Activation of PPARδ alters lipid metabolism in db/db mice

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Abstract Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors, which heterodimerize with the retinoid X receptor and bind to peroxisome proliferator response elements in the promoters of regulated genes. Despite the wealth of information available on the function of PPARα and PPARγ, relatively little is known about the most widely expressed PPAR subtype, PPARδ. Here we show that treatment of insulin resistant db/db mice with the PPARδ agonist L-165 041, at doses that had no effect on either glucose or triglycerides, raised total plasma cholesterol concentrations. The increased cholesterol was primarily associated with high density lipoprotein (HDL) particles, as shown by fast protein liquid chromatography analysis. These data were corroborated by the chemical analysis of the lipoproteins isolated by ultracentrifugation, demonstrating that treatment with L-165 041 produced an increase in circulating HDL without major changes in very low or low density lipoproteins. White adipose tissue lipoprotein lipase activity was reduced following treatment with the PPARδ ligand, but was increased by a PPARγ agonist. These data suggest both that PPARδ is involved in the regulation of cholesterol metabolism in db/db mice and that PPARδ ligands could potentially have therapeutic value.

Key words: Peroxisome proliferator-activated receptor δ agonist; Peroxisome proliferator-activated receptor; Lipid metabolism

1. Introduction

Three mammalian peroxisome proliferator-activated receptors (PPARα, PPARδ and PPARγ) are known (for review see [1,2]). PPARα, the first PPAR identified [3], regulates the expression of genes involved in lipid metabolism. PPARα agonists, such as the fibrates, are used to treat hyperlipidemia (reviewed in [1,4]). PPARγ is an important regulator of adipogenesis, lipid metabolism and glucose homeostasis (reviewed in [5]). The thiazolidinedione (TZD) PPARγ agonists, such as rosiglitazone or pioglitazone, are used as insulin sensitizers in the treatment of non-insulin-dependent diabetes mellitus [6–9] (for review see [10]). In contrast to PPARα and PPARγ, relatively little is known about the function of the most ubiquitously expressed PPAR, PPARδ [11–13]. PPARδ is also known as NUC-1 [11] or FAAR [13] and it is presently unclear whether PPARβ in *Xenopus* [14] is its functional homolog.

The absence of data relating to the physiological role of PPARδ can be explained on the one hand by the lack of specific high affinity ligands which can be used as physiological probes and also by the absence of animal models carrying mutations in the PPARδ gene. We recently described a ligand (L-165 041) that can be used to begin to explore the physiological role of PPARδ [15]. Recently, two papers described potential roles for PPARδ. First, Lim et al. [16], using L-165 041 and other techniques, have shown that PPARδ is involved in the regulation of embryo implantation in the mouse. Second, PPARδ has recently been shown to be an APC-regulated target gene [17].

2. Materials and methods

2.1. Materials

The TZD AD-5075 (5-[4-[2-(5-methyl-2-phenyl-4-oxazoly)-2-hydroxyethoxy]benzyl]-2,4-thiazolidinedione), and L-165 041 (4-[3-[2-propyl-3-hydroxy-4-acetyl]phenoxy]propyloxyphenoxy acetic acid) were kindly provided by Gerard Kieczykowski, Philip Eskola, Joseph F. Leone, Mark S. Levorse and Peter A. Cicala (Merck Research Laboratories, Rahway, NJ, USA).

2.2. In vivo studies

Male db/db mice (10–11 week old C57BLKS/J-m +/+*Lepr^{db}*, Jackson Laboratory, Bar Harbor, ME, USA) were housed 3–5/cage and allowed ad libitum access to ground Purina rodent chow and water. Lean animals were age-matched heterozygous mice maintained in the same manner. The animals, and their food, were weighed every 2 days and were dosed daily by gavage with vehicle (0.5% carboxymethylcellulose) ± PPAR agonists at the indicated doses. Drug suspensions were prepared daily. Plasma glucose, triglyceride and cholesterol concentrations were determined from blood obtained by tail bleeds into

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heparinized capillaries at 3–5 day intervals during the study. At the end of the study animals were fasted overnight and serum was prepared from the blood of animals that were exsanguinated by heart puncture. Epididymal white adipose tissue (WAT) was frozen in liquid nitrogen following exsanguination. All animal experiments were approved by the Institutional Animal Care and Use Committee.

