Biochemical Pharmacology, Vol. 33, No. 22, pp. 3661–3666, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

EFFECT OF FENOFIBRATE AND LF 2151 ON HEPATIC PEROXISOMES IN HAMSTERS

SUZANNE POURBAIX, FRANCIS HELLER* and CARL HARVENGT
Laboratoire de Pharmacothérapie, Université Catholique de Louvain, 53, Avenue E. Mounier, B-1200 Bruxelles, Belgium

(*Received* 2 *April* 1984; *accepted* 25 *June* 1984)

Abstract—Hamsters were given a diet containing fenofibrate (0.5% or 0.05%) or its metabolite, LF 2151 (0.15% or 0.015%) or a standard diet for a 3-week period. At the end of this period, the analysis of plasma lipids showed that the mean plasma triglyceride concentrations were not significantly different in the five groups of animals. The mean plasma cholesterol concentrations were significantly reduced in animals treated with both drugs but only when given at the high dosage. No consistent changes were noted in the liver weight/body weight ratio and the DNA content of the liver; the number of peroxisomes was increased in the hepatocytes of animals given fenofibrate at the high dosage. Liver homogenates were fractionated and the fractions rich in peroxisomes were used for assays of several enzymes involved in lipid metabolism. Compared with the control animals, activity of cyanide-insensitive fatty acyl-CoA (FA-CoA) oxidizing system was significantly increased by fenofibrate at the high dosage, carnitine acetyltransferase activity was markedly increased by both drugs at the high dosage and catalase activity remained unmodified. As there was a significant inverse correlation between the peroxisomal activity of FA-CoA oxidizing system and the plasma cholesterol concentrations, it is suggested that the increase of peroxisomal β-oxidation activity can be involved in the hypocholesterolemic action of fenofibrate and LF 2151. This is further substantiated by the finding that fenofibrate and LF 2151 were present in the peroxisomal fraction only in hamsters displaying hypocholesterolemia and high activity of FA-CoA oxidizing system. The presence of fenofibric acid in the plasma of hamsters given LF 2151 suggested that hepatocytes are able to generate the parent drug from this metabolite, underlining that the pharmacokinetics of fenofibrate are rather complex in hamsters.

Fenofibrate is a clofibrate-related drug which has been shown to be effective in the treatment of various hyperlipidemic states in humans [1]. The mechanism of action of fenofibrate is not fully understood, although an inhibition of hepatic hydroxymethyl-glutaryl-CoA (HMG-CoA) reductase has been described in animals [2] and an enhancement of the plasma activities of lipoprotein lipase (LPL) and lecithin:cholesterol acyltransferase (LCAT) has been demonstrated in normolipemic subjects given fenofibrate [3]. After absorption, fenofibrate is hydrolysed to fenofibric acid, fenofibric acid itself is reduced to a metabolite (Fig. 1) which is said to exhibit a threefold greater hypolipidemic action than the parent drug (C. Legendre, Fournier, personal communication), and thus could be incriminated in the mechanism of action of fenofibrate. Another matter of concern is the marked increase in the number of peroxisomes described in rodents receiving lipid-lowering agents, especially those related to clofibrate and derivatives: bezafibrate, gemfibrozil, fenofibrate. As peroxisomes have the enzymatic apparatus for β-oxidation of fatty acids, it was inferred that increased peroxisomal β-oxidation can play an important role in the mechanism of the hypolipidemic action of these compounds [4–6]. In addition, the possibility has been raised that peroxisome proliferators as a class may be carcinogenic [7, 8].

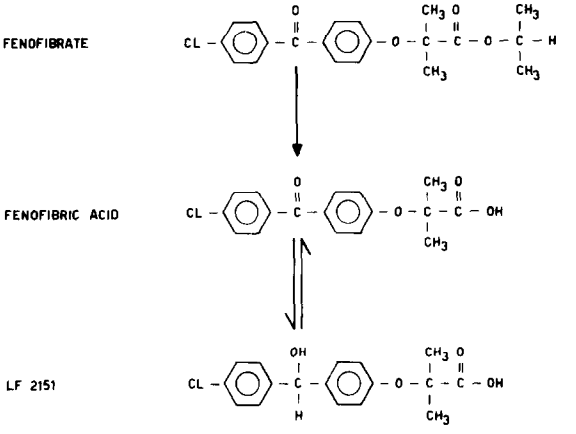


Fig. 1. Structure of fenofibrate, fenofibric acid and LF 2151.

