Circulating free fatty acids are increased independently of PPARγ activity after administration of poloxamer 407 to mice

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Abstract: Poloxamer 407 (P-407) is a copolymer surfactant that induces a dose-controlled dyslipidemia in both mice and rats. Human macrophages cultured with P-407 exhibit a concentration-dependent reduction in cholesterol efflux to apolipoprotein A1 (apoA1) linked to downregulation of the ATP-binding cassette transporter A1 (ABCA1). Activators of peroxisome proliferator-activated receptor gamma (PPARγ), as well as PPARα, increase expression of liver X receptor alpha (LXRα) in macrophages and promote the expression of ABCA1, which, in turn, mediates cholesterol efflux to apoA1. The present study investigated whether P-407 interferes with this signaling pathway. A transactivation assay was used to evaluate whether P-407 can either activate or inhibit the transcriptional activity of PPARγ. Because thiazolidinedione drugs (PPARγ agonists) improve glycemic control in type 2 diabetes by reducing blood glucose concentrations, P-407 was also evaluated for its potential to alter plasma insulin and blood glucose concentrations in wild-type (C57BL/6) and PPARγ-deficient mice. Additionally, because thiazolidinediones attenuate release of free fatty acids (FFAs) from adipocytes and, consequently, decrease circulating plasma levels of FFAs, plasma concentrations of circulating FFAs were also determined in P-407-treated mice. P-407 was unable to modulate PPARγ activity in cell-based transactivation assays. Furthermore, P-407 did not perturb plasma insulin and blood glucose concentrations after administration to mice. However, by an as yet unidentified mechanism, P-407 caused a significant increase in the serum concentration of FFAs in mice beginning 3 h after administration and lasting more than 24 h postdosing. It is concluded that P-407 does not interfere with the functional activity of PPARγ after administration to mice.

Key words: free fatty acid, glucose, insulin, peroxisome proliferator-activated receptor (PPAR), transactivation assay.

Résumé : Le poloxamère 407 (P-407) est un surfactant copolymère qui induit une dyslipidémie fonction de la dose chez les souris et les rats. Des macrophages humains cultivés en présence de P-407 présentent une réduction concentration-dépendante de l'efflux de cholestérol vers l'apolipoprotéine A1 (apoA1) associée à une diminution du transporteur ABC transporteur ABC (ATP binding cassette) A1. Les activateurs du récepteur gamma activé de la prolifération des peroxysomes (PPARγ), ainsi que du PPARα, augmentent l'expression du récepteur alpha X (LRXα) dans les macrophages et favorisent l'expression du transporteur ABCA1, qui, lui, véhicule l'efflux de cholestérol vers l'apoA1. La présente étude examine si P-407 interfère avec cette voie de signalisation. Un test de transactivation a été utilisé pour évaluer si P-407 peut activer ou inhiber l'activité transcriptionnelle de PPARγ. Comme les thiazolidinediones (agonistes de PPARγ) améliorent la régulation glycémique du diabète de type 2 en diminuant les concentrations de glucose sanguin, la capacité de P-407 de modifier les concentrations d'insuline plasmatique et de glucose sanguin après son administration à des souris de type sauvage et à des souris déficientes en PPARγ a aussi été évaluée. De plus, les triazolinediones atténuent la libération des acides gras libres (AGL) des adipocytes et, par conséquent, les taux d'AGL plasmatiques circulants. Les concentrations d'AGL plasmatiques circulants ont donc aussi été déterminées chez des souris traitées au P-407. Le P-407 n'a pu moduler l'activité de PPARγ dans les tests de transactivation cellulaire. Il n'a pas non plus perturbé les concentrations d'insuline plasmatique et de glucose sanguin après son administration aux souris. Toutefois, il a provoqué une augmentation significative de la concentration d'AGL sérique chez les souris, qui a débuté 3 h après l'administration et a duré >24 h, et ce, par un mécanisme encore inconnu. On conclut que P-407 n'interfère pas avec l'activité fonctionnelle de PPARγ après son administration aux souris.

Mots-clés : acide gras libre, glucose, insuline, récepteur activé de la prolifération des peroxysomes (PPAR), test de transactivation.

