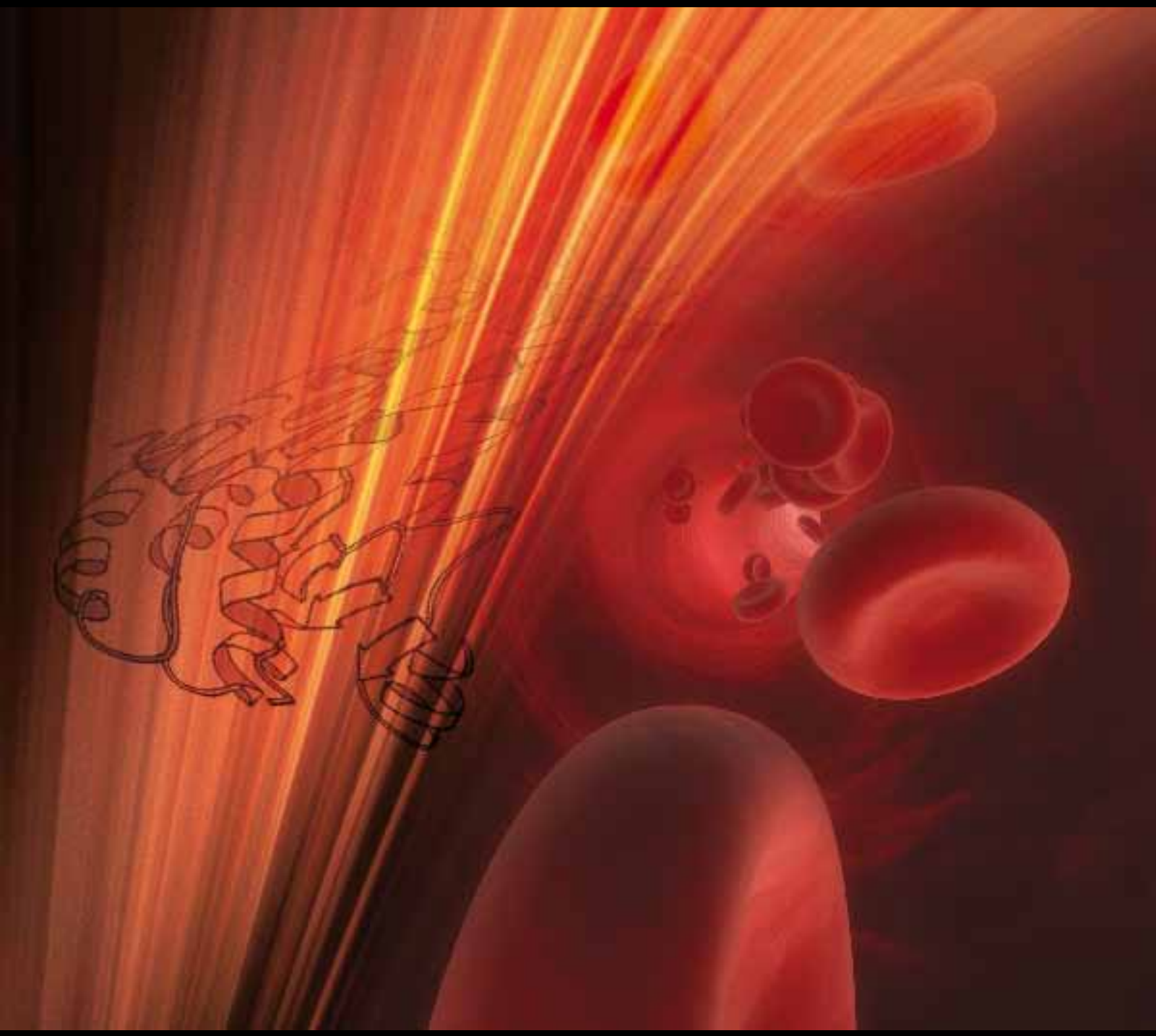
PPAR Research

The Interplay of PPARs with Parasites and Related Intracellular Pathogens

Guest Editors: Marion M. Chan and Dunne Fong



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Editorial Board

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Editorial

The Interplay of PPARs with Parasites and Related Intracellular Pathogens

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The objective of this special issue is to present current findings on how PPARs can tilt the delicate balance between host defense and parasite survival to affect the outcome of parasitic infections. The volume contains five comprehensive reviews and one original research article that describe the mechanisms of action of PPARs on several parasitic diseases which infect differently. The pathogens include the malaria parasite *Plasmodium falciparum* which invades hepatocytes and erythrocytes, the *Leishmania* species which infect macrophages, the intracellular protozoan *Trypanosoma cruzi* which invades all tissues, and the helminth *Schistosoma* species, which live in extracellular environment of specific organs.

Whereas it is well known that many parasitic diseases are exacerbated by the activation of T helper 2, recent research on the anti-inflammatory alternatively activated macrophage (AAM), which is activated by PPARγ, shows that there is another facet to the host immune response [1]. PPARγ primes the differentiation of macrophages towards AAM rather than the proinflammatory classically activated macrophage (CAM) phenotype, by promoting arginase while suppressing inducible nitric oxide synthase expression. This activity favors the survival of pathogens, which may be susceptible to the free radical nitric oxide. On the other hand, PPARγ activation becomes beneficial to the host in malaria. This special issue presents studies that, timely, characterize the interaction between parasites and these macrophages which express the PPAR nuclear factors.

Malaria is the most devastating among diseases of parasitic protozoa. L. Serghides explains how PPAR agonists relieve immunopathology in cerebral malaria. PPARγ-mediated transcription of CD36, a scavenger receptor which enhances phagocytosis and facilitates the removal of parasitized erythrocytes, leads to reduced parasitemia. In addition, the anti-inflammatory action of PPARγ protects the central nervous system from inflammation-mediated destruction. Y. Ren concurs that the strategy to augment the upregulation of CD36 is potentially therapeutic. Using PPARγ agonist rosiglitazone as adjunctive therapy during treatment of cerebral malaria has successfully gone through phase I/IIa trial in Thailand and now awaits a final randomized double-blind placebo-controlled clinical trial.

On the contrary, PPAR activation may benefit the parasite in other infections. For protozoan parasites, trypanosomatids set as examples. M. Chan et al. describe how the activation of PPARα and PPARγ by cutaneous and visceral *Leishmania* species may promote their survival within macrophages. E. Hovsepian et al. also discuss how *Trypanosoma cruzi*-mediated PPAR activation influences intracellular parasite survival. For metazoan parasites, B. Anthony et al. review how the *Schistosoma* species activate PPARα, PPARγ, and AAM to potentiate survival.

The PPARγ agonist rosiglitazone has been shown to possess anti-inflammatory, neuroprotective and neuroregenerative properties. However, although rosiglitazone (drug name Avandia, from GlaxoSmithKline) is still being used to treat type 2 diabetes in the United States, the drug is withdrawn in Europe because of adverse effects, especially to the heart. Pioglitazone may be a better alternative. Extending from rosiglitazone and PPARγ, K. Chen et al. report the protective activity of Wy14643, an agonist of PPARα, the isoform prevalent in the liver, towards hypoxia reoxygenation injury in rodent hepatocytes.

Many parasitic diseases are widespread in developing countries. Historically, they have been regarded as "neglected tropical diseases" [2]. With parasitic infections being increasingly diagnosed in developed countries due to global travel and immigration, their control is being pursued by many investigators [3, 4]. Public and private agencies, such as TDR, Special Programme for Research and Training in Tropical Diseases of World Health Organization, and Bill & Melinda Gates Foundation, using arrangements such as public-private partnerships, are determined to target such diseases of poverty. Scientists from many countries are actively researching various aspects of parasitic diseases. With this background, we sincerely thank the international cast of scientists who have contributed to this special issue on PPARs and parasites. They come from different countries: Argentina, Australia, Canada, China, United Kingdom, and United States, covering the continents Australia, Europe, North, and South America.

In summary, this special issue provides a glimpse of our contemporary understanding on PPAR involvement in parasitic diseases. Different angles have been explored, for example, while PPAR agonists may decrease immunopathology of cerebral malaria, they may enhance parasite survival in leishmaniasis. We hope the readers will find this special issue of *PPAR Research* informative and will be inspired to make their own contributions to the challenge our world faces from the diverse parasitic infections.

Acknowledgments

We are grateful to Dr. Mostafa Badr, former Editor-in-Chief of *PPAR Research*, for his invitation to Marion to organize this special issue and to Hindawi Publishing Corporation staff, whose resourceful and patient assistance are much appreciated.

Marion M. Chan
Dunne Fong

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Review Article

Role of PPARs in *Trypanosoma cruzi* Infection: Implications for Chagas Disease Therapy

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Chagas disease, which is caused by *Trypanosoma cruzi* (*T. cruzi*), remains a substantial public health concern and an important cause of morbidity and mortality in Latin America. *T. cruzi* infection causes an intense inflammatory response in diverse tissues by triggering local expression of inflammatory mediators, which results in the upregulation of the levels of cytokines and chemokines, and important cardiac alterations in the host, being one of the most characteristic damages of Chagas disease. Therefore, controlling the inflammatory reaction becomes critical for the control of the proliferation of the parasite and of the evolution of Chagas disease. The nuclear receptors known as peroxisome proliferator-activated receptors (PPARs) have emerged as key regulators of lipid metabolism and inflammation. The precise role of PPAR ligands in *T. cruzi* infection or in Chagas disease is poorly understood. This review summarizes our knowledge about *T. cruzi* infection as well as about the activation of PPARs and the potential role of their ligands in the resolution of inflammation, with the aim to address a new pharmacological approach to improve the host health.

1. Introduction

Chagas disease is widely distributed throughout Latin America, thus causing a serious public health problem. It is considered the parasitic disease that leads to the greatest economic burden in Latin America due to its prolonged chronicity. Reasonably, public control programs normally focus their resources and strategies on the elimination of insect vectors associated with human habitat and relegate the infected patient. Moreover, migration from rural to urban areas has changed the epidemiology of the disease. While in the 1930's 70% of Latin Americans lived in rural areas, currently about 70% live in urban areas. The infection was primarily rural and became urban transmissible by blood transfusion. In recent decades, the number of donors with positive serology has increased in endemic countries. Thus, there is a need for new strategies to prevent or stop the cardiac consequences of *T. cruzi* infection, with an affordable cost to the health system and patients. The host responds to invasion by the activation of inflammation and induction of innate and specific immunity. The infectious inflammatory myocarditis generated by *T. cruzi* induces an inflammatory response that affects the heart tissue and function. Moreover, the immune system can act with autoimmune reaction with infiltration of macrophages and/or cell-damaging attack. Therefore, the anti-inflammatory actions of peroxisome proliferator-activated receptors (PPARs) γ and α have received great attention because of the availability of synthetic PPAR activators. PPAR ligands emerge as attractive drug targets for lipid and glucose metabolism as well as for inflammation resolution. The aim of this article is to review the role of PPARs in *T. cruzi* infection and their potential contribution to inflammation resolution.

2. Chagas Disease

Chagas disease, also called American trypanosomiasis, is a chronic and systemic parasitic infection caused by the protozoan *Trypanosoma cruzi*. This parasite was discovered

in 1909 by the Brazilian physician Carlos Chagas (1879–1934), who described in detail the cycle of transmission and the human clinical manifestations.

