Biochemical Pharmacology, Vol. 42, No. 7, pp. 1487–1491, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

EFFECT OF THE PEROXISOME PROLIFERATOR LY171883 ON TRIGLYCERIDE ACCUMULATION IN RATS FED A FAT-FREE DIET

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(*Received* 17 *January* 1991; *accepted* 1 *June* 1991)

Abstract—LY171883 is a leukotriene D_4 antagonist that induces peroxisome proliferation in the rodent liver. Like many peroxisome-proliferating agents, it causes transient lipid accumulation and several other changes in hepatic lipid metabolism. The effect of LY171883 on lipid metabolism was studied further in rats maintained on a fat-free diet. Administration of a fat-free diet for 14 days caused a 5.6-fold increase in liver triglycerides associated with a 3.3-fold increase in fatty acid synthetase. Co-administration of 0.1% LY171883 increased liver triglycerides slightly, whereas 0.3% LY171883 prevented the accumulation of triglycerides. Furthermore, treatment with 0.3% LY171883 reversed the fatty liver in rats pretreated with the fat-free diet for 14 days. Fatty acid synthetase activity increased comparably in all treatment groups, indicating that 0.3% LY171883 did not prevent the lipogenic response to a fat-free diet. In rats treated with 0.3% LY171883, peroxisomal β-oxidation increased 9.5-fold, mitochondrial β-oxidation 4.8-fold, carnitine palmitoyltransferase I 1.9-fold, and plasma ketones 3-fold. In the 0.1% dose group the increases in these parameters were smaller. The data indicate that 0.3% LY171883 sufficiently increased mitochondrial and peroxisomal β-oxidation such that fatty acids generated by lipogenesis were preferentially oxidized rather than esterified to triglycerides. In the 0.1% dose group oxidation was only mildly increased, and the excess fatty acids continued to be esterified.

Peroxisome-proliferating agents cause numerous changes in hepatic lipid metabolism in the rat; for example, peroxisomal β-oxidation and microsomal ω-oxidation increase 20-fold or more [1, 2]. While studies of peroxisome proliferators have focused mainly on oxidative enzymes in peroxisomes and microsomes, the compounds also increase the mitochondrial oxidation of fatty acids [3, 4]. This is associated with induction of carnitine palmitoyltransferase (CPT-I), the rate-limiting step in mitochondrial β-oxidation [5, 6]. Thus, these agents greatly increase the capacity of the rodent liver to oxidize fatty acids. This change shifts the balance of fatty acid metabolism away from esterification, resulting in decreased triglyceride synthesis [7, 8].

In studies with the peroxisome proliferator LY171883, we found that increases in peroxisomal and mitochondrial fatty acid oxidation alter the disposition of triglycerides in the rat liver [6]. After the first day of treatment, the compound increases the liver triglycerides. This is attributable, at least partially, to acute inhibition of fatty acid metabolism at the level of CPT-I. With continued treatment for several days, there is sufficient induction of mitochondrial and peroxisomal β-oxidation to overcome the acute inhibition, resulting in a reversal of the triglyceride accumulation.

In this report we have further examined the ability of LY171883 to alter the balance of fatty acid metabolism between oxidation and esterification to triglycerides. The peroxisome-proliferating agent was given to rats fed a fat-free/high carbohydrate diet. This diet greatly enhances hepatic lipogenesis and causes fatty liver [9]. Thus, the effects of LY171883 on fatty acid oxidation and triglyceride disposition were examined under conditions in which the balance of fatty acid metabolism is markedly shifted towards esterification to triglycerides. We found that despite the high rate of lipogenesis in the livers of rats fed a fat-free diet, LY171883 increased fatty acid oxidation sufficiently to prevent the accumulation of triglycerides.