[Traduit par la Rédaction]

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Introduction

The peroxisome proliferator-activated receptors PPARα and PPARγ are nuclear receptors that, upon heterodimerization with the retinoid X receptor (RXR), function as ligand-activated transcriptional regulators of genes controlling lipid and glucose metabolism (Pineda et al. 1999). PPARα, which is activated by fibrates, fatty acids, and eicosanoids (Chinetti et al. 2000), is most highly expressed in liver, heart, muscle, and kidney, whereas PPARγ₁ is expressed in many tissues and cells, including white and brown adipose tissue, skeletal muscle, intestine, and macrophages (Auwerx 1999; Gonzalez 1997; Kersten et al. 2000; Saltiel and Olefsky 1996; Spiegelman and Flier 1996). A splice variant, PPARγ₂, is primarily expressed in both white and brown adipose tissue (Chawla et al. 1994; Kliewer et al. 1994; Tontonoz et al. 1994). PPARγ is also expressed in pancreatic β cells, but its level of expression is much lower than that found elsewhere (Braissant et al. 1996). Agonist-induced activation of PPARγ/RXR is known to increase insulin sensitivity (Lehmann et al. 1995; Mukherjee et al. 1997). Consequently, thiazolidinedione drugs (TZDs), which are synthetic ligands of PPARγ and have the ability to directly bind and activate PPARγ (Lehmann et al. 1995) and stimulate adipocyte differentiation (Okuno et al. 1998; Spiegelman and Flier 1996), are used clinically to reduce insulin resistance and improve hyperglycemia in type 2 diabetes mellitus (T2DM).

TZDs (PPARγ agonists) are often used to improve hyperglycemia associated with the metabolic syndrome of T2DM. This syndrome is characterized by (*i*) central obesity, (*ii*) atherogenic dyslipidemia (that is, increased plasma triglyceride (TG) and reduced high-density lipoprotein (HDL) cholesterol), (*iii*) hypertension, (*iv*) insulin resistance or glucose intolerance, (*v*) a prothrombotic state, and (*vi*) a proinflammatory state. We have developed a chemically induced alternative animal model of hyperlipidemia and atherosclerosis by using poloxamer 407 (P-407), a copolymer surfactant, that replicates one of the features observed in the metabolic syndrome, that of atherogenic dyslipidemia (Johnston et al. 1998; Johnston 2004; Palmer et al. 1997). Recently, we demonstrated that P-407 downregulates the gene expression of ATP-binding cassette transporter A1 (ABCA1) and inhibits cholesterol efflux from human macrophages in response to apolipoprotein A1 (apoA1) in cell culture (Johnston et al. 2006). Hence, we wondered whether P-407, either directly or indirectly, modulates the functional activity of PPARγ in the PPAR – liver X receptor (LXR) – ABCA1 signaling pathway. This would have important consequence with regard to cellular cholesterol homeostasis in this particular mouse model of atherogenesis (Johnston et al. 1998; Johnston 2004; Palmer et al. 1997) because TZDs are used, in part, to promote cholesterol efflux from macrophages in patients with T2DM.

The present work was conducted to further understand the pharmacologic effects of P-407 in our animal model of atherogenesis. Because P-407, or some intermediate that may potentially be activated in a biochemical or metabolic cascade after P-407 administration, could conceivably function as either a PPARγ agonist (similar to TZDs) or a PPARγ antagonist, we first used an in vitro transactivation assay to determine whether P-407 directly modulated PPARγ transcriptional activity. Next, since PPARγ agonists are used to treat hyperglycemia associated with T2DM, we determined whether blood glucose and plasma insulin levels were perturbed in wild-type and PPARγ-deficient mice after P-407 administration. Last, we explored the possibility that P-407 acts through PPARγ to effect the mobilization of free (nonesterified) fatty acids (FFAs) from adipocytes.

