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Microgram-order ammonium perfluorooctanoate may activate mouse peroxisome proliferator-activated receptor α , but not human PPAR α

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Abstract

Perfluorooctanoic acid (PFOA) is a ligand for peroxisome proliferator-activated receptor (PPAR) α , which exhibits marked species differences in expression and function, especially between rodents and humans. We investigated the functional difference in PFOA response between mice and humans, using a humanized *PPAR α* transgenic mouse line. Three genotyped mice, 129/Sv wild-type (*mPPAR α*), *Ppara*-null mice and humanized *PPAR α* (*hPPAR α*) mice (8-week-old males) were divided into three groups: the first was treated with water daily for 2 weeks by gavage (control group), and the remaining two groups were treated with 0.1 and 0.3 mg/kg ammonium perfluorooctanoate (APFO), respectively, for 2 weeks by gavage. The APFO dosages used did not influence the plasma triglyceride or total cholesterol levels in any mouse line, but the high dose increased both hepatic lipid levels only in *mPPAR α* mice. APFO increased mRNA and/or protein levels of PPAR α target genes cytochrome P450 *Cyp4a10*, peroxisomal thiolase and bifunctional protein only in the liver of *mPPAR α* mice, but not in *Ppara*-null or *hPPAR α* mice. This chemical also increased expression of mitochondrial very long chain acyl-CoA dehydrogenase only in the liver of *mPPAR α* mice. Taken together, human PPAR α may be less responsive to PFOA than that of mice when a relatively low dose is applied. This information may be very valuable in considering whether PFOA influences the lipid metabolism in humans.

Keywords

Ammonium perfluorooctanoate; Peroxisome proliferator-activated receptor; (PPAR) α ; Humanized PPAR α mouse; *Ppara*-null mouse; Lipid metabolism

1. Introduction

Perfluorooctanoic acid (PFOA) (CAS, 335-67-1), an organofluoro compound, is used in industrial surfactants, emulsifiers and numerous consumer products (U.S. Environmental Protection Agency, U.S. EPA, 2006). This chemical is very stable and incombustible at normal temperature, and when released into the environment it is not decomposed in either the ecosystem or the body. Repeated exposure to PFOA may result in accumulation in humans and wildlife (Prevedouros et al., 2006). Thus, PFOA is scheduled to be added to the list of Persistent Organic Pollutants (POPs) in the near future (World Wildlife Fund, 2005).

PFOA is thought to be resistant to clearance in humans, with a biological half-life of 3.5–4.4 years (U.S. EPA, 2002; Olsen et al., 2007). Since there are dramatic sex and species clearance (Kennedy et al., 2004), it is very difficult to evaluate the level of human risk. Several studies of the potential health risk of exposure to PFOA have been conducted. Ammonium perfluorooctanoate (APFO) causes hepatic peroxisome proliferation (Pastoor et al., 1987), liver hyperplasia, liver adenoma, pancreatic acinar cell adenomas (Biegel et al., 2001) and Leydig cell adenomas (Cook et al., 1992; Biegel et al., 2001) in rats and mice. Since PFOA is not mutagenic, the induction of these tumors most likely occurs through nongenotoxic mechanisms (Kennedy et al., 2004).

PFOA is a peroxisome proliferator-activated receptor α (PPAR α) agonist in mice and rats (Ikeda et al., 1985; Rosen et al., 2007, 2008a,b), and through PPAR α increases peroxisomal and mitochondrial β -oxidation of fatty acids, inhibits the secretion of very low-density lipoproteins and cholesterol from the liver, as well as reduces cholesterol and triglyceride in serum and the accumulation of lipids in the liver (Maloney and Waxman, 1999; Xie et al., 2003; Kennedy et al., 2004). Epidemiological studies have resulted in disparate findings. Olsen et al. (2003a), reported a positive association between PFOA and serum cholesterol (T-Chol) and triglycerides (TG) among participants in a fluorochemical medical surveillance program in some factories. In contrast, no such association was reported in another factory (Olsen et al., 2003b). Since there are species differences in the expression levels of PPAR α , it is very difficult to accurately evaluate the exact effects of PFOA on the lipid levels of human from the experiments using mice or rats. To investigate species-specific effects and mechanisms of PPAR α , PPAR α -humanized (hPPAR α) mice were recently established (Cheung et al., 2004). The mice express human PPAR α specifically in the liver under the control of the doxycycline (Tet-OFF) system in a PPAR α -null background. High-level expression of human PPAR α protein achieved in this hPPAR α mouse model proved comparable to murine PPAR α expression in wild-type mice. This mouse model was shown to be a useful tool in determining human PPAR α function in the effects of Wy-14,643 on both the serum and hepatic lipid levels (Cheung et al., 2004).

