Alkylthioacetic acids (3-thia fatty acids) as non-β-oxidizable fatty acid analogues: a new group of hypolipidemic drugs. III. Dissociation of cholesterol- and triglyceride-lowering effects and the induction of peroxisomal β-oxidation

Asle Aarsland,* Niels Aarsaether,* Jon Bremer,† and Rolf K. Berge [1,*]

Laboratory of Clinical Biochemistry,* University of Bergen, Haukeland Sykehus, N-5021 Bergen, Norway, and Institute of Medical Biochemistry,† University of Oslo, P. O. Box 1112, Blindern, Oslo 3, Norway

Abstract Previous work in this laboratory indicated that sulfur-substituted fatty acid analogues, 1,10-bis(carboxymethylthio)decane and alkylthioacetic acid, both non-β-oxidizable compounds, and the β-oxidizable alkylthiopropionic acid (1) caused, to different extents, dose-related hepatomegaly and proliferation of peroxisomes and enhanced peroxisomal fatty acid β-oxidation. In the present study, treatment of normolipidemic rats with alkylthioacetic acid resulted in a dose- and time-dependent decrease in serum cholesterol and serum and liver triglycerides to an extent comparable to that of the 3-thiadicarboxylic acid. At hypolipidemic doses, alkylthioacetic acid caused no hepatomegaly, did not significantly alter peroxisome morphology, and only marginally affected peroxisomal β-oxidation activity. Only at the highest, nonpharmacological doses of alkylthioacetic acid were these hepatic parameters increased, although to a lesser extent than by the 3-thiadicarboxylic acid. Hence, on the basis of dose- and time-related studies of the two compounds, data indicate that the hypotriglyceridemia and hypocholesterolemia were dissociated from induction of peroxisomal β-oxidation and peroxisome proliferation. Palmitic acid and hexadecanedioic acid, both β-oxidizable fatty acids, only marginally affected the serum and liver parameters. The β-oxidizable fatty acid analogue, alkylthiopropionic acid lowered the serum triglycerides in normolipidemic rats. In contrast to the 3-thiadicarboxylic acid and alkylthioacetic acid, alkylthiopropionic acid treatment at hypolipidemic doses caused accumulation of triglycerides in the liver.—**Aarsland, A., N. Aarsaether, J. Bremer, and R. K. Berge.** Alkylthioacetic acids (3-thia fatty acids) as non-β-oxidizable fatty acid analogues: a new group of hypolipidemic drugs. III. Dissociation of cholesterol- and triglyceride-lowering effects and the induction of peroxisomal β-oxidation. *J. Lipid Res.* 1989. **30:** 1711–1718.

Supplementary key words hepatic and plasma lipids • hypolipidemic drugs • peroxisomal fatty acid oxidation

Excess of lipids in blood is considered to accelerate the development of arteriosclerosis and is a risk factor for myocardial infarction. Accordingly, a reduction of high blood lipid levels by diet or by drugs is used as a preventive measure in people at risk.

Some ordinary long-chain fatty acids, particularly polyunsaturated fatty acids of fish origin, are effective in lowering plasma triglyceride levels in hypertriglyceridemic and combined hyperlipidemic human subjects (2, 3). Experimental studies in animals have shown that these fatty acids enhance fatty acid oxidation (4), partly by increased peroxisomal activity (5–7), decrease synthesis of fatty acids, and lower the hepatic secretion of VLDL (7–9).

Similar effects are obtained with hypolipidemic drugs such as tiadenol, clofibrate, ciprofibrate and fenofibrate among others, which all are known to be peroxisome-proliferating in rodents (10–13). Thus, a possible side effect associated with hypolipidemic drugs may be increased proliferation of peroxisomes concomitant with increased H_2O_2-generating peroxisomal β-oxidation.

Polyunsaturated long-chain fatty acids are relatively slowly metabolized. Tiadenol and clofibrate and its derivatives are converted to carboxylic acids that are blocked for β-oxidation (1). We found it likely, therefore, that simple non-β-oxidizable fatty acid analogues might show desirable lipid-lowering effects with minimal side effects.