Our desire to investigate the possibility that P-407 acts via PPARγ to cause the release of FFAs from adipocytes, thereby increasing the concentration of circulating FFAs, is based on the following information. PPARγ is activated by prostaglandins, leukotrienes, and TZDs and affects the expression of many genes involved in the storage of FFAs. If P-407, or some intermediate involved in a biochemical or metabolic cascade downstream of P-407 administration, functioned as a PPARγ agonist or antagonist, then the expression of genes involved in the storage of FFAs may be modulated. This potential pharmacologic action of P-407 is based on 2 previous observations. First, Wasan et al. (2003) demonstrated a significant increase in the activity of lecithin cholesterol acyltransferase (LCAT) in the plasma of P-407-treated rats relative to controls. LCAT catalyzes the formation of cholesteryl esters from lecithin (phosphatidylcholine) and cholesterol. Second, Nash et al. (1996) observed a significant decrease in the plasma concentrations of both TG and total cholesterol when nicotinic acid and P-407 were simultaneously administered to rats. Nash et al. suggested that P-407 may cause hyperlipidemia in rodents, in part, by stimulating the release of FFAs from the adipocyte for at least 24 h after its administration, although the authors did not measure circulating FFA levels in P-407-treated animals. Nicotinic acid is an effective hypolipidemic agent that functions primarily by reducing lipolysis in adipocytes, resulting in a reduction in the plasma concentration of FFAs, an essential substrate for both TG and cholesterol biosynthesis. The findings of Wasan et al. (2003) and Nash et al. (1996) suggest that P-407 may influence, either directly or indirectly, the mobilization and storage of FFAs by modulating the functional activity and (or) gene expression of PPARγ. Therefore, we determined whether P-407 treatment affected the concentration of circulating FFAs in wild-type mice.

Materials and methods

Materials

Plasmids were obtained from the same sources as previously reported (Maloney and Waxman 1999; Shipley and Waxman 2004; Shipley et al. 2004). Troglitazone (Rezulin), a potent PPARγ agonist, was obtained from Parke-Davis Pharmaceuticals (Ann Arbor, Mich.). Male wild-type mice (strain C57BL/6) and PPARγ-deficient mice (strain B6.129-Pparg[tm2Rev]/J) were purchased from The Jackson Laboratory (Bar Harbor, Me.) and weighed approximately 18 g. Test strips, which were inserted into the test strip chamber of the blood glucose monitor, were Chemstrip bG reagent strips (No. 502, Boehringer Mannheim, Indianapolis, Ind.). For determination of plasma insulin concentrations, commercially available Coat-A-Count radioimmunoassay kits were obtained from Diagnostic Products (Los Angeles, Calif.). An in vitro enzymatic, colorimetric assay kit (NEFA-C) for the

determination of serum FFAs was purchased from Wako Diagnostics (Richmond, Va.).

Transactivation assay

The transactivation assay described previously (Maloney and Waxman 1999; Shipley et al. 2004) was used to assess the effect of P-407 on PPARγ activity. Briefly, COS-1 cells (American Type Culture Collection, Rockville, Md.) were passaged in 100-millimetre tissue culture dishes (Greiner Labortechnik, Germany) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, N.Y.) and 50 U/mL penicillin–streptomycin (Gibco). Cells were cultured overnight at 37 °C and then reseeded at 2000–4000 cells/well in a 96-well tissue culture plate (Greiner Labortechnik) in DMEM containing 10% FBS. The cells were grown for 24 h and then transfected as described previously (Chang and Waxman 2005; Maloney and Waxman 1999) by using FuGENE 6 transfection reagent (Roche Diagnostics, Indianapolis, Ind.), which provides higher transfection efficiencies and more consistent results when compared with calcium phosphate transfection methods.

Twenty-four hours after drug treatment (P-407 or 3.0 μmol/L troglitazone), cells were washed once in cold phosphate-buffered saline (pH 7.4) and then lysed by incubation at 4 °C in passive cell lysis buffer for 15–30 min (Promega). Firefly and *Renilla* luciferase activities were measured in the cell lysate by using the dual luciferase activity kit (Promega).

In vivo experiments

To determine whether PPARγ was involved with any potential changes in plasma insulin and blood glucose concentrations after administration of P-407, we used 4 groups of mice. Groups 1 and 2 consisted of wild-type mice treated with saline or P-407 (0.5 g/kg), respectively. Groups 3 and 4 were PPARγ-deficient mice administered either saline or P-407 (0.5 g/kg), respectively. It should be noted that disruption of the gene for PPARγ does not cause any significant changes in blood insulin and glucose concentrations relative to these parameters in controls (He et al. 2003). All procedures for P-407 administration and subsequent blood collection were in accordance with the guide for the care and use of laboratory animals of the University of Missouri – Kansas City, and the treatment protocol was approved by the institutional animal care and use committee.

To determine whether P-407 caused any change in the concentrations of plasma insulin and blood glucose in normal mice, as well as whether any potential changes to the concentrations of plasma insulin and blood glucose were mediated through PPARγ, 12 wild-type mice and 12 PPARγ-deficient mice were randomly divided into the same 4 groups. All mice were administered 0.5 mL of either normal saline (groups 1 and 3) or P-407 (0.5 g/kg) (groups 2 and 4) by intraperitoneal injection. Blood samples were obtained from all mice by tail vein sampling at 0 h (before P-407 administration), and then at 2, 4, 8, 16, and 24 h postdosing. Fifty microlitres of blood was collected into heparinized tubes at each sampling time point. One drop was immediately used for determination of blood glucose, while the remainder of the blood sample (approximately 40 μL)

was centrifuged, the plasma obtained, and the plasma samples stored at –80 °C until the time of insulin analysis.