This infectious disease is endemic throughout Central and South America and is still recognized by the World Health Organization (WHO) as one of the most important ignored tropical diseases and as a significant public health problem. In recent decades, the increased rate of emigration from Chagas-endemic countries to the United States, Canada, and the European Union has become a new concern for the WHO [1].

T. cruzi has several instances of transmission to humans and other susceptible hosts, mainly through contact with the feces of infected blood-feeding insect vectors. However, alternative routes such as blood transfusion, organ transplant, congenital transmission and oral transmission have also been determined [2].

The clinical course of the infection has two phases: an acute and a chronic. The acute phase is characterized by evident parasitemia and parasitism in a wide variety of host cells. This phase can be confused with other infections since symptoms such as fever or hepatomegaly and/or splenomegaly are shared by different infectious diseases. Most of the patients that survive the acute phase remain in a life-long asymptomatic state (indeterminate form) during the chronic phase of infection [3].

During the acute phase of infection, most individuals usually have mild symptoms such as fever, which do not need medical attention, with few or no parasites found in circulation. Symptoms of acute infection may last up to a few weeks or months. Although the infection then remains largely asymptomatic, often for years or even decades, 30% of patients develop chronic Chagas disease [4].

Symptoms of the acute phase resolve spontaneously in about 90% of infected patients even if the infection is not treated with trypanocidal drugs. About 60–70% of these patients will never develop clinically apparent disease. These patients have the indeterminate form of chronic Chagas disease. The remaining 30–40% of patients will develop a form of chronic disease characterized by progression to cardiac disease, gastrointestinal disease, or both, over a period of years to decades [3, 5, 6].

The cardiomyopathy in South and Central America develops manifestations like cardiac arrhythmias, apical aneurysms, congestive heart failure, thromboembolism, and sudden cardiac death in disease-endemic areas [7]. As expected, Chagas disease can be reactivated in patients with HIV/AIDS or subject to chemotherapy [8].

It has been described that Chagas disease is typified by a chronic inflammatory process that causes damage to the myocardium as well as to the conduction system. The pathogenesis may involve several mechanisms, including immunologically mediated tissue damage, cardiac dysfunction, and coronary microvascular disease [9]. There are substantial evidences showing that cardiac tissue, an important target of *T. cruzi,* produces marked amounts of proinflammatory cytokines, chemokines, and enzymes including inducible nitric oxide (NO) synthase (NOS2) and metalloproteinases (MMPs), resulting in inflammation and cardiac remodeling in response to parasite infection [10–12].

3. The Peroxisome Proliferator-Activated Receptors (PPARs) Family

PPARs are members of the nuclear receptor superfamily of ligand-activated transcription factors. The PPAR subfamily (NR1C) includes PPAR-α (NR1C1), PPAR-β (also called PPAR-δ) and NUC1, NR1C2, and PPAR-γ (NR1C3) [13], each with different ligands, target genes, and biological role. Most of these PPARs share a similar structure, which includes an amino-terminal activation domain (AF-1), a DNA-binding domain, a ligand-binding domain, and a second carboxy-terminal activation domain (AF-2) [14]. In response to ligand binding, these receptors change their conformational structure recruiting coactivators and freeing corepressors. The PPAR family not only regulates metabolic processes but also participates in extrametabolic processes, including direct activation of genes, ligand-independent repression, ligand-dependent repression, and transrepression [15].

Nuclear receptors can be activated by ligand-dependent or -independent mechanisms. PPARs are activated by xenobiotics as well as by endogenous fatty acids and their metabolites. PPARs activate the transcription of their target genes as heterodimers with retinoid X receptors (RXRs), which are activated by 9-cis retinoic acid [16, 17]. Eicosanoids are some of the endogenous ligands that bind to the PPAR-RXR complex, leading to conformational changes, freeing the corepressor, and thus binding to the response element of target genes [15] (Figure 1). PPARs have been cloned in several species, including rodents, amphibians, teleosts, cyclostomes, and even humans. The PPAR subtypes (α, β, and γ) are expressed differently according to the tissue but may also be coexpressed in different relative concentrations [18].

3.1. PPAR-α. PPAR-α was identified in the early 1990s on the basis of it being a target of hypolipidemic fibrate drugs and other compounds that induce peroxisome proliferation in rodents [19]. PPAR-α is expressed in cells that have active fatty acid oxidation like hepatocytes, cardiomyocytes, enterocytes, smooth muscle cells, and kidney cells and has been implicated in the regulation of cellular energetic processes. It has been shown that PPAR-α ligands, such as fibrates, decrease triglyceride levels and reduce the incidence of cardiovascular events and atherosclerosis [20]. The first evidence indicating a potential role for PPARs in the inflammatory response was the demonstration that leukotriene B4, a proinflammatory eicosanoid, binds to PPAR-α and induces the transcription of genes involved in ω- and β-oxidation [21]. It has been described that PPAR-α is expressed in human and mouse immune cells, including lymphocytes, macrophages, and dendritic cells, and numerous studies have implicated PPAR-α in the negative regulation of inflammatory responses. Different works using PPAR-α ligands have shown a reduction in the symptoms of inflammation and disease in several models, including models of allergic airway disease, arthritis, and inflammatory intestine disease [22].

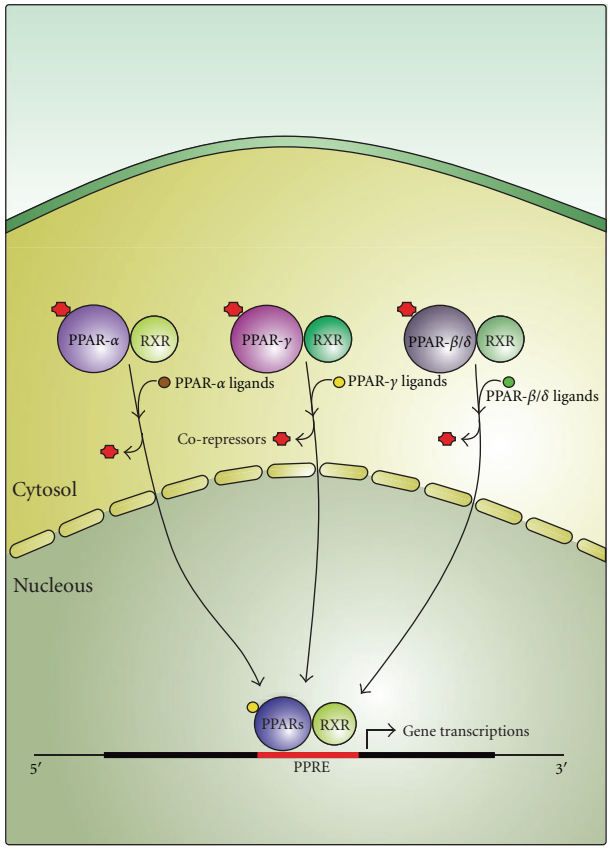

Figure 1: A proposed mechanism of ligand-mediated activation of PPARs. In response to ligand binding, PPARs undergo conformational changes in protein structure. This allows dissociation of corepressor proteins which inhibit transcription and the recruitment of co-ac-tivators. PPAR-RXR heterodimers bind to specific recognition sites of DNA, termed peroxisome proliferator activated receptor response elements (PPREs) located in the regulatory region of target genes.

Moreover, the role of PPAR-α in the heart has been shown with regards not only to the governing of myocardial energy metabolism and function (using both gain-of-function and loss-of-function murine models) but also to extrametabolic activities such as anti-inflammatory activities (see [23, 24], respectively, for a review). There are many works that have further implicated PPAR-α as an important regulator of inflammatory disease. For instance, it has been demonstrated that PPAR-α activators inhibit IL-1β-induced IL-6 secretion by human aortic smooth muscle cells in a dose-dependent manner [25]. PPAR-α activators also negatively regulate IL-1β-induced-IL-6 production at the gene expression level by inhibiting NF-κB transcriptional activity [26]. In addition, other mechanisms, including alterations in cytokine-receptor and growth-factor receptor signaling, and the upregulation of the expression of a subunit of the inhibitor of NF-κB (IκB), have been reported (see [24] for a review).

3.2. PPAR-β/δ. PPAR-β, also known as PPAR-δ, is expressed ubiquitously and often at higher levels than PPAR-α and PPAR-γ, suggesting a fundamental role for it in many tissues. PPAR-δ was first identified in *Xenopus laevis* [27], and the mouse and human receptors were subsequently cloned on the basis of sequence similarity with PPAR-α [17]. PPAR-δ target genes in metabolic tissues are broadly involved in fatty acid metabolism, mitochondrial respiration, and thermogenesis. An in vitro study in endothelial cells has indicated that PPAR-β/δ ligands inhibit TNFα-induced upregulation of the expression of VCAM-1, MCP-1, and NF-κB translocation [28]. The role of PPAR-δ in the modulation of inflammation is poorly understood. It has been proposed that, in macrophages, PPAR-β/δ also controls inflammation by its association and disassociation with the transcriptional repressor BCL-6. It has also been described that the loss of hematopoietic PPAR-δ expression protects against atherosclerosis, being the proatherogenic effects of PPAR-δ due in part to the influence of PPAR-δ on the basal expression of inflammatory mediators in the arterial wall. It has also been found that PPAR-$\delta^{-/-}$ bone-marrow-derived macrophages show reduced expression of CCL2, matrix metalloproteinase 9 (MMP9), and IL-1β and that the ligand binding to PPAR-δ releases BCL-6, resulting in the repression of inflammatory gene expression [29].

3.3. PPAR-γ. PPAR-γ is the most studied member of the PPAR family. Two distinct isoforms of PPAR-γ (PPAR-γ1 and PPAR-γ2), which are derived from the same gene but arise by differential transcription start sites and alternative splicing, have been described [30]. This receptor has been cloned from a number of species, including mice, hamsters, frogs, pigs, monkeys, and humans [17, 27, 31]. PPAR-γ has a prominent expression in brown and white adipose tissue, the colon, differentiated myeloid cells and the placenta [32]. Brown and white adipocyte tissues are major sites for PPAR-γ expression. In 1995, Greene et al. identified two transcripts corresponding to a full-length mRNA and a short form devoid of functional domains [31]. More recent studies have demonstrated that the full-length PPAR-γ is indeed expressed in activated T and B cells and monocytes/macrophages [33–35].

The main physiological function of PPARs is the modulation of the expression of specific target genes [36]. PPAR-γ is critical for the differentiation of preadipocytes to adipocytes and also participates in glucose metabolism homeostasis [37, 38]. PPAR-γ can be activated by several physiological ligands, such as docosahexaenoic acid, linoleic acid, and some synthetic ones like antidiabetic glitazones, which are used as insulin sensitizers. Other ligands include oxidized LDL, azoyle PAF, and eicosanoids, such as 5,8,11,14-eicosatetraenoic acid and the prostanoids PGA_1, PGA_2, PGD_2, and the dehydration products of the PGJ series of cyclopentanones, for example, 15-deoxy-Δ12,14-PGJ2 (15dPGJ2) [39]. In particular, the last one is recognized as an endogenous ligand for the intranuclear receptor PPAR-γ being responsible for many anti-inflammatory functions (see [40] for a review). However, previous studies have reported that PPARs inhibit inflammatory gene expression by several mechanisms, including direct interactions with AP1 and NF-κB [41–43], nuclear cytoplasmic redistribution of the p65

subunit of NF-κB [44], modulation of p38 mitogen-activated protein kinase activity [45], competition for limiting pools of coactivators [46], and interactions with transcriptional corepressors [29] (see [47] for a review).