We have recently reported that a sulfur-substituted dicarboxylic acid, bis(carboxylmethylthio)decane (3-thiadicarboxylic acid, BCMTD), which is blocked for both ω- and β-oxidation, tetradecylthioacetic acid (alkylthioacetic acid CMTTD), which is only blocked for β-oxidation, and tetradecylthiopropionic acid alkylthiopropionic acid, CETTD),

Abbreviations: BCMTD, 1,10-bis(carboxymethylthio)decane; CMTTD, 1-(carboxymethylthio)tetradecane (alkylthioacetic acid); CETTD, 1-(carboxyethylthio)tetradecane, (alkylthiopropionic acid); PMA, palmitic acid; HDDA, hexadecanedioic acid; VLDL, very low density lipoprotein; CMS, carboxymethyl cellulose.

[1]To whom correspondence should be addressed.

which can be β-oxidized (14) (Table 1), act as peroxisome proliferators with BCMTD being the most potent (1, 15). The order of potency with respect to induction of key enzymes involved in oxidation and esterification of long-chain fatty acids, including peroxisomal β-oxidation, was BCTMD > CMTTD > > CETTD.

Inasmuch as fish oil, which contains poorly metabolizable fatty acids, has a hypotriglyceridemic effect and there is an apparent mutual relationship between the hypolipidemic drugs and peroxisome proliferation, it was of interest to evaluate whether these sulfur-substituted fatty acid analogues have hypolipidemic effects in normolipidemic rats in vivo.

MATERIALS AND METHODS

Chemicals and drugs

Hexadecanedioic acid (HDDA) was obtained from Aldrich-Chemie (Steinheim, West Germany). 1·10-Bis-(carboxymethylthio)decane (BCMTD), 1-(carboxymethylthio)-tetradecane (CMTTD), and 1-(carboxyethylthio)tetradecane (CETTD) were prepared as described earlier (1, 15, 16). All other chemicals were obtained from common commercial sources and were of reagent grade.

Animals and treatments

Male Wistar rats from Möllegaard Breeding Laboratory, Ejby, Denmark, weighing 170–180 g, were housed individually in wire cages in a room maintained at 12-h light–dark cycles and a constant temperature of 20 ± 3°C. The animals were acclimatized for at least 1 week under these conditions before the start of the experiment. BCMTD, CMTTD, CETTD, HDDA, and palmitic acid were suspended in 0.5% sodium carboxymethyl cellulose (CMS). In the dose- response experiments, the individual agents were administered by gastric intubation in a volume of 1 ml once a day for 5 days and the animals were killed at the start of the sixth day after 12 h of starvation. The animals were separately treated from low to high dose levels with the fatty acids. The doses were: BCMTD, 75, 150, 250, and 500 mg/day per kg body weight; CMTTD, 75, 150, 250, 500, and 750 mg/day per kg body weight; CETTD, 150, 400, and 800 mg/day per kg body weight; HDDA, 75, 150, and 750 mg/day per kg body weight; palmitic acid, 350, 500, and 1000 mg/day per kg body weight. In the time study a daily dose of 150 mg/kg body weight of BCMTD, CMTTD, and CETTD suspended in 0.5% CMS was administered by gavage, total volume 1 ml. The control animal groups received only CMS. All animals had free excess to water and food. The food composition was as described earlier (11).

The body weights were measured daily. At the end of the experiments, the rats were fasted and weighed. Under light halothane anesthesia, cardiac puncture was performed to obtain blood samples and the livers were removed and immediately chilled on ice and weighed. Serum was prepared by centrifuging the clotted whole blood at 1000 *g* for 10 min.

Analytical methods

The livers from individual rats were homogenized in ice-cold sucrose-medium (0.25 M sucrose in 10 mM HEPES buffer, pH 7.4, and 1 mM EDTA) and the resulting nuclear plus postnuclear fraction was used as the total homogenate (6).

Protein was assayed by Bio-Rad protein assay kit (Bio-Rad, Richmond, CA).

The enzymatic activity of palmitoyl-CoA-dependent dehydrogenase (usually termed peroxisomal β-oxidation) was determined as previously described (6, 11).

Morphometric analysis was carried out as described earlier (1, 15) and enzymatic lipid analyses were performed according to the manufacturer's instructions (monotest cholesterol enzymatic kit, Boehringer Mannheim, Germany, and Biopak triglyceride enzymatic kit, Biotrol, Paris, France).

Presentation of the results

Data on lipids are presented as means and as means ± SEM; enzyme activities are means ± SD. Three animals in each experimental group and 12 controls were used.