The concentration of glucose in each blood sample was determined by using a commercially available blood glucose monitor (model 792 Accu-Chek II, Boehringer Mannheim, Indianapolis, Ind.). One drop of blood was placed on a glucose reagent strip, which was allowed to stand at room temperature for 1 min before being inserted into the test strip chamber of the monitor for determination of blood glucose, measured in milligrams per decilitre (mg/dL). Blood glucose concentrations were then expressed in units of millimoles per litre (mmol/L).
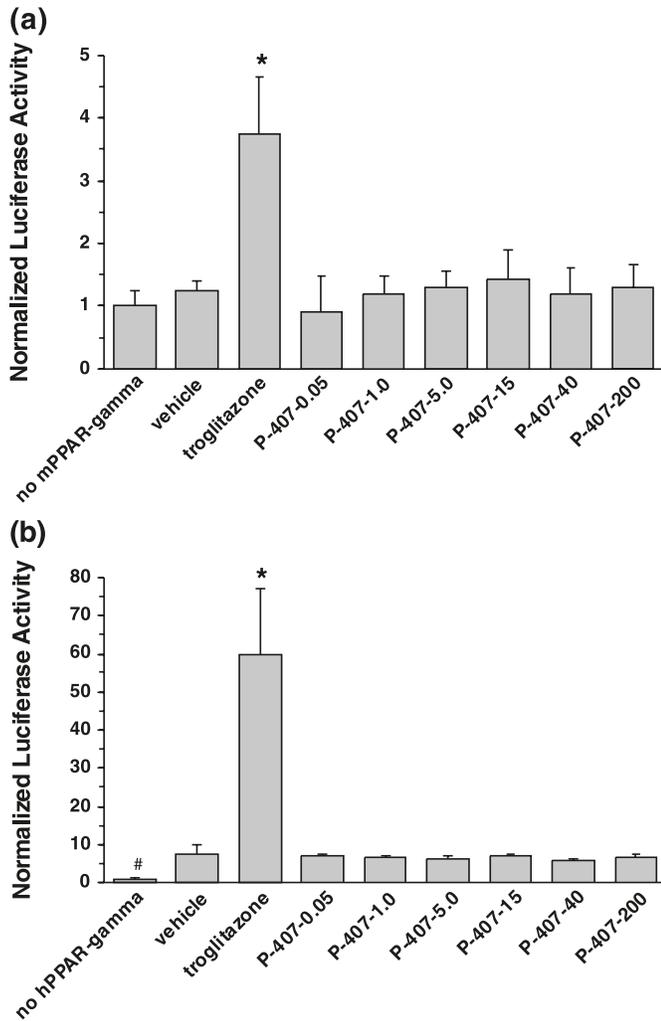
The concentration of insulin in all plasma samples was determined by using a radioimmunoassay kit according to the manufacturer's instructions. In the plasma insulin determination procedure, [^{125}I]insulin competes with insulin in the plasma sample for sites on insulin-specific antibody immobilized to the wall of the polypropylene tube. After incubation, isolation of the antibody-bound fraction was achieved by simply decanting the supernatant. The tube was then counted in a model LS 6500 Beckman liquid scintillation counter (Fullerton, Calif.), the counts being inversely related to the amount of insulin present in the plasma sample. The quantity of insulin in the sample was then determined by comparing the counts with a standard curve (package insert M-097, 5 September 1991, Diagnostic Products, Los Angeles, Calif.). Finally, the plasma insulin concentrations were calculated and expressed in picomoles per litre (pmol/L).

