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INCREASED CHOLESTEROL EPOXIDE HYDROLASE ACTIVITY IN CLOFIBRATE-FED ANIMALS

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Abstract—Cholesterol epoxide hydrolase (mCE) is a microsomal enzyme that hydrolyzes cholesterol-5,6-epoxides (CE) to cholestanetriol (CT). In the present study, hepatic mCE activity was measured in mice pretreated with several different xenobiotics known to induce a variety of hepatic drug-metabolizing enzymes. Only the phenoxyacetate hypolipidemics (clofibrate and ciprofibrate, included in the diet for 14 days) were found to increase hepatic mCE activity (1.8-fold increase). Clofibrate administration also increased rabbit hepatic (2.9-fold increase) and rat and rabbit renal mCE activity (1.5- and 2.3-fold increase respectively). On a subcellular level, mCE activities in rabbit nuclear and light mitochondrial fractions were increased (3.3- and 1.8-fold increase respectively), whereas activities in the cytosolic and heavy mitochondrial fractions were unchanged. In rabbits, clofibrate administration enhanced the hepatic microsomal hydrolysis of CE without increasing the hydrolysis of arene epoxides (benzopyrene-4,5-oxide) or fatty acid epoxides (methyl *cis*-9,10-epoxystearate). Increase of tissue mCE activity may significantly enhance tissue CT levels. In this light, it is worth noting similarities in the mechanisms of hypocholesterolemic action caused by clofibrate or CT administration.

Epoxides are highly strained three-membered cyclic ethers commonly formed through the biological oxidation of alkenes and arenes. Due to their highly electrophilic nature, many epoxides are mutagenic, carcinogenic, and cytotoxic [1]. Epoxide hydrolases (EC 3.3.2.2) are crucial detoxication enzymes which convert epoxides to the normally less reactive diols. At present, there are at least three known immunologically distinct epoxide hydrolases in mammalian tissues. A cytosolic form, termed here cTSO† (TSO = *trans*-stilbene oxide), is located mainly in the cellular cytosol and mitochondria [2]. This enzyme possesses a selectivity towards most epoxides not on hindered, cyclic systems. The major microsomal form, mCSO (CSO = *cis*-stilbene oxide) is found mainly in the microsomal and nuclear membrane and preferentially hydrolyzes mono- and *cis*-1,2-disubstituted epoxides and arene epoxides [3]. CSO and TSO are often used as diagnostic substrates to distinguish between the two enzyme activities. Extensive studies have shown that these two major enzyme forms also differ in pH optima, inhibition patterns, and molecular weight [1]. Although these hydrolases are found in virtually all mammalian tissues, *in vivo* roles involving endogenous substrates have yet to be established.

Another distinct form of membrane-bound epoxide hydrolase has been described recently [4]. This microsomal cholesterol epoxide hydrolase (mCE), which is located mainly in the microsomal membrane, catalyzes the hydrolysis of both the α- and β-5,6-epoxy-cholestan-3β-ols (CE) to cholestane-3β,5α-6β-triol (CT) with a high degree of specificity for the steroid nucleus. While mCE activity has been measured in several tissues, hepatic specific activities are approximately 10-fold higher that those measured in other organs [5]. MCE has not been characterized to the extent of the other epoxide hydrolases, but it is known to differ from them in antigenicity, pH optima, and inhibition response [4, 6]. At present, the *in vivo* role of mCE is unresolved. However, the selectivity for an endogenous substrate suggests that mCE may exist specifically to hydrolyze certain steroid-5,6-epoxides [7].

CE and CT, which have been detected in the plasma and tissues of several species [7, 8], possess significant yet very diverse biological properties. CE, formed directly from cholesterol either by cytochrome P-450- or lipid peroxide-mediated oxidation, is mutagenic and causes oncogenic transformation of mammalian cells [9–11]. CE is also tumorigenic when administered subcutaneously to rats and mice and is suspected to be a causative agent in ultraviolet light (UV)-induced skin cancer in mice [12, 13]. While CT lacks the mutagenic potential of CE, it has been shown to possess potent hypocholesterolemic properties. *In vitro*, CT inhibits incorporation of precursors into the cholesterol molecule [14]. *In vivo*, inclusion of CT in the diet significantly lowers serum and liver cholesterol, apparently by inhibiting intestinal cholesterol uptake [15].

