Activation of Peroxisome Proliferator Activator Receptor β/δ Improves Endothelial Dysfunction and Protects Kidney in Murine Lupus

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Abstract—Women with systemic lupus erythematosus exhibit a high prevalence of hypertension, endothelial dysfunction, and renal injury. We tested whether GW0742, a peroxisome proliferator activator receptor β/δ (PPARβ/δ) agonist, ameliorates disease activity and cardiovascular complications in a female mouse model of lupus. Thirty-week-old NZBWF1 (lupus) and NZW/LacI (control) mice were treated with GW0742 or with the PPARβ/δ antagonist GSK0660 plus GW0742 for 5 weeks. Blood pressure, plasma double-stranded DNA autoantibodies and cytokines, nephritis, hepatic opsonins, spleen lymphocyte populations, endothelial function, and vascular oxidative stress were compared in treated and untreated mice. GW0742 treatment reduced lupus disease activity, blood pressure, cardiac and renal hypertrophy, splenomegaly, albuminuria, and renal injury in lupus mice, but not in control. GW0742 increased hepatic opsonins mRNA levels in lupus mice and reduced the elevated T, B, Treg, and Th1 cells in spleens from lupus mice. GW0742 lowered the higher plasma concentration of proinflammatory cytokines observed in lupus mice. Aortae from lupus mice showed reduced endothelial-dependent vasodilator responses to acetylcholine and increased nicotinamide adenine dinucleotide phosphate oxidase–driven vascular reactive oxygen species production, which were normalized by GW0742 treatment. All these effects of GW0742 were inhibited by PPARβ/δ blockade with GSK0660. Pharmacological activation of PPARβ/δ reduced hypertension, endothelial dysfunction, and organ damage in severe lupus mice, which was associated with reduced plasma antidualle-stranded DNA autoantibodies and anti-inflammatory and antioxidant effects in target tissues. Our findings identify PPARβ/δ as a promising target for an alternative approach in the treatment of systemic lupus erythematosus and its associated vascular damage. (Hypertension. 2017;69:641-650. DOI: 10.1161/HYPERTENSIONAHA.116.08655.) • Online Data Supplement

Key Words: acetylcholine | blood pressure | hypertension | nephritis | oxidative stress | peroxisome | proliferator-activated receptors

Systemic lupus erythematosus (SLE) is a multisystemic chronic autoimmune inflammatory disorder that is associated with a high risk for the development of renal and cardiovascular diseases,1,2 which are major causes of mortality in these patients.3 It predominantly affects young women of child-bearing age, the same population that is at lowest relative risk of atherosclerotic heart disease. In fact, women with lupus (aged 35–44 years) are >50x as likely as healthy women without lupus to have a myocardial infarction.4 SLE is associated with a high incidence of hypertension,5,6 a well-established risk factor for the development and acceleration of atherosclerosis and ischemic heart disease. Moreover, SLE is characterized by the existence of high levels of circulating antinuclear autoantibodies, more specifically antidouble-stranded DNA (anti-dsDNA) antibodies, which can be considered as a manifestation of a loss of self-tolerance to ubiquitous nuclear autoantigens.7 The development of autoimmunity and the resultant increase of renal inflammation are important underlying factors in the high blood pressure that occurs during SLE. In fact, preventing autoimmunity with anti-CD20 therapy attenuates lupus disease progression and protects against the development of hypertension.8

Defects in apoptotic cell clearance make mice and human susceptible to the autoimmune disease SLE.9 Peroxisome proliferator activated receptors β/δ (PPARβ/δ) play a pivotal role in orchestrating the timely disposal of apoptotic cells by macrophages to promote tolerance. In fact, global or macrophage-specific deletion of PPARβ/δ delays clearance of apoptotic cells,
leading to increased production of autoantibodies and progressive lupus-like autoimmune disease. Furthermore, PPARβ/δ signaling can intrinsically regulate T-helper (Th) biology by altering proliferative capacity and expression of interferon-γ (IFN-γ) and regulating Th17 differentiation. However, whether PPARβ/δ activation would reduce the production of autoantibodies and protect against SLE progression is unknown. Moreover, it has been proposed that immune dysregulation in SLE may be responsible for the higher incidence of insulin resistance and metabolic syndrome observed in these patients, which may also contribute to vascular dysfunction. In previous studies, it has been reported that PPARβ/δ activation reduces blood pressure, improves metabolic profile and vascular function, and has anti-inflammatory and renal protective effects. In addition, preclinical evidences suggest that PPARβ/δ agonists may prevent and treat obesity-induced insulin resistance and type 2 diabetes mellitus, whereas clinical trials have highlighted their potential use in dyslipidemia. Given that SLE is a chronic inflammatory disorder with prominent hypertension, insulin resistance, inflammation, and renal disease, PPARβ/δ activation could be an attractive therapeutic option. Therefore, in this study, we propose that the treatment with a PPARβ/δ agonist can ameliorate SLE-associated hypertension and renal injury. With this aim, we have analyzed the effects of chronic treatment with the highly selective PPARβ/δ agonist GW0742 in a mouse model (NZBWFI mice) that closely mimics human SLE. Like in humans with SLE, the NZBWF1 mice produce anti-dsDNA antibodies, develop immune complex glomerulonephritis, and, more importantly, they develop hypertension.

Finally, and similarly to humans, the cause of SLE in this model is thought to be polygenic, and female NZBWF1 mice are more prominently affected than males.

Methods

Animals and Experimental Groups

The investigation conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and by the Ethic Committee of Laboratory Animals of the University of Granada (Spain; Ref. 459-his-CEEA-2012). Thirty-week-old female NZBWF1 (SLE) and NZW/LacJ (control) mice obtained from Jackson Laboratories (Bar Harbor, ME) were randomly assigned to receive by oral gavage GW0742 (5 mg kg−1 d−1) or with the PPARβ/δ antagonist GSK0660 1 mg kg−1 d−1 intraperitoneally plus GW0742 orally for 5 weeks. GSK0660 was diluted first in dimethyl sulfoxide and later in saline just before use. Urine from control mice was collected and assessed for the presence of proteinuria, as described previously. Animals were maintained at a constant temperature (24±1°C), with a 12-hour light/dark cycle, on a standard chow and water ad libitum. Mice were randomly divided into 5 groups: control untreated (Ctrl), control treated with GW0742 (GW), SLE untreated (SLE), SLE treated with GW0742 (SLE+GW), and SLE treated with GW0742 and GSK0660 (SLE+GW+GSK).