The aim of this work was to study, in hamsters, the hypolipidemic action of the reduced metabolite of fenofibric acid (LF 2151; Fournier, Dijon, France) in comparison with fenofibrate. The effects of the two compounds on hepatic peroxisomes as possible mechanism of their hypolipidemic action were also studied.

MATERIALS AND METHODS

Animals and drug treatments. Male syrian golden hamsters weighing between 90 and 115 g (mean

* To whom correspondence and reprint requests should be addressed.

105 g) were studied after a three-week period. Drug treated animals were given the standard diet to which fenofibrate or LF 2151 were added as opposed to control animals which received the standard diet without the addition of any drug. In a first series of experiments (A), the animals were given a diet containing a low concentration of fenofibrate (0.05%) (three animals) or LF 2151 (0.015%) (three animals); three other animals were used as controls.

In a second series of experiments (B), the hamsters were given a diet containing a high concentration of fenofibrate (0.5%) (five animals) of LF 2151 (0.15%) (five animals); five other animals were used as controls. The animals received diet pellets and water *ad lib*.

Preparation of homogenates. Hamsters were sacrified after light anesthesia with diethyl ether and were not fasting. Blood was withdrawn on EDTA-K from the heart; plasma was separated and frozen immediately. Liver was excised; individual liver homogenates (10%) were prepared in 0.25 M sucrose and centrifuged at 4°. The nuclear fraction (N fraction) was resuspended in 0.25 M sucrose and the post-nuclear supernatant was centrifuged (12,500 *g*) for 20 min; the pellet, corresponding to "the light mitochondrial fraction" (LM fraction) of de Duve [9] was resuspended in 0.25 M sucrose and constituted the fraction enriched with peroxisomes (P fraction). The N and the P fractions were kept frozen.

Morphology. Small pieces of liver were prepared for cytochemical reaction for catalase [10] and the samples were processed for electron microscopic examination [11, 12].

Assays. Enzyme assays were carried out on each P fraction. Activity of fatty acyl–CoA (FA–CoA) oxidizing system was measured in the presence of 1 mM KCN [13]; the NADH formed was estimated by using rezazurin and NADH oxidoreductase from *Clostridium kluyveri* [14]. Catalase (EC 1.11.1.6) was assayed according to Baudhuin [15] and carnitine acetyltransferase (EC 2.3.1.78) (CAT) was measured following Mittal *et al.* [16]. Protein concentrations [17] were measured in the P fractions. The N fractions were used for the assay of DNA [18].

The concentration of fenofibric acid and LF 2151 in plasma and in the P fractions were measured by HPLC (C. Legendre, Fournier, personal communication). To 0.5 or 1 ml of the sample were added 2 ml acetonitrile containing LF 178 (5 μg/ml) as an internal standard. After vigorously mixing, the sample was centrifuged and the supernatant was injected via a six-port valve (Rheodyne 7125). The HPLC system used was a RR015 pump (HPLC technology, Macclesfield, U.K.) with two u.v. detectors PYE LC3 (PYE UNICAM, Cambridge, U.K.) in series, working respectively at 245 nm for LF 2151 and 290 nm for LF 153. The column was filled with Merck Lichrosorb 10 RP8 (250 × 4.6 mm) (Merck Darmstadt, F.R.G.) and isocratically eluted with a solvent made of acetonitrile/water (60/40; v/v), water being adjusted to pH 3 with phosphoric acid. Retention times were 160 sec, 192 sec and 600 sec for LF 2151, LF 153, LF 178 respectively. Reproducibility reached a CV of 6% for LF 2151 and of 3% for LF 153, in the range 200 ng/ml–4 μg/ml. The flow rate was 1.3 ml/minute. Plasma triglycerides and cholesterol were assayed by automated enzymatic methods (WAKO).

