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STUDIES ON THE EFFECT OF FENOPROFEN ON THE ACTIVATION AND OXIDATION OF LONG CHAIN AND VERY LONG CHAIN FATTY ACIDS IN HEPATOCYTES AND SUBCELLULAR FRACTIONS FROM RAT LIVER

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Abstract—We studied the effect of fenoprofen on the activation of palmitic acid ($C_{16:0}$), lignoceric acid ($C_{24:0}$) and cerotic acid ($C_{26:0}$) in microsomal and peroxisomal fractions from rat liver. Fenoprofen was found to inhibit the formation of palmitoyl-CoA in both microsomal and peroxisomal fractions whereas the formation of lignoceroyl-CoA and cerotoyl-CoA was not inhibited at all. In freshly isolated rat hepatocytes palmitic acid β-oxidation was progressively inhibited at increasing concentrations of fenoprofen, most probably due to its inhibitory effect on palmitoyl-CoA synthetase activity. On the other hand, fenoprofen was also found to inhibit the β-oxidation of lignoceric acid and cerotic acid in rat hepatocytes. It is shown that the acyl-CoA oxidase activity with lignoceroyl-CoA as substrate was inhibited by fenoprofen whereas the palmitoyl-CoA and pristanoyl-CoA oxidase activities were not inhibited by fenoprofen. This finding provides an explanation for the inhibitory effect of fenoprofen on lignocerate and cerotate β-oxidation in hepatocytes.

Non-steroidal anti-inflammatory drugs such as fenoprofen, ibuprofen, naproxen and ketoprofen exist as *R* and *S* enantiomers and are administered to man as racemic compounds although the anti-inflammatory activity resides almost exclusively in the *S* enantiomer. These 2-arylpropionic acids are generally considered to be well tolerated and effective. However, benoxaprofen and pirprofen, which are structurally related to these drugs, were withdrawn from use because of severe and potentially lethal hepatotoxicity. In addition, several cases of hepatitis induced by naproxen [1] and fenoprofen [2] have been reported.

Recently, it was demonstrated that *R*-fenoprofen and *R*-ibuprofen are stereospecifically activated to their corresponding acyl-CoA thioesters by rat hepatic microsomal long chain acyl-CoA synthetase (EC 6.2.1.3) [3, 4]. This enzyme is involved in the activation of a variety of long chain fatty acids, showing optimal activity with palmitic acid ($C_{16:0}$) [5]. Pyrenedecanoic acid [6], 3-phenoxybenzoic acid [7] and hypolipidaemic drugs like ciprofibrate, clofibrate and nafenopin [8] are known to be alternative substrates for the long chain acyl-CoA synthetase. The enzyme is present in mitochondria, peroxisomes and the endoplasmic reticulum (microsomes) (see Ref. 9 and refs therein). A variety of 2-arylpropionic acids inhibit the formation of palmitoyl-CoA by the rat microsomal long chain acyl-CoA synthetase [10] as well as by the peroxisomal enzyme [11].

Apart from a long chain acyl-CoA synthetase a very long chain acyl-CoA synthetase has been reported [12, 13] which is present in peroxisomes and the endoplasmic reticulum but not in mitochondria [14, 15]. The existence of a distinct very long chain fatty acid-activating enzyme has been questioned by Kishimoto and co-workers [16–18], but as summarized by Wanders *et al.* [19] available information is strongly in favour of a distinct very long chain fatty acid-activating enzyme.

After activation to their corresponding acyl-CoA thioesters, fatty acids can be β-oxidized by mitochondria and peroxisomes. Long chain fatty acids like palmitic acid ($C_{16:0}$) are predominantly β-oxidized by mitochondria whereas the initial steps in β-oxidation of very long chain fatty acids like lignoceric acid ($C_{24:0}$) and cerotic acid ($C_{26:0}$) are exclusively peroxisomal [15, 20–22]. The first enzyme of the peroxisomal β-oxidation process is an FAD-containing acyl-CoA oxidase [23, 24]. Schepers *et al.* [25] recently described the presence of three acyl-CoA oxidases in rat liver peroxisomes: a fatty acyl-CoA oxidase that is induced 10–20-fold by treatment of rats with peroxisome proliferators, a non-inducible fatty acyl-CoA oxidase and a non-inducible trihydroxycoprostanoyl-CoA oxidase that oxidizes di- and trihydroxycoprostanoyl-CoA esters but not the CoA esters of fatty acids. The non-inducible acyl-CoA oxidase proved to be a branched chain fatty acyl-CoA oxidase (pristanoyl-CoA oxidase) [26, 27].

We have examined the effects of the 2-arylpropionic acid fenoprofen on the activation and β-oxidation of long chain and very long chain fatty acids in rat liver subcellular fractions and on the β-oxidation of these compounds in rat hepatocytes. The results are described in this paper and their implications are discussed.