Analysis of serum free fatty acids

An additional group of 6 wild-type mice were utilized to determine whether P-407 caused a change in the circulating levels of FFAs in the serum. In these experiments, the mice were fasted for 12 h before the experiment. On the day of the experiment, 50 μL of blood was collected from the tail vein of each mouse and served as the preinjection (time t = 0 h) control (baseline) FFA concentration for each mouse. Next, 0.5 mL of P-407 (0.5 g/kg) was administered by intraperitoneal injection to each mouse and blood samples (50 μL) collected from the tail vein of each fasting mouse at 3, 6, 12, and 24 h postdosing. To determine whether fasting influenced the serum levels of FFAs, another group of 6 wild-type mice were similarly fasted, then injected with normal saline (0.5 mL) at t = 0 h, and blood samples were obtained at the same time points as those of mice treated with P-407. All blood samples were placed into microcentrifuge tubes on ice and allowed to clot. Blood samples were then centrifuged at 10 000g for 20 min at 4 °C and the serum supernatant removed and frozen at –80 °C until the time of FFA analysis.

Analysis of serum samples for FFA utilized a procedure, adapted for a 96-well microtiter plate, supplied by the manufacturer. Briefly, an aliquot (5 μL) of each serum sample was placed into a separate well and 100 μL of color reagent A added to each well. Next, samples were mixed and incubated at 37 °C for 5 min, after which time, 200 μL of color reagent B was added to each well. The plate was again incubated at 37 °C for 5 min, removed from the incubator, and 5 min later, the plate read at 550 nm using a model 450 microplate reader (Bio-Rad, Richmond, Calif.). Both a reagent blank and a calibration standard, as well as

Fig. 1. Effect of P-407 on mouse (*a*) and human (*b*) PPARγ activity in vitro. First bar, no PPARγ (control); second bar, cells transfected with either mouse (*a*) or human (*b*) PPARγ expression plasmid and then treated with vehicle; other bars, as for bar 2, except cells were stimulated for 24 h with troglitazone (3.0 μmol/L) or P-407 at the concentrations indicated (0.05–200 μmol/L). Data are means ± SD. Asterisk (*) indicates that the mean value of bar 3 (troglitazone) is significantly ($p < 0.05$) greater than the mean value of all other bars. Octothorpe (#) indicates that the mean value of bar 1 (no human PPARγ) in (*b*) is significantly ($p < 0.05$) less than the mean value of all other bars. P-407, poloxamer 407; PPAR, peroxisome proliferator-activated receptor.

(a)

(b)

Fig. 2. Effect of P-407 on human PPARγ activity stimulated by the PPARγ activator troglitazone in vitro. P-407 concentrations are 0.05–200 μmol/L. Data are means ± SD. Asterisk (*) indicates that the mean value of bars 1 (no PPARγ) and 2 (vehicle) are significantly ($p < 0.05$) less than the mean values of all other bars. Octothorpe (#) indicates that the mean value of bar 2 (vehicle) is significantly ($p < 0.05$) greater than the mean value of bar 1 (no PPARγ).
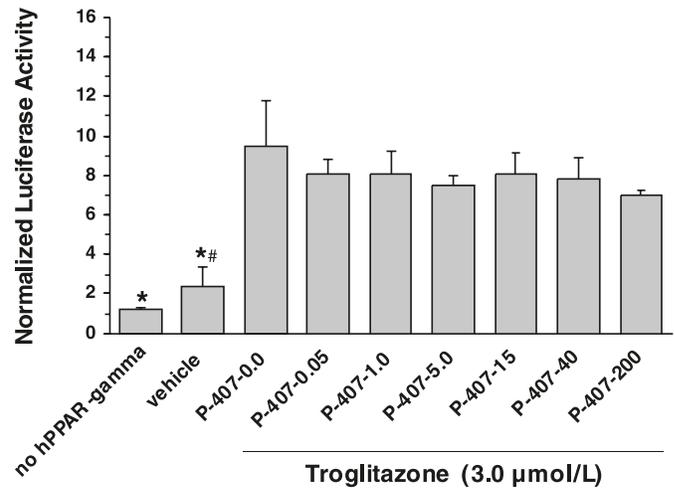
and blood glucose concentration versus time profiles. Using the blood glucose concentration versus time profiles (Fig. 3*a*) as an example, we determined whether each blood glucose concentration at a given time point was different from the rest of the blood glucose concentrations at that same time point between the 4 groups of mice. This statistical analysis was performed for the 4 blood glucose concentrations at each of the 5 sampling time points and any significant ($p < 0.05$) differences between the 4 concentrations at a specific time point were appropriately designated on the resulting graph. Plasma insulin concentration versus time profiles (Fig. 3*b*) were analyzed in a similar manner.