Parameters Determined

Systolic blood pressure (SBP) evolution was measured by tail-cuff plethysmography and final mean arterial blood pressure and heart rate by intra-arterial register. At the end of the experiment, plasmatic concentrations of anti-dsDNA antibodies, cytokines, glucose, triglycerides, high-density lipoprotein (HDL), and total cholesterol were measured, as previously described. In addition, physical characteristics, cardiac, and renal weight indices were also measured.

Renal injury was assessed by proteinuria determination and histopathologic evaluation of kidneys, which was performed in sections stained with hematoxylin–eosin, Periodic acid–Schiff, and Masson trichrome.

Descending thoracic aortic rings were mounted in a wire myograph for isometric tension measurement as previously described. Vascular reactive oxygen species (ROS) levels were estimated from the ratio of ethidium/4,6-diamidino-2-phenylindole dichlorohydryl fluorescence in sections of unfixed thoracic aortic rings incubated with dihydroethidium and counterstained with the nuclear stain 4,6-diamidino-2-phenylindole dichlorohydrate. Vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity was measured by the lucigenin-enhanced chemiluminescence assay.

Flow cytometry was used to measure B (positive to anti-B220+), and T cells (positive to anti-CD4+, total T; anti-CD4+ FOXP3+, regulatory T cells (Treg); anti-CD4+ IL17+, Th17; and anti-CD4+ IFN-γ, Th1) from spleens.

Protein and mRNA expression were measured by Western blotting analysis and reverse transcriptase-polymerase chain reaction analysis, respectively, as previously described. The sequences of the sense and antisense primers used for amplification are described in Table S1 in the online-only Data Supplement.

Statistical Analysis

Results are expressed as mean±SEM. Statistical analyses were performed using Graph Pad Prism 5 software (Methods in the in the online-only Data Supplement).

Results

Effects of GW0742 on Blood Pressure, Cardiac and Renal Hypertrophy, Spleen Lymphocytes, and Plasma Determinations

The time course of tail SBP is shown in Figure 1A and the final mean arterial blood pressure and heart rate measured by direct recordings in Figure 1B. Initial SBP was significantly higher in SLE mice when compared with controls. No significant changes in SBP were induced after 5 weeks of vehicle treatment either in SLE or in control mice. GW0742 treatment reduced BP in SLE mice (Figure 1A and 1B), without significantly affecting heart rate (Figure 1B). This effect was prevented by coadministration of the PPARβ/δ antagonist GSK0660, confirming that PPARβ/δ activation was responsible for the effects of GW0742. There is a significant increase in body weight in SLE mice compared with control animals which was unaffected by GW0742 treatment (Table S2). Heart weight/tibia length, left ventricular weight/tibia length, and kidney weight/tibia length indices were higher in SLE (Table S2) than in control mice. GW0742 treatment reduced significantly both cardiac and renal hypertrophy found in SLE mice, which was inhibited by PPARβ/δ blockade with GSK0660.

SLE disease activity, measured by plasma levels of anti-dsDNA autoantibodies, was significantly greater in SLE mice compared with control mice, as previously reported (Figure 2A). GW0742 treatment significantly reduced the levels of anti-dsDNA in SLE, being without effect in control mice. Disease progression has been associated with splenomegaly, most probably because of a lymphoproliferative disorder. We also found splenomegaly in lupus mice, which was appreciably reduced by PPARβ/δ activation with GW0742 (Figure 2B).

Increased production of autoantibodies and progressive lupus-like autoimmune disease are associated with a delay in the clearance of apoptotic cells. Opsonins, proteins secreted
by macrophages, such as C1q, thrombospondin-1, and milk fat globule-epidermal growth factor-8, enhance the recognition and phagocytosis of apoptotic cells by macrophages.28 Kupffer cells are liver resident macrophages, considered the primary source of opsonins that circulate in the serum,29 and this prompted us to investigate the opsonin expression in the livers of mice from all experimental groups. We found that in vivo pharmacological activation of PPARβ/δ enhances hepatic opsonins (C1qa, C1qb, thrombospondin-1, and milk fat globule-epidermal growth factor-8) gene expression only in lupus mice, which tended to be reduced in these animals when compared with control (Figure 2C). In the liver, the mRNA levels of PPARα (Figure S1A) were reduced in SLE mice when compared with control, whereas PPARγ (Figure S1B) and PPARβ/δ (Figure S1C) were similar. GW0742 did not modify hepatic mRNA levels of PPARα and PPARγ, but increased PPARβ/δ expression. In addition, increased mRNA levels of proinflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin-1β [IL-1β], and IL-6) and reduced levels of adiponectin were also found in liver from SLE mice. In vivo PPARβ/δ activation restored the hepatic levels of these cytokines to those found in control mice (Figure S1D). Fasting plasma glucose and total cholesterol levels were higher in SLE mice when compared with control (Table S2), with similar
Hypertension  April 2017

levels of triglycerides and HDL. GW0742 reduced the levels of glucose and increased HDL in a PPARβ/δ-dependent manner.

To determine the immunomodulatory actions of PPARβ/δ activation, we measured the levels of B and T cells in spleens from all experimental groups (Figure 3A). The number of total cells (Figure 3A) and the percentages of T (Figure 3B), and B (Figure 3C) cells were higher in spleens from SLE mice than in control group. In addition, the percentages of Treg, Th1 (Figure 3D), and CD4+ IL17A+ (Figure 3E) cells were also increased in splenocytes from SLE mice. GW0742 treatment significantly decreased the cell counts that were augmented in SLE mice. Plasma levels of IFN-γ, IL-21, TNF-α, IL-10, and IL-17 were also increased in SLE mice when compared with control, whereas plasma adiponectin levels were reduced. Again, PPARβ/δ activation normalized the levels of these parameters (Figure S2).

Effects of GW0742 on Renal Injury

The comparative study of renal injury in different mice groups is shown in representative micrographs in Figure 4A and Table S3. No renal lesions were observed in control groups. At 35 weeks, extracapillary proliferation (crescent) in moderate intensity, segmental sclerosis, and abundant immune deposits on basal membrane of tuft capillary were detected in SLE mice group glomeruli with Masson trichrome stain. Tubular dilatation with hyaline casts and patch of chronic inflammatory infiltrate were present in 80% of SLE group kidneys. The treatment of SLE mice with GW0742 resulted in a significant reduction of tubular cast and immune deposits in tuft capillary; although the percentage of glomerulosclerosis and mesangial proliferation tended to be lower, no statistical differences were obtained in comparison with SLE group. In the group treated with GW0742 plus the PPARβ/δ antagonist GSK0660, the glomerular lesions were similar to SLE group. The number of nuclei per glomerular cross-section, which is a measure of glomerular cellularity and proliferation, was significantly increased in SLE mice when compared with control group. Also, the administration of GW0742 to SLE mice decreased the glomerular proliferation in comparison with SLE group, being this effect reversed by the antagonist GSK0660, which increased the number of cells of proliferative glomeruli to similar values obtained in SLE group.

