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Relationship between peroxisome-proliferating sulfur-substituted fatty acid analogs, hepatic lipid peroxidation and hydrogen peroxide metabolism

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Abstract—The effect of the administration of three peroxisome-proliferating sulfur-substituted fatty acid analogs on hepatic antioxidant status and lipid peroxidation was studied in rats. After 14 days of treatment, the ratio of induction of peroxisomal fatty acyl-CoA oxidase to catalase was 4.2 and 3.5 in rats treated with 1,10 bis-(carboxymethylthio)decane (BCMTD) and 1-mono (carboxymethylthio)tetradecane (CMTTD), respectively, while the corresponding ratio was 1.3 in 1-mono (carboxyethylthio)tetradecane (CETTD)-treated rats. As compared to the controls an increase in hepatic hydrogen peroxide content was noted in BCMTD- and CMTTD-treated rats, but not CETTD-treated rats. Hepatic lipid peroxidation was increased in all the three treatment groups in a manner not related to the potency of the compounds to induce the peroxisomal hydrogen peroxide metabolizing enzymes. Hepatic glutathione content increased while the activities of its associated enzymes such as glutathione transferase, glutathione peroxidase and glutathione reductase decreased in all the treated rats. Taken together, our data show a relationship between the levels of hydrogen peroxide and lipid peroxidation in rat livers treated with BCMTD and CMTTD. However, increased hepatic lipid peroxidation in CETTD-treated rats cannot be accounted for by the changes in the peroxisomal enzymes.

Previous works in this laboratory have shown that the sulfur-substituted fatty acid analogues, 1,10 bis-(carboxymethylthio)decane (BCMTD*), 1-mono (carboxymethylthio)tetradecane (CMTTD), and 1-mono (carboxyethylthio)tetradecane (CETTD), act as peroxisome proliferators to different extents in rodents. At a dose of 150 mg/kg body weight the order of their potency with respect to peroxisome proliferation was BCMTD > CMTTD > CETTD [1]. In addition, both BCMTD and CMTTD have been shown to be potent hypolipidemic compounds, thus lowering the levels of both serum cholesterol and triacylglycerol [2]. In contrast, the most striking effect of CETTD was the accumulation of lipids in the liver with marginal effects on serum lipids [2].

Several studies have previously shown that hypolipidemic compounds such as clofibrate cause peroxisome proliferation when administered to rodents [3, 4]. In addition, long-term administration of these compounds is followed by increased hepatic neoplasia [3, 5, 6]. As suggested by Reddy and co-workers [3, 7], excess production of H_2O_2 and genesis of free radicals due to enhanced H_2O_2-generating peroxisomal β-oxidation is thought to lead to uncontrolled lipid peroxidation [8] and damage to DNA [9]. Ultimately, these may be important events that lead to the initiation of carcinogenesis in the rat liver treated with hypolipidemic peroxisome-proliferating compounds.

The aim of the present work was to investigate the relationship between the extent of increase in peroxisomal β-oxidation and changes in cellular antioxidant important in scavenging H_2O_2 in rat livers treated with the sulfur-substituted fatty acid analogs, BCMTD, CMTTD and CETTD. Furthermore, the effects of these changes on hepatic lipid peroxidation will be discussed.

Materials and Methods

Chemicals. BCMTD, CMTTD and CETTD were prepared as described earlier [10]. Glutathione reductase (GRD) was obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). All other chemicals were of analytical grade.

Animal and treatments. Male Wistar rats from Möllegaard Breeding Laboratory (Ejby, Denmark) weighing 170–200 g, were housed in groups of three in metal wire cages in a room maintained at 12 hr light and dark cycles and at a constant temperature of 20 ± 3°. The animals were acclimatized for 1 week under these conditions before the start of the experiments.

Stock suspensions (3%, w/v) of BCMTD, CMTTD and CETTD were prepared in 0.5% carboxymethylcellulose. The drugs were administered by oro-gastric intubation at a dose of 150 mg/kg body weight (0.85–1.0 mL) once a day for 14 days. Animals in the control group received equal volume of the vehicle carboxymethylcellulose.