While the induction responses of mCSO and cTSO activities are well-characterized, relatively little is known concerning the response of mCE to xenobiotic challenge. In rats and mice, hepatic mCSO

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† Abbreviations: mCE, microsomal cholesterol epoxide hydrolase; CE, cholesterol epoxides; CT, cholestanetriol; cTSO, cytosolic *trans*-stilbene oxide hydrolase; mCSO, microsomal *cis*-stilbene oxide hydrolase; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; and EGTA, ethyleneglycolbis(amino-ethylether)tetra-acetate.

activities are increased significantly upon administration of a wide variety of enzyme inducers [1]. A previous report suggested that rat hepatic mCE activity is not increased subsequent to administration of some classical enzyme inducers (such as phenobarbital, 3-methylcholanthrene, Aroclor, and clofibrate) [6]. It is important to characterize further the effects of various xenobiotics on mCE activity since this enzyme hydrolyzes an endogenous carcinogen to a product with potent hypocholesterolemic properties.

In this paper, we report the first measured increase of tissue mCE activity following oral dosing with a xenobiotic. Of several compounds tested in mice, only phenoxyacetate derivatives (e.g. clofibrate) significantly enhanced hepatic mCE activities. Clofibrate (ethyl *p*-chlorophenoxyisobutyrate) and its analogues are potent hypocholesterolemics commonly prescribed for patients unable to control serum cholesterol through dietary means [16]. This paper characterizes various aspects of the clofibrate–mCE response and discusses the possible implications concerning the mechanism of clofibrate-induced hypocholesterolemia.

METHODS

Chemicals. Unlabeled and [4-^{14}C]-labeled cholesterol epoxide were provided by Dr. Alex Sevanian, Institute of Toxicology, University of Southern California. CSO and tritiated TSO and CSO were prepared as previously described [17]. Unlabeled and tritiated benzopyrene-4,5-oxide (BPO) were prepared by Richard Armstrong (Department of Chemistry, University of Maryland) and the Midwest Research Institute (Kansas City, MO) respectively. Unlabeled and [1-^{14}C]-labeled methyl *cis*-9,10-epoxystearate were synthesized as previously described [18]. The radiochemical purity of all substrates was determined to be >96% by thin-layer chromatography in at least two systems. The radioactivity not cochromatographing with the authentic, unlabeled standard was not localized in any single region of the plate. Clofibrate was provided by Ayerst Laboratories, New York, NY, or synthesized in house. Ciprofibrate was provided by Sterling-Winthrop Research Institute, Rensselaer, NY. TSO was purchased from Aldrich Chemicals, Milwaukee, WI. 3-Methylcholanthrene (3-MC) was purchased from Eastman Kodak, Rochester, NY. Butylated hydroxyanisole (BHA) was purchased from the Sigma Chemical Co., St. Louis, MO.

Animal pretreatment. Female mice (Balb/C, 20–30 g) and male rats (Sprague–Dawley, 100–200 g) were obtained from Simonsen Laboratories, Gilroy, CA. Male New Zealand White rabbits (2.5 to 3.5 kg) were from Herbert's Rabbitry, Grass Valley, CA. Rats and mice were fed ground Purina lab chow *ad lib.*, while rabbits were fed 130 g of food pellets daily. Clofibrate was dissolved in acetone and mixed throughly with ground chow (rats and mice) or sprayed evenly over the daily ration of pellets (rabbits) to achieve a final concentration of 0.5% (w/w). The acetone was allowed to evaporate prior to diet administration. Animals were maintained on these diets for 14 days and then killed. The diets of control animals were treated similarly with appropriate amounts of acetone. Mice were injected with solutions of phenobarbital (PB) (in saline, 80 mg/kg/day), 3-MC (in corn oil, 50 mg/kg/day), or TSO (in corn oil, 400 mg/kg/day) for 4 days and then killed. Control animals were injected with appropriate volumes of vehicle. BHA and ciprofibrate were mixed into the diet at concentrations of 0.75% and 0.05% (w/v), respectively, and administered for 14 days.