MATERIALS AND METHODS

Animals. Male Fischer-344 rats, weighing 80–120 g at the start of treatment, were obtained from Harlan Sprague–Dawley (Indianapolis, IN). The rats were housed individually in stainless-steel cages and were maintained on a 12-hr light/dark cycle. The animals were given Purina Rodent Chow No. 5002 or a fat-free diet (ICN Biomedicals, Costa Mesa, CA) and tap water *ad lib*. Compound LY171883, the designation for 1-[-hydroxy-3-propyl-4-[4-(1*H*-tetrazol-5-yl)butoxy]phenyl]-ethanone, was mixed in the fat-free diet at concentrations of 0.1 or 0.3%. In some experiments the rats received fat-free diet alone for 14 days prior to the incorporation of LY171883 into the fat-free diet. In a pair-feeding study, rats were given the fat-free diet in amounts equal to the daily food consumption of rats fed the fat-free diet containing 0.3% LY171883.

Lipid analysis. A portion of liver was homogenized in 4 vol. of ice-cold distilled water and lipids were extracted [10]. Triglycerides were analyzed by high performance thin-layer chromatography and

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densitometry using a CAMAG TLC Scanner II as described by Schmitz *et al.* [11]. Serum triglycerides were determined by automated analysis (CentrifiChem®, Baker Instruments, Pleasantville, NY).

Peroxisomal and mitochondrial β-oxidation. A portion of liver from each rat was homogenized in ice-cold 0.25 M sucrose and peroxisomal β-oxidation was measured in the 600 *g* supernatant as the cyanide-insensitive reduction of NAD^+ using 50 μM palmitoyl CoA as substrate [12]. Mitochondria were prepared as described by Johnson and Lardy [13]. Mitochondrial β-oxidation was determined under the conditions described by Turnbull *et al.* [14], except that L-[1-^{14}C]palmitoylcarnitine (40 μM) was used as substrate and acid-soluble metabolites were measured [15]. The oxidation of palmitoylcarnitine was inhibited more than 98% in the presence of 2 mM cyanide in all treatment groups, indicating that the reaction was specific for mitochondrial β-oxidation.

Carnitine palmitoyltransferase I. CPT-I activity in mitochondria was determined by measuring the conversion of palmitoyl CoA to palmitoylcarnitine [16, 17] as previously described [15].

Fatty acid synthetase. A portion of liver was homogenized in 3 vol. of 0.25 M sucrose and fatty acid synthetase was assayed in the 27,000 *g* supernatant [18].

Plasma ketones. Plasma β-hydroxybutyrate and acetoacetic acid were assayed according to Mellanby and Williamson [19, 20].

Statistics. The statistical method of Dunnett [21] was used in the analysis of differences between control and treated group means.

RESULTS

Administration of the fat-free diet for 14 days resulted in a 5.6-fold increase in liver triglycerides over rats fed standard chow (Fig. 1a). In animals receiving the fat-free diet containing 0.1% LY171883, liver triglycerides were slightly higher than in fat-free diet controls, though the differences were not significant. In contrast, animals treated with 0.3% LY171883 had no accumulation of liver triglycerides compared to rats fed standard chow. LY171883 in fat-free chow caused a dose-related decrease in serum triglycerides [control, 88 ± 6 mg/dL; 0.1% LY171883, 59 ± 3 ($P \leq 0.05$); 0.3% LY171883, 34 ± 2 ($P \leq 0.05$)].

Administration of the fat-free diet increased fatty acid synthetase activity 3.3-fold over standard chow controls (Fig. 1b). LY171883-treated animals showed comparable increases in fatty acid synthetase. The direct addition of 100 μM LY171883 to homogenates from rats on the fat-free diet only slightly decreased fatty acid synthetase activity (1082 ± 38 μmol/g liver/min in the absence of LY171883; 1005 ± 40 in the presence of LY171883). The data indicate that treatment with LY171883 did not block the lipogenic effect of a fat-free diet.

In rats treated with 0.3% LY171883 in the fat-free diet, peroxisomal β-oxidation increased 9.5-fold, mitochondrial β-oxidation 4.8-fold, and CPT-I activity 1.9-fold over fat-free controls (Table 1); 0.1% LY171883 increased peroxisomal and mitochondrial β-oxidation 3- and 2.7-fold, respectively. There was also a slight increase in CPT-I activity (1.4-fold). Plasma ketones increased in a dose-related manner [control, 94.4 ± 9.6 nmol/mL; 0.1% LY171883, 161.5 ± 37.4 ($P \leq 0.05$); 0.3% LY171883, 307.5 ± 25.0 ($P \leq 0.05$)].