Preparation of tissue homogenates. At the end of the experiment, the livers from individual rats were homogenized in ice-cold sucrose medium (0.25 sucrose in 10 mM HEPES buffer and 1 mM EDTA, pH 7.4) using a Potter–Elvehjem homogenizer with a loosely fitting Teflon pestle. The resulting total homogenates were used for the enzyme assays. Liver homogenates (10%) in 1.15% KCl and 5% salfoslicylic acid were prepared for the determination of lipid peroxidation and glutathione, respectively.

Enzyme assays and other analytical methods. The activity of peroxisomal fatty acyl-CoA oxidase [11], catalase [12], glutathione peroxidase (GPx) [13], glutathione transferase (GST) [14], glutathione reductase [15], reduced glutathione (GSH) [16], H_2O_2 [9] and lipid peroxidation [17] was determined as described in the literature. Protein was assayed by Bio-Rad protein assay kit (Bio-Rad, Richmond, CA, U.S.A.).

Presentation of results. Data are presented as means ± SD from three rats. Statistical analysis was performed using a one-way analysis of variance and Scheffe's *F*-test for multiple comparison. $P < 0.05$ was considered as statistically significant.

Results and Discussion

Our previous work has shown [1, 2] at a fixed dose of 150 mg/kg body weight BCMTD, CMTTD and CETTD act as hepatic peroxisomal proliferators and enhance peroxisomal β-oxidation in descending order. In line with

* Abbreviations: BCMTD, 1,10 bis-(carboxymethylthio)decane; CETTD, 1-mono (carboxyethylthio)tetradecane; CMTTD, 1-mono (carboxymethylthio)tetradecane; GPx, glutathione peroxidase; GRD, glutathione reductase; GSH, reduced glutathione; GST, glutathione transferase; TBARS, thiobarbituric acid reactive substances.

Table 1. Changes in hepatic peroxisomal H_2O_2-metabolizing enzyme activities in rats treated with sulfur-substituted fatty acid analogs for 14 days

Enzyme activity	Control	CETTD	CMTTD	BCMTD
Acyl-CoA oxidase	5.8 ± 0.3	12.2 ± 0.6*	36.5 ± 3.0*	49.6 ± 4.7*
(μmol/min/mg protein) (%)	(100)	(210)	(629)	(855)
Catalase	24.2 ± 5.3	38.2 ± 3.3*	43.8 ± 4.2*	49.5 ± 3.8*
(μmol/min/mg protein) (%)	(100)	(158)	(181)	(205)
Acyl-CoA oxidase/catalase				
(ratio of induction in %)	1	1.3	3.5	4.2

Values are means ± SD of three animals.
* $P < 0.05$ compared to controls.

this observation, the activity of hepatic fatty acyl-CoA oxidase, the marker enzyme for peroxisomal β-oxidation, was significantly increased in all rats treated with the sulfur-substituted fatty acid analogs (Table 1). The increases were by about 8-, 6- and 2-fold in BCMTD, CMTTD and CETTD fed rats, respectively. Similarly, catalase activity was induced significantly in all the treated rats (Table 1). However, the ratio of induction of fatty acyl-CoA oxidase (H_2O_2-producing enzyme) to catalase (H_2O_2-degrading enzyme) was 4.2 and 3.5 in BCMTD- and CMTTD-treated rats, while the corresponding ratio was 1.3 in CETTD-treated groups. The response obtained with BCMTD and CMTTD is similar to that seen with other hypolipidemic peroxisome-proliferating compounds [4, 8, 18, 19]. CETTD, a structurally similar compound to both BCMTD and CMTTD but which has a non-hypolipidemic effect [2], caused no such large differential induction of the two peroxisomal enzymes.