RESULTS

Rats were given different amounts of sulfur-substituted fatty acid analogues; alkylthiopropionic acid (CETTD),

TABLE 1. Structural formulas, names, and abbreviations of sulfur-substituted fatty acid analogues and ordinary fatty acids

Structure of Compound	Systematic Names and Abbreviations	Trivial Names
$HOOC-CH_2-S-(CH_2)_{10}-S-CH_2COOH$	1.10-bis(carboxymethylthio)decane (BCMTD) (non-β-oxidizable, non-ω-oxidizable)	3-thiadicarboxylic acid
$CH_3-(CH_2)_{13}-S-CH_2COOH$	1-(carboxymethylthio)tetradecane (CMTD) (non-β-oxidizable)	alkylthioacetic acid
$CH_3-(CH_2)_{13}-S-CH_2-CH_2COOH$	1-(carboxyethylthio)tetradecane (CETTD) (β-oxidizable)	alkylthiopropionic acid
$CH_3-(CH_2)_{13}-CH_2-COOH$	hexadecanoic acid (PMA) (β-oxidizable)	palmitic acid
$HOOC-CH_2-(CH_2)_{12}-CH_2COOH$	hexadecanedioic acid (HDDA) (β-oxidizable)	

which represents a β-oxidizable fatty acid, 3-thiadicarboxylic acid (BCMTD) and alkylthioacetic acid (CMTTD), which both are non-β-oxidizable (**Table 1**), and ordinary fatty acids (palmitic acid, PMA, and hexadecanedioic acid, HDDA) for 5 days. All animals treated with the fatty acid analogues at various doses and as a function of time gained body weight at the same rate as controls. Rats in each experimental group consumed similar amounts of food (20 g/day) irrespective of the dietary regime, indicating that appetite was not affected and the drugs were well tolerated. Drug-treated rats appeared healthy and looked and behaved like the normal animals.

Serum-lipids

Repeated administration of 3-thiadicarboxylic acid and alkylthioacetic acid to rats caused a dose-related reduction of serum cholesterol (60–75% decrease, **Fig. 1A**) and trigly-