Urinary albumin excretion was increased in SLE mice compared with controls and significantly reduced after GW0742 treatment, being this effect abolished by the coadministration of GSK0660 (Figure 4B).

The gene expression of the proinflammatory cytokines, TNF-α, IL-1β, and IL-6, in the renal cortical was increased in SLE mice when compared with control. PPARβ/δ activation significantly reduced these mRNA levels of these genes, being this effect also counteracted when the antagonist GSK0660 was concurrently administered to SLE mice (Figure 4C).

The mRNA levels of PPARα and PPARγ in renal cortex were significantly lower in SLE mice compared with control mice, whereas PPARβ/δ expression was similar in both groups. Treatment with GW0742 did not affect renal cortical PPARα and PPARγ expression in either control or SLE mice, but increased PPARβ/δ mRNA levels (Figure S3). In addition, GW0742 increased mRNA level of PPARβ/δ target genes, carnitine palmitoyltransferase-1, UCP2 (uncoupling protein-2), and pyruvate dehydrogenase kinase-4. This increase was abolished by blocking PPARβ/δ with GSK0660, involving PPARβ/δ activation.

Figure 3. Effects of chronic peroxisome proliferator activator receptor β/δ (PPARβ/δ) activation on total numbers of splenocytes (A), total T (CD4+; B) and B (B220+; C) lymphocytes, regulatory T cells (Treg; CD4+ FOXP3), Th17 (CD4+ IL17+), and Th1 (CD4+ interferon-γ [IFN-γ]) cells (D), and CD4+ IL17+ cells (E), measured in spleens from all experimental groups measured by flow cytometry. Experimental groups: Ctrl (n=8), GW (GW0742, 5 mg kg−1 d−1 by oral gavage, n=8), systemic lupus erythematosus (SLE; n=8), SLE+GW (n=10), SLE−GW+GSK (GSK0660 1 mg kg−1 d−1 intraperitoneally plus GW0742 orally, n=10). Values are expressed as mean±SEM. **P<0.01 vs control group. *P<0.05 and #P<0.05 vs SLE group. *P<0.05 vs SLE+GW group.
Effects of GW0742 on Endothelial Function

Aorta from SLE mice showed strongly reduced endothelial-dependent vasodilator responses to acetylcholine. The treatment of SLE mice with GW0742 showed an increase in the acetylcholine-induced vasodilation when compared with vehicle-treated SLE mice (Figure 5A). These relaxant responses were suppressed by incubation for 30 minutes with the nitric oxide synthase (NOS) inhibitor *N*-ω-nitro-l-arginine methyl ester (L-NAME) in all experimental groups (Figure 5B), suggesting a higher NO formation in vessels from control mice compared with those from SLE mice. No differences were observed among all experimental groups in the endothelium-independent relaxant response to sodium nitroprusside (Figure 5C).

Aortic endothelial NOS (eNOS) mRNA levels (Figure 5D) and protein expression (Figure 5E) were similar among all experimental groups. However, eNOS phosphorylation at Ser-1177 was significant reduced in SLE mice when compared with control. PPARβ/δ activation restored Ser-1177-eNOS phosphorylation at values similar to control (Figure 5E).

The mRNA levels of PPARβ/δ were significantly lower in aorta from SLE mice compared with control mice, whereas the expression of their target genes UCP2, carnitine palmitoyltransferase-1, and pyruvate dehydrogenase kinase-4 was similar in both groups. Treatment with GW0742 increased the mRNA levels of both PPARβ/δ and their target genes. These changes induced by GW0742 were inhibited by PPARβ/δ.
Hypertension April 2017

blockade with GSK0660, showing that the effects of GW0742 involved PPARβ/δ activation in the aorta (Figure S4).

**Effects of GW0742 on Vascular ROS Levels and NADPH Oxidase Activity**

Aortic rings from SLE group showed marked increased aortic staining when compared with control group, which was significantly reduced by GW0742 treatment (Figure 6A). In addition, NADPH oxidase activity was increased in aortic rings from SLE mice when compared with control mice (Figure 6B), which were associated with significant mRNA increase of NADPH oxidase subunits (Figure S5) NADPH oxidase 1 (NOX1), NOX2, p22phox (phagocytic oxidase [phox]), and p47phox. Chronic in vivo PPARβ/δ activation by GW0742 reduced significantly both the upregulation of NADPH oxidase subunits and the increased NADPH oxidase activity in SLE mice, but not in control mice.

To evaluate the role of NADPH oxidase-driven ROS production in endothelial function, we tested endothelium-dependent relaxation to acetylcholine in the presence of Nω-nitro-L-arginine methyl ester (L-NAME; B). Endothelium-independent vasodilator responses to sodium nitroprusside (SNP) in arteries previously contracted by U46619 (10−8 mol/L; C). Aortic mRNA levels by reverse transcriptase-polymerase chain reaction (D), and protein expression by Western blots (E) of endothelial nitric oxide synthase (eNOS), and eNOS phosphorylation at Ser-1177.

Figure 5. Effects of chronic peroxisome proliferator activator receptor β/δ (PPARβ/δ) activation on endothelial function. Endothelium-dependent vasodilator responses to acetylcholine (Ach) in intact aortic rings precontracted with U46619 (10−8 mol/L) in the absence (A) or in the presence of Nω-nitro-L-arginine methyl ester (L-NAME; B). Endothelium-independent vasodilator responses to sodium nitroprusside (SNP) in arteries previously contracted by U46619 (10−8 mol/L; C). Aortic mRNA levels by reverse transcriptase-polymerase chain reaction (D), and protein expression by Western blots (E) of endothelial nitric oxide synthase (eNOS), and eNOS phosphorylation at Ser-1177. Experimental groups: Ctrl (n=8), GW (GW0742, 5 mg kg−1 d−1 by oral gavage, n=8), systemic lupus erythematosus (SLE; n=8), SLE+GW (n=10), SLE-GW+GSK (GSK0660 1 mg kg−1 d−1 intraperitoneally plus GW0742 orally, n=10). Values are expressed as mean±SEM. **P<0.01 and *P<0.05 vs control group. #P<0.01 and #P<0.05 vs SLE group. &P<0.01 and &P<0.05 vs SLE+GW group. p-eNOS (Ser1177) indicates eNOS phosphorylation at Ser-1177.