In this study, we compared the effects of relatively low doses of APFO (0, 0.1, and 0.3 mg/kg) on the PPAR α and target gene expression as well as on lipid levels between mouse and human PPAR α using wild-type, *Ppara*-null and hPPAR α mice, in order to determine the possible effects of this chemical or its principal metabolite, PFOA, on the lipid metabolism in humans.

2. Materials and methods

2.1. Chemicals

APFO was purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

2.2. Experimental animals

This study was conducted according to the Guidelines for Animal Experiments of the Nagoya University Animal Center. Three genotyped male and female mice, i.e., wild-type (mPPAR α),

Ppara-null (Lee et al., 1995; Akiyama et al., 2001) and *hPPARα*^{Tet-Off} (Cheung et al., 2004) with a Sv/129 genetic background were provided by Dr. Gonzalez (U.S. National Institutes of Health), and each genotyped male and female was mated in our animal center. All mice were housed in a temperature- and light-controlled environment (25 °C, 12 h light/dark cycle), and maintained on stock rodent chow and tap water *ad libitum*. At 8 weeks old, the offspring male mice of each strain were assigned to treatment groups as follows: treated with distilled water daily for 2 weeks by gavage (control group); treated with 0.1 or 0.3 mg/kg APFO, respectively, for 2 weeks by gavage (Table 1). On the day following the last dose (18–20 h later), all mice were killed by decapitation, and the blood and livers were removed. The liver samples were stored at –80 °C until use. Plasma was collected after centrifuging blood 3500×g for 10 min and stored at –80 °C until use. All samples were used in all measurements, and the measurements were done in duplicate or triplicate except for the histopathology.

2.3. Nuclear fraction

A nuclear fraction was extracted from a part of the frozen liver using a CellLytic® NuCLEAR® Extraction Kit (SIGMA, Tokyo, Japan).

2.4. Analysis of protein concentrations

Each tissue was homogenized with a threefold volume of 10 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose. Protein concentrations of the homogenate and nuclear fraction samples were measured using a Protein Assay Kit (Bio-Rad, Tokyo, Japan).

2.5. Western blotting

Liver homogenate or nuclear fraction samples for electrophoresis were subjected to 10% SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 3% skim milk, each membrane was incubated with the respective primary antibody, followed by incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG (Jackson Immuno Research, West Grove, PA). The primary polyclonal antibody was prepared using purified medium-chain acyl-CoA dehydrogenase (MCAD) (Furuta et al., 1981), very long chain acyl-CoA dehydrogenase (VLCAD) (Izai et al., 1992), peroxisomal thiolase (PT) (Furuta et al., 1980) and peroxisomal bifunctional protein (PH) (Osumi and Hashimoto, 1980). These antibodies have been described earlier (Aoyama et al., 1998; Nakajima et al., 2000). The primary polyclonal antibodies of *PPARα* and *RXRα* were purchased from Santa Cruz Biotechnology, Inc. (CA). Each band was quantified using the densitometry with the Lane & Spot Analyzer version 5.0 (ATTO Corporation, Tokyo, Japan).