MATERIALS AND METHODS

Materials. Fenoprofen, lignoceroyl-CoA and

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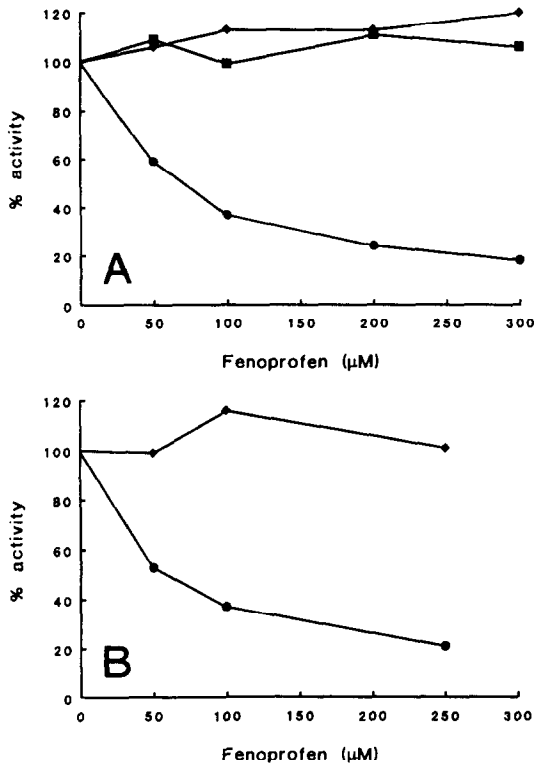

Fig. 1. Effect of different concentrations of fenoprofen on the activation of [1-^{14}C]palmitic acid (●), [1-^{14}C]lignoceric acid (◆) and [1-^{14}C]cerotic acid (■) in rat liver microsomal fraction (A) and purified rat liver peroxisomes (B). The rates of fatty acid activation in the absence of fenoprofen were in (A) palmitoyl-CoA synthetase 77 nmol/min/mg protein; lignoceroyl-CoA synthetase 1.84 nmol/min/mg protein and cerotoyl-CoA synthetase 1.23 nmol/min/mg protein; and in (B) palmitoyl-CoA synthetase 99 nmol/min/mg protein and lignoceroyl-CoA synthetase 0.97 nmol/min/mg protein.

homovanillic acid were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). FAD, NAD^+, horseradish peroxidase, coenzyme A, ATP and palmitoyl-CoA were purchased from Boehringer (Mannheim, Germany). Radiochemicals were purchased from New England Nuclear (Dreieich, Germany). All other chemicals were of the highest purity available and obtained from various commercial sources. Long chain acyl-CoA synthetase purified from rat liver microsomes was a gift from Prof. T. Hashimoto (Matsumoto, Japan).

Preparation of liver microsomes and peroxisomes. Livers from male Wistar rats (200–250 g) were finely minced and homogenized in a medium containing 250 mM sucrose, 2.5 mM EDTA, 0.1% (v/v) ethanol and 5 mM morpholinopropane sulphonic acid (Mops)–NaOH (pH 7.4) using a glass Potter homogenizer and a loosely fitting Teflon pestle. Fractions rich in microsomes or peroxisomes were prepared by differential centrifugation according to standard procedures [28, 29] using a SORVALL-RC5B-superspeed centrifuge with a SS-34 rotor (8 × 50 mL). The total homogenate was first centrifuged at $600\,g_{av}$ for 10 min. The resulting supernatant (postnuclear supernatant) was collected and centrifuged at $3600\,g_{av}$ for 10 min. The resulting pellet contained the bulk of mitochondria. The $3600\,g_{av}$-supernatant was subsequently centrifuged at $25{,}000\,g_{av}$ for 10 min. The pellet was taken up in sucrose medium again, gently homogenized and pelleted by centrifugation at $25{,}000\,g_{av}$ (peroxisomal or light mitochondrial fraction). Finally, the $25{,}000\,g_{av}$-supernatant was centrifuged at $40{,}000\,g_{av}$ for 120 min giving rise to a microsomal fraction and a soluble fraction. Purified peroxisomes were prepared according to Ref. 30 with slight modifications [31] by subjecting the fraction richest in peroxisomes ($25{,}000\,g_{av}$ pellet) to density gradient centrifugation in a discontinuous Nycodenz gradient. Measurement of marker enzymes indicated that the peroxisomal preparations were routinely more than 95% pure (see Ref. 31). The same procedures were followed for the preparation of human liver peroxisomal fractions. The preparations were stored at −80° until further use.

Isolation of rat hepatocytes. Rat hepatocytes were isolated from rat liver by collagenase perfusion according to Berry and Friend [32] with modifications as described by Groen *et al.* [33]. In the final step the isolated hepatocytes were washed with Krebs–Henseleit buffer without bovine serum albumin and kept on ice until further use.