2.3. Biochemical analysis

Glucose, triglyceride and/or cholesterol determinations were performed on either an Alpkem RFA/2 320 Micro-Continuous Flow Analyzer (Astoria-Pacific International, Clackamas, OR) or a Boehringer Mannheim Hitachi 911 automatic analyzer (Boehringer Mannheim, Indianapolis, IN, USA) using heparinized plasma diluted 1:6 (v/v) with normal saline and commercially available reagents (Boehringer Mannheim). Lipoprotein cholesterol profiles were obtained by fast protein liquid chromatography (FPLC) size fractionation of lipoproteins. Pooled mouse serum samples (150 or 200 μl) were injected onto a Superose 6 HR 10/30 prepacked column (Pharmacia, Uppsala, Sweden) and eluted at a constant flow rate of 0.2 ml/min with 10 mM phosphate-buffered saline, pH 7.2. The effluent was collected in 0.27 ml fractions and cholesterol and triglyceride concentrations were determined in 0.1 ml of each fraction. For the analysis of lipoprotein composition, the lipoprotein fractions were isolated from serum according to their hydrated density by sequential ultracentrifugation and analyzed for protein, cholesterol, triglyceride and phospholipid content as described [18]. The corresponding density ranges were as follows: very low density lipoproteins (VLDL), $d < 1.006$; low density lipoproteins (LDL), $1.006 < d < 1.063$ and high density lipoproteins (HDL), $1.063 < d < 1.21$.

2.4. Lipoprotein lipase (LPL) activity

LPL activity was measured in epididymal WAT extracts according to the procedure of Ramirez et al. [19]. One unit of enzyme activity was defined as the amount of enzyme that released 1 μmol oleate/min at 25°C.

3. Results and discussion

We recently described a synthetic PPAR ligand, L-165 041 that binds to both PPARδ and PPARγ; while L-165 041 binds to and activates both PPARδ and PPARγ, it has a substantially lower affinity for PPARγ than PPARδ (Table 1) [15]. The compound does not activate mouse PPARα. To characterize the effects of PPARδ activation we compared the effects of L-165 041 treatment to those of the TZD AD-5075, a selective PPARγ agonist, in the insulin-resistant db/db mouse, a commonly used animal model for metabolic studies. As a result of a defective leptin receptor, db/db mice are obese, hyperglycemic and hypertriglyceridemic (for review see [20]). PPARγ binding affinity has been shown to be correlated with the hypoglycemic activity of both TZD and non-TZD PPARγ agonists in db/db mice [8,9,15]. Ten week old male db/db mice were dosed daily by gavage with the γ selective agonist AD-5075 (2 mg/kg body weight/day (mg/kg)), or the PPARδ agonist L-165 041 (10 or 30 mg/kg) for 31 days. AD-5075 reduced plasma glucose and triglyceride concentrations essentially to those of age-matched lean mice. At both doses, L-165 041 produced little effect on either plasma glucose or triglycerides (Fig. 1a,b) and [15]. Higher doses of L-165 041 (100 mg/kg) will lower both glucose and triglycerides in db/db mice, as expected based upon its binding affinity for PPARγ, not PPARδ (data not shown).

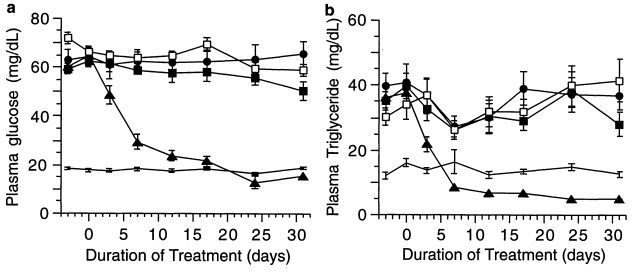

Fig. 1. Mean (±S.E.M.) plasma glucose (a) and triglyceride (b) concentrations of db/db mice treated with the indicated compounds; vehicle (●), 2 mg/kg body weight AD-5075 (▲), 10 or 30 mg/kg body weight L-165 041 (□,■). Values for age-matched lean mice dosed with vehicle (-).