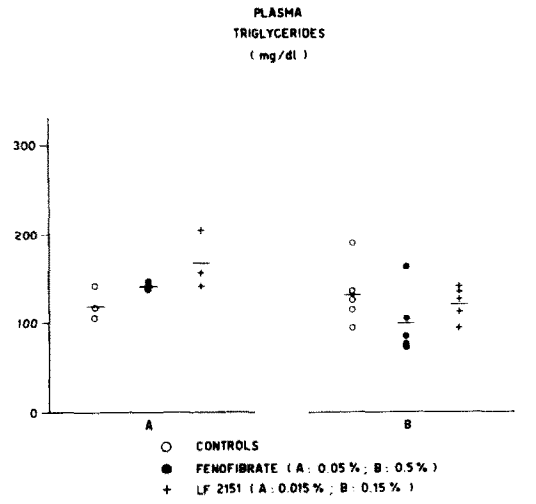


Fig. 2. Plasma triglyceride concentrations in control hamsters and in hamsters given fenofibrate and LF 2151. Bars (—) represent the mean values. A: low dosage of fenofibrate (0.05%) and LF 2151 (0.015%); B: high dosage of fenofibrate (0.5%) and LF 2151 (0.15%).

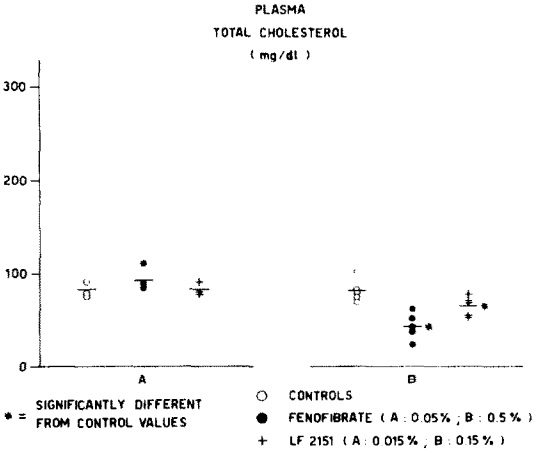


Fig. 3. Plasma cholesterol concentrations in control hamsters and in hamsters given fenofibrate and LF 2151. Bars (—) represent the mean values. A: low dosage of fenofibrate (0.05%) and LF 2151 (0.015%); B: high dosage of fenofibrate (0.5%) and LF 2151 (0.15%).

Materials. Golden syrian hamsters were purchased from Proefdierencentrum, KUL (Heverlee, Belgium). The diet was prepared by UCB (Brussels, Belgium). Fenofibrate and LF 2151 were given by Fournier (France). Palmitoyl–CoA and DL-carnitine were from Sigma U.S.A. NADH oxidoreductase was from Boehringer F.R.G. All other chemicals and solvents were standard commercial high purity material.

RESULTS

Plasma lipids (Figs. 2 and 3)

The mean plasma triglyceride concentrations of the animals given the drugs at the two dosages were

not significantly different from those of the control animals although at the high dosages, the values (mean + S.E.M.) tended to be lower than those of the control animals (143.0 ± 2.0 and 101.4 ± 16.3 mg/dl for fenofibrate, respectively in experiment A and experiment B; 168.3 ± 19.4 and 122.2 ± 6.5 mg/dl for LF 2151, respectively in experiment A and experiment B; 123.0 ± 9.9 and 131.0 ± 16.1 for control animals, respectively in experiment A and experiment B).

The mean (±S.E.M.) plasma cholesterol concentration of the animals given the drugs at the low dosages (experiment A) (92.7 ± 9.9, and 84.0 ± 3.0 mg/dl, respectively for fenofibrate and LF 2151) were not significantly different from those of control animals (83.0 ± 5.0 mg/dl). In animals treated with fenofibrate and LF 2151 at the high dosages (experiment B), the mean (±S.E.M.) plasma cholesterol concentration was significantly lower than that of control animals (43.4 ± 6.2, 66.6 ± 4.6 and 82.2 ± 5.3 mg/dl, respectively for animals treated with fenofibrate and LF 2151 and control animals; $P < 0.0025$ in the first case and $P < 0.025$ in the second case).

