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Lack of PPAR α exacerbates lipopolysaccharide-induced liver toxicity through STAT1 inflammatory signaling and increased oxidative/nitrosative stress^{1,2}

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Abstract

Peroxisome proliferator-activated receptor- α (PPAR α) has been implicated in a potent anti-inflammatory activity. However, no information is available on whether PPAR α can affect signal transducers and activator of transcription proteins (STATs) in acute liver damage. Thus, this study was aimed to investigate the *in vivo* role of PPAR α in elevating STATs as well as oxidative/nitrosative stress in a model of lipopolysaccharide (LPS)-induced acute hepatic inflammatory injury. Using age-matched *Ppara*-null and wild-type (WT) mice, we demonstrate that the deletion of PPAR α aggravates LPS-mediated liver injury through activating STAT1 and NF- κ B-p65 accompanied by increased levels of pro-inflammatory cytokines. Furthermore, the activities of key anti-oxidant enzymes and mitochondrial complexes were significantly decreased while lipid peroxidation and protein nitration were elevated in LPS-exposed *Ppara*-null mice compared to WT. These results indicate that PPAR α is important in preventing LPS-induced acute liver damage by regulating STAT1 inflammatory signaling pathways and oxidative/nitrosative stress.

Keywords

Peroxisome proliferator-activated receptor- α ; Lipopolysaccharide; Acute liver damage; Cytokines; Signal transducer and activator of transcription; Oxidative/nitrosative stress

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1. Introduction

Sepsis is an acute syndrome associated with a high rate of morbidity and mortality. A systemic gram-negative bacterial infection discharges endotoxins such as lipopolysaccharide (LPS)³, which elevates various pro-inflammatory cytokines, eventually contributing to multi-organ failures (Cohen, 2002). In livers from animal models, LPS activates Toll-like receptors (TLR), whose stimulation leads to the induction of various signaling pathways including tumor necrosis factor- α (TNF α)/nuclear factor- κ B (NF- κ B), mitogen activated protein kinase (MAPK) family members, and signal transducers and activators of transcription (STATs), as well as the release of pro-inflammatory cytokines and oxygen free radicals (Kim et al., 2003; Su, 2002).

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily of ligand-activated transcription factors that regulate lipid/lipoprotein metabolism, glucose homeostasis, amino acid metabolism, inflammation, and cell differentiation (Desvergne and Wahli, 1999; Francis et al., 2003). So far three isoforms of the PPARs have been identified: PPAR α , PPAR β/δ , and PPAR γ (Desvergne and Wahli, 1999). In both humans and rodents, PPARs are widely expressed in many metabolically

³Abbreviations used:

LPS	lipopolysaccharide
TLR	Toll-like receptors
TNFα	tumor necrosis factor- α
NF-κB	nuclear factor- κ B
MAPK	mitogen activated protein kinase
STAT	signal transducers and activators of transcription
ALT	alanine aminotransferase
WT	wild-type
SOD	superoxide dismutase
H&E	hematoxylin and eosin
MPO	myeloperoxidase
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
MDA	malondialdehyde
CAT	catalase
GPx	glutathione peroxidase
TE buffer	50 mM Tris-HCl, pH 7.5, and 1 mM EDTA
IL-6	interleukin-6
MCP-1	Monocyte chemotactic protein-1
IFN-γ	interferon- γ
TNFR1	tumor necrosis factor receptor-1
ROS	reactive oxygen species
3-NT	3-Nitrotyrosine

active tissues including liver, heart, kidney, skeletal muscle, and intestine (Desvergne and Wahli, 1999).

Accumulating evidence links PPARs with the regulation of immune and inflammatory reactions (Alleva et al., 2002; Delerive et al., 2001). PPAR γ agonists have been reported to decrease the inflammatory responses through the NF- κ B pathways (Chen et al., 2003; Cuzzocrea et al., 2004; Diab et al., 2004). Recent evidence has demonstrated that PPAR α also exhibits a potent anti-inflammatory activity through suppressing NF- κ B (Delerive et al., 2002) and/or modulating the activation (phosphorylation) of the STAT1-related inflammatory signaling in cultured neuronal cells (Lee et al., 2005). Indeed, *Ppara*-null mice exhibited an aggravated reaction to various inflammatory stimuli in the skin, blood vessels, intestine and lung (Delayre-Orthez et al., 2005; Delerive et al., 1999; Di Paola et al., 2006; Sheu et al., 2002). Furthermore, an important role of PPAR α was reported in a model of alcoholic liver disease (Okiyama et al., 2009) and in acute hepatic damage observed after exposure to various toxic chemicals including carbon tetrachloride, cadmium, and paraquat (Anderson et al., 2004). PPAR α has also been implicated in the expression or activation of anti-oxidant enzymes such as catalase and Cu²⁺, Zn²⁺-superoxide dismutase (SOD1) (Inoue et al., 2001; Toyama et al., 2004).