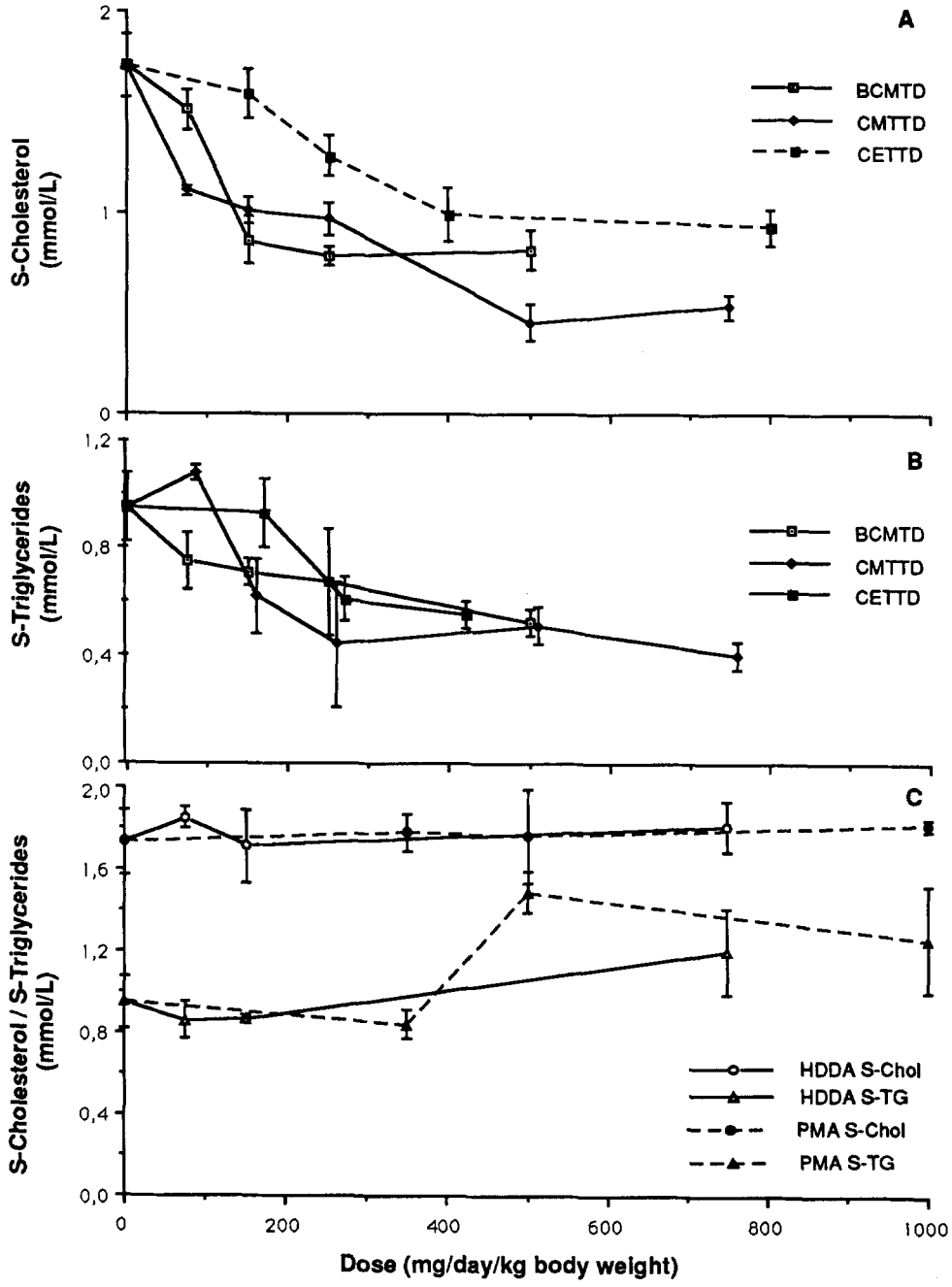

Fig. 1. Dose-dependent changes of serum cholesterol and triglycerides in animals treated with 3-thiadicarboxylic acid (⊡-⊡) (A, B), alkylthioacetic acid (♦-♦) (A, B), alkylthiopropionic acid (■-■) (A, B), palmitic acid (●-●, ▲-▲) (C) and hexadecanedioic acid (O-O, △-△) (C) for 5 days.

TABLE 2. Time-dependent changes of serum lipids in rats treated with sulfur-substituted fatty acid analogues

Days of Treatment	Nontreated		BCMTD		CMTTD		CETTD	
	Chol	TG	Chol	TG	Chol	TG	Chol	TG
				mmol/l				
0	1.81 ± 0.13	1.02 ± 0.13						
0.5			1.65 ± 0.12	1.15 ± 0.08	1.50 ± 0.11**	1.32 ± 0.30	1.94 ± 0.37	1.47 ± 0.63
1			1.24 ± 0.14	0.90 ± 0.15	1.27 ± 0.10*	0.89 ± 0.02**	1.79 ± 0.10	1.31 ± 0.50
1.5			0.91 ± 0.21*	1.11 ± 0.06	1.34 ± 0.13*	1.02 ± 0.32	1.55 ± 0.24	1.46 ± 0.38
2			0.98 ± 0.02*	0.77 ± 0.12*	1.29 ± 0.16*	1.19 ± 0.32	1.45 ± 0.15**	0.97 ± 0.25
3			0.92 ± 0.12*	0.58 ± 0.06*	1.08 ± 0.07*	0.65 ± 0.17*	1.61 ± 0.18	1.38 ± 0.08**
7			0.85 ± 0.11*	0.65 ± 0.15*	1.06 ± 0.06	0.55 ± 0.05*	1.95 ± 0.02	2.03 ± 0.22*
10			0.78 ± 0.10*	0.66 ± 0.08*	0.90 ± 0.11*	0.65 ± 0.15*	1.64 ± 0.11	2.12 ± 0.19*
14			0.85 ± 0.05*	0.81 ± 0.12*	0.60 ± 0.08*	0.48 ± 0.02*	1.83 ± 0.25	2.20 ± 0.08*

The tabulated values (mmol/l) are means ± SEM of 12 control animals and three rats in each treatment group at a dose of 150 mg/day per kg body weight; $*P < 0.01$; $**P < 0.05$. Abbreviations: Chol, cholesterol; TG, triglyceride; BCMTD ($HOOC-CH_2-S-(CH_2)_{10}-S-CH_2COOH$), the thiadicarboxylic acid; CMTTD ($CH_3(CH_2)_{13}-S-CH_2COOH$), the alkylthioacetic acid; CETTD ($CH_3(CH_2)_{13}-S-CH_2-CH_2COOH$), the alkylthiopropionic acid.

cerides (50–60% decrease, Fig. 1B). Hypocholesterolemic and hypotriglyceridemic effects were already established during the first 2 days of treatment of rats with 3-thiadicarboxylic acid and alkylthioacetic acid (150 mg/day per kg body weight) (**Table 2**).