Subcellular preparation. Rats and mice were killed by cervical dislocation and rabbits were exsanguinated. Tissues were removed (and pooled in the case of mice) and placed in 0.9% saline at 4°. Homogenates (10%, w/v) were prepared in 0.25 M sucrose containing 0.3 M mannitol, 10 mM HEPES, and 1 mM EGTA at pH 7.2. Differential sedimentation was used to prepare nuclear, microsomal, cytosolic, and light and heavy mitochondrial fractions (20%, w/v) exactly as described by Kaur and Gill [19]. Briefly, liver homogenates were centrifuged (Beckman VTi50 vertical rotor) at 600 *g* for 5 min. The pellet was rehomogenized in the same quantity of buffer and the process repeated two more times to give the nuclear fraction. The 600 *g* supernatant fraction was centrifuged at 6,000 *g* for 10 min. The resulting pellet was rehomogenized in the same quantity of buffer and the process repeated twice more to yield the heavy mitochondrial fraction. The 6,000 *g* supernatant fraction was centrifuged at 12,000 *g* for 15 min. The resulting pellet was washed twice and finally resuspended in buffer to give the light mitochondrial fraction. The 12,000 *g* supernatant fraction was centrifuged at 105,000 *g* for 60 min to obtain the microsomal pellet and cytosolic fraction. The cytosolic fraction was used without further purification, whereas the microsomal pellet was washed twice before use. It is important to note that administration of some xenobiotics can change the stability and sedimentation properties of organelles in differential centrifugation schemes.

Enzyme and cholesterol assays. Cholesterol epoxide hydrolase activities were assayed as described by Sevanian and McLeod [20] using the β oxide unless otherwise indicated. mCSO and cTSO activities were measured by partition methods as described previously [17]. mBPO activity was determined as described by Jerina *et al.* [21]. The rate of hydrolysis of methyl epoxystearate was determined using the methods of Gill and Hammock [18]. Enzyme activities in tissues of treated animals were compared with controls using Student's *t*-test with significance defined as $P < 0.05$.

Serum cholesterol. Blood from rats and rabbits was obtained at the time of sacrifice. Serum cholesterol concentrations were then determined according to the colorimetric assay described by Zlatkis *et al.* [22]. Absolute levels of cholesterol and its oxidation products were determined in perfused rabbit liver with and without exposure to dietary clofibrate. Levels were determined by Dr. L. Gruenke of the Pharmaceutical Chemistry Department, U.C.S.F., using slight modifications of the gas chromatography–mass spectrophotometric method described in Ref. 23.

Table 1. Epoxide hydrolase activities in tissues of female Balb/C mice subsequent to 14 days treatment with (Clof) and without (control) 0.5% clofibrate (w/w) included in the diet*

	Activity (nmol/mg·min)					
	mCSO		cTSO		mCE	
	Control	Clof	Control	Clof	Control	Clof
Liver	1.85 ± 0.25	4.35 ± 0.19†	1.22 ± 0.06	4.79 ± 0.30†	0.21 ± 0.02	0.37 ± 0.07†
Lung	1.10 ± 0.12	0.92 ± 0.26	0.022 ± 0.010	0.024 ± 0.010	0.0031 ± 0.0020	0.0061 ± 0.0030†
Kidney	0.30 ± 0.05	0.29 ± 0.01	0.38 ± 0.06	0.91 ± 0.04†	0.0052 ± 0.0020	0.0035 ± 0.0010

* Values are the mean ± SD of at least three values from each of at least three different animal groups. Abbreviations: CSO, *cis*-stilbene oxide, TSO, *trans*-stilbene oxide, and CE, cholesterol-5,6-β-epoxide. The c and m prefixes indicate rates of hydrolysis measured in cytosol and microsomes respectively.

† Significantly increased over control value ($P < 0.05$).

RESULTS

Table 1 contains the epoxide hydrolase values measured in various tissues of clofibrate-treated mice. Hepatic mCSO and cTSO activities were increased significantly by clofibrate treatment (2.4- and 3.9-fold respectively) as was renal cTSO activity (2.4-fold). These substrate- and tissue-specific induction responses confirm previously reported values [24]. Hepatic and lung mCE activities were increased significantly (1.8- and 2.0-fold, respectively), whereas renal mCE activity was unchanged. It should be noted that the levels of enzymatic activity reported in the lung were near the limits of detection for the assay. mCE responded independently from either of the other major hydrolase forms.