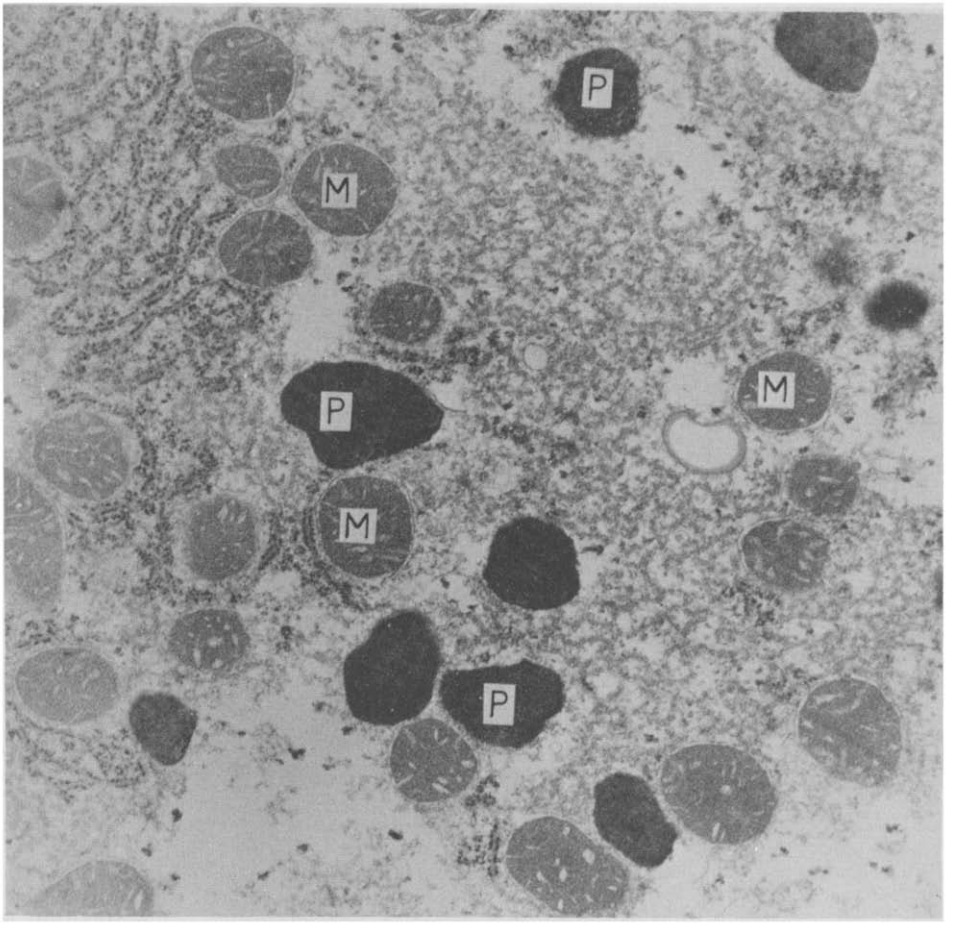
Liver morphology and DNA content

In hamsters given fenofibrate at a high dosage, the liver/body weight ratio (0.059 ± 0.005) was slightly but not significantly increased when compared to control animals (0.034 ± 0.04) and the number of peroxisomes was found to be enhanced (Fig. 4). No change was found with fenofibrate at the low dosage and LF 2151 whatever the dosage. It must be emphasized that the dietary intake of animals given fenofibrate at the high dosage (about 4.75 g per animal per day) was lower than that of control animals (about 7.05 g per animal per day) and of animals given LF 2151 at the high dosage (about 7.05 g per animal per day).