Recently, Crisafulli and Cuzzocrea (2009) reported the effects of LPS on promoting liver injury in *Ppara*-null mice. In that study, they observed marked changes in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase, and bilirubin after LPS administration, indirectly suggesting that PPAR α is related to LPS-mediated hepatic inflammation. However, the underlying mechanism and direct role of PPAR α in LPS-mediated liver injury are still largely unknown. Particularly, no information is available on the relationship between PPAR α and the activation of STATs or increased oxidative/nitrosative stress in LPS-induced liver damage. Therefore, this study was aimed to investigate the roles of STAT1- or STAT3-related inflammatory signaling as well as oxidative/nitrosative stress in a model of LPS-induced acute hepatic injury using *Ppara*-null mice compared to wild-type (WT) mice.

2. Materials and methods

2.1 Animals

Animal experiments were performed on age- and gender-matched inbred *Ppara*-null mice (129/Svj background) and WT mice [stock number 2448 from the Jackson Laboratory (Bar Harbor, Maine, USA)]. All female mice were housed in a controlled environment and fed standard rodent chow and water. At the age of 8-10 weeks, the animals were randomly divided into six groups (n=4~5/group). The first and second groups (WT) were treated with LPS (*Escherichia coli*, serotype 0111:B4, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 10 mg/kg body weight intraperitoneally. The third group (WT) was injected with an equivalent volume of vehicle (0.9% NaCl solution) intraperitoneally. In the fourth, fifth, and sixth groups, *Ppara*-null mice received LPS and 0.9% NaCl solution, respectively. Liver tissues were rapidly excised from each mouse, which was killed by asphyxiation at 4 or 24 h after LPS injection, and 24 h for the saline-treated groups (the third and sixth groups). All animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee of the NIAAA, NIH.

2.2 Measurements of serum ALT and cytokine levels

Serum ALT levels were determined using a clinical chemistry analyzer (IDEXX Vet Test; IDEXX Laboratories, West brook, ME, USA). Serum cytokine levels were determined using cytometry beads array (BD Bioscience) following the manufacturer's protocol.

2.3 Liver histology and immunohistochemistry

A small portion from each mouse liver was fixed in 10% neutral formalin and embedded in paraffin before 4- μ m thick sections were stained with hematoxylin and eosin (H&E). Anti-myeloperoxidase (MPO) antibodies (Biocare Medical, Concord, CA, USA) were used for immunohistostaining of neutrophils according to the manufacturer's protocol. Apoptotic cell death in liver tissues was determined using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon International Inc., Temecula, CA, USA). Numbers of MPO-positive neutrophils and TUNEL-positive hepatocytes were counted in 10 high-power (X200) microscope fields, and the mean was evaluated. To quantify necrotic areas, 20 fields (X200) were randomly selected and calculated.

2.4 Activity measurements of anti-oxidant enzymes and mitochondrial complexes

The catalytic activities of SOD1, SOD2, catalase (CAT), and glutathione peroxidase (GPx) were analyzed using the commercial kits from Calbiochem (San Diego, CA, USA) by the manufacturer's protocols, as described [Abdelmegeed et al., 2009]. ATP synthase activity was determined using an ATP bioluminescence assay kit (Roche, Mannheim, Germany) following the manufacturer's instructions. The activity of mitochondrial complex I (NADH-ubiquinone oxidoreductase) was determined by measuring the OD at 340 nm and subtracting the OD values in the presence of rotenone, an inhibitor of NADH-ubiquinone oxidoreductase (Moon et al., 2008). One unit represents the reduction of 1 nmol NADH/min/mg protein. Other methods such as lipid peroxide, not specifically described here, are performed as previously described (Moon et al., 2008; Abdelmegeed et al., 2009).