Values listed in Table 2 indicate that, of the species tested, rabbits exhibited the largest increase in hepatic mCE activity (2.9-fold increase) subsequent to clofibrate treatment. Rat hepatic mCE activities were unchanged, as has been reported by Levin *et al.* [6]. Renal mCE activities were increased in both rats and rabbits (1.5- and 2.3-fold increase respectively). Testis mCE activities did not respond in either species. Thus, in every species tested, at least one major tissue had enhanced mCE activity subsequent to clofibrate treatment. Clofibrate treatment may have resulted in true enzyme induction (an increase in amount of mCE protein) or an activation of the native enzyme, or a combination of both. In microsomal preparations, addition of clofibrate or clofibric acid (up to 1 mM) had no effect on mCE activity (data not shown). This result suggests that the presence of clofibrate does not directly influence mCE activity and that the increases in mCE activity measured in this study may be the result of enhanced synthesis of the mCE enzyme. Clofibrate acted as an effective hypocholesterolemic in the rat and rabbit, producing significant decreases in free serum cholesterol after 14 days of inclusion in the diet. In the rabbit, serum cholesterol was lowered from a control value of 98 ± 3 mg/100 ml to a value of 45 ± 5 mg/100 ml, while in the rat, serum cholesterol was lowered from 74 ± 5 mg/100 ml to 47 ± 6 mg/100 ml.

Table 2. Response of microsomal cholesterol epoxide hydrolase activity (mCE) to 14-day treatment with (Clof) and without (control) 0.5% clofibrate (w/w) included in the diet*

	mCE activity (nmol/mg·min)	
	Control	Clof
Rabbit		
Liver	0.18 ± 0.03	0.52 ± 0.11†
Kidney	0.0052 ± 0.0010	0.012 ± 0.007†
Testis	0.019 ± 0.004	0.017 ± 0.004
Rat		
Liver	0.27 ± 0.04	0.23 ± 0.04
Kidney	0.011 ± 0.003	0.017 ± 0.003†
Testis	0.0058 ± 0.002	0.0069 ± 0.002

* Values are the mean ± SD of at least three values from each of at least three animals.

† Significantly increased over control value ($P < 0.05$).

Of major interest is the fact that only those compounds with recognized hypocholesterolemic properties increased mCE activities in mice (Fig. 1). Clofibrate and ciprofibrate (2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropionic acid) both produced a 1.8-fold increase in hepatic mCE activity, whereas PB, TSO, BHA, and 3-MC had no effect. Ciprofibrate was administered at one-tenth the concentration of clofibrate, yet caused apparent induction of an equal magnitude. This observation is consistent with the higher hypolipidemic potency of the drug. As has been reported previously [6], hepatic mCSO activities were responsive to administration of all of the above xenobiotics.

Clofibrate treatment of rabbits increased hepatic mCE activity without enhancing the microsomal hydrolysis of *cis*-substituted epoxides (CSO) or arene epoxides (BPO) (Table 3). In addition, hydrolysis of methyl epoxystearate was unaffected. This result apparently conflicts with the conclusions from the finding of Sevanian *et al.* [25] of competitive inhibition between methyl epoxystearate and CE hydrolysis in microsomes of rat lung. The present induction data suggest that methyl epoxystearate and CE are hydrolyzed by separate microsomal epoxide hydrolases. In contrast to an earlier rat study [4], rates of hydrolysis of the α- and β-5,6-epoxides of cholesterol were similar in rabbits. The rates of