Significant reduction of serum cholesterol (Fig. 1A) and triglycerides (Fig. 1B) after alkylthiopropionic acid treatment for 5 days was observed at a dose of 400 mg/day per kg body weight. Treatment of rats maintained on a standard pellet diet with 150 mg/day per kg body weight alkylthiopropionic acid, however, resulted in a 2.2-fold increase in serum triglycerides, whereas the serum cholesterol level was only marginally affected (Table 2).

In palmitic acid- and hexadecanedioic acid-treated rats, no changes of the serum cholesterol and triglyceride levels were observed (Fig. 1C).

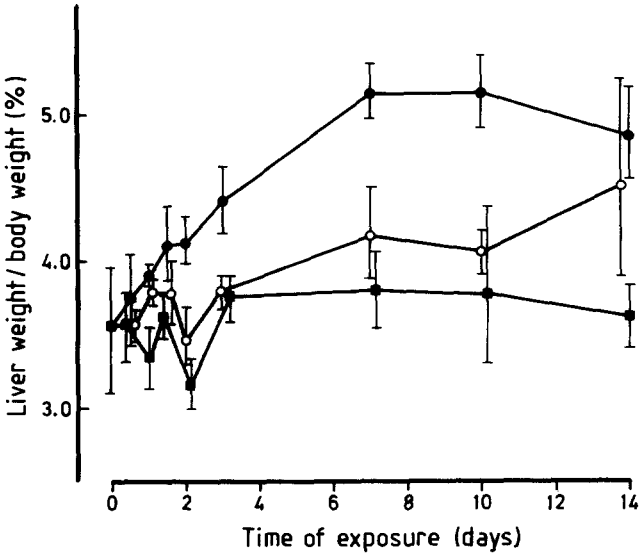

Fig. 2. Effect of 3-thiadicarboxylic acid (●-●), alkylthioacetic acid (○-○), and alkylthiopropionic acid (■-■), (150 mg/day per kg body weight) on hepatomegaly as a function of time.

Hepatic pleiotropic response

Hepatomegaly. Previous work in this laboratory indicated that BCMTD, characterized both as a non-β- and non-ω-oxidizable fatty acid derivative, caused dose-related hepatomegaly (1). In the present study feeding 3-thiadicarboxylic acid at a dose of 150 mg/day per kg significantly increased the liver weight within 24 h; the weight continued to increase up to 7 days, when a 1.5-fold increase was obtained (**Fig. 2**). With alkylthioacetic acid feeding at a dose of 150 mg/day per kg body weight, only a modest trend toward increases in liver weight was seen after 7 days of feeding; however, the increases were not statistically significant (Fig. 2). No hepatomegaly resulted with alkylthiopropionic acid feeding (Fig. 2) or in PMA- and HDDA-treated animals (data not shown), all of which are characterized as β-oxidizable fatty acids.

Liver lipids

Administration of increasing amounts of 3-thiadicarboxylic acid, palmitic acid, and hexadecanedioic acid to rats for 5 days only marginally affected the hepatic cholesterol content (**Table 3**). Repeated administration of alkylthioacetic acid and alkylthiopropionic acid, however, tended to decrease the hepatic cholesterol (Table 3); this was already evident during the first 2 days of treatment (**Fig. 3A**). At that time a 50% reduction of hepatic cholesterol was observed in the alkylthioacetic acid- and alkylthiopropionic acid-treated animals. Subsequently, with longer feeding periods, the cholesterol content of liver returned to normal values (Fig. 3A) in agreement with the dose-dependent experiments (Table 3).

The hepatic triglyceride content was reduced by feeding 3-thiadicarboxylic acid, palmitic acid, and hexadecanedioic acid (Table 3). Repeated administration of alkylthioacetic acid caused a dose-related reduction of liver triglycerides (Table 3), although to a lesser extent than that caused by 3-thiadicarboxylic acid. Treatment of rats with 3-thiadicar-

TABLE 3. Dose-dependent changes of hepatic lipids in rats treated with sulfur-substituted fatty acid analogues, palmitic acid, and hexadecanedioic acid for 5 days

