OA. The concentration of GAGs in serum was reportedly unchanged following ingestion (Baici *et al.*, 1993). Morrison (1977) has indicated that the intact absorption of CS was extremely low, estimating the absorption rate to be between 0 and 8%. The complexity of this issue is based on the fact that CS is found in a wide range of molecular weights, chain lengths, charge distributions, with positional isomers of sulfo groups, and containing variable percentages of similar disaccharide residues comprised of sulfated glucuronic acid (GlcA) and *N*-acetylgalactosamine (GalNAc) as shown in Fig. 1. A further complication occurs because low-molecular weight derivatives of CS have also been pharmacologically produced and utilized in some of the pharmacokinetic and therapeutic studies and trials (Conte *et al.*, 1991; Ronca *et al.*, 1998). It is quite possible that the contrasting metabolic fates of orally administered CSs are a direct reflection

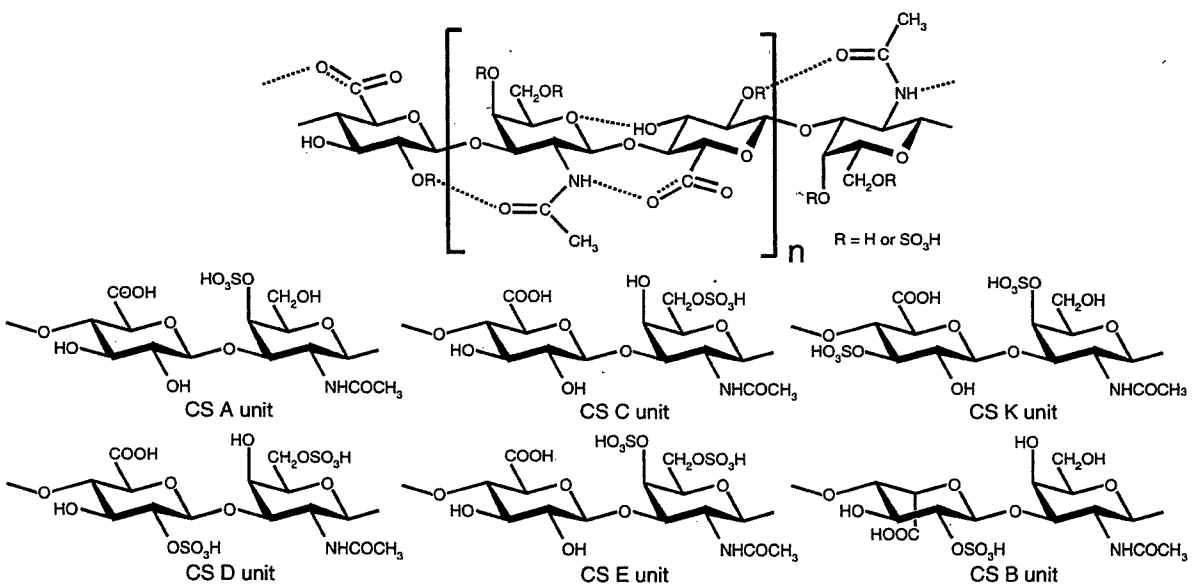

FIGURE 1 Disaccharide structures found in chondroitin sulfate.