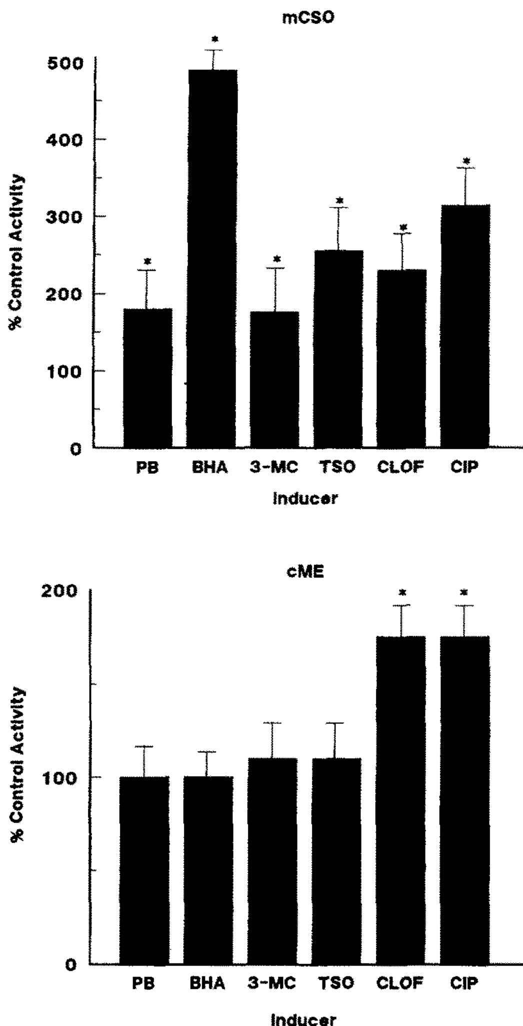

Fig. 1. Response of hepatic mCSO and mCE activities in female Balb/C mice subsequent to xenobiotic pretreatment. Abbreviations: PB, phenobarbital; BHA, butylated hydroxyanisole; 3-MC, 3-methylcholanthrene; TSO, *trans*-stilbene oxide; CLOF, clofibrate; CIP, ciprofibrate; mCSO, microsomal *cis*-stilbene oxide hydrolase; and mCE, microsomal cholesterol epoxide hydrolase. Each value is the mean ± SD of three values from three animals. An asterisk indicates a significant increase over control values ($P < 0.05$). Control rates were 1.85 ± 0.25 nmol/mg·min for mCSO activity and 0.21 ± 0.02 nmol/mg·min for mCE activity.

hydrolysis of the α- and β-5,6-epoxides of cholesterol also were increased to a similar extent following treatment with clofibrate.

The procedures used to prepare and isolate the subcellular fractions have been shown previously to afford good separation based on marker enzyme assays [19] although microsomes commonly contaminate other particulate fractions. However, since marker enzyme assays were not used in the present study, subcellular fractions are referred to as the centrifugation speed required for isolation (Table 4). mCE activities in nuclei (600 *g* sediment) and light mitochondria (LM, 12,000 *g* sediment) were increased (3.3- and 1.8-fold respectively) in hepatic preparations from clofibrate-treated rabbits. Activities in cytosol (105,000 *g* supernatant) and heavy mitochondria (6,000 *g* sediment) were not increased. Values obtained for nuclear and mitochondrial mCE activities were similar to previously reported values [5]. The LM fraction, as prepared here, also contains the "peroxisomal" fraction. Clofibrate is a well-known peroxisome-proliferator [26], and the possibility exists that enhanced mCE activities associated with the LM fraction are, in fact, due to increased peroxisomal hydrolysis of CE.

The data in Table 5 indicate that clofibrate, as expected, causes a marked reduction in hepatic cholesterol as well as three of its oxidation products. By 14 days the absolute amount of triol as well as the percentage of triol relative to the cholesterol epoxides had decreased.

DISCUSSION

In this study, we report the first measured increase of tissue mCE activity in an animal subsequent to oral xenobiotic administration. Inclusion of clofibrate in the diet raised hepatic, lung, and renal mCE activities within the various species tested. This response was independent from the response of either of the other major hydrolase forms, and it occurred only upon administration of compounds known to be effective in lower serum cholesterol. In mouse and rabbit, clofibrate administration significantly increased hepatic mCE activity, whereas rat liver was unresponsive as previously reported [6]. However, rat renal mCE activity was increased (1.5-fold) in the present study.