2.5 Immunoblot analyses

Liver tissues were homogenized in TE buffer (50 mM Tris-HCl, pH 7.5, and 1 mM EDTA) with protease inhibitor and phosphatase inhibitor cocktails (Calbiochem). Western immunoblot analyses were performed with whole liver lysates (40 μ g proteins) using anti-STAT3 (1:1000 dilution), anti-STAT1 (1:1000 dilution), anti-pSTAT3 (1:1000 dilution), anti-pSTAT1 (1:1000 dilution), anti-NF- κ B-p65, anti-phospho-NF- κ B-p65 (1:1000, Cell signaling, Beverly, MA, USA), anti-3-nitrotyrosine (1:1000 dilution, Abcam, Cambridge, MA, USA), and anti- β actin (1:2000 dilution Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.6 Statistical analysis

The results are expressed as mean $\pm$ standard deviation. Student's *t*-test was performed to compare quantitative data obtained from two different groups. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Exacerbation of LPS-induced acute hepatic injury in *Ppara*-null mice

Preliminary results, performed with a single ip injection at 2, 5, or 10 mg LPS/kg, $n \geq 3$ /group exposed for 4 and 24 h post-LPS treatment, showed greater, more potent liver damage with 10 mg/kg LPS than with lower doses. Therefore, we used 10 mg/kg LPS for this study. Our data revealed that *Ppara*-null mice in the LPS-24 h group had significantly higher serum ALT levels (3713 ± 87.19 IU/L) than those of the corresponding WT mice (1311 ± 121.7 IU/L) (Fig. 1A). In histological analyses, no obvious difference was observed between *Ppara*-null and WT mice under saline treatment (data not shown) or between the groups 4 h after LPS injection (Fig. 1B). At 24 h after LPS administration, necrosis was found in both mouse groups. However, the parenchymal necrosis in *Ppara*-null mice was much more severe and extensive than that of WT mice (Fig. 1B). Quantifications for necrotic area from

multiple sections of each slide revealed an approximately 2.5-fold larger area in *Ppara*-null mice than in WT mice (Fig. 1C). TUNEL analysis, a marker for the degree of apoptotic cells, also demonstrated the presence of significantly increased TUNEL-positive hepatocytes in LPS-exposed *Ppara*-null mice compared to the corresponding WT mice at 4 and 24 h (Figs. 1B and D). MPO staining, reflecting the degree of neutrophil infiltration, also revealed that *Ppara*-null mice had 40 times more MPO-positive neutrophils than WT mice at 4 h post-LPS injection (Figs. 1B and E). The amount of neutrophil infiltration in *Ppara*-null mice exposed to LPS for 4 h was 2-fold higher than that of the corresponding mice exposed for 24 h (Fig. 1E). Furthermore, the LPS-mediated severe hepatic damage in *Ppara*-null mice was not alleviated by pretreatment with a PPAR α agonist Wy-14643 (dissolved in corn oil and injected intraperitoneally at a single dose of 50 mg/kg for 2 h before LPS administration). In contrast, Wy-14643 pretreatment significantly prevented the hepatic damage in the LPS-exposed WT mice as determined by histological analysis of necrosis areas and serum ALT activity measurement, supporting the important role of PPAR α in hepatic damage (data not shown but provided to the reviewers).

3.2 Changes in the levels of cytokine and STAT1 activation in LPS-exposed mice

To elucidate the mechanism of massive infiltration of neutrophils in the LPS-exposed *Ppara*-null mice compared to the corresponding WT mice, we analyzed the serum levels of pro-inflammatory cytokines including TNF α , IL-6, MCP-1, and IFN- γ . At 4 h after LPS treatment, serum levels of TNF α and IFN- γ were significantly increased in *Ppara*-null mice compared to those of WT mice (Fig. 2A). The elevated level of TNF α was persistent and also observed in LPS-24 h *Ppara*-null mice, although its peak level was markedly diminished compared to that of the 4-h group (Fig. 2A). The serum level of MCP-1 was significantly increased at 24 h after LPS administration compared to the corresponding WT, while its serum levels increased in both mouse groups at 4 h post-LPS injection (Fig. 2A).