2.6. Real-time quantitative PCR

mPPARα (Accession No. NM_011144) -, *hPPARα* (BC000052) -, *RXRα* (NM_011305) -, *CYP4A10* (AB018421) -, *MCAD* (NM_007382) -, *VLCAD* (NM_017366) -, *PT* (NM_130864) -, *PH* (NM_023737) -, diacylglycerol acyltransferase (*DGAT*) 1 (NM_010046) - and 2 (NM_02638), *p65* (XM_342346) -, *p50* (AY521463) - and *p52* (AF155372) -, tumor necrosis factor (*TNF*) α (NM_013693) -, *TNF* receptor (*TNFR*) 1 (NM_011609) -, and *TNFR2* (NM_011610) -mRNA levels were monitored by the ABI PRISM 7000 Sequence Detection system (Applied Biosystems, Foster City, CA). Total RNA was isolated using a RNeasy Protect Mini Kit (QIAGEN, Tokyo, Japan). Complementary DNA (cDNA) was synthesized from 1 μ g of total RNA using Oligo(dT)₂₀ primer. RNA quantity and quality were checked by a GeneQuant II RNA/DNA Calculator (Pharmacia Biotech, Framingham, MA). The sequences for the forward and reverse primers were as follows: *hPPARα* forward: 5'-GCGATCTAGAGAGCCCGTTATC-3' and reverse: 5'-GCCAAAGCTTCCAGAACTATCC-3'; *RXRα* forward: 5'-CAGTTGCTGCGCATCCAA-3' and reverse: 5'-GTGCTGCTAAATAACTCTTGCATGA-3'; *DGAT1* forward: 5'-

GGCGGTCCCCAACCAT-3' and reverse 5'-GCTCTGCCACAGCATTGAGA-3'; DGAT2 forward: 5'-TGGCCTGCAGTGTCATCCT-3' and reverse: 5'-GGGCGTGTTCCAGTCAAATG-3'. The sequences of other genes have been described elsewhere (Ito et al., 2007; Ramdhan et al., 2008).

The PCR mixture (25 µl) contained 1×SYBR Green PCR master Mix (Applied Biosystems), 0.1 µM of each primer, and cDNA diluted by TE including 1 µg/ml transfer-RNA. PCR amplification was as follows: an initial step for 2 min at 50 °C, then 10 min at 95 °C, followed by 50 cycles at 95 °C for 15 s and at 60 °C for 1 min. Each mRNA level was normalized to the level of GAPDH (BC096440) mRNA expression in the same preparation.

2.7. Lipid concentrations in plasma and liver

Lipid from livers was extracted using the method of Folch. TG and T-Chol in the liver and plasma were measured using kits of TG-IE and T-Chol IE (Wako, Osaka, Japan), respectively.

2.8. Histopathological analysis

The organs fixed in 10% neutral buffered formalin were embedded in paraffin and sliced into 4 µm sections. Tissue sections of the livers were stained with hematoxylin and eosin, and examined under a light microscope using the BZ-8000 (Keyence, Osaka, Japan). Necrotic cells were measured in a blinded fashion according to the method of Soni et al. (1998). Inflammatory cells, and lipid accumulation were measured in a blinded fashion referring to the methods of Brunt et al. (1999) and Okiyama et al. (2009): (1) grade of steatosis: 0, none; 1, mild (5–33% of parenchymal involvement by steatosis); 2, moderate (33–66%); 3, severe (>66%); (2) intra-acinar (lobular) and portal tract inflammations: 0, none; 1, mild; 2, moderate; 3, severe.

2.9. Alanine aminotransferase (ALT) measurement

Plasma ALT was measured using a Transaminase C II Test kit purchased from Wako (Osaka, Japan).

2.10. Statistical analysis

Comparisons were made using two-way analysis of variance (ANOVA) and the Tukey–Kramer HSD post hoc test among each treated group of each kind of genotyped mice, or also between *mPPARα* or *Ppara*-null mice and *hPPARα* mice with the same dose. Values of *P* < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Body and liver weights

Body and liver weights of *hPPARα* mice were lower than those of *mPPARα* and *Ppara*-null mice, but APFO treatment did not influence the increase in the body weight of all genotyped mice (Table 1). The low dose (0.1 mg/kg) of APFO did not influence either absolute or relative liver weights in any mouse line, while the APFO high dose (0.3 mg/kg) increased the liver weight and the ratio of liver/body weight only in *mPPARα* mice.