Tissue-specific increases in mCE activity and other enzymes [23] following clofibrate treatment suggest either that certain tissues lack a response capacity

Table 3. Rates of epoxide hydrolysis in rabbit hepatic microsomes subsequent to 14 days of treatment with (Clof) and without (control) 0.5% clofibrate (w/w) included in the diet*

	Activity (nmol/mg·min)				
			Substrate		
	CSO	Methyl epoxystearate	BPO	α-CE	β-CE
Control	44.1 ± 2.8	14.5 ± 1.8	56.8 ± 7.8	0.18 ± 0.03	0.19 ± 0.04
Clof	43.8 ± 5.6	12.7 ± 2.9	52.4 ± 10.6	0.52 ± 0.14†	0.50 ± 0.12†

* Values are the mean ± SD of at least three values from each of at least three animals. Abbreviations: CSO, *cis*-stilbene oxide; BPO, benzopyrene-4,5-oxide; α-CE, cholesterol-5,6α-epoxide; and β-CE, cholesterol-5,6-β-epoxide.

† Significantly increased over control value ($P < 0.05$).

Table 4. Cholesterol epoxide hydrolase activity measured in subcellular fractions of rabbit liver subsequent to inclusion of 0.5% clofibrate (w/w) in the diet for 14 days.*

Subcellular fraction	Activity (pmol/mg·min)	
	Untreated	Clofibrate-treated
600 *g* Sediment (nuclei)	7.5 ± 2.0	25.0 ± 5.0†
6,000 *g* Sediment (heavy mitochondria)	4.5 ± 1.0	4.9 ± 1.0
12,000 *g* Sediment (light mitochondria)	4.4 ± 2.0	8.0 ± 2.0†
105,000 *g* Supernatant (cytosol)	2.0 ± 0.5	2.0 ± 0.9

* Values are the mean ± SD of at least three values from each of at least three animals. The "g" value associated with each fraction indicates the centrifugal force used to isolate the individual fractions.

† Significantly increased over control value ($P < 0.05$).

or that different dose–response relationships exist among tissues. These differences in response may merely be the result of differences in tissue sensitivities or tissue accumulation of clofibrate and its metabolites. The differences also may be due to the presence of tissue specific receptors [27]. The dose of clofibrate administered in the present study (0.5% of diet, 14 days) is often used to provide a model for peroxisome proliferation and hypolipidemia in rats and mice. Dose–response relationships between clofibrate intake and increase in tissue mCE activity were not investigated in the present study. However, it is interesting to note that ciprofibrate, administered as 0.05% of the diet, increased mouse hepatic mCE activity to the same extent (1.8-fold increase) as did clofibrate. Ciprofibrate is approximately eight to ten times more potent than clofibrate (on a weight basis) as a hypolipidemic agent [16]. Thus, a relationship may exist between the ability of a xenobiotic to increase mouse hepatic mCE activity and the hypolipidemic properties of that xenobiotic.

On a subcellular level, there was variation in response among microsomal, cytosolic, and mitochondrial mCE activities. In rabbit, rates of CE hydrolysis were increased in the microsomal, nuclear, and LM fractions, but were unchanged in the cytosolic and HM fractions. Sevanian and McLeod have measured large differences in K_m values for hepatic microsomal (3.69 μM) and mitochondrial (100 μM) mCE activities [20], and the present induction data support the idea that different mCE isozymes exist within different subcellular locations.

Table 5. Levels of cholesterol and cholesterol oxidation products in tissues of rabbits subsequent to 14 days of treatment with (Clof) and without (control) 0.5% clofibrate (w/w) included in the diet

Compound	Amount per 100 g* wet weight	
	Control	Clof
Cholesterol	1126 mg	204 mg
α-CE	137 μg	38 μg
β-CE	402 μg	99 μg
Cholesterol triol	137 μg	23 μg

* Data were provided courtesy of Drs. L. D. Gruenke, J. C. Craig, and N. L. Petrakis at the University of California, San Francisco (cited with permission). Values are averages from four separate livers with standard deviations of less than 20% of the reported value. Data were obtained as described in Ref. 23.

Chan and Black [13] reported that daily UV-irradiation of mouse skin results in a dramatic increase in skin CE content, presumably due to photooxidation of the lone carbon–carbon double bond of cholesterol. The increase in CE content was followed by an increase in skin mCE activity, which returned the skin CE levels to normal. This suggests that mCE activities are responsive to substrate levels and that changes in mCE activities can influence endogenous CE and CT levels. In the present study, clofibrate administration may have enhanced tissue CE levels so as to trigger mCE induction. Clofibrate, a potent peroxisome proliferator, has been shown to increase hepatic lipid peroxide levels, due to peroxisomal production of H_2O_2 [28]. It would seem possible that increased cellular peroxidation could enhance CE levels through increased oxidation of the 5,6-double bond in cellular cholesterol. Indeed, Sevanian *et al.* [29] reported that the increased lipid peroxidation of rat lung tissue that occurred upon exposure to nitrogen dioxide is accompanied by a 1.5-fold increase in tissue CE concentration.