Enzyme assays. The activity of palmitoyl-CoA synthetase and lignoceroyl-CoA/cerotoyl-CoA synthetase was measured as described before [6]. Acyl-CoA oxidase activities were measured in a medium of the following standard composition: 50 mM Mops–NaOH (pH 7.6), 1 mM homovanillic acid, 18 U/mL horseradish peroxidase, 0.1 mM NaN_3 and 0.01 mM FAD. Reactions were started by addition of palmitoyl-CoA (final concentration: 50 μM) or lignoceroyl-CoA (final concentration: 200 μM). Fluorescence was followed in time at 30 sec intervals for 10 min using a Cobas Centrifugal Analyzer [excitation wavelength: 327 nm, emission filter: 410–490 nm; Hoffman-La Roche (Basel, Switzerland)].

Fatty acid oxidation reactions in peroxisomal fractions were carried out at 37° using a medium of the following composition: 150 mM Tris–HCl (pH 8.5), 10 mM $MgCl_2$, 10 mM ATP, 50 μM FAD, 1 mM CoA, 0.05% Triton X-100, 1 mM NAD^+, 2 mM NaN_3 and 10 μM 1-^{14}C-labelled palmitic acid or lignoceric acid dissolved in α-cyclodextrin (final concentration of α-cyclodextrin in the reaction mixture: 1 mg/mL). β-Oxidation activities in rat hepatocytes were measured in Krebs–Henseleit buffer supplemented with 10 μM 1-^{14}C-labelled fatty acid in the absence of cofactors. After 15 min the reactions were terminated and the radioactivity of $^{14}CO_2$ and ^{14}C-labelled acid-soluble products was assayed as described in Ref. 34.

Inhibition of enzyme activities. Fenoprofen was dissolved (5–100 mM) in dimethyl sulphoxide. The concentrations used in the inhibition studies were 50–1000 μM with a final concentration of dimethyl sulphoxide of 1% (v/v). Dimethyl sulphoxide (1% v/v) did not inhibit the acyl-CoA synthetase, acyl-CoA oxidase and β-oxidation activities (results not shown).

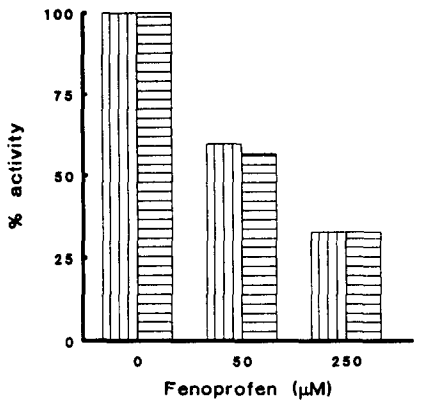

Fig. 2. Effect of different concentrations of fenoprofen on the activation of [1-^{14}C]palmitic acid (vertical striped bars) and [1-^{14}C]lignoceric acid (horizontal striped bars) by purified long chain acyl-CoA synthetase. The rates of fatty acid activation in the absence of fenoprofen were: palmitoyl-CoA synthetase 1.30 μmol/min/mg protein and lignoceroyl-CoA synthetase 0.84 nmol/min/mg protein.

RESULTS

Previous studies showed that fenoprofen is able to inhibit the formation of palmitoyl-CoA by microsomal and peroxisomal subcellular fractions [10, 11]. In the experiment shown in Fig. 1 we studied the effect of fenoprofen on the activation of palmitate, lignocerate and cerotate in microsomal fractions and purified peroxisomes from rat liver. Fenoprofen was found to inhibit the formation of palmitoyl-CoA in both microsomal (Fig. 1A) and peroxisomal (Fig. 1B) fractions, with comparable patterns of inhibition. In both subcellular fractions the formation of cerotoyl-CoA (Fig. 1A) and lignoceroyl-CoA (Fig. 1A, B) was not inhibited at all. It is known that the purified long chain acyl-CoA synthetase exhibits reactivity with very long chain fatty acids such as lignoceric acid [16] and cerotic acid [35]. Figure 2 shows that fenoprofen inhibits not only the activation of palmitic acid but also that of lignoceric acid by the purified rat liver microsomal palmitoyl-CoA synthetase.

In the experiment described in Fig. 3 we studied the effect of fenoprofen on the β-oxidation of palmitate, lignocerate and cerotate in freshly isolated rat hepatocytes. For this purpose isolated hepatocytes were incubated in the standard reaction medium supplemented with palmitic acid, lignoceric acid or cerotic acid plus fenoprofen at the concentrations indicated. Reactions were carried out for 15 min during which time period fatty acid oxidation was found to proceed linearly (results not shown). Figure 3A shows that palmitate β-oxidation was progressively inhibited at increasing concentrations of fenoprofen reaching 80% inhibition at 1 mM fenoprofen. Unexpectedly, fenoprofen also inhibited the β-oxidation of lignocerate and cerotate (Fig. 3B and C). These findings can not be ascribed to a deleterious effect of fenoprofen on hepatocytes, since intracellular ATP and CoASH levels were essentially unaffected by fenoprofen up to concentrations of 500 μM (results not shown). The underlying basis for the observed inhibition of very long chain fatty acid oxidation was therefore studied in more detail.