3.2. mRNA and protein levels of PPARα and related genes

In all groups, the expressions of PPARα protein and mRNA were higher in *hPPARα* mice than in *mPPARα* mice (Figs. 1 and 2). APFO treatments did not influence either PPARα mRNA or protein levels in any of the mice regardless of genotype, nor did they influence RXRα, which forms a heterodimer with PPARα in the nucleus, protein and mRNA levels, though a significant increase was observed in RXR-mRNA in *hPPARα* mice between 0.1 and 0.3 mg/kg APFO exposure.

We then analyzed the effects of APFO treatment on the induction of the PPAR α target genes, PH, PT, VLCAD, MCAD and CYP4A10. Among these genes, PH was the most sensitive to APFO treatment; high dose APFO significantly increased PH mRNA and protein expression in mPPAR α mice (Figs. 1 and 2). However, APFO increased neither PH mRNA nor protein levels in *Ppara*-null or hPPAR α mice. APFO treatment influenced PT-mRNA and protein levels similarly to PH in mPPAR α mice. A high dose of APFO increased mitochondrial VLCAD-mRNA and protein only in mPPAR α mice, but the increases seemed to be less than those of the peroxisomal enzymes, PH and PT. MCAD-mRNA and protein levels were slightly increased only in hPPAR α mice. CYP4A10-mRNA levels were not detectable in *Ppara*-null mice. In contrast, CYP4A10 expression was clearly detected in the livers of mPPAR α and hPPAR α mice (Fig. 1). APFO, however, clearly induced mRNA in the former, but not in the latter.

When the above mentioned gene expressions were compared at the same dosages, there were no significant differences in PH- and MCAD-mRNA and protein expressions among controls of three genotyped mice. In contrast, a significant difference was observed in the expression of VLCAD-mRNA and protein between the control mPPAR α or hPPAR α mice and *Ppara*-null mice, whereas that of CYP4A10-mRNA was higher in hPPAR α mice than in mPPAR α or *Ppara*-null mice. PT-mRNA expression was significantly higher in hPPAR α mice than mPPAR α and *Ppara*-null mice, while the protein expression was higher in mPPAR α and hPPAR α mice than *Ppara*-null mice.

APFO treatments did not increase hepatic NF- κ B subunits, p65-, p50- and p52-mRNAs, TNF α and the receptors, suggesting that the dose used could not cause inflammation (data not shown).

3.3. TG and T-Cho levels in plasma and liver

TG and T-Cho are typically decreased upon PPAR α activation (Peters et al., 1997). TG and T-Cho in the liver and plasma were measured to investigate whether enzymes involved in lipid metabolism were induced by APFO treatment (Fig. 3). Although plasma TG levels in *Ppara*-null mice and T-Cho levels in *Ppara*-null and hPPAR α mice were significantly higher than those in mPPAR α mice, APFO did not influence the levels in any mouse lines (Fig. 3(A)). In hPPAR α mice, plasma T-Cho levels of all dosage groups were higher than those of the mPPAR α group.

In the liver, TG and T-Cho levels were also significantly higher in *Ppara*-null mice than in mPPAR α mice (Fig. 3(B)). Interestingly, a high dose of APFO significantly increased hepatic TG and T-Cho levels only in mPPAR α mice. In *Ppara*-null mice, hepatic TG levels of all dosage group were higher than those of the mPPAR α group.

We also measured mRNA levels of DGAT1 and 2 that are involved in TG synthesis. APFO treatments did not influence expression of these enzymes in any group (data not shown).

3.4. Histopathological changes

In the control animals, there were no obvious changes in the grade of steatosis, and the inflammatory cells in the lobular and portal area of liver in all genotyped mice, though little or no lipid accumulation was seen in the liver of PPAR α -null mice (Fig. 4, score grade of data not shown). A high APFO dose did not cause any histopathological changes in liver except for the quite mild hepatocellular enlargements seen in mPPAR α mice and hPPAR α mice, and appeared to increase cytoplasmic vacuoles morphologically consistent with the lipid accumulation in zone 2 of hepatic lobule from *Ppara*-null mice as pointed out by Wolf et al. (2008a).

3.5. ALT levels in plasma

APFO treatment did not affect the ALT levels in any group (data not shown).