The DNA content of the N fraction was slightly but not significantly increased in animals given LF 2151 at the high dosage (155.2 ± 15.9 μg/mg protein) when compared to control animals (116.1 ± 10.5 μg/mg protein); with LF 2151 at the low dosage, and fenofibrate whatever the dosage, there was no significant change in the DNA content.

Peroxisomal enzymes (Figs. 5–7)

Cyanide-insensitive activity of FA–CoA oxidizing system of the P fraction was nearly double in hamsters given fenofibrate at the high dosage (185.8 ± 25.3 mU/mg protein) compared with control animals (106.7 ± 15.4 mU/mg protein) (experiment B) ($P < 0.0005$). With LF 2151 at the high dosage, the difference was not found to be significant (137.7 ± 11.3 mU/mg proteins) ($P > 0.1$).



(a)

Fig. 4. Electron micrographs of liver from one control animal (a) and one animal given fenofibrate (0.5%) (b). M: mitochondria; P: peroxisomes (*overleaf*).

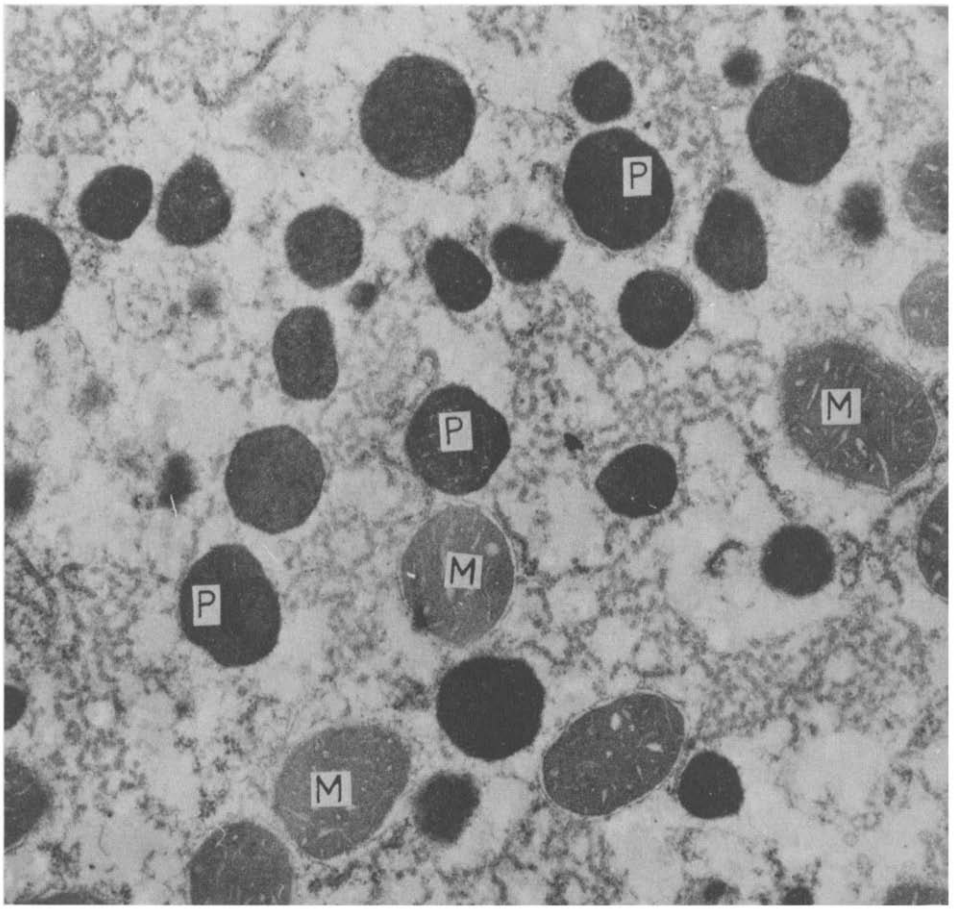


Fig. 4. (b)

No effect was shown with any drug at the low dosage (experiment A: 89.3 ± 7.3, 107.9 ± 23.9 and 102.0 ± 20.8 respectively for animals treated with fenofibrate, LF 2151 and control animals).