Since the initial steps in β-oxidation of very long chain fatty acids proceed exclusively in peroxisomes [15, 20–22], we investigated whether fenoprofen was also able to inhibit lignoceric acid β-oxidation in peroxisomal fractions. Figure 4 shows that this is indeed the case. No inhibitory effect was observed on the β-oxidation of palmitoyl-CoA (Fig. 4). In order to identify which step in the pathway of lignoceric acid β-oxidation is affected by fenoprofen, we studied the initial step in lignoceroyl-CoA β-oxidation as catalysed by acyl-CoA oxidase since this step is considered to be rate limiting [36]. We measured the acyl-CoA oxidase activities in rat liver peroxisomal fractions with palmitoyl-CoA, lignoceroyl-CoA and pristanoyl-CoA (a branched chain fatty acid) as substrates. Palmitoyl-CoA oxidase and pristanoyl-CoA oxidase were not inhibited by fenoprofen (Fig. 5). The oxidase activity with lignoceroyl-CoA as substrate, however, was inhibited up to 85% by 500 μM of fenoprofen (Fig. 5). Analogous results were obtained with ketoprofen, ibuprofen and naproxen (results not shown). The inhibition of rat liver lignoceroyl-CoA oxidase activity by fenoprofen was found to be competitive

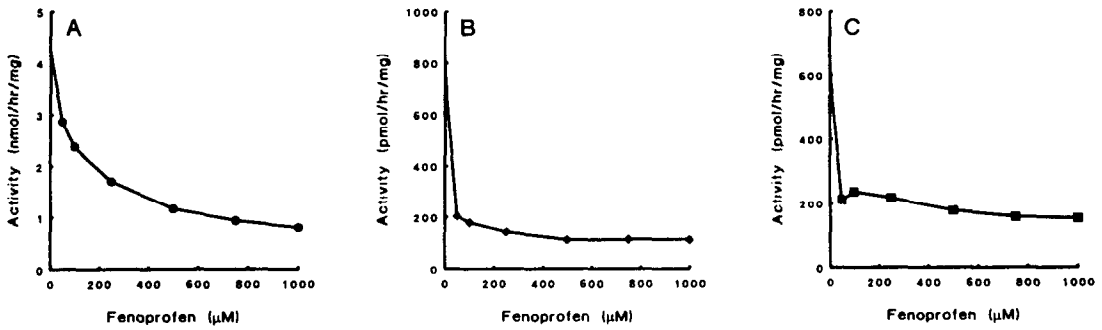

Fig. 3. Effect of fenoprofen on the rate of β-oxidation of [1-^{14}C]palmitic acid (A), [1-^{14}C]lignoceric acid (B) and [1-^{14}C]cerotic acid (C) in rat hepatocytes.

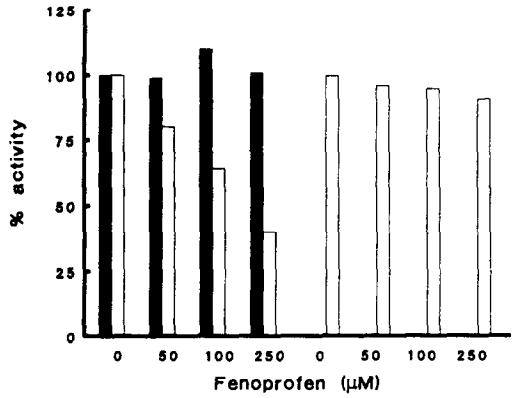

Fig. 4. Different effects of fenoprofen on the activation (closed bars) and subsequent β-oxidation (open bars) of [1-^{14}C]lignoceric acid (left panel) and on the β-oxidation of [1-^{14}C]palmitoyl-CoA (right panel) in peroxisomal fractions.

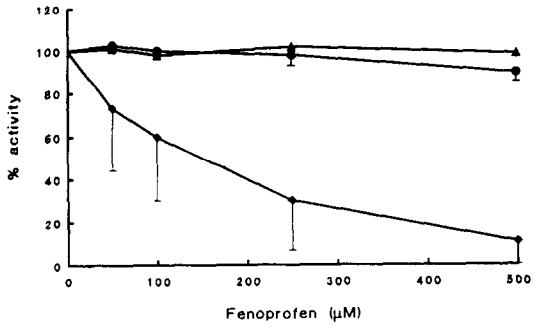

Fig. 5. Effect of fenoprofen on rat liver acyl-CoA oxidase activities. The activities of palmitoyl-CoA oxidase (●), pristanoyl-CoA oxidase (▲) and lignoceroyl-CoA oxidase (◆) were measured in rat liver peroxisomal fractions as described in Materials and Methods. The results are the means ± SD of nine separate experiments for lignoceroyl-CoA, three separate experiments for palmitoyl-CoA and of a single experiment for pristanoyl-CoA. Specific activities in the absence of fenoprofen of palmitoyl-CoA, lignoceroyl-CoA and pristanoyl-CoA oxidase activities were 7.45 ± 0.76, 0.94 ± 0.31 and 1.55 nmol/min/mg protein, respectively.