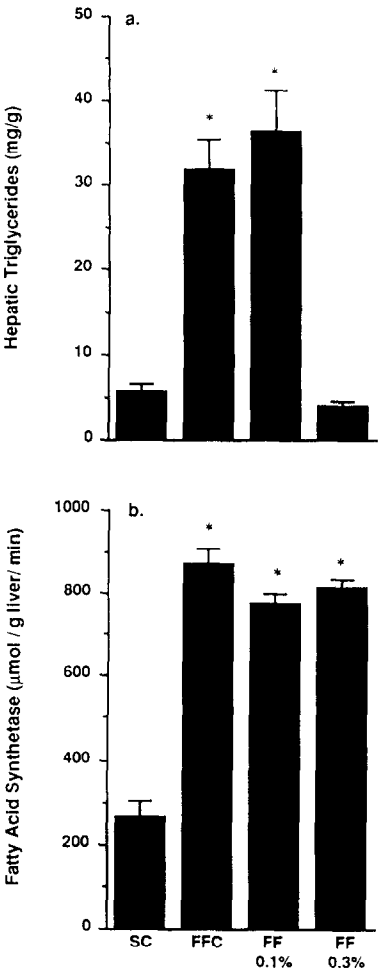

Fig. 1. Effect of LY171883 on liver triglycerides (a) and fatty acid synthetase (b) in rats fed a fat-free diet. Rats received standard chow (SC), fat-free diet alone (FFC), or fat-free diet containing 0.1% (FF 0.1%) or 0.3% (FF 0.3%) LY171883 for 14 days. Values are the means ± SEM of ten rats. Key: (*) significantly different from control, $P \leq 0.05$.

Daily food consumption in rats receiving 0.3% LY171883 was slightly less than in fat-free controls during the first week of the study [control, 14.62 ± 0.32 g/day; 0.3% LY171883, 12.16 ± 0.44 ($P \leq 0.05$)]. By the end of the study food consumption in rats receiving 0.3% LY171883 was comparable to controls (control, 15.12 ± 0.30 g/day; 0.3% LY171883, 14.31 ± 0.25). To determine if the slight reduction in food consumption contributed to the effects ascribed to 0.3% LY171883, a separate pair-feeding study was conducted. Rats fed the fat-free diet *ad lib.* were compared to rats fed the fat-free diet in the daily quantities consumed by rats receiving

Table 1. Effect of LY171883 on peroxisomal β-oxidation, mitochondrial β-oxidation and carnitine palmitoyltransferase I activity in rats fed a fat-free diet for 14 days

Dietary treatment	Peroxisomal β-oxidation*	Mitochondrial β-oxidation†	Carnitine palmitoyl-transferase I‡
Standard chow	0.69 ± 0.07	38.41 ± 6.4	33.65 ± 0.74
Fat-free chow	0.64 ± 0.05	45.03 ± 0.30	31.17 ± 0.75
Fat-free chow + 0.1% LY171883	1.92 ± 0.15§	120.79 ± 4.60§	42.15 ± 0.49§
Fat-free chow + 0.3% LY171883	6.05 ± 0.44§	214.29 ± 15.12§	59.66 ± 4.33§

Values are means ± SEM of three (mitochondrial β-oxidation and carnitine palmitoyltransferase I) or ten (peroxisomal β-oxidation) rats.
* Values expressed as μmol NAD^+ reduced per g liver per min.
† Values expressed as nmol palmitoylcarnitine oxidized per g liver per min.
‡ Values expressed as nmol palmitoylcarnitine produced per g liver per min.
§ Significantly different from fat-free control, $P \leq 0.05$.