Dose	Nontreated		BCMTD		CMTTD		CETTD		PMA		HDDA	
	Chol	TG	Chol	TG	Chol	TG	Chol	TG	Chol	TG	Chol	TG
mg/day/kg body weight	*μmol/g liver*											
0	9.79 ± 0.38	6.14 ± 0.66										
75			10.57 ± 0.18	3.02 ± 0.10*	9.71 ± 0.18	5.21 ± 0.52					9.57 ± 0.12	2.12 ± 0.11*
150			9.94 ± 0.15	2.93 ± 0.19*	9.07 ± 0.05**	5.42 ± 0.34	10.03 ± 0.18	28.60 ± 4.95*			9.18 ± 0.37	2.60 ± 0.34*
250			9.48 ± 0.23	2.47 ± 0.56*	8.37 ± 0.14*	4.80 ± 0.67*						
350									9.47 ± 0.53	4.84 ± 0.41		
400							8.60 ± 0.23**	48.57 ± 6.12*				
500			10.33 ± 0.48	3.30 ± 0.63*	8.15 ± 0.35*	5.60 ± 0.88			10.55 ± 0.25	3.62 ± 0.38*		
750					9.09 ± 0.77	6.06 ± 0.40					9.67 ± 0.41	5.37 ± 0.18
800							8.52 ± 1.06	70.42 ± 0.80				
1000									7.44 ± 0.55**	4.04 ± 0.85		

The tabulated values (μmol/g liver) are means ± SD of 12 control animals and three rats in each treatment group; $^*P < 0.01$; $^{**}P < 0.05$. Abbreviations: Chol, cholesterol; TG, triglyceride; BCMTD ($HOOC-CH_2-S-(CH_2)_{10}-S-CH_2COOH$), the thiadicarboxylic acid; CMTTD ($CH_3(CH_2)_{13}-S-CH_2COOH$), the alkylthioacetic acid; CETTD ($CH_3(CH_2)_{13}-S-CH_2-CH_2COOH$), the alkylthiopropionic acid; PMA, palmitic acid; HDDA, hexadecanedioic acid.

boxylic acid and alkylthioacetic acid at 150 mg/day per kg body weight resulted in acute reduction of the hepatic triglyceride level which was already established in 1–3 days of treatment and amounted to a 50% decrease in total liver triglycerides (Fig. 3B).

Noteworthy, in alkylthiopropionic acid-fed animals, the triglyceride content of liver increased in dose-related manner and at a dose of 800 mg/day per kg body weight a 12-fold increased triglyceride level was observed (Table 3). Upon dissection, the steatosis was macroscopically evident. The liver had a yellow-brown tincture, and its viscous consistence made it difficult to remove. With a dose of 150 mg/day per kg body weight daily, however, the lipotrophic nature of alkylthiopropionic acid was not evident before the 7th day (Fig. 3C).

Peroxisomal β-oxidation

The time-course pattern showed that 3-thiadicarboxylic acid was more effective than alkylthioacetic acid in inducing peroxisomal β-oxidation in the total liver homogenates from 12 h to 14 days at daily doses of 150 mg/kg body weight (**Fig. 4**). It is interesting to note that the peroxisomal β-oxidation in the 3-thiadicarboxylic acid-treated animals began to increase after only 12 h and continued to rise for up to 36 h, when a 5-fold stimulation over the basa value (control animals) was obtained (Fig. 4). The peroxisomal β-oxidation of the alkylthioacetic acid dosage groups also tended to increase within hours; however, the increases were not statistically significant. Significant peroxisomal β-oxidation stimulation was observed in the animals treated with alkylthioacetic acid for 2 days when about a 2-fold stimulation ($P < 0.01$) was observed (Fig. 4). Subsequently, the level of the peroxisomal β-oxidation activity tended to level off at 7- and 3-fold increases in the 3-thiadicarboxylic acid- and alkylthioacetic acid-treated animals, respectively. Alkylthiopropionic acid only marginally affected the peroxisomal activity (Fig. 4).

DISCUSSION

The present results clearly show that sulfur-substituted fatty acid analogues, especially 3-thiadicarboxylic acid and alkylthioacetic acid, possess hypocholesterolemic and hypotriglyceridemic capacities (Table 2, Fig. 1). In dose- and time-dependent studies, alkylthioacetic acid was able to reduce serum cholesterol and triglyceride to the same extent, if not even more, as compared to 3-thiadicarboxylic acid. Noteworthy, alkylthioacetic acid and 3-thiadicarboxylic acid were more active in lowering serum cholesterol than triglyceride levels. This is clearly shown in the time-course study where maximal lowering of serum cholesterol levels was observed within 36 to 48 h and maximal lowering of serum triglyceride content was obtained after 3 days (Table 2).