Based on the significantly elevated level of serum IFN- γ in LPS-4 h *Ppara*-null mice compared to WT, we investigated the hepatic levels of pSTAT1 and STAT1 proteins (Fig. 2B). Immunoblot analyses confirmed that the increased pSTAT1 protein was detected 4 h post-LPS treatment in both mouse groups with statistically higher levels in *Ppara*-null mice (Fig. 2C). Although STAT3 activation, determined by pSTAT3 immunoblot, was observed in both LPS-4h groups, there was no significant difference in the immunoreactive levels of pSTAT3 between the two mouse groups (Figs. 2B and C).

3.3 Differential activation of NF- κ B in LPS-exposed WT mice and *Ppara*-null mice

To determine the level of activated NF- κ B-p65, a key regulator of immune reaction, we performed immunoblot analysis using an anti-phospho-NF- κ B-p65 antibody. The level of phosphorylated NF- κ B-p65 was significantly higher in *Ppara*-null mice than in WT 4 h after LPS exposure (Figs. 3A and B), which corresponded well with the high serum level of TNF α at the same time point in *Ppara*-null mice.

3.4 Changes in the activities of hepatic anti-oxidant enzymes and mitochondrial complexes in LPS-exposed WT mice and *Ppara*-null mice

We also evaluated the activities of anti-oxidant enzymes such as cytosolic SOD1, mitochondrial SOD2, GPx and CAT in all groups since the level of reactive oxygen species (ROS) positively correlated with the severity of liver injury during sepsis (Jaeschke et al., 1999; Leach et al., 1998) and because these enzymes could be inactivated by elevated oxidative/nitrosative stress (Abdelmegeed et al., 2010). SOD activity measurements revealed a significantly lower SOD1 activity in *Ppara*-null mice compared with WT 4 h after LPS treatment, whereas no significant difference in SOD2 activity was observed in any group (Fig. 4A and B). Although the level of CAT activity in saline-treated *Ppara*-null mice

was significantly higher than that of the WT counterparts, CAT activities significantly declined in *Ppara*-null mice compared with WT following LPS treatment (Fig. 4C). Moreover, an approximately 66% reduction of GPx activities was observed in *Ppara*-null mice in the LPS-4-h group compared with that of corresponding WT (Fig. 4D).

Increased oxidative stress can negatively affect mitochondrial function through oxidative modifications to one of its components, as we reported (Moon et al., 2008). Therefore, we measured the activities of 2 enzymes in the mitochondrial electron transport chain such as ubiquinone-NADH-dehydrogenase (complex I) and ATP synthase (complex V) in all groups. The activities of hepatic ubiquinone-NADH-dehydrogenase and ATP synthase were significantly inhibited in *Ppara*-null mice 24 h after LPS injection compared with WT mice (Figs. 4E and F). These results suggest that the acute liver damage observed in LPS-exposed *Ppara*-null mice is likely accompanied by mitochondrial dysfunction.

3.5 Changes in the levels of malondialdehyde (MDA) and protein nitration in LPS-exposed WT mice and *Ppara*-null mice

To assess the levels of oxidative stress, we measured the levels of MDA, as a marker of lipid peroxidation, in each group. MDA levels (Fig. 5A) were significantly elevated in LPS-exposed *Ppara*-null mice at 4 and 24 h compared to those of the corresponding WT. We also determined the levels of 3-nitrotyrosine (3-NT), a footprint of peroxynitrite and nitrosative stress, by immunoblot analysis. The levels of 3-NT (Fig. 5B) were increased in *Ppara*-null mice compared to WT both 4 h and 24 h after LPS treatment.

4. Discussion

It is known that LPS-induced acute liver injury develops in a stepwise fashion. Apoptosis, caused by inflammatory cytokines and observed earlier, progresses toward focal and subsequent massive necrosis, which are induced by neutrophils and mononuclear cells at later time points (Kato et al., 1995; Leist et al., 1995). Our results revealed significantly increased levels of neutrophil infiltration and apoptotic hepatocytes at the earlier time (i.e., 4 h), and extensive necrosis at 24 h in LPS-treated *Ppara*-null mice compared to the corresponding WT mice. This discrepancy of time-dependent progression to severe liver injury between the two mouse strains can be explained by multiple molecular mechanisms. First, increased levels of secreted TNF α and concomitantly elevated chemokines including MCP-1 promote hepatocyte apoptosis and neutrophil infiltration through the NF- κ B pathway. Our data showed that NF- κ B-p65 was markedly activated (phosphorylated) in *Ppara*-null mice at 4 h after LPS treatment. In addition, the time-dependent changes in hepatic damage positively correlated with the alterations of serum levels of MCP-1 and TNF α . Serum TNF α levels were significantly elevated in *Ppara*-null mice 4 h and 24 h after LPS injection compared with the corresponding WT. Serum MCP-1 levels were elevated in both LPS-4 h groups. However, significantly elevated MCP-1 levels were still observed in LPS-24 h *Ppara*-null mice. The persistent elevation of TNF α and MCP-1 likely contributed to extensive necrosis in *Ppara*-null mice exposed to LPS for 24 h.