Table 2. Liver triglycerides, fatty acid synthetase, peroxisomal β-oxidation, mitochondrial β-oxidation, and carnitine palmitoyltransferase I in rats fed a fat-free diet *ad lib.* or pair-fed

Hepatic parameter	Dietary regimen (fat-free diet) *ad lib.*	Pair-fed
Triglycerides (mg/g)	46.95 ± 5.15	53.27 ± 6.14 (113)
Fatty acid synthetase (μmol/g/min)	1082 ± 38	1208 ± 13* (112)
Peroxisomal β-oxidation (μmol/g/min)	0.90 ± 0.06	1.01 ± 0.06 (112)
Mitochondrial β-oxidation (nmol/g/min)	23.74 ± 0.81	24.32 ± 1.12 (102)
Carnitine palmitoyltransferase I (nmol/g/min)	49.44 ± 0.70	46.94 ± 1.60* (97)

Pair-fed rats received the fat-free diet equal to the daily quantities eaten by rats receiving the fat-free diet containing 0.3% LY171883. Values are means ± SEM of six (*ad lib.*) or five (pair-fed) rats. Numbers in parentheses are percent of control.
* Significantly different from control, $P \leq 0.05$.

0.3% LY171883. There were no major differences in the hepatic parameters measured (Table 2). Thus, the slight reduction in food consumption did not account for the effects on hepatic lipid metabolism.

The effect of 3-, 7-, and 14-day co-administration of 0.3% LY171883 on rats pretreated with the fat-free diet for 14 days was tested to determine if the compound could reverse a pre-existing fatty liver. Liver triglycerides were not affected appreciably on day 3 of LY171883 treatment, but decreased progressively thereafter (Fig. 2). On day 14 liver triglyceride levels were comparable to those found in rats fed standard chow. There was a time-related increase in peroxisomal and mitochondrial β-oxidation and carnitine palmitoyltransferase on days 3 and 7 (Table 3). There was little or no additional induction on day 14.

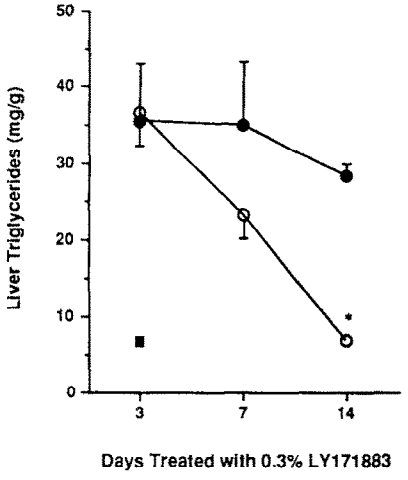

Fig. 2. Effect of LY171883 on fatty liver induced by 14-day administration of a fat-free diet. Rats received the fat-free diet for 14 days. At this time half of the animals were placed on the fat-free diet containing 0.3% LY171883 (–○–) and half remained on the fat-free diet (–●–) alone. The closed square (■) represents the value for rats maintained on standard chow. Values are means ± SEM of five rats. Key: (*) significantly different from control, $P \leq 0.05$.

DISCUSSION

In previous studies we found that 0.3% LY171883 causes transient lipid accumulation that reverses with continued treatment [6]. The reversal of the lipid accumulation was attributed to the induction of mitochondrial and peroxisomal β-oxidation. However, the lipid accumulation was relatively mild. In the present studies we examined the effects of

Table 3. Effect of 3-, 7-, or 14-day treatment with 0.3% LY171883 on peroxisomal β-oxidation, mitochondrial β-oxidation and carnitine palmitoyltransferase I activity in rats pretreated with a fat-free diet

Days treated with LY171883	Treatment	Peroxisomal β-oxidation*	Mitochondrial β-oxidation†	Carnitine palmitoyl-transferase I‡
3	Control	0.71 ± 0.07	26.09 ± 2.13	50.32 ± 1.28
	0.3% LY171883	5.71 ± 0.17§	74.96 ± 3.15§	76.56 ± 1.58§
7	Control	0.66 ± 0.04	22.18 ± 1.72	50.27 ± 1.94
	0.3% LY171883	8.33 ± 0.49§	108.62 ± 6.49§	112.84 ± 2.79§
14	Control	0.82 ± 0.05	20.88 ± 1.29	54.58 ± 2.65
	0.3% LY171883	12.03 ± 0.64§	102.82 ± 5.13§	134.99 ± 4.44§

Rats received the fat-free diet for 14 days, at which time half of the animals were placed on the fat-free diet containing 0.3% LY171883 and half remained on the fat-free diet alone. Values are mean ± SEM of four to five rats.