As is the case with the two major hydrolase forms, little is known concerning the physiological role of mCE. Presumably, additional substrates for the enzyme may exist. At present, pregnenolone-epoxide is the only other endogenous steroid-5,6-epoxide that has been reported to be hydrolyzed by microsomal preparations [30].

It has been suggested that a major function of mCE may be to prevent accumulation of the carcinogenic epoxides of cholesterol [31]. If this hypothesis is true, maintenance of cellular mCE activity would be essential for defense against interaction of CE with cellular targets. Indeed, it has been shown that, in the hyperplastic nodules of rats fed the carcinogen 2-acetylaminofluorene (AAF), there is a large decrease in microsomal and nuclear mCE activities as compared to normal tissue [32]. These authors [32] noted that decreased mCE activities may have enhanced or potentiated AAF-induced hepatocarcinogenesis through increased CE contact with nuclear DNA. The converse of this situation concerns the possible physiological consequences of

enhanced mCE activity, as measured in the present study. Chan and Black reported that an increase in tissue mCE activity resulted in increased hydrolysis of tissue CE [13], and this study clearly shows that clofibrate treatment reduces the amount of three cholesterol oxidation products. However, mCE is not necessarily involved in this process. Significant enhancement of mCE activities may result in elevated tissue CT levels if the amount of this enzyme is the rate-limiting step in the production of CT. Due to the potent hypocholesterolemic properties of CT, it follows that mCE induction may elicit a hypocholesterolemic response. In this light, it is worth noting the association of mCE induction with administration of the hypocholesterolemic agents clofibrate and ciprofibrate. A detailed time course correlating the levels of CT and mCE following clofibrate administration could shed light on this hypothesis.

Clofibrate is commonly prescribed to lower serum cholesterol in genetic hypercholesterolemias [16]. Detailed sterol balance studies in humans have suggested that clofibrate causes an increased output of cholesterol into the feces while inhibiting any compensatory increase in hepatic cholesterol synthesis [33]. However, clofibrate itself does not directly inhibit cholesterol synthesis or absorption, and, at present, the precise means through which it exerts its hypocholesterolemic action are unknown. The sterol-balance results are remarkably compatible with the known mechanisms of CT-induced hypocholesterolemia. Addition of CT to liver preparations directly inhibits cholesterol biosynthesis at several points. In rat liver homogenates, 5 μM CT produced a 50% inhibition in cholesterol formation [14], apparently at a point between lanosterol and cholesterol [34]. CT also directly inhibits hydroxymethylglutaryl-CoA (HMG-CoA) reductase activity in mouse hepatocyte preparations [35]. HMG-CoA reductase, which converts HMG-CoA to mevalonic acid, is the rate-determining enzyme in hepatic cholesterol biosynthesis. Liver preparations from clofibrate-treated rats show a reduced ability to synthesize cholesterol, due to a 2-fold decrease in the activity of this enzyme [36]. In addition to the inhibitory effects on cholesterol biosynthesis, CT also blocks intestinal cholesterol uptake when included in the diet. Aramaki *et al.* [37] reported that inclusion of CT (0.5%, w/w) in an atherogenic diet of hypercholesterolemic rabbits significantly lowered serum and liver cholesterol levels while also reducing the degree of aortic atherotoma. CT is not absorbed from the intestine to any extent, and probably retards cholesterol uptake as in the manner of the resin compounds [37]. Clofibrate also has been shown to increase fecal excretion of cholesterol in humans [33]. Conceivably, this could result from blocked intestinal absorption via elevated CT levels.

A species- and tissue-dependent increase in the rates of hydrolysis of the 5,6-epoxides of cholesterol has been demonstrated following dietary exposure to hypocholesterolemic agents. A review of the literature suggests that the mechanisms by which CT and clofibrate induce hypocholesterolemia are not incompatible, but the testing of a hypothetical cause–effect relationship will require further investigation.

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