Data obtained from the analysis of serum FFAs were first corrected by using the reagent and specimen blanks as per the manufacturer's instructions. The resulting mean value of the serum FFA concentration at each time point for P-407-treated mice was then individually compared with the mean serum FFA concentration at $t = 0$ h (that is, the preinjection level of 0.83 mEq/L) by using the Student's t test, results being deemed statistically significant if $p < 0.05$. Additionally, the mean value of the serum FFA concentration at each time point for P-407-treated mice was compared with the corresponding mean serum FFA concentration for saline-treated mice by using the Student's t test and deemed significantly different if $p < 0.05$.

Results

Transactivation assay

As determined in a cell-based transactivation assay, P-407 did not directly modulate the activity of either mouse or human PPARγ relative to vehicle (Figs. 1*a* and 1*b*, respectively). Additionally, Fig. 2 demonstrates that P-407, over a concentration range of 0.05–200 μmol/L, did not inhibit a known PPARγ agonist (troglitazone) from activating human PPARγ.
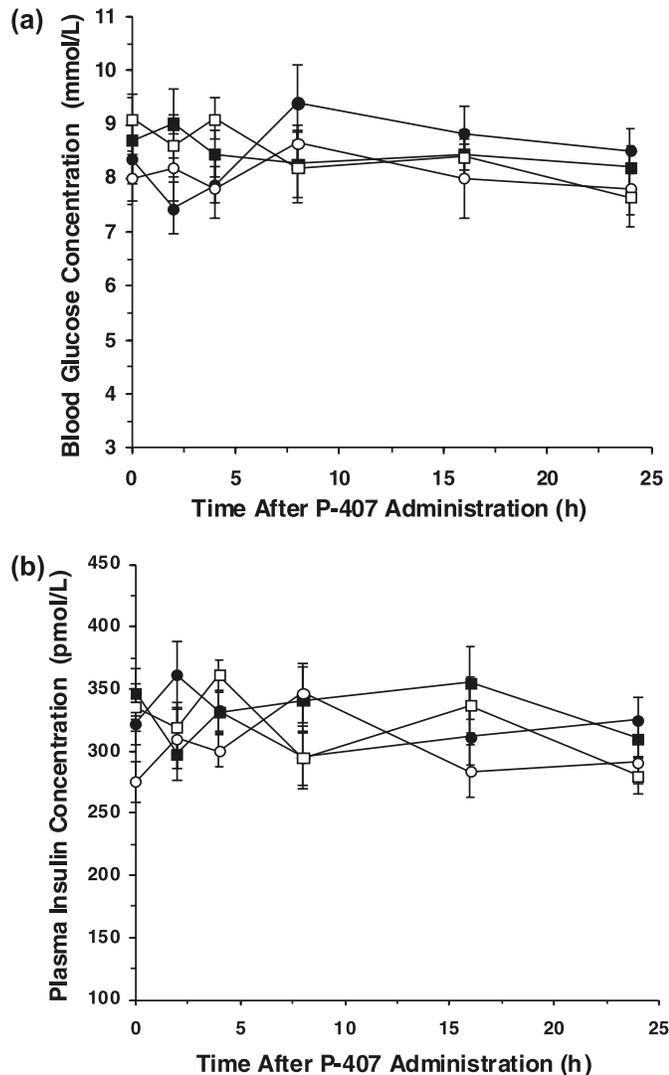
a specimen blank (to correct for lipemic samples), were included in the assay.

Data analysis

To determine whether P-407 modulated mouse and human PPARγ in the transactivation assays, we utilized a classic one-way analysis of variance (ANOVA) to uncover any significant differences in the mean values associated with the individual P-407 concentrations tested relative to the vehicle (Fig. 1). ANOVA was also utilized to determine whether increasing concentrations of P-407 inhibited troglitazone's capacity to activate human PPARγ (Fig. 2). Last, similar to the treatment of the data obtained from the transactivation assays, ANOVA was used to compare the plasma insulin

Fig. 3. Blood glucose (*a*) and plasma insulin (*b*) concentrations after administration of normal saline (squares) to wild-type (C57BL/6) (■) or PPARγ-deficient (□) mice and after administration of 0.5 g/kg P-407 (circles) to wild-type (●) or PPARγ-deficient (○) mice. Data are means ± SD.