Discussion

In this study, we investigated whether the PPARβ/δ agonist therapy reduces cardiovascular complications in an experimental model of this autoimmune disease accompanying with hypertension, the NZBWF1 mice, which similar features to human SLE. This study revealed that, in SLE mice, chronic PPARβ/δ activation with GW0742 treatment (1) markedly attenuated lupus disease progression as evidenced by reduced splenomegaly, B cells accumulation, and lower anti-dsDNA autoantibody activity, (2) significantly reduced the elevated SBP, (3) ameliorated heart and kidney hypertrophy, (4) improved renal morphological lesions, renal inflammation, and albuminuria, (5) restored endothelial function in SLE, and (6) exerted vascular protective effects that seem to be related to decreased ROS production as a result of NADPH oxidase subunit downregulation and increased NO bioavailability.

SLE is characterized by the production of autoantibodies, being the presence of anti-dsDNA antibodies, the hallmark in SLE. Kidney is one of the most frequently involved organs in SLE; deposition of autoantibodies in the kidneys triggers inflammation resulting in lupus nephritis, which may progress to end-stage renal failure. In fact, transgenic overexpression of anti-dsDNA autoantibody in mice induced proteinuria and increased the sensitivity of Toll-like receptor 4 activation to induce severe SLE syndrome. In the experimental model of SLE used in this study, the NZBWF1 mice, we also found increased anti-dsDNA activity. This correlated with albuminuria, inflammation, and morphological alterations in renal cortex, including immune complex deposition, which were ameliorated after chronic GW0742 treatment, in association with reduced plasma anti-dsDNA titer. All these features in
this genetic model of SLE are in agreement with previous data, showing that genetic deficiency of PPARβ/δ increased both autoantibodies in sera and deposits of immune complexes in the glomeruli of pristane (agent which induced lupus-like autoimmune disease by activating B cells)-injected mice. Interestingly, renal damage in SLE mice was associated with increased expression of inflammatory cytokines in kidney that was almost abolished by GW0742, despite only a partial reduction in plasma autoantibodies and immune complex deposition was obtained with the agonist treatment. These data suggested that GW0742 activated PPARβ/δ in the kidney, as denoted by increased PPARβ/δ mRNA levels induced by GW0742 treatment in SLE mice that might be related to the reduced expression of opsonins, proteins that facilitate the recognition and uptake of apoptotic cells by macrophages, as it has been detected in our experiment in the liver from lupus mice. In fact, the activation of PPARβ/δ increased the expression of hepatic opsonins only in SLE mice, in association with reduced plasma anti-dsDNA activity. In addition, humoral immune system activation plays a central role in the pathogenesis of SLE because there is evidence that B cells, which differentiate into antibody-producing plasma cells, are increased during SLE. Furthermore, IL-21 drives B cells maturation and autoantibody production in rodent models of lupus. Accordingly, we found a higher number of spleen B cells and increased plasma levels of IL-21 in SLE mice than in the control group, which were counteracted after GW0742 treatment in SLE mice, thus contributing to attenuate antibody activity in these treated mice. Moreover, despite their elevated proportion in aged lupus mice, Treg cells are ultimately unable to control cumulative impact of multiple genetic elements driving lymphocyte activation and autoactivity. The reduction induced by GW0742 in Treg cell counts might also reduce these processes, leading to a decrease of autoantibodies. However, it has been reported that B-cell depletion with anti-CD20 antibody, starting at 30 weeks of age in NZBWF1 female mice, once the inflammatory process has begun and antibodies are being produced, did not affect blood pressure. Remarkably, in our experiment, pharmacological PPARβ/δ activation, which started at 30 weeks of age, reduced the levels of autoantibodies and attenuated hypertension, thus providing data that the improvement of the autoimmune in SLE may be an important factor underlying in the control of hypertension, although other mechanisms induced by the PPARβ/δ agonist could be also involved.

Hypertension is often associated with impaired endothelial function, but whether this is causative in the progression of hypertension is difficult to prove. Numerous studies suggest that the endothelium is prominently affected during SLE, as demonstrated by the high risk for the development of hypertension in this model. Thus, blockade of TNF-α biological activity with etanercept attenuates the hypertension in this model.
of atherosclerosis. Endothelial cell dysfunction represents the earliest indication of the development of cardiovascular disease and is also a principal element of SLE. In NZBWF1 mice, we observed an impaired aortic endothelium-dependent relaxation response to acetylcholine. Interestingly, Ryan et al found that the impaired response to acetylcholine begins before the development of proteinuria and results in increased blood pressure, suggesting that early changes in vessel function may contribute to the development of hypertension during SLE. Our present results extend previous findings of our group linking endothelial dysfunction and hypertension in SLE because PPARγ activation increased acetylcholine relaxation and reduced BP. Moreover, hypertensive SLE mice develop cardiac and renal hypertrophy when compared with control mice. Pharmacological PPARγ activation in SLE mice was accompanied by significant amelioration in these structural alterations.

Inflammatory responses in the endothelium induced by circulating autoantibodies and other inflammatory mediators are known to contribute to the pathogenesis of endothelial dysfunction, and numerous studies have implicated the release of cytokines in the progression of SLE. Therefore, we found increased plasma levels of proinflammatory cytokines TNF-α, IFN-γ, IL-17, and IL-21 in SLE, which correlate with changes in cell populations in the spleen. As it has been previously reported, GW0742 reduced Th1 cells, as well as IFN-γ and IL-21 levels in plasma, thus reducing their deleterious effects in the vasculature. However, the molecular mechanisms involved in endothelial dysfunction in SLE mice have never been analyzed in detail. It is well known that NO secretion is required for normal endothelium-dependent vasodilatation, and SLE patients display a defect of eNOS function in endothelial cells. We found that endothelium-dependent relaxations induced by acetylcholine were abolished by eNOS inhibition with L-NAME, suggesting a defect in NO pathway in SLE mice. However, no significant changes in eNOS expression were detected in SLE mice, whereas reduced eNOS phosphorylation at the activation site Ser-1177 indicates reduced NO production. Interestingly, the improvement in acetylcholine relaxation induced by GW0742 in SLE was suppressed by L-NAME, suggesting that GW0742 improved the eNOS pathway in aortic tissue, which was associated with the restoration of Ser-1177-eNOS phosphorylation by PPARγ activation. In addition, the protective effects seem to be unrelated to changes in the sensitivity to the NO–cGMP pathway because the vasodilator response to the NO donor, nitroprusside, was unaffected by GW0742 treatment.