4. Discussion

In general, administration of peroxisome proliferators to rodents increases liver weight and the levels of PPAR α target genes such as PT, PH, VLCAD (Aoyama et al., 1998; Nakajima et al., 2000) and CYP4A10 (Ramdhan et al., 2008). The present study clearly shows that a relatively low dose of APFO increased liver weight and induced these genes only in mPPAR α , but not in Ppara-null or hPPAR α mice. These results were quite distinct from an earlier report that another mPPAR α ligand, Wy-14,643, induced some of these genes not only in mPPAR α mice but also in hPPAR α mice (Cheung et al., 2004). One reason for this discrepancy may lie in the different dosage used; the dose of Wy-14,643 was roughly estimated to be 0.37 mmol/kg when mice weighed 25 g and were consuming 3 g feed a day, whereas the dose of APFO (0.3 mg/kg) was calculated to be 0.70 μ mol/kg in our study. Indeed, in another study using hPPAR α mice, 1 mg/kg APFO, which is about 3 times higher than that in the current study, clearly activated human PPAR α judging from the induction of the target genes (in preparation). Recently, Abbott and colleagues measured the lowest effective concentrations of PFOA at which mouse and human PPAR α were activated in an in vitro system and showed them to be 10 μ M and 30 μ M, respectively (Takacs and Abbott, 2007). Wolf et al. (2008b) also reported that activation of mouse PPAR α by APFO was generally higher compared to that of human PPAR α . Taken together, hPPAR α may have a weaker affinity for PFOA than does mPPAR α . Foreman et al. investigated the activation of mouse PPAR α and human PPAR α , by perfluorobutyrate, which has a similar structure to PFOA and is a PPAR α agonist, and showed that dosages higher than 35 mg/kg caused activation in both wild-type and humanized mouse strains, and this was the same hPPAR α strain as used in the present study. Thus, when a relative high dosage of APFO or perfluorobutyrate is used, PPAR α activation may be observed not only in mPPAR α mice but also in hPPAR α mice.

Expression of PPAR α protein was thought not to differ between mPPAR α and hPPAR α mice, although the mRNA level was greater in the latter than in the former (Cheung et al., 2004). In the current experiment, expression of PPAR α protein and mRNA appeared to be greater in hPPAR α than in mPPAR α mice. Nevertheless, the fact that PFOA-activated mPPAR α but not hPPAR α suggested that the functional activation of hPPAR α itself may also be weak compared to that of mPPAR α .

As noted above, although APFO-induced peroxisomal and mitochondrial lipid β -oxidation enzymes in mPPAR α mice, it did not reduce TG and T-Cho levels in plasma, and only slightly increased levels in the liver. In contrast, APFO treatment did not increase either of these enzyme levels or plasma and hepatic lipid levels in hPPAR α mice. Loveless et al. (2006) reported that the same dose of APFO as the one used in the current study did not affect the plasma TG levels. On the other hand, Kudo et al. (1999) showed that 0.02% APFO in the diet for a week (which corresponded to 20 mg/kg/day for rats with 200 g body weight) reduced plasma TG levels, but increased hepatic TG levels of rats. Similar results were also obtained in our recent study using 1 and 5 mg/kg APFO in mice. In such case, APFO exposure dose-dependently reduced plasma TG level but slightly increased TG in livers of mPPAR α mice. Thus, a relatively high dose or long exposure duration might influence both plasma and hepatic TG levels. In the study by Kudo et al., APFO increased the expression levels of glycerol 3-phosphotransferase and DGAT, which are involved in TG synthesis and are thought to induce an increase in hepatic TG levels (Kudo et al., 1999). The expression of these genes may be important for their influence on hepatic TG levels. In the current study, however, APFO affected neither DGAT1 nor DGAT2 expression. After APFO treatment, TG increase in the liver was seen only in mPPAR α mice, suggesting that some PPAR α target gene(s) might also be involved in the

increase. Since fatty acid transport protein is a PPAR α target gene (Martin et al., 1997) involved in lipid transport to the liver, the involvement of this protein in TG accumulation should be also be investigated.