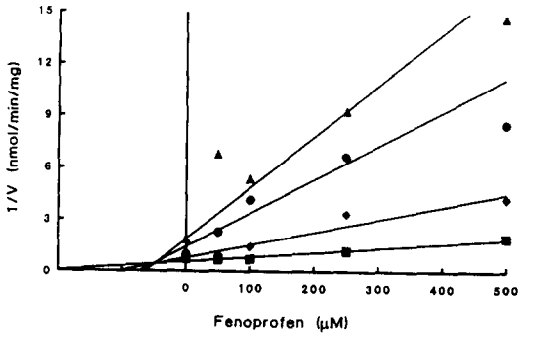

Fig. 6. Representative Dixon plot for the inhibition of lignoceroyl-CoA oxidase activity by fenoprofen (50–500 μM). Substrate concentrations of lignoceroyl-CoA: 25 (▲), 50 (●), 100 (◆), 200 (■) μM.

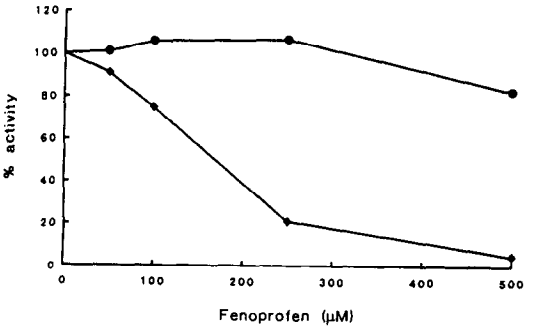

Fig. 7. Effect of fenoprofen on human liver acyl-CoA oxidase activities. The activities of palmitoyl-CoA oxidase (●) and lignoceroyl-CoA oxidase (◆) were measured in human liver peroxisomal fractions as described in Materials and Methods.

with an inhibition constant of 50 μM (Fig. 6). The competitive character of the inhibition was confirmed by means of Lineweaver–Burk plots (not shown).

Since fenoprofen is used as a non-steroid anti-inflammatory drug in humans we investigated its effect on the palmitoyl-CoA and lignoceroyl-CoA oxidase activities in a human liver peroxisomal fraction. A similar sensitivity of the acyl-CoA oxidase activities to that in the rat was found (Fig. 7).

DISCUSSION

In this study we investigated the effects of fenoprofen on the activation and oxidation of long chain and very long chain fatty acids. Fenoprofen was found to inhibit the formation of palmitoyl-CoA by rat liver microsomal and peroxisomal fractions, in accordance with earlier reports by Knights and co-workers [10, 11]. Half-maximal inhibition was observed at fenoprofen concentrations of 60–70 μM (Fig. 1). Roberts and Knights [11], however, found that much higher concentrations of fenoprofen were required to inhibit the rat liver peroxisomal palmitoyl-CoA synthetase (55% inhibition with 2.5 mM of fenoprofen). The reason for this discrepancy is not clear. Roberts and Knights did not investigate the effect of fenoprofen on the activation of very long chain fatty acids. We show that the microsomal as well as the peroxisomal very long chain fatty acyl-CoA synthetase activity is not inhibited at all by fenoprofen (Fig. 1A, B).

Recently, we showed that pyrenedecanoic acid inhibits the activation of cerotic acid more strongly than the activation of palmitic acid in microsomes and peroxisomes [6]. Moreover, the activities have different sensitivities towards detergents [37] and

have a different subcellular distribution pattern [14, 15]. This, together with the results described in this paper showing that fenoprofen inhibits the activation of long chain but not of very long chain fatty acids, provides strong support for the existence of separate enzymes activating long chain fatty acids and very long chain fatty acids.

An important finding is that fenoprofen was able to inhibit the β-oxidation of both long chain and very long chain fatty acids in freshly isolated rat hepatocytes. With regard to palmitic acid the inhibition of palmitic acid β-oxidation in hepatocytes by fenoprofen may be explained by the inhibition of palmitoyl-CoA synthetase. Indeed, fenoprofen was about equally potent in inhibiting palmitic acid β-oxidation in hepatocytes and palmitoyl-CoA synthetase (compare Figs 1–3). The finding that the activation of very long chain fatty acids is not inhibited by fenoprofen suggests the involvement of a different kind of mechanism for the inhibition of lignoceric acid and cerotic acid β-oxidation in hepatocytes. There are at least two possible explanations: (1) fenoprofen lowers the intracellular ATP and/or CoASH concentration, thereby diminishing the activation of fatty acids or (2) fenoprofen inhibits the β-oxidation (but not the activation) of very long chain fatty acids. Fenoprofen had only a limited effect on the intracellular ATP levels. Knights and Drew [38] showed recently that 1–3 mM ibuprofen, another 2-arylpropionic acid, was able to reduce the CoASH concentration in hepatocytes. In our experiments 50–500 μM fenoprofen had no effect on the intracellular CoASH concentration. We can not, however, exclude a possible sequestration of other cofactors in intact hepatocytes. The finding that fenoprofen inhibits the β-oxidation of lignoceric acid in peroxisomal fractions from rat liver (Fig. 4) provides suggestive evidence for the second possibility although it must be stressed that fenoprofen affected lignoceric acid β-oxidation in hepatocytes much more than in peroxisome-enriched fractions.