A key mechanism of endothelial dysfunction involves the vascular production of ROS, particularly O₂⁻, which reacts rapidly with and inactivates NO. We found that ROS levels are increased in aorta from SLE and that GW0742 reduced ROS content. The activity of the NADPH oxidase, considered the major source of O₂⁻ in the vascular wall, was markedly increased in SLE mice, accompanied with an increase in mRNA level of the subunits NOX1, NOX2, p22(phox), and p47(phox). NADPH oxidase-driven ROS production is a key event in endothelial dysfunction in SLE because incubation with the NADPH oxidase inhibitor apocynin increased the aortic endothelium-dependent relaxation to acetylcholine in SLE to similar level that found in control. GW0742 treatment inhibited the upregulation of these NADPH oxidase subunits and its activity in SLE mice. The normalization of ROS also seems to contribute to the restoration of endothelial function. Overall, these beneficial effects of GW0742 on endothelial function might be the results of the reduction in plasma cytokines and direct effects on the vasculature increasing eNOS phosphorylation and reducing NADPH oxidase expression.

Metabolic factors, such as hyperglycemia, insulin resistance, hypertriglyceridemia, and low HDL, can impair endothelial function. In fact, the metabolic syndrome is highly prevalent in patients with SLE, and it has been associated with increased cardiovascular risk. In these patients, metabolic syndrome may contribute to the development of atherosclerosis by increasing inflammation levels, arterial stiffness and decreasing circulating endothelial progenitor cells. PPARγ activation reduced hyperglycemia and increased HDL in SLE mice. We also showed that hepatic mRNA levels of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, were higher in SLE mice compared with control mice. Hepatic PPARγ activation reduced these markers of liver inflammation and consequently improved glucose metabolism. Moreover, it has been reported that GW0742 was also able to restore the hepatic production and plasma levels of adiponectin, an insulin-sensitizing adipokine, with potent protective effects against endothelial dysfunction and auto-antibodies production. The ability showed by GW0742 to increase HDL and to reduce glucose in plasma might also play a role in preserving endothelial function in SLE mice.

Finally, a reduction in the number of endothelial progenitor cells and the deficiency in their function have been reported to occur in both human and murine SLE. In particular, the capacity of endothelial progenitor cell for differentiation into mature endothelial cells is significantly impaired. IL-10 has received attention as a genetic risk factor for SLE. Although IL-10 can play a protective role in atherosclerotic plaque formation in mice, in SLE, IL-10 serves as an intermediary of the deleterious effects of type 1 interferons to induce endothelial progenitor cell dysfunction. The administration of GW0742 to SLE mice was able to reduce plasma level of IL-10, which might also contribute to improve endothelial function in SLE.

Because of the experimental design used, this study has several limitations, and some questions remain to be explored. First, which genes are activated or repressed to mediate the protective effects of the PPARγ agonist in SLE? Second, does it occur primarily in immune cells or in the vascular endothelium, or is it a combination of both?

In conclusion, our study demonstrates that pharmacological PPARγ activation in SLE mice improves endothelium-dependent relaxation, essentially by preserving the NO-mediated component and reduces SBP. This protective effect may be attributable to a decrease in the vascular oxidative stress by normalizing the expression of NADPH oxidase subunits, as a result of reduced plasma levels of autoantibodies and other plasmatic insults (hyperglycemia and proinflammatory cytokines) and direct anti-inflammatory and antioxidant tissue actions.

Perspectives

SLE treatments have improved over the last decades, resulting in better control of organ damage associated to this
disease. However, current therapies are not completely optimal because they can promote significant side effects, and the premature cardiovascular complications frequently develop in this patient population. Therefore, the search of new strategies that prevent or improve endothelial dysfunction and decrease metabolic abnormalities associated with premature vascular damage in SLE is considered as an important priority in the health care for these patients. Our findings identify PPARβ/δ as a promising target for an alternative approach in the treatment of SLE.

Sources of Funding

This work was supported by Grants from Ministerio de Economía y Competitividad (SAF2010-22066-C02-01, SAF2011-28150, SAF2014-55523-R and AGL2015-67995-C3-3-R), by Junta de Andalucía (Proyecto de excelencia, P12-CTS-2722 and CTS 164) with funds from the European Union (Fondo Europeo de Desarrollo Regional FEDER), and by the Instituto de Salud Carlos III (Red de Investigación Cardiovascular [RIC], RD12/0042/0011, CIBER-ED, Spain). M. Romero is a postdoctoral fellow of RIC, M. Sánchez de Investigación Cardiovascular [RIC], RD12/0042/0011, CIBER-ED, Spain. M. Romero is a postdoctoral fellow of Junta de Andalucía, A. Rodríguez-Nogales is a postdoctoral fellow, and I. Robles-Vera is a predoctoral fellow of the Plan Propio of Investigación, University of Granada, Spain.

Disclosures

None.

References

Novelty and Significance

**What Is New?**

- We found for the first time that chronic peroxisome proliferator activator receptor β/δ activation with GW0742 treatment markedly attenuated lupus disease progression and the cardiovascular complications.

**What Is Relevant?**

- These studies demonstrate that pharmacological peroxisome proliferator activator receptor β/δ activation reduced hepatic opossums and B-cell population with the subsequent decrease in autoimmunity.

- These studies confirm the potentially important underlying role that autoimmunity can have in the pathogenesis of hypertension in systemic lupus erythematosus.