One of the principal arguments on the mechanism of toxicity by hypolipidemic peroxisome-proliferating compounds is whether the H_2O_2-degrading capacity of catalase is exceeded by the H_2O_2-generating capacity of acyl-CoA oxidase during such treatment [19]. As illustrated in Table 2, hepatic H_2O_2 content was increased only in BCMTD- and CMTTD-treated rats (Table 2). This finding thus shows an association between an imbalance in the induction of the peroxisomal enzymes and the accumulation of H_2O_2 in the liver. But, contrary to our expectation, the extent of increase in hepatic H_2O_2 was more in rats treated with the less potent peroxisome proliferator CMTTD than those given BCMTD. There is no apparent explanation to this finding at present.

It was also of interest to study whether the changes in cellular antioxidants and parameters of oxidative stress such as lipid peroxidation parallel the peroxisomal events.

Hepatic lipid peroxidation was elevated in all the treated rats in comparison to controls (Table 3). The extent of increase was highest in rat livers treated with CMTTD followed by BCMTD. This parallels the extent of increase in H_2O_2. Thus, increased lipid peroxidation in BCMTD- and CMTTD-treated rats may, at least in part, be accounted for by H_2O_2-mediated oxidative stress. However, increased lipid peroxidation in CETTD-treated animals looks somewhat paradoxical as it cannot be attributed to the events in the peroxisomes or to the changes in hepatic H_2O_2 content. Our previous study has shown that the administration of CETTD is followed by the formation of fatty liver and inhibition of mitochondrial β-oxidation [1], a phenomenon which was also observed in rat livers exposed to ethionine [20]. It is, therefore, likely that the

Table 2. Changes in hepatic GSH, H_2O_2 content and lipid peroxidation levels measured as thiobarbituric acid reactive substance (TBARS) in rats treated with sulfur-substituted fatty acid analogs for 14 days

Treatment group	GSH (μmol/g liver)	H_2O_2 content (nmol/g liver)	TBARS (nmol/g liver)
Control	5.71 ± 0.48	385 ± 32	197 ± 27
CETTD	7.43 ± 0.89*	367 ± 59	308 ± 43*
CMTTD	7.18 ± 0.66*	710 ± 82*	578 ± 37*
BCMTD	7.25 ± 0.44*	590 ± 67*	416 ± 52*

Values shown are means ± SD from three animals.
* $P < 0.05$ as compared to controls.

Table 3. Changes in glutathione associated enzyme activities in rats treated with sulfur-substituted fatty acid analogs for 14 days

Enzyme activity	Control	CETTD	CMTTD	BCMTD
Glutathione peroxidase	650 ± 34	514 ± 44*	527 ± 39*	520 ± 41*
(nmol/min/mg protein) (%)	(100)	(79)	(81)	(80)
Glutathione transferase	2.50 ± 0.17	2.0 ± 0.14*	1.9 ± 0.21*	1.3 ± 0.15*
(μmol/min/mg protein) (%)	(100)	(80)	(76)	(52)
Glutathione reductase	110 ± 12.7	89 ± 9.2*	85 ± 7.8*	79 ± 6.4*
(nmol/min/mg protein) (%)	(100)	(81)	(77)	(72)

Values are means ± SD of three animals.
* $P < 0.05$ compared to controls.

increase in lipid peroxidation in CETTD-treated rats is linked to a decrease in the ATP-generating process in the mitochondria rather than the events in the peroxisomes.

Hepatic GSH level was increased (Table 2), while the activities of GST, GPx and GRD were decreased significantly in all the treated rats (Table 3). Except for the extent of decrease in activity of GST in rats treated with BCMTD, no correlation has been found between extent of alteration in these antioxidant parameters and the potency of the compounds to induce the peroxisomal enzymes.

In conclusion, the present study shows that peroxisomal β-oxidation/catalase ratio increased 3.5–4 in total liver homogenates of rats treated with the two hypolipidemic compounds BCMTD and CMTTD and this was accompanied by increased hepatic H_2O_2 content and lipid peroxidation. However, lipid peroxidation was also increased in CETTD-fed rats where the ratio of induction was 1.3 and there was no increase in hepatic H_2O_2 content. Thus, the increase is unlikely to be due to the events in the peroxisomes. Furthermore, alteration in the level of glutathione and in the activities of the glutathione-associated enzymes has no pattern with the potency of the compounds inducing the peroxisomal enzymes.

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