When the individual reactions involved in the peroxisomal β-oxidation of long chain acyl-CoA esters and very long chain acyl-CoA esters are considered, it is generally believed that the conversion of the enoyl-CoA esters to the chain-shortened products is catalysed by the same set of enzymes. This conclusion is primarily based on the findings in two patients with a deficiency of the peroxisomal multifunctional protein and peroxisomal thiolase, respectively (see Ref. 39 for review). Accordingly, the differential effects of fenoprofen on the peroxisomal oxidation of palmitoyl-CoA and lignoceroyl-CoA are probably due to distinct effects on the formation of the enoyl-CoA esters. Recent results from van Veldhoven *et al.* [40] and Wanders *et al.* [41] have shown that both palmitoyl-CoA oxidase and pristanoyl-CoA oxidase react with palmitoyl-CoA and lignoceroyl-CoA, although the quantitative contribution of the two oxidases to the total peroxisomal β-oxidation of palmitic acid and lignoceric acid remains to be established. Our finding that fenoprofen has no inhibitory effect on the palmitoyl-CoA and pristanoyl-CoA oxidase activities in rat liver peroxisomes would suggest the involvement of a distinct acyl-CoA oxidase (preferentially reacting with very long chain fatty acyl-CoA esters) different from any of the hitherto described acyl-CoA oxidases. In this respect, it may be relevant to mention that mitochondria contain at least four different acyl-CoA dehydrogenases with different substrate specificities for the oxidation of saturated fatty acyl-CoA esters.

Although the precise mechanism of the action of fenoprofen remains to be established, it is clear that it drastically inhibits the oxidation of long chain and very long chain fatty acids. This has important implications since fenoprofen and other non-steroid anti-inflammatory drugs are used in humans. So far attention has only been paid to the potential inhibition by 2-arylpropionic acid-derived non-steroid anti-inflammatory drugs on mitochondrial β-oxidation [42]. Our finding that the peroxisomal oxidation of very long chain fatty acids is also affected by fenoprofen might be of even greater pathophysiological consequence, since very long chain fatty acids seem to be very toxic to man. This can be concluded from studies on a recently recognized group of inherited diseases in man in which there is an impairment in one or more peroxisomal functions. The cerebro-hepato-renal syndrome of Zellweger is best known among these disorders. In several of these disorders there is an accumulation of very long chain fatty acids. In some disorders this is due to the complete absence of peroxisomes *per se* resulting from a defect in the correct assembly of the organelle. In other disorders the accumulation of very long chain fatty acids is due to a functional deficiency of one of the enzymes involved in peroxisomal β-oxidation. All these disorders are associated with severe clinical abnormalities and in most cases with early death. Accordingly, very long chain fatty acids are generally considered to be highly toxic to humans. In fact, a therapeutic trial is currently being carried out in X-linked adrenoleukodystrophy patients (in which there is a deficiency of the peroxisomal very long chain acyl-CoA synthetase activity [14]) which is aimed at reducing the plasma levels of very long chain fatty acids by means of dietary restriction and administration of erucic acid ($C_{22:1}$). Rizzo *et al.* [43] have shown that erucic acid reduces very long chain fatty acid levels in such patients [43]. Furthermore, reduction of very long chain fatty acid levels by erucic acid in X-linked adrenoleukodystrophy patients seems to be highly beneficial, provided therapy is initiated before the onset of symptoms [44]. It will therefore be important to verify the levels of very long chain fatty acids in patients receiving fenoprofen or one of the other structurally related non-steroid anti-inflammatory drugs for long periods of time.