It has been proposed that the increase in hepatic peroxi-

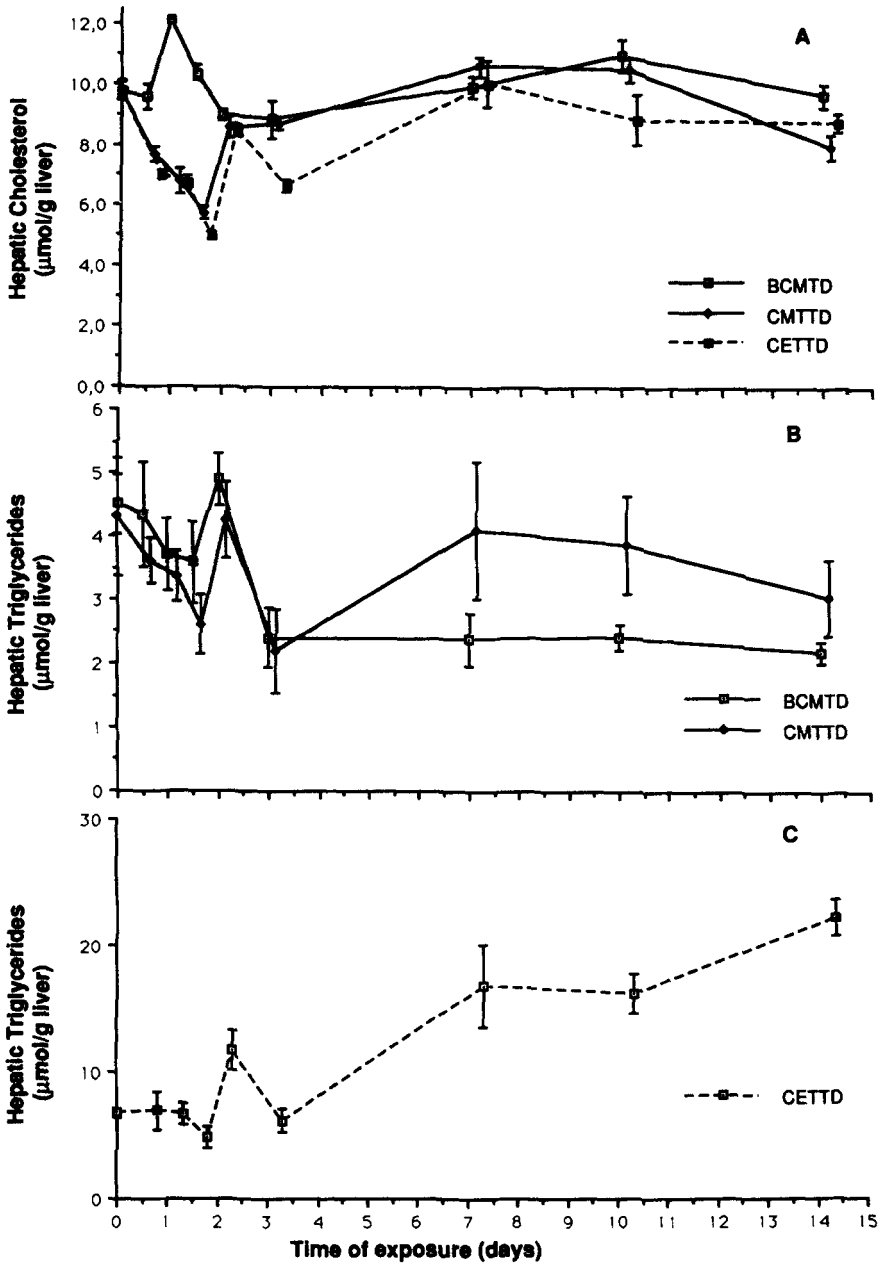

Fig. 3. Time-dependent changes of hepatic lipids in rats treated with BCMTD (□-□) (A, B), CMTTD (♦-♦) (A, B), and CETTD (■-□) (A, C).

somal oxidation of fatty acids in rats is responsible for the reduction in serum lipids (7). Previous work in this laboratory indicated that at pharmacological doses of alkylthioacetic acid (75–150 mg/kg body weight) no hepatomegaly and marginal effects on peroxisomal activities were observed (1). At a daily dose of 150 mg/kg body weight, the peroxisomal β-oxidation was not significantly increased within 24 h of feeding (Fig. 4). Hence, the increase in peroxisomal β-oxidation is not a prerequisite for the hypolipidemic effect of alkylthioacetic acid.