PPAR-γ has been reported to regulate inflammatory responses, both in vivo and in vitro, being involved in the regulation of macrophages and endothelial cells, both crucial to the inflammatory response. The presence of PPAR-γ in macrophages was first described in studies using human atheromas [48–51]. A role for PPAR-γ in T-lymphocyte regulation has also been described [52]. Furthermore, PPAR-γ is involved in the differentiation and activation of monocytes and in the regulation of their inflammatory responses. Previous works have suggested that different stimuli increase monocytes/macrophages PPAR-γ expression [53, 54]. Anti-inflammatory effects of macrophage PPAR-γ activation have been demonstrated in a number of studies [55, 56].

The roles of 15dPGJ2 and PPAR-γ in the regulation of human autoimmune diseases or in animal models of autoimmunity have been studied by several groups.

15dPGJ2 and other PPAR-γ ligands inhibit inflammation in models of arthritis [57–59], ischemia-reperfusion injury [60], inflammatory bowel disease (IBD) [61–63], Alzheimer's disease [64–66], and lupus nephritis [67, 68].

4. Can PPAR Promote or Prevent the Inflammatory Response after *T. cruzi* Infection?

Chagas disease is caused by infection with the protozoan kinetoplastid parasite *Trypanosoma cruzi*. Acute *T. cruzi* infection is accompanied by an intense inflammatory reaction in many tissues, being usually asymptomatic. When symptoms occur, they include prolonged fever, enlargement of the liver, spleen, and lymph nodes, subcutaneous edema (chagoma) or edema of the ocular mucous membranes (Romaña's sign).

There are substantial evidences showing that the cardiac tissue is an important target of *T. cruzi* infection. Controlling the inflammatory reaction is critical for the control of the parasite proliferation in all the tissues, especially in the heart since it may progress to fibrosis and remodeling, resulting in a dilated cardiomyopathy accompanied by myocardial dysfunction [69]. In the context of inflammatory response, in a review of parasitic infections, Chan et al. (2010) argue that PPAR activation might favor the establishment of a chronic parasitic infection, making symbiotic survival between the host and parasite more probable [70]. In this sense, the adipose tissue has been identified as one of the main sites of inflammation during Chagas disease progression when cultured adipocytes are infected with the Tulahuen strain of *T. cruzi,* demonstrating an increase in the expression of proinflammatory mediators [71]. Fnu Nagajyothi et al. (2008) described an infection-associated decrease in adiponectin and PPAR-γ in infected adipocytes. They also showed that PPAR-γ is highly expressed in adipose tissue and that, together with the adipokine adiponectin, represses the inflammatory process although the mechanism by which adiponectin exerts an anti-inflammatory effect is unclear [71]. However, it is clear that a reduction in the level of adiponectin is associated with an increase in inflammation and that there is an inverse relationship between PPAR-γ and inflammation [71]. Another group of researchers has recently described in a mouse model of infection with the Colombian strain of *T. cruzi* (MHOM/CO/00/Colombian) that the treatment with 15dPGJ2 reduces the inflammatory infiltrate in the skeletal muscle at the site of infection and decreases the number of lymphocytes and neutrophils in the blood. These researchers also found that 15dPGJ2 also decreases the relative volume density of parasitic nests in cardiac muscle [72]. Many works have described that PPAR-α and PPAR-γ agonists play an important role in regulating inflammation in different models in vivo and in vitro. Recently, the role of rosiglitazone, a PPAR-γ synthetic agonist, in the modulation of the innate immune response has been demonstrated in an experimental cerebral malaria model (Reviewed in [70]). Also, it has been reported that rosiglitazone together with antischistosomal drugs improves the symptoms of liver fibrosis induced by *Schistosoma japonicum* in mice [70]. Besides, by linking metabolism and immunity, Gallardo-Soler et al. proposed that PPAR activity induces macrophage activation toward a more Th2 immune phenotype in a model of *Leishmania major* infection. These authors showed that PPAR-γ and PPAR-δ ligands promote intracellular amastigote growth in infected macrophages, and that this effect is dependent on both PPAR expression and arginase activity, suggesting that Arginase I is a key marker of the alternative program triggered by PPAR in macrophages [73].

Trypanosoma cruzi infection causes an intense inflammatory response in diverse tissues, including the heart. The inflammatory reaction is critical for the control of the parasites' proliferation and evolution of Chagas disease. 15dPGJ2 can repress the inflammatory response in many experimental models. However, the precise role of PPAR-γ ligands in *T. cruzi* infection is poorly understood. Hovsepian et al. (2011) have recently reported the first evidence that 15dPGJ2 treatment increases the number of intracellular parasites and inhibits the expression and activity of different inflammatory enzymes such as inducible nitric oxide synthase (NOS2), matrix metalloproteinases 2 and 9 (MMP-2, MMP-9), as well as proinflammatory cytokine (TNF-a and IL-6) mRNA expression in neonatal mouse cardiomyocytes after *T. cruzi* infection [12] (Figure 2). They also observed that transfection of cardiomyocytes with small interfering RNA (siRNA) induces silencing of PPAR-γ, impairing the effects of 15dPGJ2 on the modulation of proinflammatory enzymes. In addition, they found that transfection restores the ability of these cells to control the intracellular growth of *T. cruzi* [12]. Like other nuclear receptors, PPARs are phosphoproteins and their transcriptional activity is affected by crosstalk with kinases and phosphatases. In addition, 15dPGJ2 can act in a ligand-dependent or -independent manner through mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) [74]. Hovsepian et al. (2011) demonstrated in *T. cruzi*-infected neonatal cardiac cells that PPAR-γ-independent pathways are involved, since

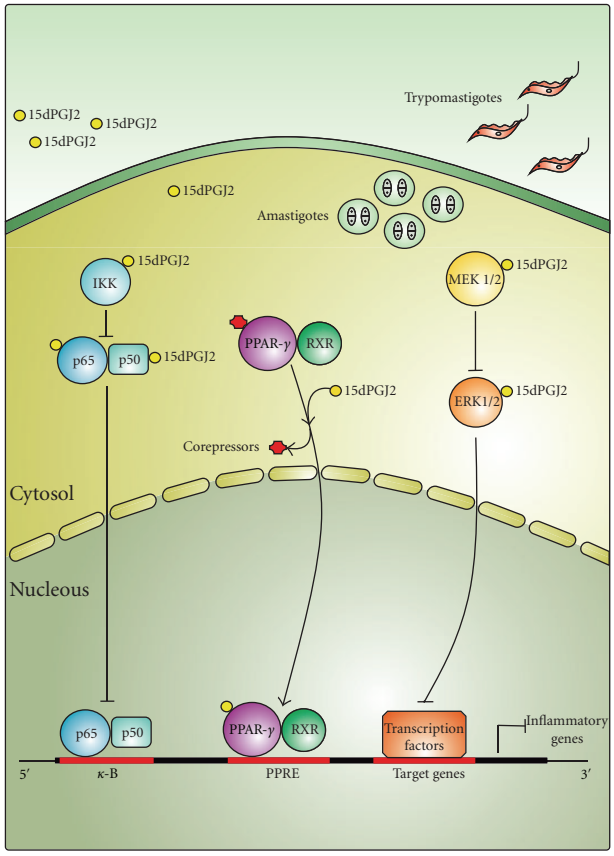

Figure 2: Anti-inflammatory actions of 15dPGJ2 in *T. cruzi*-infected cardiomyocytes. 15dPGJ2 can exert its effects by binding to PPAR-γ or through interaction with intracellular targets like NF-κB-signaling pathway and Erk MAP kinase cascade. By PPAR-γ-dependent mechanisms, the 15dPGJ2-PPAR-γ complex forms a heterodimer with nuclear retinoid X receptor (RXR) to recognize PPAR-response elements (PPREs) in the promoter region of the target genes thereby stimulating their transcription. In the cytosol, 15dPGJ2 can also bind specific residues in IKK, p50, or p65 of the NF-κB-signaling pathway, or MEK1/2 and Erk1/2 in the MAP kinase pathway leading to functional inactivation of inflammatory target genes. The consequent inhibition of inflammatory factors/mediators by 15dPGJ2 promotes an increase in the number of intracellular parasites.

15dPGJ2 also exerts its effect through extracellular signal-regulated kinases-mitogen-activated protein kinase (Erk-MAPK) and NF-κB. The use of specific pharmacological inhibitors confirmed these findings [12] (Figure 2). Our group has recently found evidence about the role of 15dPGJ2 in the regulation of inflammation parameters in a mouse-experimental model of *T. cruzi* infection, confirming all the results assayed in neonatal cardiomyocytes (data not shown, sent manuscript). In this manuscript, we report evidences that 15dPGJ2 treatment inhibits TNFα and IL-6 mRNA levels as well as the expression and activity of inflammatory enzymes like NOS2 and MMP-2. We found that 15dPGJ2 participates in both parasitemia and amastigote nest regulation in hearts of infected mice and that it does not modify the mortality rate in acute infection. In the presence of GW9662, a specific PPAR-γ antagonist, 15dPGJ2 partially inhibited NOS2 expression and MMP-2 activity, denoting the participation of some other signaling pathway. We also found that NF-κB was activated by means of p65 nuclear translocation in hearts of infected mice and inhibited after 15dPGJ2 treatment. These results highlight that both PPAR-γ and NF-κB are implicated in the inhibitory effects of 15dPGJ2 on the inflammatory response of the heart in an acute model of *T. cruzi* infection (data not shown).

5. Perspectives

To date, the accurate role of PPAR ligands in *T. cruzi* infection remains essentially unexplored. In this sense, some authors argue that the expression of PPAR-γ decreases after *T. cruzi* infection, while others argue that this expression increases. However, in general, most research groups highlight the role of PPARs in resolving inflammation and collaborating with tissue repair in some cases. Therefore, we hope that PPARs can potentially contribute to address a new pharmacological approach to improve the host health.

Authors' Contribution

E. Hovsepian and F. Penas contributed equally in the paper.

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Review Article

Peroxisome Proliferator-Activated Receptor-γ-Mediated Polarization of Macrophages in *Leishmania* Infection

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Infection is the outcome of a contest between a pathogen and its host. In the disease leishmaniasis, the causative protozoan parasites are harbored inside the macrophages. *Leishmania* species adapt strategies to make the infection chronic, keeping a balance between their own and the host's defense so as to establish an environment that is favorable for survival and propagation. Activation of peroxisome proliferator-activated receptor (PPAR) is one of the tactics used. This ligand-activated nuclear factor curbs inflammation to protect the host from excessive injuries by setting a limit to its destructive force. In this paper, we report the interaction of host PPARs and the pathogen for visceral leishmaniasis, *Leishmania donovani*, *in vivo* and *in vitro*. PPAR expression is induced by parasitic infection. Leishmanial activation of PPARγ promotes survival, whereas blockade of PPARγ facilitates removal of the parasite. Thus, *Leishmania* parasites harness PPARγ to increase infectivity.