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REFERENCES

1. Victorino RM, Baptista A, Silveira JC and De Moura MC, Jaundice associated with naproxen. *Postgrad Med J* **56**: 368–370, 1980.
2. Stennett DJ, Simonson W and Hall AC, Fenoprofen induced hepatotoxicity. *Am J Hosp Pharm* **35**: 901, 1978.
3. Knights KM, Drew R and Meffin PJ, Enantiospecific formation of fenoprofen coenzyme A thioesters in vitro. *Biochem Pharmacol* **37**: 3539–3542, 1988.
4. Knihinicki RM, Williams KM and Day RO, Chiral inversion of 2-arylpropionic acid non-steroidal anti-inflammatory drugs—1. *Biochem Pharmacol* **38**: 4389–4395, 1989.
5. Tanaka T, Hosaka K, Hoshimaru M and Numa S, Purification and properties of long chain acyl-coenzyme A synthetase from rat liver. *Eur J Biochem* **98**: 165–172, 1979.
6. Lageweg W, Wanders RJA and Tager JM, Long chain acyl-CoA synthetase and very long chain acyl-CoA synthetase activities in peroxisomes and microsomes from rat liver. *Eur J Biochem* **196**: 519–523, 1991.
7. Imhof DA, Logan CJ and Dodds PF, Synthesis of 3-phenoxybenzoic acid-containing lipids via the monoacylglycerol pathway. *Biochem Pharmacol* **34**: 3009–3010, 1985.
8. Bronfman M, Amigo L and Morales MN, Activation of hypolipidaemic drugs to acyl-coenzyme A thioesters. *Biochem J* **239**: 781–784, 1986.
9. Miyazawa S, Hashimoto T and Yokata S, Identity of long chain acyl-CoA synthetase of microsomes, mitochondria and peroxisomes in rat liver. *J Biochem* **98**: 723–733, 1985.
10. Knights KM and Jones ME, Inhibition kinetics of hepatic microsomal long chain fatty acid-CoA ligase by 2-arylpropionic acid non-steroidal anti-inflammatory drugs. *Biochem Pharmacol* **43**: 1465–1471, 1992.
11. Roberts BJ and Knights KM, Inhibition of rat peroxisomal palmitoyl-CoA ligase by xenobiotic carboxylic acids. *Biochem Pharmacol* **44**: 261–267, 1992.
12. Singh I, Singh R, Bhushan A and Singh AK, Lignoceroyl-CoA ligase activity in rat brain microsomal fraction: topographical localization and effect of detergents and α-cyclodextrin. *Arch Biochem Biophys* **236**: 418–426, 1985.
13. Bhushan A, Singh R and Singh I, Characterization of rat brain microsomal acyl-coenzyme A ligases: different enzymes for the synthesis of palmitoyl-coenzyme A and lignoceroyl-coenzyme A. *Arch Biochem Biophys* **246**: 374–380, 1986.
14. Wanders RJA, van Roermund CWT, van Wijland MJA, Schutgens RBH, Heikoop JC, van den Bosch H, Schram AW and Tager JM, Peroxisomal fatty acid β-oxidation in relation to the accumulation of very long chain fatty acids in cultured skin fibroblasts from patients with Zellweger syndrome and other peroxisomal disorders. *J Clin Invest* **80**: 1778–1783, 1987.
15. Singh H, Derwas H and Poulos A, Very long chain fatty acid β-oxidation by rat liver mitochondria and peroxisomes. *Arch Biochem Biophys* **259**: 382–390, 1987.
16. Nagamatsu K, Soeda S and Kishimoto Y, Change of substrate specificity of rat liver microsomal fatty acyl-CoA synthetase activity by Triton X-100. *Lipids* **21**: 328–332, 1986.
17. Soeda S and Kishimoto Y, Immobilization of fatty acyl-CoA synthetase: effect on its stability and substrate specificity. *Biochem Int* **12**: 225–233, 1986.
18. Kishimoto Y, Soeda S and Nagamatsu K, Substrate specificity of fatty acyl-CoA synthetase; effect of detergent. *Fed Proc* **44**: 1413, 1985.
19. Wanders RJA, van Roermund CWT, Lageweg W, Jakobs BS, Schutgens RBH, Nijenhuis AA and Tager JM, X-linked adrenoleukodystrophy: biochemical diagnosis and enzyme defect. *J Inher Metab Dis* **15**: 634–644, 1992.
20. Jakobs BS and Wanders RJA, Conclusive evidence that very long chain fatty acids are oxidized exclusively in peroxisomes in human skin fibroblasts. *Biochem Biophys Res Commun* **178**: 842–847, 1991.
21. Jakobs BS and Wanders RJA, Resolution of the subcellular site of very long chain fatty acid β-oxidation in human skin fibroblasts using a novel approach. In: *New Developments in Fatty Acid Oxidation* (Eds. Coates PM and Tanaka K), pp. 231–238. Wiley–Liss, New York, 1992.
22. Lageweg W, Sykes JEC, Lopes-Cardozo M and Wanders RJA, Oxidation of very long chain fatty acids in rat brain: cerotic acid is β-oxidized exclusively in rat brain peroxisomes. *Biochim Biophys Acta* **1085**: 381–384, 1991.
23. Lazarow PB and de Duve C, A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement of clofibrate, a hypolipidaemic drug. *Proc Natl Acad Sci USA* **73**: 2043–2046, 1976.
24. Osumi T, Hashimoto T and Ui N, Purification and properties of acyl-CoA oxidase from rat liver. *J Biochem (Tokyo)* **87**: 1735–1746, 1980.
25. Schepers L, van Veldhoven PP, Casteels M, Eyssen HJ and Mannaerts GP, Presence of three acyl-CoA oxidases in rat liver peroxisomes. *J Biol Chem* **265**: 5242–5246, 1990.
26. Veldhoven van PP, Vanhove G, Vanhoutte F, Dacremont G, Parmentier G, Eyssen HJ and Mannaerts GP, Identification and purification of a peroxisomal branched chain fatty acyl-CoA oxidase. *J Biol Chem* **266**: 24676–24683, 1991.
27. Wanders RJA, Denis S, Jakobs C and ten Brink HJ, Identification of pristanoyl-CoA oxidase as a distinct, clofibrate non-inducible enzyme in rat liver peroxisomes. *Biochim Biophys Acta* **1124**: 199–202, 1992.
28. De Duve C, Pressmann MC, Gianetto R, Wattiaux R and Appelmans F, Tissue fractionation studies: 6. Intracellular distribution pattern of enzymes of rat liver tissue. *Biochem J* **60**: 604–617, 1955.
29. Leighton F, Poole B, Beaufay H, Baudhuin P, Coffey JW, Fowler S and de Duve C, The large scale separation of peroxisomes, mitochondria, and lysosomes from the livers of rats injected with Triton WR-1339. Improved isolation procedures, automated analysis, biochemical and morphological properties of fractions. *J Cell Biol* **37**: 482–513, 1968.
30. Ghosh MK and Hajra AK, A rapid method for the isolation of peroxisomes from rat liver. *Anal Biochem* **159**: 169–174, 1986.
31. Wolvetang EJ, Wanders RJA, Schutgens RBH, Berden JA and Tager JM, Properties of the ATPase activity associated with peroxisome-enriched fractions from rat liver: comparison with mitochondrial F1F0-ATPase. *Biochim Biophys Acta* **1035**: 6–11, 1990.
32. Berry MN and Friend DS, High yield preparation of isolated rat liver parenchymal cells. *J Cell Biol* **43**: 506–520, 1969.
33. Groen AK, Sips HJ, Vervoorn RC and Tager JM, Intracellular compartmentation and control of alanine metabolism in rat liver parenchymal cells. *Eur J Biochem* **122**: 87–93, 1982.
34. Wanders RJA, van Roermund CWT, van Wijland MJA, Schutgens RBH, Schram AW, van den Bosch H and Tager JM, Studies on the peroxisomal oxidation of palmitate and lignocerate in rat liver. *Biochim Biophys Acta* **919**: 21–25, 1987.
35. Wanders RJA, van Roermund CWT, Schelen A, Schutgens RBH, van den Bosch H and Tager JM, X-