There was a significant inverse correlation between the values of plasma cholesterol concentration and those of cyanide-insensitive activity of FA–CoA oxidizing system of the P fraction when all hamsters (treated animals plus those untreated, animals of experiment A plus those of experiment B) are taken

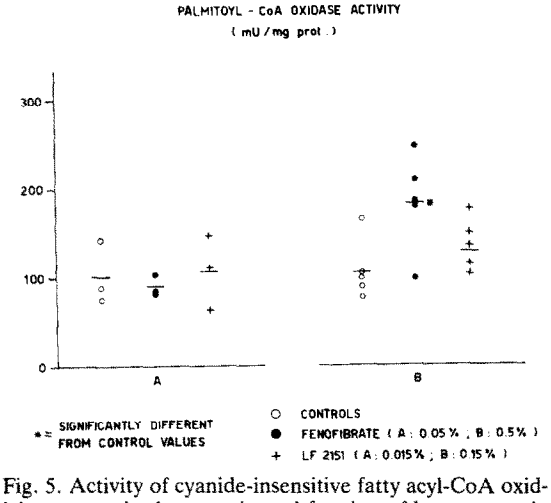


Fig. 5. Activity of cyanide-insensitive fatty acyl-CoA oxidizing system in the peroxisomal fraction of hepatocytes in control hamsters and in hamsters given fenofibrate A: 0.05%; B: 0.5% and LF 2151 A: 0.015%; B: 0.15%. Bars (—) represent the mean values.

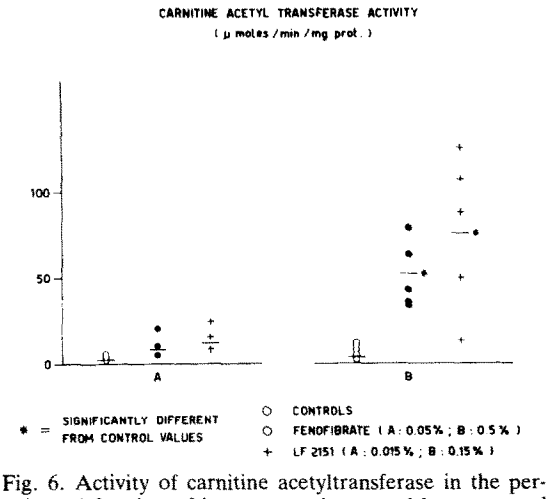


Fig. 6. Activity of carnitine acetyltransferase in the peroxisomal fraction of hepatocytes in control hamsters and in hamsters given fenofibrate A: 0.05%; B: 0.5% and LF 2151 A: 0.015%; B: 0.15%. Bars (—) represent the mean values.

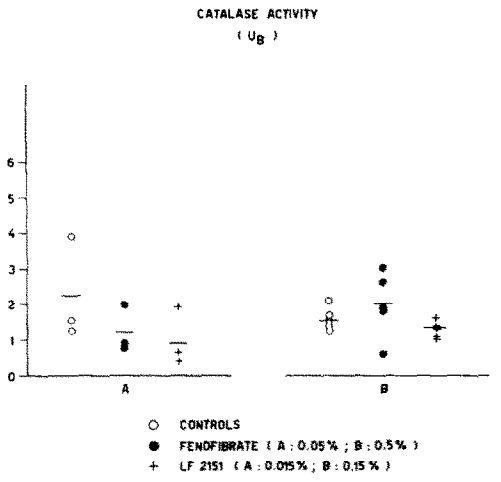


Fig. 7. Activity of catalase in the peroxisomal fraction of hepatocytes in control hamsters and in hamsters given fenofibrate A: 0.05%; B: 0.5% and LF 2151 A: 0.015%; B: 0.15%. Bars (—) represent the mean values.