* Values expressed as μmol NAD^+ reduced per g liver per min.

† Values expressed as nmol palmitoylcarnitine oxidized per g liver per min.

‡ Values expressed as nmol palmitoylcarnitine produced per g liver per min.

§ Significantly different from control, $P \leq 0.05$.

0.1 and 0.3% LY171883 on a more severe fatty liver induced by a fat-free diet. After 14-day administration of the fat-free diet, liver triglycerides increased nearly 6-fold. The increase is attributable to increased lipogenesis, as indicated by a 3-fold increase in fatty acid synthetase activity. Co-administration of 0.3% LY171883 in the fat-free diet prevented the accumulation of liver triglycerides. However, the increase in fatty acid synthetase was comparable to that of fat-free controls, indicating that 0.3% LY171883 did not interfere with the lipogenic effect of the fat-free diet.

Peroxisomal and mitochondrial β-oxidation were increased markedly (9.5- and 4.8-fold, respectively) in rats treated with 0.3% LY171883. Because mitochondria are the primary site of hepatic fatty acid oxidation [3], the increases in mitochondrial β-oxidation may be the more important factor in preventing the lipid accumulation. Indeed, the increases in plasma ketones corresponded with the increased rates of β-oxidation in isolated mitochondria. Thus, it appears that 0.3% LY171883 increased the oxidative capacity of the liver sufficiently to catabolize the high levels of newly synthesized fatty acids. It is interesting to note that in rats receiving 0.3% LY171883 in the fat-free diet, the synthesis and oxidation of fatty acids were induced simultaneously. Generally, these opposing pathways are reciprocally regulated [22, 23].

Administration of 0.1% LY171883 in the fat-free diet did not prevent the lipid accumulation. In fact, liver triglycerides tended to be slightly higher in the 0.1% dose group than in fat-free controls, which is consistent with studies in rats fed standard chow [6]. Peroxisomal and mitochondrial β-oxidation were increased only modestly, as were plasma ketones. Under these conditions fatty acids from lipogenesis and serum continued to be esterified to triglycerides.

The studies above demonstrated that administration of 0.3% LY171883 prevented the formation of fatty liver by the fat-free diet. By pretreating rats with the fat-free diet, we have also demonstrated that 0.3% LY171883 can reverse a pre-existing fatty liver. Maximal increases in peroxisomal and mitochondrial β-oxidation were achieved after 7 days of co-treatment. However, liver triglycerides did not return to standard chow levels until day 14. The lag between maximal increases in oxidation and reversal of liver triglycerides may represent the time required for the existing triglycerides to be cleared from the liver.

Similar effects on hepatic lipid metabolism have been demonstrated with other peroxisome proliferators. Clofibrate has been shown to both prevent and reverse the accumulation of lipids caused by a high sucrose diet containing orotic acid [24, 25] or by polychlorinated biphenyls [26]. Likewise, ciprofibrate reduces the accumulation of lipids caused by a hyperlipidemic diet [27] or partial hepatectomy in rats [28].

Peroxisome-proliferating agents such as LY171883 clearly have dramatic effects on lipid metabolism in rodents. The 0.3% dose of LY171883 caused marked increases in fatty acid oxidation and reversed the accumulation of liver triglycerides. Although it is possible that other factors may be involved in the diminished level of triglycerides, the data suggest that the increases in fatty acid oxidation shifted the balance of fatty acid metabolism away from esterification. Results from the 0.1% dose group, in which oxidation was only nominally increased and triglycerides continued to accumulate, support this conclusion.

Acknowledgements—The authors would like to thank Dave Perry, Kim Holmes, and Kim DeGroot for their contributions to this research.

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