This was reinforced in studies with 3-thiadicarboxylic acid. In the dose- and time-related studies there was no correlation between the hypolipidemic action (Table 2) and induction of peroxisomal β-oxidation (Fig. 4).

At hypolipidemic doses (Fig. 1) alkylthiopropionic acid did not cause hepatomegaly (Fig. 2) and only marginally affected peroxisomal (Fig. 4) and extra-peroxisomal enzyme activities (1, 15). Hence, on the basis of dose- and time-related studies of the three sulfur-substituted fatty acids, the data indicate that the hypotriglyceridemia and hypocholes-

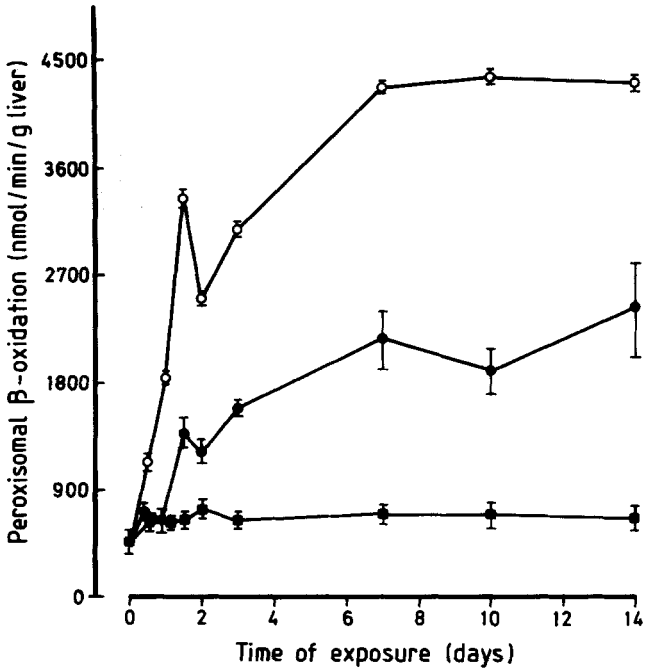

Fig. 4. Time-dependent changes of peroxisomal β-oxidation in total liver homogenates of rats by the 3-thiadicarboxylic acid (O-O), alkylthioacetic acid (●-●), and alkylthiopropionic acid (■-■). The tabulated values represent the means ± SD of three treated animals and six controls.

terolemia are dissociated from induction of peroxisome β-oxidation and peroxisome proliferation. This is strengthened by the fact that induction of the hepatic pleiotropic response, i.e., hepatomegaly, and peroxisome proliferation was pronounced only with higher doses of alkylthioacetic acid, and to a lesser extent than with 3-thiadicarboxylic acid.

Alkylthiopropionic acid had triglyceride-lowering potential (Table 2, Fig. 1). However, a side effect associated with the consumption of alkylthiopropionic acid was an induction of fatty liver (Table 3, Fig. 3) and a delayed hypertriglyceridemia (Table 2). 3-Thiadicarboxylic acid at hypolipidemic doses caused hepatomegaly, induced altered peroxisome morphology, and increased activities of key enzymes involved in oxidation and esterification of long-chain fatty acids, including peroxisomal β-oxidation (1, 14). Whether acute induction of peroxisomal β-oxidation (Fig. 4) is a potential side effect of administration of 3-thiadicarboxylic acid at hypolipidemic doses is yet to be considered.

Repeated administration of the more recent members of the fibrate family (fenofibrate, bezafibrate, gemfibrosil) to animals and humans reduces plasma triglyceride, whereas plasma cholesterol is essentially unchanged (13, 17, 18). In normal subjects, a moderate supplement of n-3 (ω-3) fatty acids reduces plasma triglyceride without changing plasma cholesterol (19). In contrast, the HMG-CoA reductase inhibitors (lovastatin, simvastatin, and pravastatin) are potent hypocholesterolemic drugs (20, 21).

Alkylthioacetic acid has been found to increase fatty acid oxidation and inhibit liver lipogenesis (22) and cholesterogenesis (R. K. Berge, J. Skorve, and A. Aarsland, unpublished results). Thus, the lowering of plasma triglycerides and cholesterol by the alkylthioacetic acid may reflect diminished lipogenesis and cholesterogenesis, increased fatty acid oxidation, and, as a consequence, diminished secretion of hepatic triglycerides. Whether such mechanisms are important and/or are new ways to lower triglyceride and cholesterol levels by the use of poorly β-oxidizable sulfur-substituted fatty acid analogues is under investigation. ■

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