1. Leishmaniasis

Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania.* The disease is found worldwide, with an estimated prevalence of 12 million cases, 50,000 annual deaths, and 350 millions of the world's population at risk [1]. *Leishmania* has two stages in its life cycle: flagellated promastigotes that live within the alimentary canal of the insect vector and amastigotes that multiply within the phagolysosomes of mammalian macrophages. Infected female sandflies introduce saliva and promastigotes into the mammalian host during blood meals. The promastigotes are taken by leukocytes and differentiate into intracellular amastigotes within the macrophages. Then, infected macrophages carry the parasites to different organs. Over twenty species are known to infect humans. The cutaneous species reside and multiply within the skin tissue, whereas the visceral species predominantly accumulate in the liver, spleen, and bone marrow. These diverse species cause different clinical manifestations, varying from self-healing or metastasizing skin lesions to enlargement of visceral organs including the liver and spleen. The disease symptoms are classified as cutaneous, mucocutaneous, or visceral leishmaniasis.

2. Resistance versus Susceptibility

Immunity against all species of *Leishmania* uniformly relies on a type 1 immune response that produces interferon γ (IFNγ). Produced by T helper 1 cells, IFNγ activates macrophages to generate nitric oxide (NO), a free radical that can kill *Leishmania*. Type 2 immune response, on the other hand, is ineffective [2]. Production of interleukin-4 (IL-4) in *Leishmania major* infection, regulatory T cells in *L. mexicana* infection, and IL-10 in infection of various species are all associated with susceptibility [2–6]. It is well established that IL-4 exacerbates leishmaniasis when added exogenously, and IL-10 mutant mice become resistant to infection. However, to date, the reason why this cytokine promotes the pathogenesis of *Leishmania* infection remains partially understood [7–9].

3. M1 versus M2 in Disease Pathogenesis

Macrophages, the host of *Leishmania* parasites, are markedly heterogeneous. When stimulated by IFNγ, these macrophages differentiate into the classically activated (M1) phenotype, with inducible nitric oxide synthase (iNOS) which produces NO from arginine. Intracellular *Leishmania* parasites are eliminated by this subpopulation. On the contrary, IL-4 differentiates macrophages towards the alternatively activated (M2) phenotype, which promotes humoral immunity and tissue repair. This subpopulation produces IL-10, and transforming growth factor-β (TGF-β), [10].

In terms of signaling, IL-4 induces the expression of PPARγ and PPAR gamma coactivator-1 (PGC-1) β protein through the STAT-6 pathway [12]. This nuclear regulator polarizes the monocytes into alternatively activated (M2) macrophages with anti-inflammatory properties. By its transcriptional activity, it mediates the expression of arginase-1 (Arg1) and CD36 [13, 14]. Arginine metabolism away from production of NO compromises the ability of infected macrophage to clear the intracellular pathogens [2, 11]. CD-36 is a scavenger receptor that mediates phagocytosis and facilitates the removal of apoptotic cells. By its transrepressive action, PPARγ blocks the expression iNOS as well as nuclear factor kappa B (NFκB)-mediated transcription of proinflammation mediators [15].

4. *Leishmania donovani* Infection Induces Host PPAR Gene Expression *In Vivo* and *In Vitro*

Our laboratory has been studying the pathogenesis of *Leishmania*, with particular interest in *L. donovani*. We investigated whether susceptibility to infection is associated with the activation of PPAR [16]. Mice of the susceptible BALB/c strain were infected with stationary phase promastigotes of *L. donovani*. After four weeks, their liver and spleen were excised, and PPARα and γ mRNA levels were analyzed, using the technique of quantitative real-time RT-PCR. Infection of *Leishmania* leads to increase in PPAR gene expression. We detected 3-fold increase in mRNA of PPARα in the liver (Figure 1(a)) and 3-fold increase for PPARγ in the spleen (Figure 1(b)), as compared to the uninfected control organs. At the cellular level, when residential macrophages from peritoneal exudates of BALB/c mice were infected, PPARγ gene expression was also increased. The increase of PPARγ mRNA was 2-fold for *Leishmania*-infected peritoneal exudate cells (PECs) (Figure 1(c)). Kinetics study was performed to examine whether PPAR expression correlates to parasite burden (Figure 2). The expression of PPARγ in the liver was found to be slightly ahead of the increase in parasites burden (Figure 2(a)). Both PPARγ and parasite number peaked at 4 weeks, which is the time when granuloma will form and parasite growth will be quenched. As the infection in the liver decreases, the expression of PPARγ subsides in coordination. In the spleen where the parasites will persist, the rise in PPARγ expression correlates closely with the increase in parasite number (Figure 2(b)). Both parameters followed a logarithmic increase between weeks 2 to 4 until reaching a plateau at week 6.

5. Possible Mechanisms by Which *Leishmania* Induces PPAR Gene Expression

PPAR is a genetic sensor of fatty acids, and its ligands are produced during the course of *Leishmania* infection. Cyclooxygenase-2 (COX-2) is an enzyme that converts arachidonic acid into various bioactive lipids, including prostaglandin (PG) D_2, PGE_2, PGF_2, thromboxane (TX) B_2, 15d-PGJ_2, and prostacyclin. Studies in the murine model of *L. donovani* infection have demonstrated that production of these bioactive lipids is enhanced upon infection [18, 19], and studies with *L. amazonensis* have revealed that COX-2 is needed for establishing infection [20]. Blockade of COX with indomethacin inhibits *L. amazonensis* infection of peritoneal macrophages *in vitro* and reduces the size of lesions in susceptible BALB/c mice.

Among the COX-2 products 15d-PGJ_2 is a potent endogenous ligand for PPAR [21]. Moreover, *in vitro* addition of PGE_2 increases the number of amastigotes within macrophages [22]. PGE_2 can activate the generation of lipoxins, a relatively new class of eicosanoids that are also derived from arachidonic acid, but through lipoxygenase or acetylated COX instead [23, 24]. Lipoxin will shut off inflammatory response when bound to its receptor [25]. In *L. major* infection, addition of exogenous lipoxin A_4 increases infectivity; this effect has been confirmed by receptor inhibition studies [26]. The eicosanoid downregulates inflammation by promoting clearance of apoptotic neutrophils [25]. *Leishmania* parasites (*L. major, L. donovani, L. mexicana*, etc.) are covered with phosphatidylserine (PS), a major surface characteristic of apoptotic cells, and engulfment of apoptotic cells leads to induction of PPAR [27, 28]. For these reasons, the parasitized macrophages would have activated PPAR and are likely to express an alternatively activated (M2) phenotype. Furthermore, PPAR turns on the expression of CD36, and this scavenger receptor would bind to thrombospondin and facilitate phagocytosis of the apoptotic neutrophils [29], the so-called Trojan horses for *Leishmania* parasites at the site of inoculation, reciprocally in a positive feedback manner [30–32]. Ligand activation of PPARγ augments phagocytic capacity of the alternatively activated macrophages.

6. Blockade of PPAR Reduces *Leishmania* Infection

Since PPAR is upregulated with *Leishmania* infection, we proceeded to determine whether the activation of PPAR is essential for infection. Studies assessing the effect of PPAR blockade on *Leishmania* infectivity have been conducted with *L. major*. Our laboratory studied *L. donovani* using PECs from the C57BL/6 mice, a strain that is susceptible to *L. donovani* infection though not *L. major*. A reason for selecting this strain is that it does not have a deficiency in T helper 1 cells and thus is capable of producing IFNγ, which is necessary for generation of the parasiticidal NO molecule. Nonelicited residential macrophages from the peritoneum were infected with *L. donovani* promastigotes, and IL-4 was added to activate PPARγ [33]. Then, PPARγ transcriptional

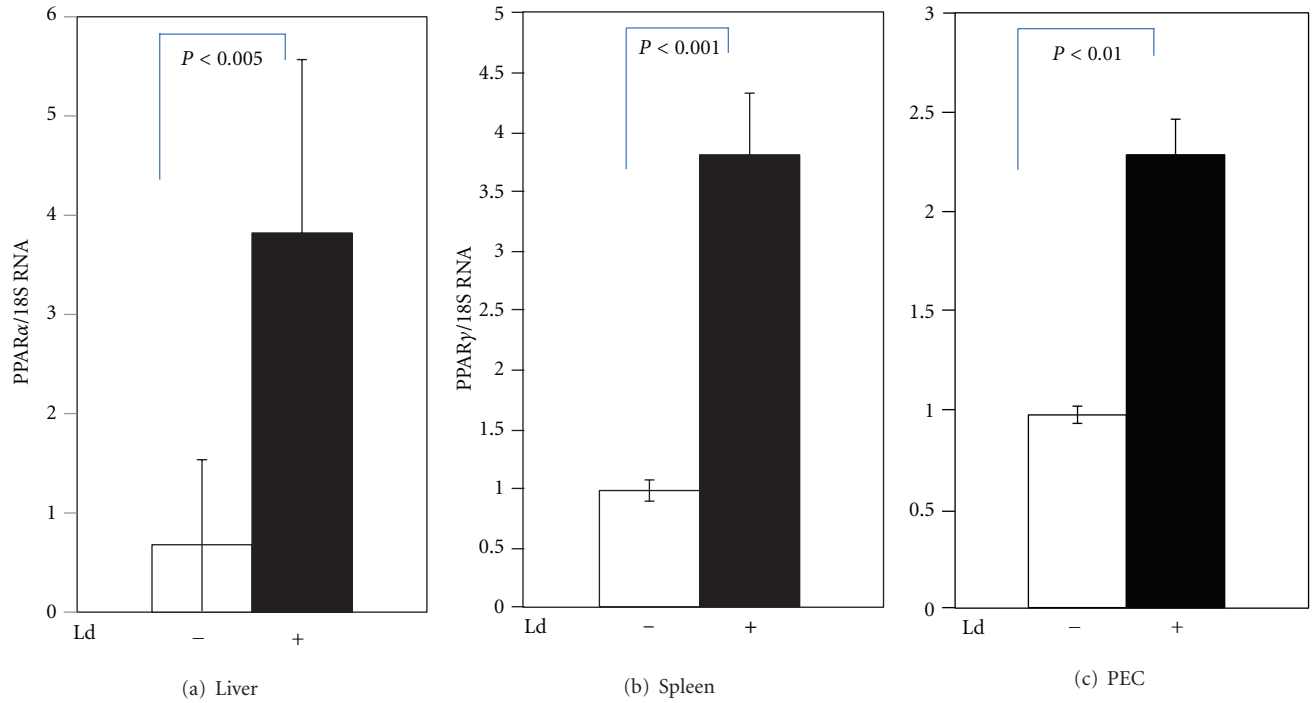


Figure 1: *Leishmania* infection activates PPAR gene expression. In (a) and (b), BALB/c mice were infected i.v. with 10^7 stationary phase promastigotes of *L. donovani* for 4 weeks, and then liver and spleen, respectively, were harvested. In (c), peritoneal exudate cells (PECs) were obtained from the peritoneum of normal BALB/c mice, infected with *L. donovani* at 1 : 10 ratio, and then harvested for RNA isolation after 2 days. PPAR activation was measured by real-time RT-PCR with normalization to 18S or actin RNA, and modulation was compared and expressed relative to the uninfected control using the delta-delta Ct method. The infected groups (black bars) showed higher levels of gene expression in liver (a), spleen (b), and PECs (c) in comparison to uninfected controls (open bars). Statistical analysis was performed by Mann-Whitney *U* test, and a *P value* of less than 0.05 was considered as significant.

activity was blocked with SR202, an antagonist which efficacy and specificity have been shown in adipocytes [34–36]. The effect of PPARγ on parasite survival and proliferation in the host macrophages was assessed by enumerating the number of amastigotes per macrophage. In the absence of IL-4, the number of amastigotes per infected macrophage was 4.94 ± 0.44 (Figure 3(b)). IL-4 activated PPARγ (Figure 3(a)), and this resulted in an increase in infectivity (Figure 3(b)). There were 7.10 ± 1.82 amastigotes per macrophage. This enhancement by IL-4 was reversed by blocking PPARγ (Figure 3(b)). Addition of SR202, at 25 and 50 μM, in a dose-dependent manner, reduced the number of amastigotes per macrophage to 5.05 ± 1.38 and 2.02 ± 0.81, respectively. This reduced infection was further correlated to an increase in nitric oxide level in the cultures (Figure 3(c)). The effect of SR202 at 50 μM or lower is specific to the infectious process for, at these concentrations, the compound did not affect the survival of promastigotes or mammalian cells (Figures 3(d) and 3(e)). Figure 4 shows the cultures that had been stained with Diff-Quik for microscopic enumeration. The macrophages that had been treated with 50 μM of SR202 presented a healthy morphology with many empty parasitophorous vacuoles freed of parasites.