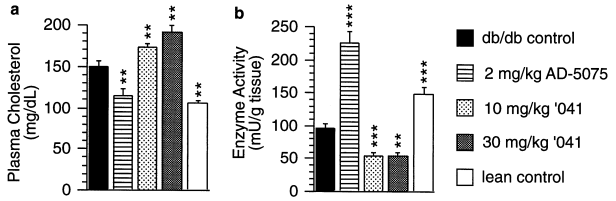

Fig. 2. a: Plasma cholesterol values (mean ± S.E.M.) from db/db or lean mice treated for 31 days, as indicated. Asterisks indicate values statistically different from db/db control (Student's *t*-test, $^{**}P < 0.01$). b: LPL activity in epididymal WAT obtained from db/db or lean animals treated for 31 days. Asterisks indicate values statistically different from db/db control (Student's *t*-test, $^{**}P < 0.01$; $^{***}P < 0.001$).

In contrast to the effects on glucose or triglycerides, the determination of total plasma cholesterol concentrations showed that the PPARδ agonist produced significant, dose-dependent increases in total plasma cholesterol (Fig. 2a). The PPARγ agonist significantly lowered plasma cholesterol in db/db mice (Fig. 2a). We next examined the size distribution of cholesterol-containing lipoprotein particles in a single pool of serum from the animals treated with either 10 or 30 mg/kg of L-165 041. Each pool was fractionated by FPLC and the distribution of cholesterol is shown in Fig. 3a. Almost all of the cholesterol is contained in small, dense particles that contain little triglyceride (data not shown) and are presumably HDL particles. Treatment with L-165 041 produced a dose-dependent increase in HDL cholesterol. This observation is in sharp contrast to the decrease in HDL cholesterol levels observed in rodents after treatment with fibrate PPARα agonists [21,22]. Both doses of L-165 041 produced an equivalent, small increase in LDL cholesterol.

Table 1
PPAR ligands

Compound	PPAR Binding* hPPARγ K_i (nM)	hPPARδ K_i (nM)
AD-5075	1	No Activity
L-165,041	730	6

*binding based upon [15].

*Binding based on [5].

In a more detailed experiment we examined both the distribution of cholesterol-containing lipoprotein particles by FPLC and determined the chemical composition of lipoproteins, isolated by ultracentrifugation according to their hydrated density. We first compared the distribution of cholesterol-containing lipoproteins by FPLC analysis of pooled serum from db/db mice dosed with AD-5075 (2 mg/kg) or L-165 041 (30 mg/kg) for 14 days (Fig. 3b). Treatment with AD-5075 reduced the HDL cholesterol peak and produced a dramatic increase in LDL. In contrast, treatment with L-165 041 produced an increase in HDL cholesterol with little change in the LDL fraction. We determined the chemical composition of lipoproteins isolated by ultracentrifugation (Fig. 4). None of the treatments altered the composition of HDL particles, suggesting that L-165 041 raised HDL cholesterol by increasing the number of HDL particles. This reflects a true increase in the HDL/(VLDL+LDL) ratio. AD-5075 treatment, however, dramatically altered the composition of both VLDL and LDL particles, producing a decrease in the triglyceride content of VLDL and generating a cholesterol-enriched LDL fraction. This observation was consistent with the increase in LDL levels observed following FPLC fractionation of lipoprotein particles (Fig. 3b) and could be the consequence of enhanced lipolysis (Fig. 2b).

Since PPARγ agonists have been shown to increase WAT LPL activity, via a direct transcriptional effect on the LPL promoter [23], we wanted to determine whether a change in LPL activity could explain some of the changes in either lipoprotein distribution or composition described above. Consistent with our previous observation [23], the PPARγ agonist AD-5075 significantly increased total WAT LPL activity in tissues taken from db/db mice dosed for 31 days (Fig. 2b). This increased LPL activity most likely contributes to the changes in lipoprotein characteristics, observed in an independent experiment, after AD-5075 treatment (Fig. 3b). On the other hand, both the 10 and 30 mg/kg doses of L-165 041 significantly lowered total LPL activity. These data strongly suggest differential regulation of LPL activity by activation of PPARδ or PPARγ.