Second, significantly increased levels of IFN- γ in the *Ppara*-null mice induce hepatic damage via activating the STAT1 inflammatory signaling pathway. The noxious effect of IFN- γ is mediated by the activation of STAT1, which not only enhances the susceptibility of hepatocytes to IFN- γ or IFN- γ + TNF α -induced apoptosis but also induces inflammatory cells infiltration by the expression of leukocyte-attracting chemokines (Kim et al., 2003). In addition, various PPAR α agonists can suppress phosphorylation (activation) of STAT1, thus leading to prevention of STAT1-mediated neuronal inflammation (Lee et al., 2005), although the effects of these PPAR α agonists on the STAT1 activation in other non-neuronal tissues are unknown. In our study, serum levels of IFN- γ and the hepatic level of

phosphorylated (activated) STAT1 in the LPS-4 h *Ppara*-null mice were significantly elevated compared to WT counterparts. Although STAT1 might promote compensatory cell proliferation as recently suggested (Wang et al., 2010), this STAT1 activation could also play a major role in severe inflammatory damage observed in LPS-24 h *Ppara*-null mice. In contrast, there was no significant difference in the serum IL-6 levels and subsequent activation (phosphorylation) of hepatic STAT3 between both groups. Together, our results indicate that PPAR α attenuates the activation (phosphorylation) of STAT1, but not STAT3, suggesting that STAT3-mediated survival and regeneration might have played a minor role during STAT1-dependent acute liver injury in LPS-exposed *Ppara*-null mice.

Third, in the absence of PPAR α , oxidative stress was increased during LPS-induced liver injury. Anti-oxidant enzymes such as SODs, CAT, and GPx coordinately eliminate potentially toxic ROS to protect the cells/tissues. Therefore, inhibition of CAT, GPx, and the SODs would promote deteriorating injury through induction of oxidative stress. In a model of alcohol-induced liver damage in *Ppara*-null mice, CAT, SOD and GPx activities were significantly inhibited, suggesting that the inhibition of these proteins could be directly and indirectly regulated by increased oxidative/nitrosative stress, as demonstrated (Abdelmegeed et al., 2010). Our results of significantly decreased activities of SOD1, CAT, and GPx in the LPS-4 h *Ppara*-null mice indicate that PPAR α plays a role in cytoprotection against LPS-induced acute hepatic injury through regulating these anti-oxidant enzymes and oxidative/nitrosative stress. Our data also revealed the lowest activities of mitochondrial complex I and complex V in the LPS-24 h *Ppara*-null mice among all the groups determined in our study, suggesting a potential implication of PPAR α in preventing oxidative inactivation of mitochondrial complexes (i.e., mitochondrial dysfunction) and the subsequent production of additional ROS through the dysfunctional mitochondrial electron transport chain. Consistent with these results, the levels of MDA, which can participate in acute liver injury and chronic fibrosis (Mantena et al., 2008), were elevated in *Ppara*-null mice at 4 and 24 h after LPS treatment compared with the corresponding WT mice. Furthermore the levels of 3-NT, which is a footprint of peroxynitrite produced by the reaction between NO and superoxide ($O_2^{\bullet-}$), were markedly increased in *Ppara*-null mice at 4 h and 24 h after LPS treatment compared to the WT counterparts. The different levels of MDA and nitrated proteins between *Ppara*-null and WT mice suggest that the absence of PPAR α is likely to be implicated in oxidative/nitrosative tissue damage caused by potentially toxic agents such as LPS.