(a)

(b)

Fig. 4. Serum nonesterified (free) fatty acid concentration after administration of P-407 (0.5 g/kg) (●) or saline (■) to fasted (12 h) wild-type (C57BL/6) mice. Data are means ± SD. Asterisk (*) indicates a significant ($p < 0.05$) increase in the serum concentration of FFAs relative to both the preinjection level (0.83 mEq/L) in P-407-treated mice and the average serum FFA concentration in saline-treated (control) mice.
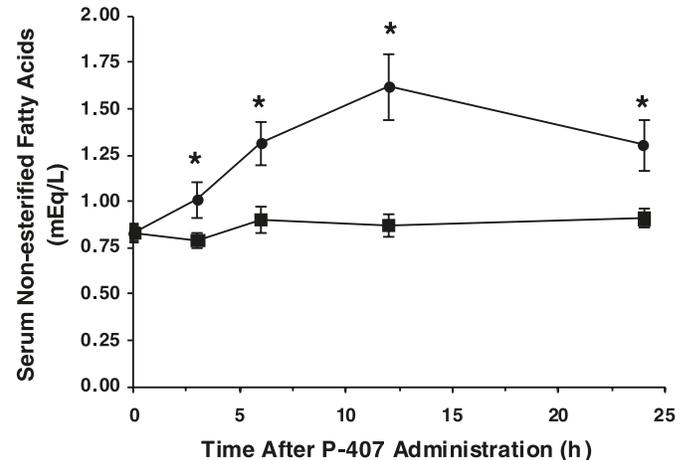
Serum FFA concentrations

Administration of P-407 to wild-type mice caused a significant ($p < 0.05$) increase in the serum concentration of FFAs as soon as 3 h after injection when compared with corresponding FFA concentrations in saline-treated controls (Fig. 4). The serum FFAs appeared to reach an apparent maximal concentration of 1.61 mEq/L at 12 h after P-407 administration, FFA concentrations remaining significantly elevated for as long as 24 h postdosing. The baseline (preinjection) FFA concentration in fasted wild-type mice administered P-407 was 0.83 mEq/L, not significantly different from the average serum FFA concentration in saline-treated mice (approximately 0.87 mEq/L).

Discussion

The present study demonstrated that P-407 does not directly activate either mouse or human PPARγ in vitro. Moreover, as assessed by a transactivation assay, P-407 does not inhibit the capacity of the known PPARγ agonist troglitazone to activate human PPARγ. These observations suggest that our previous finding of reduced cholesterol efflux by macrophages cultured with P-407 is a result of downregulation in the gene expression of ABCA1 as proposed (Johnston et al. 2006), and not the result of interference with the functional activity of PPARγ. Additionally, it should be noted that we recently demonstrated with a transactivation assay that P-407 neither modulated PPARα activity in vitro, nor altered the plasma concentrations of total cholesterol, HDL-cholesterol, non-HDL-cholesterol, or triglycerides in PPARα-deficient mice relative to P-407-treated wild-type mice (Johnston and Waxman 2008).

The second portion of this study focused on whether P-407 perturbed plasma insulin and blood glucose concentrations in mice and, if so, whether this outcome was mediated through PPARγ. Although the transactivation assays demonstrated that P-407 was unable to activate mouse or

PPARγ-deficient mouse experiments

These experiments were designed to assess whether P-407 indirectly modulated PPARγ activity and thereby altered plasma insulin and blood glucose concentrations. As shown in Fig. 3a, blood glucose concentration–time profiles for both wild-type and PPARγ-deficient mice treated with P-407 were no different than corresponding profiles obtained when mice were treated with saline. The concentration–time profiles were overlapping and grouped around an average blood glucose concentration of approximately 8.5 mmol/L. Similar to the blood glucose results, plasma insulin concentration–time profiles were also overlapping and appeared to be grouped around an average plasma insulin concentration of approximately 328 pmol/L (Fig. 3b). No significant differences were noted for concentration–time profiles in Figs. 3a and 3b when analyzed using an ANOVA.

human PPARγ activity in vitro, we still wished to know whether P-407 indirectly modulated PPARγ in vivo. This concern was based on the fact that a compound's ability to modulate PPARs is not always predicted from the results of an in vitro transactivation assay. For example, Peters et al. (1996) demonstrated that dehydroepiandrosterone-3-beta-sulfate (DHEA-S) does not modulate PPARα in vitro as assessed by a transactivation assay, yet in studies using PPARα-knockout mice, PPARα was obligatory for DHEA-S-stimulated hepatic peroxisomal gene induction.