Complementary results were obtained with *Leishmania major* in bone marrow-derived macrophages from the resistant C57BL/6 mice in a study by Gallardo-Soler et al. (2008). The PPAR antagonists, GW9662 and GW5393, reduce infectivity [13]. Whereas we correlated PPAR activity in our *L. donovani* infection to the transrepressive action on iNOS-mediated NO production (Figure 3), this study correlated infectivity to the transcription of arginase. The enzyme is a bona fide marker for PPAR-mediated transcription and alternatively activated (M2) macrophages. Its mRNA level was decreased in coordination to the decrease in infection. In addition to pharmacological inhibitors, Odegaard et al. (2007) have examined *L. major* infectivity in mice with macrophages that do not express PPARγ (Mac-PPARγ KO, PPAR$\gamma^{fl/fl}$ LysM^{cre}) [37]. The mutant mice have impaired M2 macrophage activation, a delayed disease progression, and a lower parasite load (less footpad swelling) compared to the wild type [37]. Henceforth, PPARγ plays an essential role in the pathogenic process of both *L. major* and *L. donovani*. In the absence of PPARγ activity, the balance shifts from the arginase producing M2 phenotype to that of nitric oxide producing, type 1, response.

7. PPAR Activation Enhances *Leishmania* Infection

Conversely, Gallardo-Soler et al. (2008) also demonstrated that the PPAR agonists GW1929 and GW7845 (for PPARα) and GW0742 (for PPARγ), at 1 μm concentration, increased

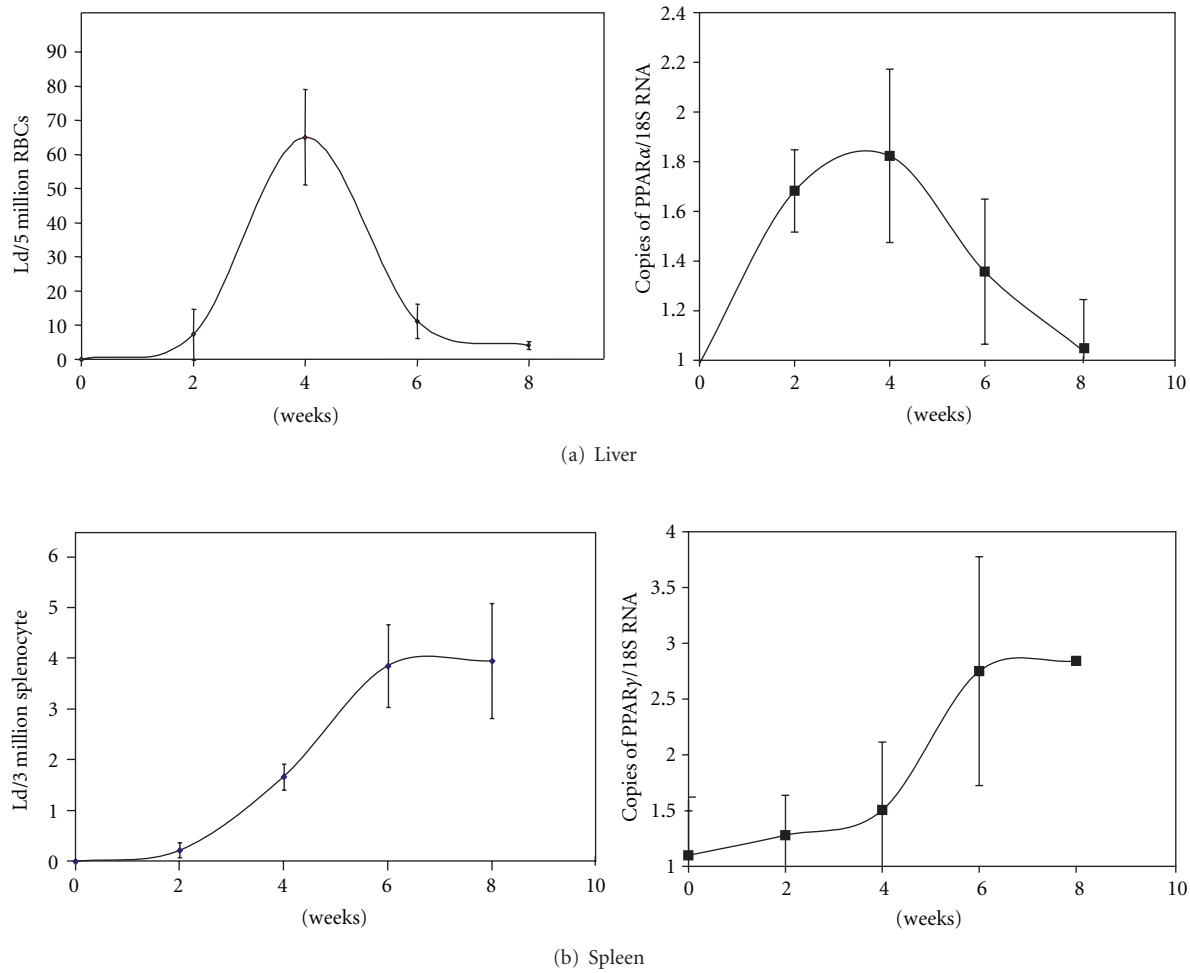


Figure 2: Kinetics of PPAR expression and parasitic infection. BALB/c mice were intravenously injected with 10^7 stationary phase promastigotes of *L. donovan*i 1S. At two-week intervals, groups ($n = 5$) were sacrificed, and their liver and spleen were harvested. PPAR expression was determined by real-time RT-PCR as described in legend of Figure 1. The amount of parasites in the organs was determined by limiting dilution analysis according to the procedure of Titus and colleagues [17]. Cells were plated into wells of 96-well plates at a range of concentrations, according to the number of red blood cells in the liver and splenocytes in the spleen suspensions and then incubated at 27°C to allow the parasites to transform from intracellular amastigotes into promastigotes. After 2-3 weeks of proliferation, the number of wells that shows parasite growth was scored under a microscope, and the L-Calc software for limiting dilution analysis (provided by Stem Cell Technology, Vancouver, Canada) was used to determine the frequency of parasite. Normalization was based on the number of red blood cells in the liver cultures (a) and the number of splenocytes in the spleen cultures (b).

intracellular growth of *Leishmania major* in bone marrow-derived macrophages [13]. When both PPAR/RXR ligands were coadministered, the degree of infection was similar to those infected in the presence of IL-4. This increased number of intracellular amastigotes can be correlated to the levels of arginase activity.

PPAR is also regarded as dietary-sensing nuclear receptors; many activators of PPARγ have been identified in foods [38]. Our laboratory is interested in the effect of curcumin (Figure 5), a dietary activator of PPARγ on visceral leishmaniasis [16, 39]. It is the active principle in the spice turmeric, which is used abundantly in India, where visceral leishmaniasis is endemic in the Bihar region. Curcumin is well known for its anti-inflammatory effect, and there is ample evidence that the activity can be attributed to the activation of PPARs [40–43]. Zheng and Chen (2007) have suggested that there is a curcumin-responsive element residing in the regulatory region of the PPARγ gene [44]. We examined the effect of curcumin on PPAR activation and *Leishmania* infection *in vivo* [16]. Susceptible BALB/c mice and resistant C3H mice were infected with *L. donovani*; immediately following inoculation, the mice were fed curcumin or phosphate-buffered saline (PBS) every other day. Then, at 4 weeks after infection, the livers and spleens were harvested and quantified for PPARγ, iNOS, cytokines, and parasite load. Parasite load was quantified by two complementary methods, limiting dilution analysis and real-time PCR detection, and compared by the parametric test ANOVA after data transformation. Figure 6 shows the results on PPARγ, iNOS, and *Leishmania* kinetoplast DNA