In control animals, plasma T-Cho levels were reported to be greater in *Ppara*-null mice than in *mPPAR α* mice (Peters et al., 1997), a finding which was reconfirmed in the present study. It is thought that endogenous PPAR α ligands such as leukotriene B4 and palmitic acid could activate PPAR α (Desvergne and Wahli, 1999), which might result in increased cholesterol metabolism, followed by a reduction of T-Cho levels in *mPPAR α* mice. Since human PPAR α may be less responsive to these endogenous fatty acids, similar to PFOA, it might contribute to higher levels of plasma T-Cho in *hPPAR α* mice.

Although we did not measure the serum PFOA concentration in mice, Loveless et al. (2006) reported that the serum concentration in mice after a 0.3 mg/kg administration of APFO for 14 days was 13 ± 2.4 $\mu\text{g/ml}$. In our experiment, we administered the same concentration of APFO for the same period, so that the serum PFOA level might be comparable with those reported by Loveless et al. On the other hand, the mean blood concentration of PFOA among workers engaged in its production was 2.21 $\mu\text{g/ml}$ (95% confidence interval, 1.66–2.77 $\mu\text{g/ml}$) (Olsen and Zobel, 2007). Because durations of exposure to PFOA differ considerably between animal and epidemiological studies, and half-life of PFOA is 12 days in ddY male mice (Kudo and Kawashima, 2003), which is significantly lower than that of human (U.S. EPA, 2002; Olsen et al., 2007), mouse and human PFOA levels could not be directly compared. Nevertheless, both PFOA levels were not markedly different. Since 0.3 mg/kg APFO did not influence either plasma TG or T-Cho levels and did not affect expression of PPAR α and its target genes in *hPPAR α* mice, blood PFOA levels in the workers may not induce the functional activation of PPAR α nor alter lipid levels in their blood. The finding that the expression level of PPAR α was 10 times lower in humans than in mice or rats (Tugwood et al., 1996; Palmer et al., 1998) may further support our conclusion as noted above. Indeed, although Olsen et al. (2003a) found a relationship between serum PFOA and the TG or T-Cho levels in the workers in two factories, they did not find such an association in another factory (2003b). Then, they re-analyzed the data obtained in two studies (2003a, b) excluding subjects who took antilipemic agents, and reported that serum PFOA concentrations were not associated with T-Cho in the workers (2007). On the other hand, a positive association was seen in the analysis between serum PFOA concentration and TG levels, but they concluded that this association might have been due, at least partially, to residual confounding as a consequence of the disproportionate number of employees in the two factories with higher PFOA concentrations than in a remaining factory (Olsen and Zobel, 2007).

Here, we focused on an interaction between PFOA and PPAR α in relation to the lipid metabolism. However, Rosen et al. (2008b) demonstrated that PFOA could also activate constitutive androstane receptor, which also has an important role in the lipid metabolism (Maglich et al., 2009). Further study may be required to determine whether or not the dosages used in the current study activate CAR.

In conclusion, the 0.3 mg/kg dose of APFO significantly activated the function of PPAR α in wild-type mice, but not in humanized PPAR α mice. In addition, APFO increased the hepatic TG and T-Cho in *mPPAR α* mice, but not in *hPPAR α* mice. Since human PPAR α expression in *hPPAR α* mice was equal to or even higher than that of mice, the function of human PPAR α was significantly lower than that of mice. Thus, PFOA levels such as 1.66–2.77 $\mu\text{g/ml}$ in serum may not influence serum TG or T-Cho levels in human.

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Fig. 1.

mRNA expression of hepatic PPARα and related genes ($n = 4-6$) in duplicate or triplicate analyses. Expression of mRNA was analyzed by quantitative real-time PCR. Each mRNA was normalized to the level of GAPDH-mRNA expression in the same preparation, and mean of control in *mPPARα* mice was assigned a value of 1.0. Each column and bar represents mean and $\pm$ S.D., respectively. *Significantly different from respective controls ($P < 0.05$). #Significantly different from respective low dose groups ($P < 0.05$). †Significantly different from *mPPARα* mice treated with same dose ($P < 0.05$). §Significantly different from *Ppara-null* mice treated with same dose ($P < 0.05$).

Fig. 2.