When we initiated the present study, we had no a priori knowledge whether the administration of P-407 to wild-type mice would cause any changes in the plasma insulin and blood glucose concentrations. Our data suggest that P-407 has no capacity to modulate either plasma insulin or blood glucose concentrations after administration to wild-type mice. To assess whether any potential P-407-induced changes in insulin and glucose concentrations were mediated through PPARγ, we also included a group of P-407-treated PPARγ-deficient mice and determined the plasma insulin and blood glucose concentrations versus time postdosing. Our findings revealed no P-407-mediated perturbations in the plasma insulin and blood glucose concentration–time profiles for P-407-treated PPARγ-deficient mice when compared with P-407-treated wild-type mice. This was also true when the profiles were individually compared with the corresponding profiles for saline-treated PPARγ-deficient and saline-treated wild-type mice. Therefore, this strongly suggests that P-407 has no indirect capacity to activate or inhibit PPARγ, and thus corroborates our in vitro data obtained from the transactivation assays. As an example, if P-407 had acted as a PPARγ agonist similar to TZDs, then blood glucose concentrations would have been significantly reduced after the administration of P-407, but this outcome did not occur in the present study.

In accordance with the idea that PPARγ ligands elicit their effects primarily through adipose tissue, it has been demonstrated that PPARγ agonists alter the expression of genes that are involved in lipid uptake, lipid metabolism, and insulin action in adipocytes (Rangwala and Lazar 2004). As a result, they enhance adipocyte insulin signaling, lipid uptake, and anabolic lipid metabolism and also attenuate lipolysis and FFA release. Consequently, lipid levels in adipose tissue increase, whereas the concentration of circulating FFAs decrease (Bays et al. 2004). By repartitioning lipids away from liver and muscle, the 2 primary tissues that are responsible for insulin-mediated glucose disposal and metabolism, PPARγ agonists improve glycemic control by reversing lipotoxicity-induced insulin resistance (Berger et al. 2005). Because of these multiple adipocentric actions, PPARγ agonists (for example, TZDs) decrease blood glucose concentrations. As shown in the present study, P-407 did not affect either the plasma insulin or blood glucose concentrations after administration to wild-type or PPARγ-deficient mice. Therefore, P-407 is neither functioning as a PPARγ agonist, nor does it appear to be functioning as a PPARγ antagonist, since P-407 was not able to block the action of the PPARγ agonist troglitazone from activating PPARγ in the transactivation assays.

Finally, as stated earlier, PPARγ agonists cause a reduction in the circulating levels of FFAs. In the present investigation, we demonstrated that P-407 increased the level of FFAs in the serum for up to 24 h postdosing. Because P-407 causes a decrease in cellular cholesterol efflux (Johnston et al. 2006), which is opposite to the action of both PPARα and PPARγ agonists (Chawla et al. 2001; Chinetti et al. 2001), perhaps cholesterol homeostasis is maintained, in part, by a P-407-mediated release of FFAs (an essential substrate for both TG and cholesterol synthesis) from adipocytes, as well as by an increase in cholesterol synthesis due to a temporary (up to 48 h after P-407 administration) upregulation in the activity of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (Johnston and Palmer 1997; Leon et al. 2006). Future work will examine potential mechanisms responsible for the elevation of serum FFAs after P-407 administration to mice.

In conclusion, P-407 neither activated nor inhibited PPARγ in vitro, nor did it interfere with activation of human PPARγ by a known PPARγ agonist (troglitazone). Furthermore, after administration to both wild-type and PPARγ-deficient mice, P-407 did not perturb plasma insulin and blood glucose concentrations, suggesting that P-407 does not indirectly activate or inhibit PPARγ activity in vivo. Last, in contrast to a PPARγ agonist, P-407 administration to mice increased the concentration of circulating FFAs by an as yet undetermined mechanism, but one that is probably unrelated to the inhibition of PPARγ activity. Therefore, because our previous work has shown that (i) P-407 does not interfere with an LXRα agonist's ability to enhance cholesterol efflux from human macrophages (Johnston et al. 2006), and (ii) ABCA1 gene expression is significantly reduced by P-407 (Johnston et al. 2006), and because our present work has demonstrated that P-407 is unable to activate or inhibit PPARγ in vitro or to perturb plasma insulin and blood glucose levels after administration to mice, we conclude that (i) P-407 does not modulate cellular cholesterol efflux at the level of PPARγ in the PPAR–LXR–ABCA1 signaling pathway, and (ii) although P-407 increases the level of FFAs in the serum for up to 24 h postdosing, it does not interfere with the functional activity of PPARγ after administration to mice.

Acknowledgements

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