linked adrenoleukodystrophy and other inborn errors of peroxisomal β-oxidation: clinical and biochemical aspects. In: *Adrenoleukodystrophy and other Peroxisomal Disorders* (Eds. Uziel G, Wanders RJA and Cappa M), pp. 17–36. Elsevier Science Publishers B.V., Amsterdam, 1990.

36. Inestrosa NC, Bronfman M and Leighton F, Detection of peroxisomal fatty acyl-CoA oxidase activity. *Biochem J* **182**: 779–788, 1979.
37. Singh H and Poulos A, Distinct long chain and very long chain fatty acyl-CoA synthetases in rat liver microsomes and peroxisomes. *Arch Biochem Biophys* **266**: 486–495, 1988.
38. Knights KM and Drew R, The effects of ibuprofen enantiomers on hepatocyte intermediary metabolism and mitochondrial respiration. *Biochem Pharmacol* **44**: 1291–1296, 1992.
39. Wanders RJA, van Roermund CWT, Schutgens RBH, Barth PG, Heymans HSA, van den Bosch H and Tager JM, The inborn errors of peroxisomal β-oxidation: a review. *J Inher Met Dis* **13**: 4–36, 1990.
40. Veldhoven van PP, Vanhove G, Assselberghs S, Eyssen HJ and Mannaerts GP, Substrate specificities of rat liver peroxisomal acyl-CoA oxidases: palmitoyl-CoA oxidase (inducible acyl-CoA oxidase), pristanoyl-CoA oxidase (non-inducible acyl-CoA oxidase), and trihydroxycoprostanoyl-CoA oxidase. *J Biol Chem* **267**: 20065–20074, 1992.
41. Wanders RJA, Denis SW and Dacremont G, Studies on the substrate specificity of the inducible and non-inducible acyl-CoA oxidases from rat kidney peroxisomes. *J Biochem*, in press.
42. Geneve J, Hayat-Bonan B, Degott C, Letteron P, Freneaux E, Le Dinh T, Larrey D and Pessayre D, Inhibition of mitochondrial β-oxidation of fatty acids by pirprofen. Role in microvascular steatosis due to this nonsteroidal anti-inflammatory drug. *J Pharmacol Exp Ther* **242**: 1133–1137, 1987.
43. Rizzo WB, Leshner RT, Odone A, Dammann AL, Craft DA, Jensen ME, Jennings SS, Davis S, Jaitly R and Sgro JA, Dietary erucic acid therapy for X-linked adrenoleukodystrophy. *Neurology* **39**: 1415–1422, 1989.
44. Uziel G, Bertini E, Bardelli P, Rimoldi M and Gambetti M, Experience on therapy of adrenoleukodystrophy and adrenomyeloneuropathy. *Dev Neurosci* **13**: 274–279, 1991.