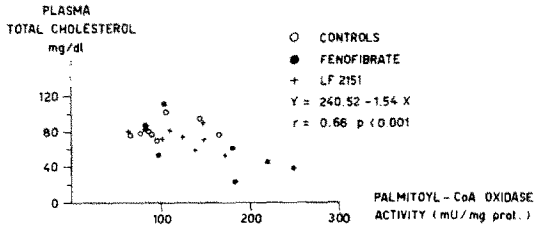


Fig. 8. Relationship between concentrations of plasma cholesterol and activity of cyanide-insensitive fatty acyl-CoA oxidizing system of the peroxisomal fraction of hepatocytes in control hamsters and in hamsters given fenofibrate and LF 2151.

into account ($r = 0.66$; $P < 0.001$; $N = 24$) (Fig. 8). The catalase activity of the P fraction was not modified by any drug whatever the dosage.

The CAT activity of the P fraction was slightly but not significantly higher in animals treated by both drugs at the low dosage (experiment A: 8.84 ± 3.9, 12.4 ± 3.6 and 2.9 ± 1.1 μmoles/min/mg protein, respectively for animals treated with fenofibrate, LF 2151 and control animals) and markedly increased (10–100 times) by fenofibrate and overall by LF 2151 at the high dosage (experiment B: respectively 53.1 ± 10.3, 77.2 ± 22.9 μmoles/min/mg protein vs 4.1 ± 0.8 μmoles/min/mg protein for control animals) ($P < 0.0005$ in both cases).

Drug concentrations (*Table* 1)

At the low dosage of fenofibrate and LF 2151, substantial amount of LF 2151 was found in the plasma. At the high dosage, when fenofibrate is given, much more fenofibric acid is found in the plasma than LF 2151; when LF 2151 is given, an unexpectedly substantial amount of fenofibric acid is also found together with LF 2151. For reference, it has to be noted that in patients given fenofibrate 300 mg daily for treating hyperlipidemic states, the plasma level of fenofibric acid lies between 10 and 20 μg/ml [1].

In the P fraction, no drug was detected at the low dosages of fenofibrate and LF 2151 and at the high dosage of LF 2151. At the high dosage of fenofibrate, nearly equal amounts of fenofibric acid and of LF 2151 were found in this fraction.

DISCUSSION

The hypolipidemic action of fenofibrate is well known and is attributed to its metabolite (fenofibric acid) which is formed after absorption. The reduced metabolite of fenofibric acid (LF 2151) is considered to exhibit a threefold greater hypolipidemic action than fenofibrate (C. Legendre, Fournier, personal communication), but our data are not sufficient to make a precise evaluation of the magnitude of the hypolipidemic action of LF 2151 as compared to fenofibrate; indeed, LF 2151, although given at a concentration in the diet equal to nearly one third of the concentration of fenofibrate, may be less well absorbed by the gastrointestinal tract than fenofibrate, because it was administered as free acid.

Various hypolipidemic drugs induce in rodents, liver enlargement due partly to a striking increase in peroxisome number, accompanied by alterations in the peroxisomal enzyme activities [19]. A link between the changes in peroxisomes and the hypolipidemic activity of these compounds has been suggested [4–6]. Fenofibrate is a hypolipidemic drug which is extensively used in Europe [1] and has been reported to produce proliferation of peroxisomes in rats [20, 21]. Thus, it was of great interest to study the effects of fenofibrate and its metabolite on plasma lipid levels and on peroxisomes in hamsters. We have found that increases in the number and in some

Table 1. Levels of fenofibric acid (F.A.) and LF 2151 in plasma (μg/ml) and in the hepatic peroxisomal fraction (μg/mg protein) of hamsters given fenofibrate (0.05 or 0.5%) and LF 2151 (0.015 or 0.15%) for 3 weeks

		Plasma		Hepatic peroxisomal fraction	
		F.A.	LF 2151	F.A.	LF 2151
Fenofibrate	0.05%	9.3 ± 0.7	5.3 ± 1.9	N.D.	N.D.
LF 2151	0.015%	N.D.*	5.8 ± 0.7	N.D.	N.D.
Fenofibrate	0.5%	175.9 ± 20.6	8.6 ± 0.6	0.25 ± 0.10	0.27 ± 0.13
LF 2151	0.15%	2.3 ± 0.4	1.5 ± 0.4	N.D.	N.D.