**Peroxisome proliferator activator receptor β/δ activation is a new strategy that improves endothelial dysfunction and decreases metabolic abnormalities associated with premature vascular damage in systemic lupus erythematosus.**

**Summary**

Pharmacological activation of peroxisome proliferator activator receptor β/δ reduced hypertension, endothelial dysfunction, and organ damage in severe lupus mice, which was associated with reduced plasma antibody-damaged DNA autoantibodies and anti-inflammatory and antioxidant effects in target tissues.
Activation of Peroxisome Proliferator Activator Receptor \(\beta/\delta\) Improves Endothelial Dysfunction and Protects Kidney in Murine Lupus

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Hypertension. 2017;69:641-650; originally published online February 27, 2017;
doi: 10.1161/HYPERTENSIONAHA.116.08655

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ACTIVATION OF PEROXISOME PROLIFERATOR ACTIVATOR RECEPTOR 
β/δ IMPROVES ENDOTHELIAL DYSFUNCTION AND PROTECTS KIDNEY 
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Methods

Plasma determinations

At the end of the treatment, mice were killed under isoflurane anesthesia. Blood samples were chilled on ice and centrifuged for 20 min at 5000 g at 4 °C, and the plasma frozen at −70 °C. Plasma glucose, triglycerides, HDL and total cholesterol concentrations were measured by colorimetric methods using Spinreact kits (Spinreact, S.A., Spain). Plasma anti-dsDNA antibodies were measured as described previously (1), and was presented as a positive antibody activity index per the manufacturer’s instructions. Plasma cytokines were measured by a multiplex assay using luminex technology (Merck Millipore, Darmstadt, Germany), and plasma adiponectin by an Elisa Kit (Abcam, Cambridge, UK).

Blood pressure measurements

Systolic blood pressure (SBP) was measured in conscious mice by tail-cuff plethysmography (Digital Pressure Meter LE 5001, Panlab) as described previously (2). Briefly, mice were held in a plastic tube, and their tail was put through a rubber cuff, and the cuff was inflated with air. The pressure level at which the first pulse appeared, after blood flow had been interrupted with the inflated cuff, was designated SBP. At least seven determinations were made in every session and the mean of the lowest three values within 5 mmHg was taken as the SBP. At the end of the experimental period, a polyethylene catheter containing 100U heparin in isotonic, sterile, NaCl solution was inserted into the left carotid artery in some mice. Twenty-four hours after implantation of the catheter, intra-arterial BP and heart rate (HR) were recorded continuously for 60 min with a sampling frequency of 400/s (McLab; AD Instruments, Hastings, UK). BP and HR values obtained during the last 30 min were averaged for intergroup comparisons.

Physical characteristics, cardiac and renal weight indices

Body weight (in grams) was measured for all the mice. At the end of the experimental period, the animals were euthanized and dissected. The hearts were excised; the atria and the right ventricle were then removed and the remaining left ventricles weighed. The left ventricular, liver, spleen, gonadal and mesenteric fat, and kidney weight indices were calculated by dividing their weights by the tibia length. All tissue samples were frozen in liquid nitrogen and then stored at -80ºC.

Assessment of renal injury.

Albuminuria

Mice were placed in metabolic cages to collect urine for 24 h. Albuminuria was determined using a commercially available kit (mouse albumin ELISA kit, Abcam, Cambridge, UK), and the results were expressed as mg of albumin
excreted, normalized by body weight (per 100 g of mice), and time (during 24 h) (3). The means of the values obtained during the 2 experimental days were used for statistical analysis between groups.

**Histopathological techniques**

Kidney biopsies were fixed in 10% buffered formalin for 24 hrs, and embedded in paraffin. Then, 4-μm sections were cut along the central axis of the biopsies and dewaxed and hydrated for staining with hematoxylin-eosin, Peryodic Acid Schiff, and Masson’s trichrome. The presence of SLE-like lesions, such as glomerular sclerosis, mesangial proliferation, glomerular hypercellularity, extracapillar proliferation (crescent formation), capillary wire-loop lesion, fibrinoid necrosis in glomerular capillary and arterioles, interstitial inflammatory infiltrate and tubular casts were studied. Morphological study on light microscopy was done in a blinded fashion (MR and FO). Results were calculated semiquantitatively using a 0 to 3 scale (0, absence; 1, mild [<10% of glomeruli or tubules injured]; 2, moderate [10 to 25%]; 3, severe [>25%]) (2).

**Vascular contractility in vitro**

Descending thoracic aortic rings were dissected from animals and were suspended in a wire myograph (model 610M, Danish Myo Technology, Aarhus, Denmark) for isometric tension measurement as previously described (2). The organ chamber was filled with Krebs solution (composition in mM: NaCl 118, KCl 4.75, NaHCO3 25, MgSO4 1.2, CaCl2 2, KH2PO4 1.2 and glucose 11) at 37 ºC and gassed with 95% O2 and 5% CO2 (pH ∼7.4). Length–tension characteristics were obtained via the myograph software (Myodaq 2.01) and on the basis of these, aorta arteries were loaded to a tension equivalent to 0.5 g.

In endothelium-intact aorta, cumulative concentration-response curves to acetylcholine (10⁻⁹ M - 10⁻⁵ M) were performed in intact rings precontracted by U46619 (10⁻⁸ M) in the absence or in the presence of N⁵-nitro-L-arginine methyl ester (L-NAME, 100 μM), or apocynin (10 μM). The relaxant responses to sodium nitroprusside (10⁻⁹ M-10⁻⁵ M) were studied in the dark in endothelium-denuded vessels precontracted by U46619 (10⁻⁸ M). Relaxant responses to acetylcholine and sodium nitroprusside were expressed as a percentage of precontraction induced by U46619. Aorta was denuded of endothelium by gently rubbing the intimal surface with a needle. The absence of functional endothelium was tested by observing no relaxant response to acetylcholine (10⁻⁶ M).

**In situ detection of vascular reactive oxygen species (ROS) levels**

Unfixed thoracic aortic rings were cryopreserved (phosphate buffer solution 0.1 mol/L, PBS, plus 30% sucrose for 1-2h), included in optimum cutting temperature compound medium (Tissue-Tek; Sakura Finetechnical, Tokyo, Japan), frozen (-80ºC), and 10 μm cross sections were obtained in a cryostat (Microm International Model HM500 OM). Sections were incubated for 30 min in Hepes buffered solution containing dihydroethidium (DHE, 10⁻⁵ M), counterstained
with the nuclear stain 4,6-diamidino-2-phenylindole dichlorohydrate (DAPI, 3 x 10^-7 M) and in the following 24 h examined on a fluorescence microscope (Leica DM IRB, Wetzlar, Germany). Representative DHE staining fields of each slide were photographed at x 400 magnification, and ethidium and DAPI fluorescence were quantified using ImageJ (version 1.32j, NIH, http://rsb.info.nih/ij/). ROS level was estimated from the ratio of ethidium/DAPI fluorescence (2).

Vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity

The lucigenin-enhanced chemiluminescence assay was used to determine NADPH oxidase activity in intact aortic rings, as previously described (4). Aortic rings from all experimental groups were incubated for 30 minutes at 37 °C in HEPES-containing physiological salt solution (pH 7.4) of the following composition (in mM): NaCl 119, HEPES 20, KCl 4.6, MgSO4 1, Na2HPO4 0.15, KH2PO4 0.4, NaHCO3 1, CaCl2 1.2 and glucose 5.5. Aortic production of O2- was stimulated by addition of NADPH (100 μM). Rings were then placed in tubes containing physiological salt solution, with or without NADPH, and lucigenin was injected automatically at a final concentration of 5 μM to avoid known artifacts when used a higher concentrations. NADPH oxidase activity was determined by measuring luminescence over 200 s in a scintillation counter (Lumat LB 9507, Berthold, Germany) in 5-s intervals and was calculated by subtracting the basal values from those in the presence of NADPH. Vessels were then dried, and dry weight was determined. NADPH oxidase activity is expressed as relative luminescence units (RLU)/min/mg dry aortic tissue.

Flow Cytometry

Spleens were collected from mice. The tissues were smashed with wet slides very well to decrease friction and then the solutions were filtered through a cell strainer of 70µM. Cells were isolated followed by lysis of red blood cells with Gey’s solution. 1×10^6 cells were counted and submitted to FcγR blocking by treatment with the incubation in a plate prepared one day in advance with a solution of anti-CD3 (clone17A2, eBioscience) and anti-CD28 (clone 37.51, eBioscience). After 24 hours, a protein transport inhibitor (BD GolgiPlug™), was added to the plate for an optimum detection of intracellular cytokines by flow cytometry. After that, the cells were transferred to polystyrene tubes for the surface staining with mAbs anti-CD4 (PerCP-Cy™, clone RM4-5 BD Pharmigen™) and anti-B220 (APC, clone RA3-6B2, BD Pharmigen™) for 15 min at 4°C in the dark. The splenocytes were then fixed, permeabilized with the Fix/Perm Fixation/Permeabilization kit (eBioscience) and intracellular staining was made with mAbs anti-Foxp3 (PE,clone FJK-16s, eBioscience), anti-IL-17A (PE-Cy7, clone eBio17B7, eBioscience) and anti-IFNγ (Alexa Fluor® 488, clone XMG1.2, eBioscience) for 30 min at 4°C in the dark. Data collection was performed using a flow cytometer CANTO II (BD Biosciences) (2).
Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

For RT-PCR analysis, total RNA was extracted from the aorta, liver and renal cortex by homogenization and converted to cDNA by standard methods. Polymerase chain reaction was performed with a Techne Techgene thermocycler (Techne, Cambridge, UK). A quantitative real-time RT-PCR technique was used to analyze mRNA expression. The sequences of the sense and antisense primers used for amplification are described in Table S1. Preliminary experiments were carried out with various amounts of cDNA to determine nonsaturating conditions of PCR amplification for all the genes studied. Therefore, under these conditions, relative quantification of mRNA was assessed by the RT-PCR method used in this study. The efficiency of the PCR reaction was determined using a dilution series of a standard tissue sample. Quantification was performed using the ∆∆Ct method. The housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GADPH) and Ribosomal protein L13 (RPL13) were used for internal normalization (4).

Western blotting analysis

Aortic homogenates were run on a sodium dodecyl sulphate (SDS)-polyacrilamide electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes (PVDF), incubated with primary rabbit monoclonal anti-p-eNOS-ser-1177 antibody (Cell Signalling Technology, MA, USA), or mouse monoclonal anti-eNOS antibody (Transduction Laboratories, San Diego, California, USA) overnight and with the correspondent secondary peroxidase conjugated antibody. Antibody binding was detected by an ECL system (Amersham Pharmacia Biotech, Amersham, UK) and densitometric analysis was performed using Scion Image-Release Beta 4.02 software (http://www.scioncorp.com) (4). Samples were re-probed for expression of smooth muscle α-actin.

Statistical analysis

Results are expressed as means ± SEM. Statistical analyses were performed using Graph Pad Prism 5 software. A two-factor ANOVA was used to test for drug or group interactions. When a significant interaction was detected, one-way ANOVA with a Student-Newman-Keuls post hoc test was used to discern individual differences between groups. Significance was accepted at p<0.05.

References


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<th>mRNA targets</th>
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<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
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Table S1. Oligonucleotides for real-time RT-PCR.
Table S2. Morphological and plasma determinations in all experimental groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ctrl (n = 8)</th>
<th>Ctrl-GW (n = 8)</th>
<th>SLE (n = 8)</th>
<th>SLE+GW (n = 10)</th>
<th>SLE+GW+GSK (n = 10)</th>
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<td>BW (g)</td>
<td>27.1 ± 0.5</td>
<td>26.9 ± 0.8</td>
<td>32.1 ± 2.4*</td>
<td>31.1 ± 2.5</td>
<td>32.4 ± 1.0</td>
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<td>HW/TL (mg/cm)</td>
<td>60.3 ± 2.1</td>
<td>57.9 ± 1.8</td>
<td>71.9 ± 4.7*</td>
<td>59.4 ± 1.2‡</td>
<td>64.5 ± 1.7¶</td>
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<td>LVW/TL (mg/cm)</td>
<td>39.1 ± 2.1</td>
<td>36.6 ± 0.8</td>
<td>54.1 ± 5.5*</td>
<td>42.1 ± 1.9‖</td>
<td>47.0 ± 1.4‖</td>
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<td>Mesenteric fat/ BW (%)</td>
<td>0.34 ± 0.04</td>
<td>0.38 ± 0.06</td>
<td>0.63 ± 0.15</td>
<td>0.57 ± 0.11</td>
<td>0.57 ± 0.09</td>
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<td>Gonadal fat/BW (%)</td>
<td>0.96 ± 0.17</td>
<td>1.14 ± 0.22</td>
<td>1.28 ± 0.15</td>
<td>1.25 ± 0.24</td>
<td>1.38 ± 0.19</td>
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<td>KW/TL (mg/cm)</td>
<td>77.4 ± 3.3</td>
<td>74.2 ± 3.9</td>
<td>107.0 ± 8.0†</td>
<td>90.7 ± 2.0‖</td>
<td>104.2 ± 5.2‖</td>
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<td>Fasting glucose (mg/dl)</td>
<td>116 ± 15</td>
<td>110 ± 18</td>
<td>261 ± 29†</td>
<td>147 ± 11‖</td>
<td>187 ± 14‖</td>
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<td>Total cholesterol (mg/dl)</td>
<td>17.0 ± 4.9</td>
<td>12.8 ± 1.8</td>
<td>56.5 ± 14.9*</td>
<td>50.9 ± 12.6</td>
<td>43.3 ± 8.5</td>
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<td>Triglyceride (mg/dl)</td>
<td>43.8 ± 3.0</td>
<td>38.7 ± 4.2</td>
<td>38.9 ± 3.1</td>
<td>40.6 ± 3.7</td>
<td>38.7 ± 5.9</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>53.5 ± 7.2</td>
<td>51.6 ± 8.9</td>
<td>63.3 ± 4.1</td>
<td>72.7 ± 2.0‡</td>
<td>54.3 ± 6.6¶</td>
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Results are shown as mean ± SEM. All parameters were assessed in mice treated with vehicle or GW0742 (5 mg kg⁻¹ per day, oral gavage) or GW0742 plus GSK0660 (1 mg kg⁻¹ per day ip). *P < 0.05 and †P < 0.001 vs control; ‡P < 0.05 and ‖P < 0.01 vs SLE; ¶P < 0.05 vs SLE-GW0742 group. BW, Body weight; HW, Heart weight; KW, Kidney weight; LVW, Left ventricular weight; TL, Tibia length
Table S3. Effects on renal injury.