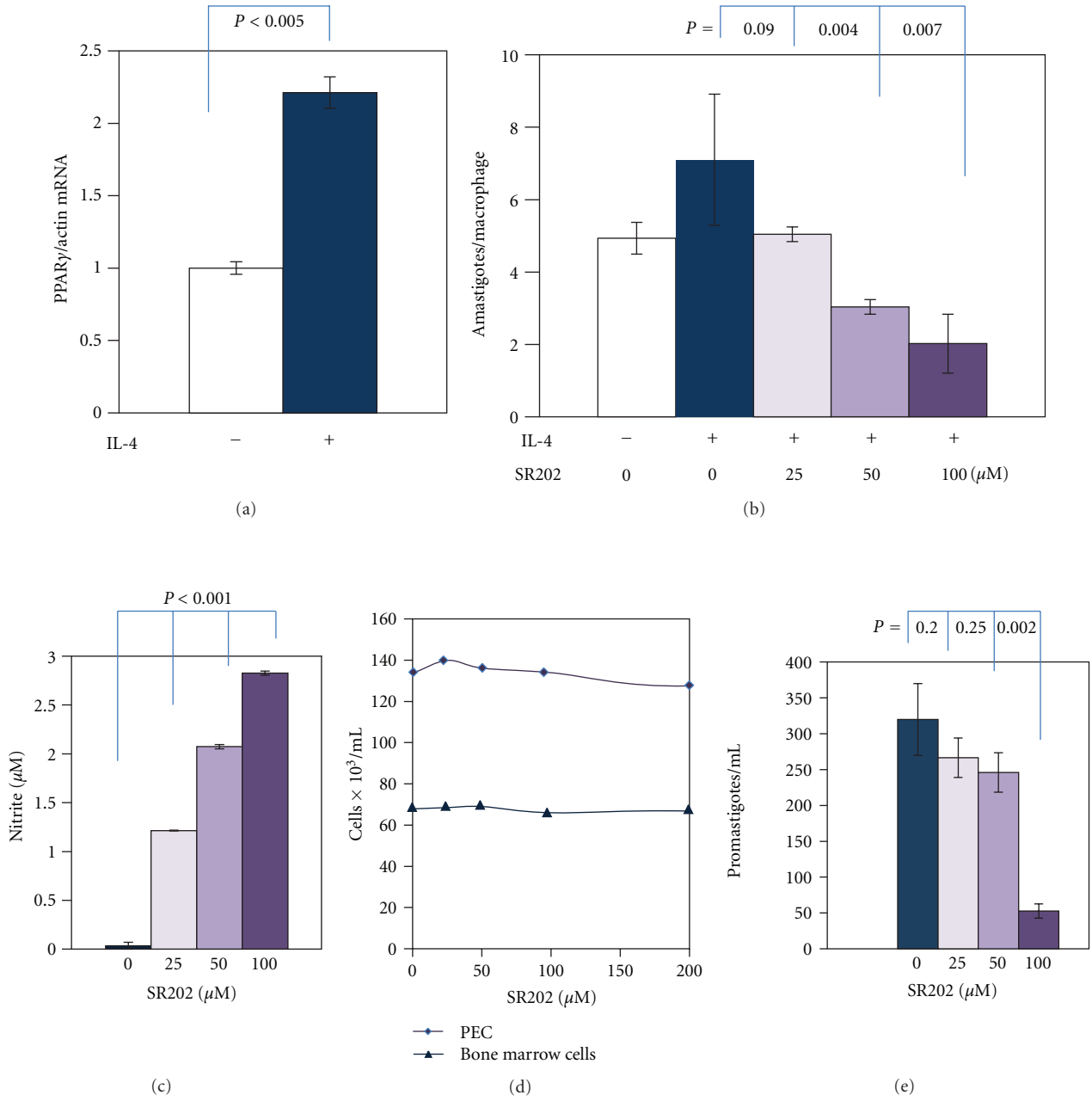


Figure 3: Blocking PPARγ activation with an antagonist reduces *L. donovani* infectivity. In (a), peritoneal exudate cells were infected by *L. donovani* using a 1 to 10 : PEC to promastigote ratio. The cultures were incubated with 4 ng/mL of IL-4 for 24 hours, then total RNA was harvested, and RT-PCRs were performed to quantify the copies of PPARγ and β-actin mRNA as described in Adapala and Chan [16]. In (b), PECs from C57/BL6 mice were attached to cover slips and infected with *L. donovani* promastigotes at 1 : 5 ratio. After 20 to 24 hours, 5 ng/mL of IL-4 and various concentration of SR202 were added. The infection was allowed to develop at 37°C in a 5% CO_2 incubator for another 3 days. Then, the cover slips were fixed in methanol and stained with Diff-Quik. The degree of parasite burden was determined by enumeration under a microscope in a double-blind manner by at least two individuals. Uninfected macrophages, infected macrophages, and the number of amastigotes in these macrophages were counted. The result is reported as amastigotes/macrophage, and each data point was derived from counting at least about one hundred macrophages or one hundred infected macrophages, as appropriate. (c) shows the levels of nitric oxide in PECs that were similarly infected with *L. donovani* promastigotes, except that IFNγ was added instead of IL-4 to stimulated inducible nitric oxide synthase expression. On day 5, the amount of nitric oxide was determined with Griess reagent. Shown are the relative levels of nitrite, oxidized form of nitric oxide, in the culture supernatants. In (d), SR202 was added to freshly harvested, uninfected PECs and bone marrow cells at various concentrations. After 3 days, the number of live cells was determined by counting with trypan blue. In (e), SR202 was added to promastigotes, and after 5 days of proliferation, the number of parasites was determined by counting under a microscope. The results shown are representative of three independent experiments.

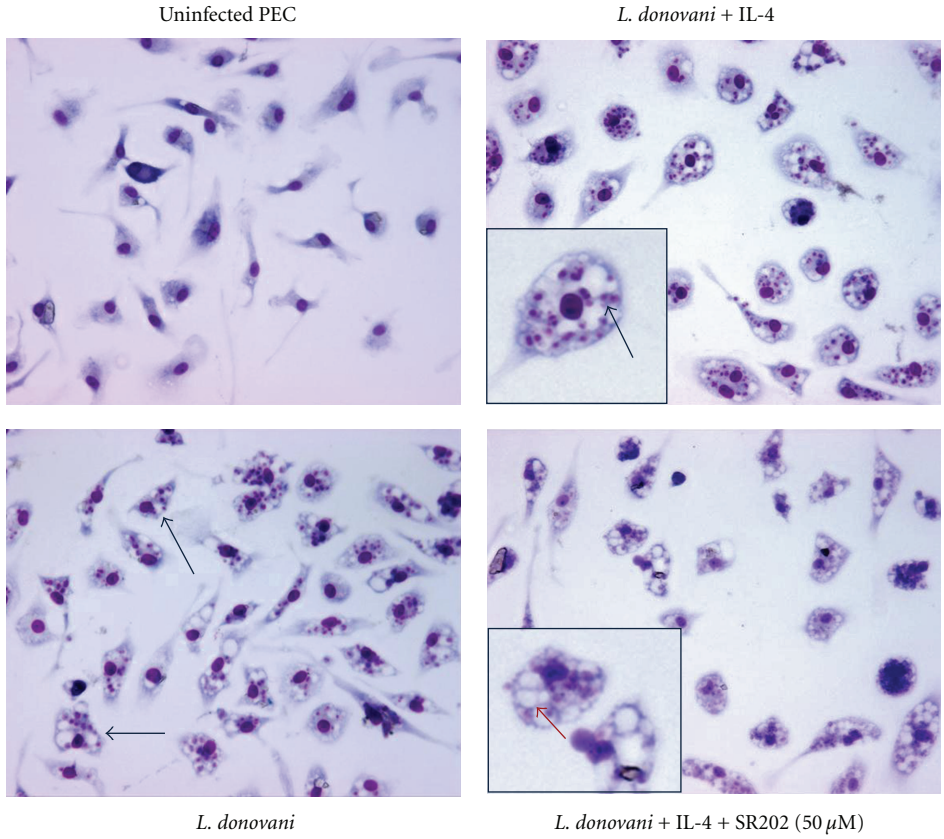


Figure 4: Morphology of the peritoneal macrophages after SR202 treatment. Micrograph of cover slips from the cultures described in Figure 3(b). The black arrows point to macrophages with phagolysosomes filled with amastigotes. The black arrows point to infected macrophages and the red arrow points to infected macrophages with phagolysosomes cleared of amastigotes.

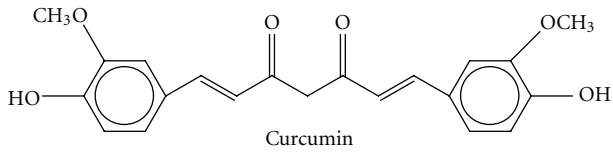


Figure 5: Chemical structure of curcumin.

quantification. Curcumin treatment led to 5-fold increase in the gene expression for PPARγ and 2-fold increase in the gene expression for PPARγ in the spleen. It also caused an 80% decrease in the expression of iNOS in the liver and 68% in the spleen (Figures 6(a) and 6(b)). Concomitant with these modulations, parasite burden was elevated compared to the untreated vehicle control, (results from limiting dilution were not shown).

Corresponding to the feeding studies, we found that curcumin increased PPARγ and decreased iNOS gene expression in infected macrophages. At 10 μm, curcumin increased PPARγ mRNA levels in infected peritoneal macrophages from BALB/c by 1.5-fold Figure 7(a). The dose dependency of the curcumin actions was demonstrated by iNOS gene expression and nitric oxide production. The level of gene expression is shown in Figure 7(b). At 10 μm, curcumin reduced the level of steady-state RNA by 70%. The level of nitric oxide in the culture supernatants was also reduced. At 5, 7.5, and 10 μm of curcumin, the reduction was 18, 39.3, and 61.4%, respectively [16]. In parallel to the reduction, parasite infectivity increased. The number of infected macrophages increased dose dependently from 28 to 37% in the resistant C3H strain and from 35 to 48% in the susceptible BALC/c strain. The number of amastigotes per macrophage also increased dose dependently, as shown in the table in Figure 7(c).

8. Conclusion: Mechanisms of PPARs on Leishmaniasis

Taken together, these cumulative data from *L. donovani* and *L. major* infections indicate that PPAR plays a role in leishmaniasis, no matter in the liver where the PPARα forms predominate, in the spleen and residential macrophages from the peritoneum or the bone marrow where the PPARγ forms predominate [46]. Our perspective on how the nuclear factor is activated during infection and how its activation enhances the survival of *Leishmania* parasites is as follows. When the infected sandflies bite, an inflammatory reaction initiates

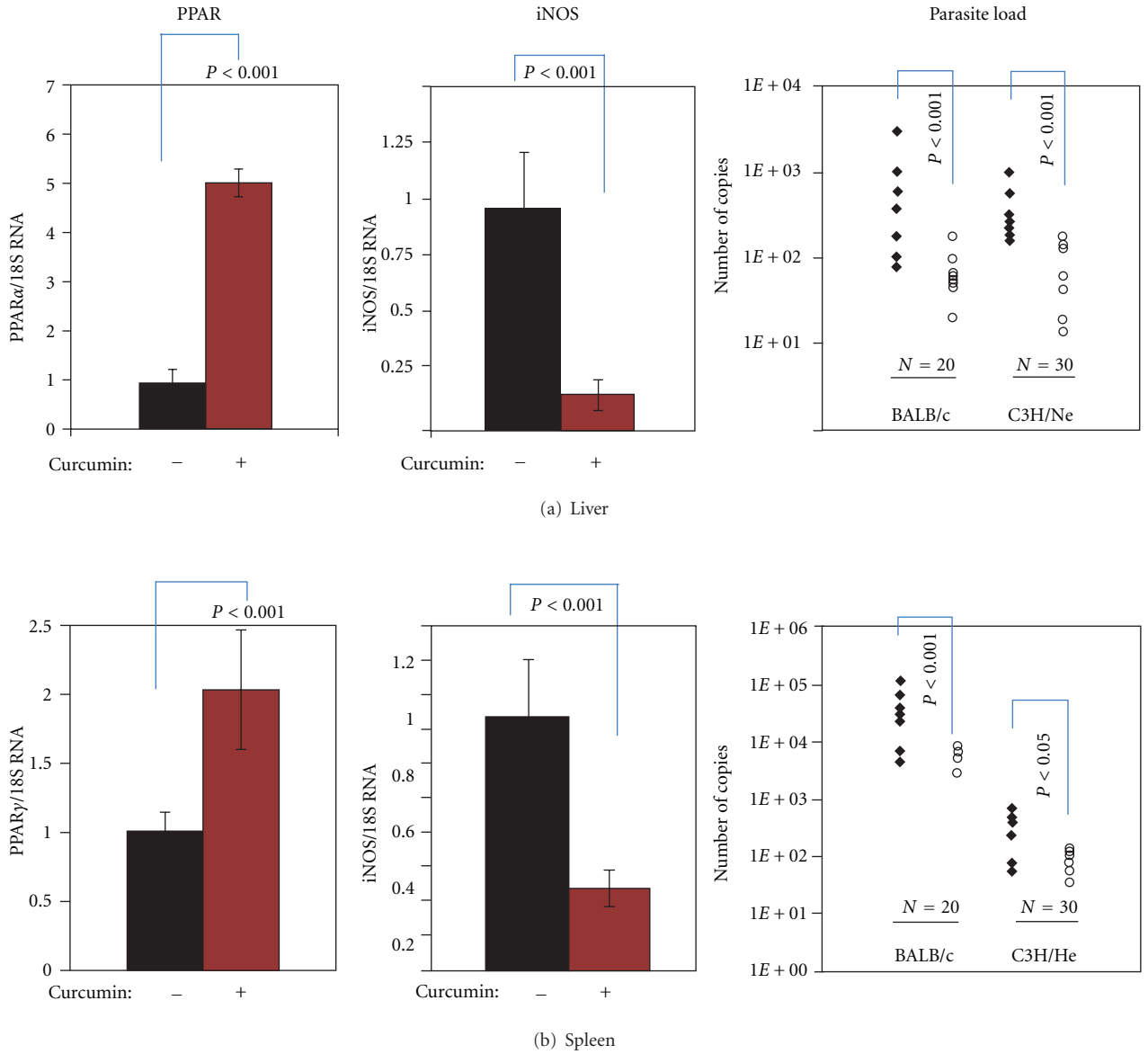
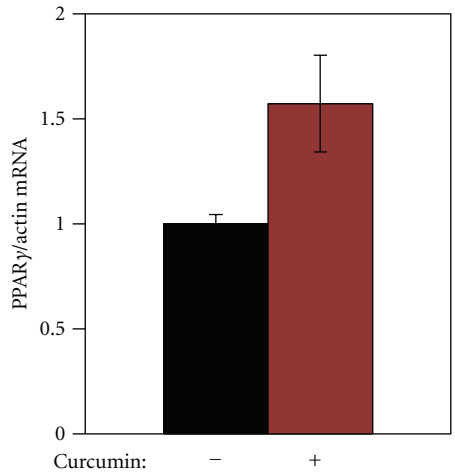
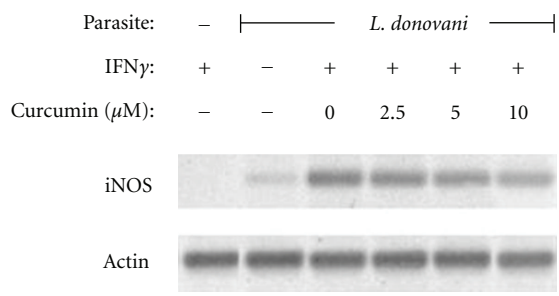