We have shown that doses of a PPARδ agonist (L-165 041) that produce significant increases in plasma cholesterol do not alter plasma glucose or triglycerides in db/db mice. In addition, this increase in cholesterol is associated with HDL cholesterol and an increase in the ratio of HDL to non-HDL

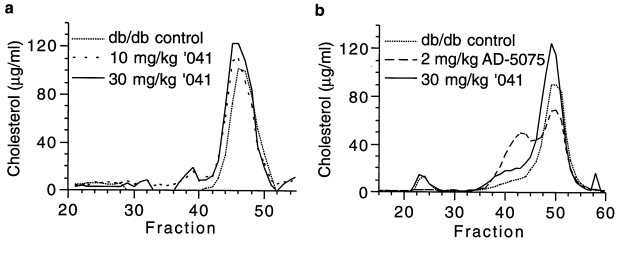

Fig. 3. a: Serum cholesterol distribution in pooled samples from db/db mice treated for 31 days with vehicle, 10 or 30 mg/kg body weight L-165 041 after gel filtration chromatography. b: Serum cholesterol distribution in pooled samples from db/db mice treated for 14 days with vehicle, 2 mg/kg body weight AD-5075 or 30 mg/kg body weight L-165 041 after gel filtration chromatography. Panels (a) and (b) are from independent experiments using different sets of db/db mice.

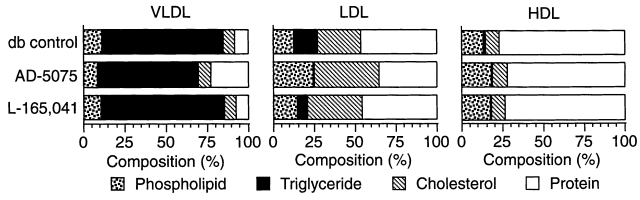

Fig. 4. Mass composition of lipoproteins (VLDL, $d < 1.006$; LDL, $1.006 < d < 1.063$; HDL, $1.063 < d < 1.21$) from serum of db/db mice treated with vehicle, 2 mg/kg body weight AD-5075 or 30 mg/kg body weight L-165 041 for 14 days (same experiment as Fig. 3b).

cholesterol. While the increase in HDL produced by PPARδ agonists is relatively small, modest HDL-raising effects can be clinically important. The most widely used drugs for HDL-raising in man are the fibrates, although they lower HDL in rodents [21,22]. In man the fibrates raise HDL 15–20% [24,25] and have been shown to decrease coronary heart disease [25]. In contrast, PPARγ agonists lower plasma glucose, triglycerides, cholesterol and apo A-I in rodents. Furthermore PPARγ, but not PPARδ, agonists induce a dramatic increase in LDL particles, caused by an LPL-mediated lipolysis of triglyceride-rich lipoproteins. Combined with our previous observations of a dramatic HDL lowering effect of PPARα agonists [21,22], the effects observed in the current study concerning PPARδ and PPARγ activation on lipid parameters suggest a distinct pharmacology associated with activation of the respective receptors.

The exact molecular mechanisms by which PPARδ activation achieves its effects are unclear. Future experiments are designed to determine specifically which metabolic pathways are being directly effected by PPARδ activation. In this context it is interesting to note that all PPARs seem to control pivotal aspects of intracellular lipid handling; whereas PPARα controls fatty acid β-oxidation, PPARγ seems to favor lipid storage. Through these activities both PPARα and γ have important effects on extracellular lipid homeostasis [1]. Our data suggest that PPARδ also fits this paradigm, since its activation markedly affects lipid homeostasis. Previous studies suggested that PPARδ activation could counteract the activity of other PPARs, such as PPARα [26]. Although this could explain the L-165 041-mediated inhibition of LPL activity, which is normally stimulated by PPARα and PPARγ activation [23], multiple other direct effects could also be invoked.

Cholesterol metabolism is regulated differently in man and rodents. Therefore, one must exercise caution in any extrapolation from the current data to the human situation. In addition, the current observations are in db/db mice that have alterations in lipid metabolism. Additional experiments should include examination of L-165 041-induced effects in non-diabetic animals. Nevertheless, these observations suggest that PPARδ plays a role in lipid metabolism in db/db mice and suggest that PPARδ ligands could be novel therapeutic agents, if our observations in db/db mice are recapitulated in non-diabetic rodents and ultimately in man.

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