Finally, Anderson et al. (2004) reported that activation of PPAR α with an agonist Wy-14643 is important in up-regulating the genes encoding the proteins responsible for adequate maintenance of healthy proteomes. For instance, both oral (gavage) and dietary administrations of Wy-14643 up-regulated the genes encoding various heat-stress inducible chaperone proteins and proteasome components in WT mice in a PPAR α -dependent manner, since up-regulation of these genes was not observed in the corresponding *Ppara*-null mice. Based on the results of this report, it is expected that WT mice, containing the PPAR α receptor, albeit not activated under normal conditions, have basal levels of these critical genes/proteins, thus preventing the aggregation of various proteins and/or efficiently removing the damaged proteins. In contrast, *Ppara*-null mice may have a deficiency in properly maintaining cellular proteomes and eliminating the damaged proteins due to the absence of the PPAR α receptor. Therefore, it is likely that the absence of the PPAR α receptor and the critical genes/proteins needed for maintaining healthy proteomes in the *Ppara*-null mice may aggravate the tissue damage especially when exposed to LPS or other toxic agents such as carbon tetrachloride, cadmium, and paraquat, as demonstrated (Anderson et al., 2004). Furthermore, insufficient compensatory proliferation in the *Ppara*-null mice may also contribute to accelerated liver damage.

In summary, at 4 h post-injection, LPS activated STAT1 and NF- κ B accompanied by the elevated levels of pro-inflammatory cytokines. Additionally, anti-oxidant enzymes and mitochondrial complexes were inactivated while the levels of lipid peroxidation and protein nitration were increased in LPS-exposed *Ppara*-null mice compared to the corresponding WT mice. These concurrent events likely explain the underlying mechanisms of severe liver damage in the LPS-exposed *Ppara*-null mice compared to the corresponding WT mice. Although a clear linkage between genetic mutations in the *PPAR α* gene and a reduced receptor function has not been established, to our knowledge, our data represents a first report that *PPAR α* is involved in regulating the STAT1 inflammatory signaling pathways and oxidative/nitrosative stress during LPS-induced acute hepatic injury. Based on all these data, *PPAR α* agonists might have therapeutic potential against acute liver damage caused by LPS or other hepatotoxic agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Histological and immunological analyses of LPS-exposed liver tissues from WT and *Ppara*-null mice. (A) Serum ALT levels, (B) H&E-stained liver sections, (C) summary of necrotic area, (D) TUNEL-positive hepatocytes (marked with arrows), and (E) MPO-positive hepatocytes in WT and *Ppara*-null mice are presented. Data are represented as means ± SD of $n=4\sim5$ /group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, compared with the WT counterparts.

Fig. 2.

Increased levels of cytokines and STAT1 activation in LPS-exposed *Ppara*-null mice. Time-dependent changes in: (A) the levels of 4 serum cytokines, (B) phosphorylated-STAT1, and (C) densitometry in LPS-exposed *Ppara*-null and WT mice are presented. Data are represented as means $\pm$ SD of $n=4\sim5$ /group. * $p<0.05$, ** $p<0.01$, compared with the WT counterparts.

Fig. 3. Time-dependent activation of NF-κB in LPS-exposed *Ppara*-null mice. (A) The relative levels of phospho-NFκB-p65 and NFκB-p65 and (B) subsequent densitometry in LPS-exposed *Ppara*-null and WT mice are presented. Data are represented as means $\pm$ SD of n=4~5/group. * p <0.05, compared with the WT counterparts.

Fig. 4. Time-dependent decreases in the activities of hepatic anti-oxidant and mitochondrial enzymes after LPS treatment. (A-D) Activities of anti-oxidant enzymes including SOD1, SOD2, catalase, and glutathione peroxidase and (E,F) mitochondrial ubiquinone-dependent NADH dehydrogenase and ATP synthase in LPS-exposed *Ppara*-null and WT mice are presented. Data are represented as means $\pm$ SD of $n=4\sim5$ /group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, compared with the WT counterparts.

Fig. 5. Elevated levels of malondialdehyde and protein nitration in LPS-exposed *Ppara*-null mice. (A) Hepatic levels of MDA and (B) nitrated proteins in LPS-exposed *Ppara*-null and WT mice are presented. Data are represented as means $\pm$ SD of $n=4\sim5$ /group. ** $p<0.01$, *** $p<0.001$, compared with the WT counterparts.