* N.D. = Not detectable.

enzyme activities (FA–CoA oxidizing system and CAT) of the peroxisomes are better indicators of an eventual hypocholesterolemic action of the drugs, than other indexes such as liver weight and DNA content.

It must be noted that, whatever the effect on plasma cholesterol, hepatic catalase activity remained unchanged. These results are in accordance with the recent findings of Lazarow *et al.* [22] who demonstrated that, in rats, clofibrate and bezafibrate increased 3- to 4-fold the activity of the peroxisomal beta-oxidation system in the liver, with modest or no effects on catalase activity, liver weight or peroxisome abundance. The absence of peroxisome proliferation noted in our experiments with fenofibrate, given at 0.05% in the diet (corresponding to approximately 30 mg/kg/day) is not surprising since in rats, an effect is demonstrated only at and above 60 mg/kg/day [19]. However, the present results are at some variance with recent studies in hamsters in which fenofibrate, given at a concentration somewhat in the range of our study (0.2%), but for a longer period of time (6 weeks), not only induced a marked proliferation of hepatic peroxisomes and an enhancement of peroxisomal activities of fatty acid β-oxidation and carnitine acetyltransferase but also exerted a marked hypotriglyceridemic action, caused a significant increase in the liver weight and induced an increase in hepatic catalase activity [6].

Can the hypocholesterolemia observed in hamsters with fenofibrate and LF 2151 at high doses be explained by an action on peroxisomal enzymes? In the peroxisomes, the fatty acids undergo β-oxidation through the FA–CoA oxidizing system [23]; as it has been outlined above, its hepatic activity remained unmodified when no effect on cholesterolemia was observed, it was slightly (although not significantly) increased when cholesterolemia was found to be decreased by LF 2151, and nearly double that of controls with fenofibrate which induced the greatest lowering effect on plasma cholesterol. Moreover, there was a significantly inverse correlation between the activity of FA–CoA oxidizing system and the plasma cholesterol concentrations. Finally, it is also interesting to emphasize that, in the peroxisomal fraction of liver cells, fenofibric acid and LF 2151 are found in hamsters in which a decrease in plasma in plasma cholesterol and an increase in the activity of FA–CoA oxidizing system were observed. Taken together, these features tend to suggest that an increase of peroxisomal β-oxidation activity can be incriminated in the mechanism of the hypocholesterolemic action of fenofibrate and LF 2151 and are in the line of the hypothesis put forward first by Reddy and Krishnakantha [4] and more recently by Lazarow [24], suggesting that the peroxisomes play an important role in the lowering of plasma lipid levels caused by hypolipdemic drugs in rodents.

Finally, substantial amount of reduced metabolite (LF 2151) is found in the plasma when fenofibrate is given but relatively more at the low than at the high concentration of fenofibrate in the diet, suggesting that production of reduced metabolite from the parent drug is a rate limiting process. On the other hand, when the reduced metabolite (LF 2151) is given, fenofibric acid can be found in the plasma, overall when high concentrations of LF 2151 is used in the diet; that suggests that hepatocyte is able to generate the parent drug from its reduced metabolite.

These results substantiate that, in rodents, the mechanism of the hypolipidemic action of fenofibrate and LF 2151 involves peroxisomes as it was noted for other drugs [22]. The pharmacokinetics of fenofibrate seems to be complex in hamsters and would need to be more extensively studied in humans particularly concerning the exact metabolic fate of the reduced metabolite.

Acknowledgements—Professor P. Maldague (Unité de Cytologie) is acknowledged for his helpful suggestion for processing the hepatic samples for electron microscopy and for the analysis of the pictures. We wish to thank J. P. Desager for the determinations of drugs in biological materials, Y. Van Nieuwenhuyze and J. Costermans for their valuable laboratory assistance and Mrs N. Bouve for preparing the manuscript. This work was supported by a grant of the Fonds National de la Recherche Scientifique, Belgium (Crédit aux Chercheurs).

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