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<tr>
<th>Variables</th>
<th>Ctrl (n = 8)</th>
<th>GW (n = 8)</th>
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<th>SLE+GW (n = 10)</th>
<th>SLE+GW+GSK (n = 10)</th>
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<tr>
<td>Cells Nº/glomerulus</td>
<td>26.7±1.6</td>
<td>25.6±2.3</td>
<td>54.8±3.2†</td>
<td>38.2±1.9‖</td>
<td>55.9±4.2#</td>
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<td>Glomerulosclerosis (%)</td>
<td>0.13±0.18</td>
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<td>2.33±0.58†</td>
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<td>Crescents (%)</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>2.77±1.13†</td>
<td>3.22±1.98</td>
<td>1.00±0.73</td>
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<td>Immunocomplex (%)</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>20.9±5.1†</td>
<td>8.2±1.6†</td>
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<td>Mesangioproliferation (%)</td>
<td>2.5±1.7</td>
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<td>24.1±5.5*</td>
<td>11.3±3.4</td>
<td>18.0±9.6</td>
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<td>Tubular casts**</td>
<td>0.20±0.14</td>
<td>0.43±0.22</td>
<td>1.88±0.31†</td>
<td>0.87±0.28‡</td>
<td>1.53±0.25‖</td>
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<td>Inflammatory infiltrate**</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>1.66±0.17†</td>
<td>1.77±0.15</td>
<td>1.62±0.57</td>
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Values are expressed as mean±standard error mean. *P<0.05 and †P<0.01 control group versus systemic lupus erythematosus (SLE) group Bonferroni test. ‡P<0.05 and ‖P<0.01 SLE group vs. SLE+GW0742 (GW) group; §P<0.05 and #P<0.01 SLE-GW group vs. SLE+GW+GSK0660 (GSK) group Bonferroni test. ** semiquantitative scale (0-3).
Figure S1. Effects of chronic PPARβ/δ activation on hepatic mRNA levels of PPARs (A, B, C), and cytokines (D) measured by RT-PCR in control (Ctrl) and systemic lupus erythematosus (SLE) mice. Experimental groups: Ctrl (n = 8), GW (GW0742, 5 mg/kg/day by oral gavage, n = 8), SLE (n = 8), SLE+GW (n = 10), SLE-GW+GSK (GSK0660 1 mg/kg/day intraperitoneally plus GW0742 orally, n = 10). Values are expressed as mean ± SEM. **p<0.01, and *p<0.05 vs control group. ##p<0.01 and #p<0.05 vs SLE group. ++p<0.01 and +p<0.05 vs SLE+GW group.
Figure S2. Effects of chronic PPARβ/δ activation on plasma cytokines measured by ELISA in control (Ctrl) and systemic lupus erythematosus (SLE) mice. Experimental groups: Ctrl (n = 8), GW (GW0742, 5 mg/kg/day by oral gavage, n = 8), SLE (n = 8), SLE+GW (n = 10), SLE-GW+GSK (GSK0660 1 mg/kg/day intraperitoneally plus GW0742 orally, n = 10). Values are expressed as mean ± SEM. **p<0.01, and *p<0.05 vs control group. #p<0.05 vs SLE group. +p<0.05 vs SLE+GW group.
Figure S3. Effects of chronic PPARβ/δ activation on renal cortex mRNA levels of PPARs (A, B, C) and PPARβ/δ-target genes CPT-1, UCP-2 and PDK-4 (D) measured by RT-PCR in control (Ctrl) and systemic lupus erythematosus (SLE) mice. Experimental groups: Ctrl (n = 8), GW (GW0742, 5 mg/kg/day by oral gavage, n = 8), SLE (n = 8), SLE+GW (n = 10), SLE-GW+GSK (GSK0660 1 mg/kg/day intraperitoneally plus GW0742 orally, n = 10). Values are expressed as mean ± SEM. *p<0.05 vs control group. #p<0.05 and ##p<0.01 vs SLE group. +p<0.05 and ++p<0.01 vs SLE+GW group.
**Figure S4.** Effects of chronic PPARβ/δ activation on aortic mRNA levels of PPARβ/δ (A), and PPARβ/δ-target genes (B, C, D) measured by RT-PCR in control (Ctrl) and systemic lupus erythematosus (SLE) mice. Experimental groups: Ctrl (n = 8), GW (GW0742, 5 mg/kg/day by oral gavage, n = 8), SLE (n = 8), SLE+GW (n = 10), SLE-GW+GSK (GSK0660 1 mg/kg/day intraperitoneally plus GW0742 orally, n = 10). Values are expressed as mean ± SEM. *p<0.05 vs control group. **p<0.01 and *p<0.05 vs SLE group. **p<0.01 and *p<0.05 vs SLE+GW group.
Figure S5. Effects of chronic PPARβ/δ activation on mRNA levels of NADPH oxidase subunits in aortic rings. Experimental groups: Ctrl (n = 8), GW (GW0742, 5 mg/kg/day by oral gavage, n = 8), SLE (n = 8), SLE+GW (n = 10), SLE-GW+GSK (GSK0660 1 mg/kg/day intraperitoneally plus GW0742 orally, n = 10). Values are expressed as mean ± SEM. **p<0.01 vs control group. ##p<0.01 and #p<0.05 vs SLE group. ++p<0.01 and +p<0.05 vs SLE+GW group.