(A) Western blot analysis of hepatic PPAR α and related genes. All mice from each treatment and genotype were examined across three numbers of gels, and one of them is shown here. (B) Western blot analysis of hepatic PPAR α and the related genes ($n = 4-6$). Each band was quantified by densitometric analysis as described in Section 2, and mean strength of control in *mPPAR α* mice was assigned a value of 1.0. Each column and bar represents mean and +S.D., respectively. A normalizing transformation was applied to PH value before Tukey-Kramer analysis. *Significantly different from respective controls ($P < 0.05$). #Significantly different from respective low dose groups ($P < 0.05$). †Significantly different from *mPPAR α* mice treated with same dose ($P < 0.05$). §Significantly different from *Ppara-null* mice treated with same dose ($P < 0.05$).

Fig. 3.

Plasma and hepatic TG and T-cho levels. (A) Plasma, (B) liver ($n = 4-6$). Each column and bar represents mean and +S.D., respectively. *Significantly different from control in *mPPARα* mice ($P < 0.05$). †Significantly different from *mPPARα* mice treated with same dose ($P < 0.05$). §Significantly different from *Ppara-null* mice treated with same dose ($P < 0.05$).

Fig. 4.

Histological findings were scored by an independent pathologist in a blinded fashion according to the following criteria: (1) grade of steatosis: 0, none; 1, mild (5–33% of parenchymal involvement by steatosis); 2, moderate (34–66%); 3, severe (66%>); (2) activity of inflammation: 0, none; 1, mild; 2, moderate; 3, severe.

Table 1

Body and liver weight.

Total mice		APFO		
	n	0mg/kg (n)	0.1 mg/kg (n)	0.3 mg/kg (n)
Body weight (g) at start of APFO treatments				
mPPARα	13	21.9 ± 0.7 (4)	23.3 ± 2.1 (5)	21.2 ± 1.0 (4)
Ppara-null	18	21.5 ± 2.4 (6)	21.4 ± 1.1 (6)	19.4 ± 2.0 (6)
hPPARα	15	18.4 ± 2.0 (5) [§]	20.6 ± 1.0 (5)	19.5 ± 1.2 (5)
Body weight (g) after APFO treatments				
mPPARα	13	24.2 ± 0.3 (4)	24.9 ± 1.6 (5)	23.8 ± 1.5 (4)
Ppara-null	18	24.3 ± 1.7 (6)	22.1 ± 0.8 (6)	22.7 ± 1.3 (6)
hPPARα	15	20.0 ± 2.2 (5) [§]	21.4 ± 1.4 (5)	19.7 ± 1.9 (5)
Body weight change (g)				
mPPARα	13	2.3 ± 0.6 (4)	1.6 ± 0.7 (5)	2.6 ± 2.9 (4)
Ppara-null	18	2.8 ± 0.6 (6)	0.7 ± 3.4 (6)	3.2 ± 2.2 (6)
hPPARα	15	1.6 ± 0.5 (5)	0.8 ± 1.0 (5)	0.1 ± 1.4 (5)
Liver weight (g)				
mPPARα	13	1.17 ± 0.11 (4)	1.24 ± 0.05 (5)	1.38 ± 0.15 (4) [*]
Ppara-null	18	1.24 ± 0.07 (6)	1.05 ± 0.14 (6) [*]	1.27 ± 0.15 (6)
hPPARα	15	0.90 ± 0.14 (5) [§]	0.94 ± 0.10 (5)	0.90 ± 0.20 (5)
Liver/body ratio (%)				
mPPARα	13	4.85 ± 0.47 (4)	5.02 ± 0.18 (5)	5.78 ± 0.33 (4) [*]
Ppara-null	18	5.12 ± 0.30 (6)	4.75 ± 0.53 (6)	5.56 ± 0.43 (6) [#]
hPPARα	15	4.48 ± 0.50 (5)	4.41 ± 0.30 (5)	4.55 ± 0.67 (5)

Data represent mean ± S.D.

^{*} Significantly different from respective control (0 mg/kg) group ($P < 0.05$).

[#] Significantly different from respective low-dose (0.1 mg/kg) group ($P < 0.05$).

[§] Significantly different from control of Ppara-null mice ($P < 0.05$).