Figure 6: Activation of PPAR enhances parasitic infection. Mice were infected with *L. donovani*. One group was fed with 0.2 mL freshly prepared curcumin solution, given by oral gavage every other day throughout the course of the study. Curcumin was dissolved to a solution of 7.52 mg/mL in 0.1 N NaOH and immediately brought to pH 7.2 by diluting to a concentration of 11.1 μg/mL in PBS. The second group received phosphate buffered saline (PBS) in the same manner. At 4 weeks, the peak of hepatic infection (as shown in Figure 2), livers, and spleens were harvested for DNA and RNA extractions. PPAR and iNOS expression were determined by real-time RT-PCR and normalized to 18S RNA. Parasite load in the liver and spleen was determined by the real-time PCR procedure which was that of Nicolas and colleagues [45], except the reaction occurred in SYBR Green I PCR master mix (from Superarray). DNA, at 40 ng per reaction, was denatured at 95°C for 10 min, and then *Leishmania* kinetoplast DNA (kDNA) was amplified in a thermal cycler (Rotor-gene 6.0, from Corbett). The number of copies of kDNA per μg of DNA was determined using a standard curve that was established with the cloned PCR product. Filled diamonds were curcumin-treated, and clear circles were saline controls. N indicates the number of mice in each group. Statistic analysis was performed by ANOVA after the data underwent natural log transformation as described in Adapala and Chan [16]. A P value of <0.05 was considered as significant.

innate and adaptive immune response for protection against the parasites. Neutrophils and macrophages are recruited to the injection site, and promastigotes enter the phagocytes. Launching a type 1 immune reaction, with production of nitric oxide, would resist infection. However, the parasites and infected host cells can synthesize ligands that activate PPARγ. Phagocytosis of apoptotic neutrophils and IL-4 from T helper 2 cells can do so as well. With the activation of PPARγ, *Leishmania* parasites would benefit from infiltration of macrophages, inactivation of the destructive inflammatory response, and promotion of the resolution of inflammation. Activation of PPAR promotes differentiation



(a)



(b)

	Curcumin (μM)	Infected macrophages (%)	Parasites/macrophage
RAW264.7	0	69.4 + 2.8	7.0 + 0.9
	2.5	73.0	7.9
	5.0	96.3 + 0.1	12.8 + 5.0
	10	96.0 + 5.6	21.2 + 9.3
C3H/He	0	28.0 + 5.5	1.4 + 0.3
	5	31.6 + 8.4	1.6 + 0.2
	7.5	39.0 + 4.4	1.5 + 0.2
	10	37.0 + 11.2	1.8 + 0.1
BALB/c	0	35.6 + 2.5	1.4 + 0.2
	5	26.0 + 5.2	1.4 + 0.2
	7.5	39.3 + 5.0	1.6 + 0.1
	10	48.0 + 10.5	1.6 + 0.2

(c)

Figure 7: Curcumin induces PPARγ mRNA expression and reduces iNOS mRNA expression in infected macrophages. In (a), PECs of BALB/c mice were infected with *L. donovani* promastigotes, and then 10 μM of curcumin (brown bar) or vehicle control (0.1% acetone) was added. After 2 days, the cells were harvested for RNA isolation to determine the level of PPARγ and β-actin expression by real-time RT-PCR. In (b), murine RAW264.7 cells were infected with *L. donovani* for 16–20 hours. Then, different concentrations of curcumin were added. Thirty minutes after curcumin treatment, IFNγ was added to activate the macrophages. At 5 hours after the addition of IFNγ, the cells were harvested, mRNA was extracted, and conventional RT-PCR was performed. The gel shows the end point PCR-amplified iNOS (496 bp) and β-actin cDNAs products. In (c), nonelicited PECs from resistant C3H and susceptible BALB/c strains were cultured with *L. donovani* promastigotes in wells that contained cover slips for a period of 16–20 hours; then curcumin and IFNγ and TNFα were added. On day 4-5, the coverslips were stained to enumerate the percent of infected macrophages and number of amastigotes per macrophage under a microscope, similar to steps described in Figure 3.

of the host macrophages into the alternatively activated (M2) macrophages, which have a type 2 phenotype and would produce arginase to interfere with enzymatic activity of iNOS [47]. As such, the parasites can survive and multiply within the host's macrophages, and the infection becomes chronic (Figure 8).

Currently, whether antagonists of PPAR would be therapeutic for leishmaniasis remains to be investigated. SR202, the antagonist that we used in our study, has been shown to prevent obesity in rats and therefore has *in vivo* efficacy. Ligands for PPARγ are drugs for type 2 diabetes, and ligands for PPARα are also currently in clinical use for obesity. How

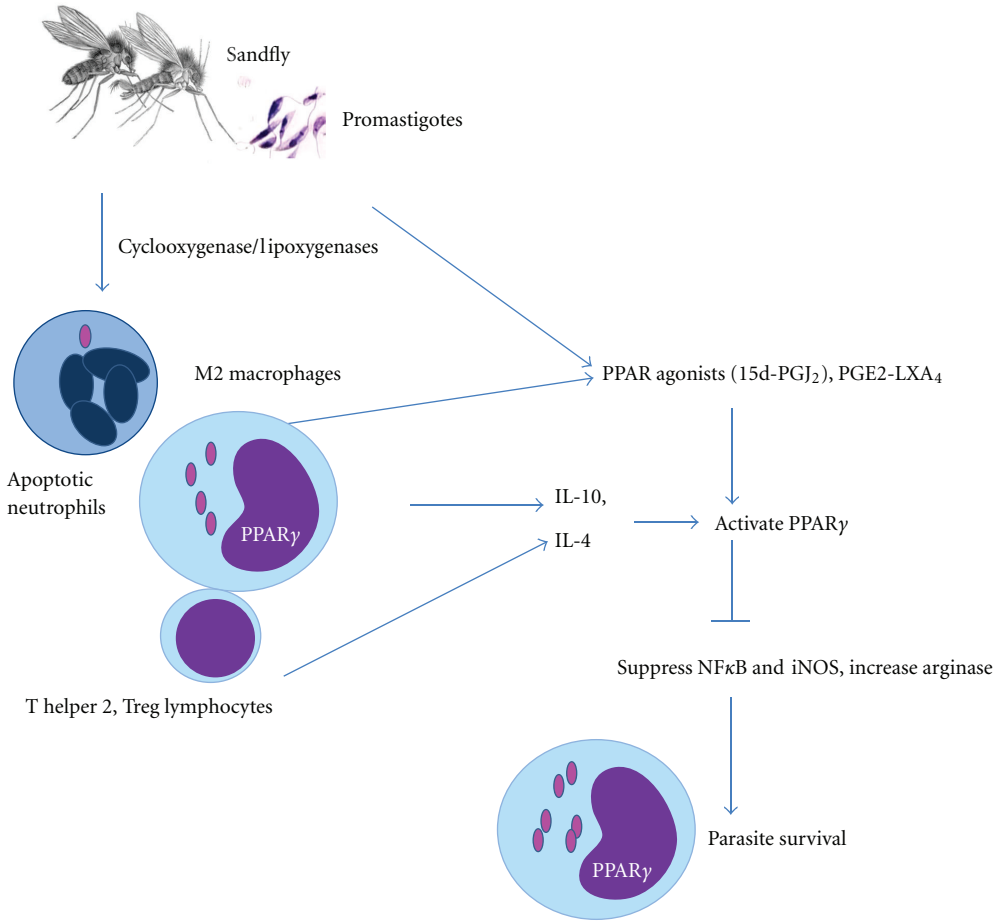


Figure 8: Scheme of *Leishmania* interaction with mediators of the resolution process during inflammation. When infected sandflies bite, promastigotes enter the neutrophils and macrophages that are recruited to the inflamed site of injection. The parasite can, by itself, activate the infected host cells to produce PPAR activators. This includes PPARγ agonists such as the bioactive lipids 15d-PGJ_2 and LXA_4 from the arachidonic acid pathways. Engulfment of apoptotic neutrophils and IL-4 from T helper 2 cells can activate PPARγ as well. Activation of PPARγ polarizes the host macrophage towards the alternatively activated macrophage (M2) phenotype, which would produce arginase to divert substrate from iNOS and thus reduce the production of nitric oxide. As such, the parasite can survive and multiply within the host's macrophages, and the infection becomes chronic.

would these agents affect the outcome of leishmaniasis and whether PPAR affects the survival of other parasites are interesting questions [46].

Acknowledgments

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Review Article

Peroxisome Proliferator-Activator Receptor γ: A Link between Macrophage CD36 and Inflammation in Malaria Infection

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Severe malaria infection caused by *Plasmodium falciparum* is a global life-threatening disease and a leading cause of death worldwide. Intensive investigations have demonstrated that macrophages play crucial roles in control of inflammatory and immune responses and clearance of *Plasmodium-falciparum*-parasitized erythrocytes (PE). This paper focuses on how macrophage CD36 recognizes and internalizes PE and participates the inflammatory signaling in response to *Plasmodium falciparum*. In addition, recent advances in our current understanding of the biological actions of PPARγ on CD36 and malaria clearance from the hosts are highlighted.

1. Introduction

Macrophages play a crucial role in the innate immunity [1] and are essential components of defense against the malaria infection. Macrophages can take up nonopsonic or opsonic *Plasmodium-falciparum*-parasitized erythrocytes (PE) by using CD36 and Fc receptors, respectively. Nonopsonic PE phagocytosis by macrophage CD36 plays a major role for PE clearance especially in the acute phase of primary malaria infection or nonimmune hosts [2, 3]. Therefore, upregulation of CD36 on macrophages could greatly trigger the capacity to clear parasites during acute malaria infection and thus is likely to be effective treatment for malaria infection. The intensive investigations demonstrate that peroxisome proliferator-activator receptor γ (PPARγ) plays an important role in the immune response via inhibiting the expression of inflammatory cytokines and control macrophage alternative activation [4–6] and has potential as a novel anti-inflammatory target for many inflammatory diseases [7] including parasitic infection [8–10]. CD36 can be induced by PPARγ ligands, and activation of PPARγ enhances the clearance of PE and inhibits the proinflammatory response [6, 11]. Here, we highlight recent advances in our current understanding of the biological actions of PPARγ on CD36 and malaria clearance from the hosts.

2. CD36 and Malaria Clearance

Macrophages play a crucial role in innate immunity to malaria infection because they can phagocytose infected erythrocytes that limit the malaria density in the absence of cytophilic or opsonizing malaria-specific antibody [2]. Macrophage pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs) and scavenger receptors such as CD36, are important components in the regulation of immune and inflammatory responses [12]. PRRs are a class of innate immune response-expressed proteins that recognize a wide range of molecules known as pathogen-associated molecular patterns (PAMPs) exposed on the pathogens or pathogen-infected cells but absent from healthy host cells and activate proinflammatory responses to infection. CD36, a member of the class B scavenger receptor family, has been identified as a PRR [12]. CD36 is an 88-kDa membrane glycoprotein that expressed on a wide range of cells such as platelets, monocytes/macrophages, endothelial cells, smooth muscle cells, and other types of cells. CD36 expressed on macrophages is involved in recognition and engulfment of endogenously derived ligands such as apoptotic and senescent cells [13–16], thrombospondin-1 [17, 18], nonopsonized bacteria [19], β-amyloid [20], and oxidized low-density lipoproteins (oxLDL) [21]. Because

CD36 is expressed by broad range of cells and involved in uptake of many self- or non-self-particles, it contributes to a varied list of physiological and pathologic processes such as apoptotic cell clearance, angiogenesis, atherosclerosis, Alzheimer's disease and infectious diseases [22].

Macrophage CD36 is also involved in recognizing and internalizing nonopsonized *Plasmodium falciparum* parasitized erythrocytes (PE) and help control replication of blood-stage parasites, which is a critical component of host defense mechanisms against blood-stage parasites [3, 23–28]. African populations contain high frequency of mutations in CD36. These mutations that cause CD36 deficiency are associated with susceptibility to severe malaria and cerebral malaria [29]. Nonopsonized PE by antibodies or complements can be recognized and internalized by macrophages via CD36 because *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the surface of PE is the major parasite ligand for CD36 [2]. CD36 null mice show the importance of CD36 in PE phagocytosis [2]. Macrophages from CD36 null mice or rat lacking CD36 internalize significantly fewer PE compared to wild-type mice or control rats [2]. CD36-null mice experience more severe and fatal malaria when challenged with *Plasmodium chabaudi chabaudi* as compared with wild-type mice [30]. CD36-null mice also display defect in parasite clearance, earlier peak parasitemias, higher parasite densities, and higher mortality rates compared to wild-type mice [30]. These results suggest that selectively triggering of malaria clearance by modulating CD36 expression may contribute to control of acute blood-stage malaria infection *in vivo*.

Current data suggest that TLRs do not function directly as phagocytic receptors [31]. Erdman et al. applied selective, receptor-targeted strategies to show that macrophage pretreatment with TLR agonists markedly stimulates PE uptake via CD36, suggesting that CD36 and TLRs cooperate functionally to promote internalization of PE by macrophages [27]. The TLR2 activation-enhanced phagocytosis capacity for PE is unlikely via TLR-mediated transcription of scavenger receptors because surface CD36 levels do not increase upon TLR2 activation [27]. The role of TLR2 for the promotion of malaria clearance by macrophages may be beneficial in the control of acute blood stage parasite replication. Increasing understanding of the molecular mechanisms involved may lead to development of strategies for manipulating phagocytic clearance of *Plasmodium falciparum* for the treatment malaria infection.

3. CD36 and Inflammatory Responses

Macrophage CD36 plays a very important role in the physiological process including apoptotic cell clearance and pathogenesis, of many diseases such as atherosclerosis, Alzheimer's disease and *Plasmodium falciparum* malaria infection. In addition to clearance of altered self- or non-self-components such as apoptotic cells, oxLDL, β-amyloid, and pathogens, macrophage CD36 may be involved in inflammatory signaling cascade by interacting with other PRRs such as TLRs, which may further increase macrophage phagocytosis capacity and dispose invaded pathogens or trigger the chronic inflammation in these diseases. However, the interaction between CD36 and TLRs is complex and the consequences of the interaction are not fully understood. Macrophage CD36 recognition and internalization of apoptotic cells fails to stimulate proinflammatory response from ingesting phagocytes but triggers an anti-inflammatory response which is mediated by the release of interleukin (IL)-10, transforming growth factor (TGF)-β [32, 33] and inhibition of tumor necrosis factor-α (TNF-α), IL-12, IL-1β, and IL-8 [33]. It was also demonstrated that macrophages recognition and phagocytosis of late apoptotic cells does not trigger the release of IL-8 and TNF-α. By contrast, macrophages response to necrotic cells, including secondarily necrotic cells derived from uncleared apoptotic cells, are perceived as proinflammatory [34]. Macrophage CD36-dependent signaling is involved in the pro-inflammatory effects of internalizing β-amyloid, oxLDL, and bacteria [35–37]. CD36 has been shown to cooperate with TLR2 in innate sensing and induceing of inflammatory cytokines in response to TLR2 agonists such as intact bacteria and bacterial ligands [38–40]. By activation of src-family kinases, MAP kinases, transcription factor nuclear factor κB (NF-κB), macrophage CD36 recognition and internalization of oxLDL activates pro-inflammatory signals such as release of cytokines and production of reactive oxygen species (ROS) and inhibits macrophage migration, which results in the formation of foam cells and atherosclerotic plaque [12, 41]. For a comprehensive review on CD36-induced signaling pathways, see Moore and Freeman and Silverstein et al. [22, 41]. Microglia cell CD36 for β-amyloid uptake also boosts pro-inflammatory response and may contribute to the pathogenesis of Alzheimer's disease [12]. CD36 recognition of oxLDL and β-amyloid triggers assembly of a heterotrimeric complex composed of CD36, TLR4 and TLR6, leading to the induction of pro-inflammatory mediators implicated in the deleterious effects oxLDL and amyloid-β *in vivo* [42]. TLR2 also requires CD36 for inflammatory signaling [12]. CD36 is necessary for the component of ischemic brain injury attributable to the inflammatory response triggered by TLR2/1 activation [43]. The inflammatory response in brain induced by TLR2/1 activation, but not TLR2/6 or TLR4 activation, is suppressed in CD36-null mice. In contrast to brain inflammation, in systemic inflammation CD36 is involved in TLR2/6 activation, but not TLR2/1 activation [39, 40, 43]. This finding suggests that CD36 signaling in neuroinflammation differs from systemic inflammation; the mechanism for such differences is unclear [43].

Macrophage CD36 also contributes to the induction of innate immunity to malaria infection [30]. Macrophage CD36 interaction with malaria and malaria products, such as *Plasmodium falciparum* glycosylphosphatidylinositol (*pf*GPI), hemozoin and *Plasmodium falciparum* DNA, known as pathogen-associated molecular patterns (PAMPs), has been found to stimulate macrophage cytokine production via collaboration with TLR family [44–46]. *pf*GPI is the primary parasite-derived bioactive molecule that activates macrophages and induces the release of inflammatory cytokines such as TNF-α and IL-1 from macrophages and, therefore, contributes to severe malarial pathogenesis

and morbidity [11, 47]. *pf*GPI interacts with macrophage TLR2 by activation of JNK, P38, c-Jun and ERK1/2 in a CD36-dependent manner [30, 48]. Hemozoin, also known as malaria pigment, is insoluble crystal generated from hemoglobin proteolysis by *Plasmodium falciparum*. Natural hemozoin is coated with both proteins and plasmodial DNA and a potent activator of the inflammatory response via triggering TLR9 dependent on MyD88 [49, 50]. However, some studies show that the inflammatory effect of hemozoin is dependent on the cells, duration of incubation time, and the method for hemozoin preparation [51]. A recent study demonstrated that a PAMP, AT-rich DNA in the genome of *Plasmodium falciparum*, couples to stimulator of interferon genes (STING) and TANK-binding kinase (TBK) to induce interferon regulatory factor 3–7 (IRF3-IRF7) dependent on type I IFN production in a TLR9-independent manner. Mice lacking IRF3, IRF7, the kinase TBK1, or the type I IFN receptor were resistant to lethal cerebral malaria [46]. This study provides evidence of a unique DNA sensing pathway that may contribute to immunopathology in plasmodial infections [52].

The role of CD36 in inflammatory signals in response to malaria infection remains controversial. Several lines of evidence suggest that selective ligation of CD36 does not lead to proinflammatory cytokine production by macrophages [3, 28]. Erdman et al. applied selective, receptor-targeted strategies to assess whether CD36 and TLRs can cooperatively mediate immune response to PE. They demonstrate that targeted activation and internalization of CD36 fail to stimulate proinflammatory cytokine production [27]. CD36-mediated intact PE internalization is also noninflammatory even in the presence of TLR agonists [27]. These data suggest that it is possible that CD36 is not directly involved in regulation of pro-inflammatory response to *Plasmodium falciparum* but rather presents or concentrates ligands for recognition by other signaling receptors such as TLRs [30], similar to the role of CD14 in presenting or concentrating LPS signal to TLR4 [53]. Taken together, the contradictory data about CD36 on inflammatory response to parasite and parasite produce indicate that further investigation is needed for the role of CD36 in parasitic infection.

4. PPARγ Ligand

PPARs are nuclear receptors which are ligand-activated transcription factors. To date, three different PPAR subtypes have been identified PPARα, PPARβ/δ, and PPARγ. PPARγ was first identified for its role in lipid and glucose metabolism. PPARγ is critical in a variety of biological processes. Natural and synthetic ligands bind to PPARγ, resulting in conformational change and activation of PPARγ.

Natural Ligands. Prostaglandin 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2) was the first discovered endogenous ligand for PPARγ [54, 55]. 15d-PGJ_2 is a potent activator for PPARγ and responsible for many of anti-inflammatory actions [56]. The variety lipophilic ligands can bind and activate PPARγ. The essential fatty acids, arachidonic acid, gamolenic acid, docosahexanoic acid, eicosapentaenoic acid, and components of oxLDL, such as 9,13-hydroxyoctadecadienoic acid (HODE) and 8,15-hydroxyeicosatetraenoic *acid* (HETE) are also potent endogenous activators of PPARγ [57]. These PPARγ ligands can be regulated by lipoxygenase and cytokines. For example, 13-HODE and 15-HETE can be generated from linoleic and arachidonic acids, respectively, by a 12/15-lipoxygenase that is upregulated by interleukin-4 [5, 58]. *Plasmodium falciparum* may itself activate PPARγ. *Plasmodium falciparum* produces hemozoin, which induces the release of lipoxin A_4 (LXA_4), 5,15-diHETE, and 15-HETE that can activate PPAR [59].

Synthetic PPARγ Ligands. In addition to natural ligands, many synthetic ligands have been identified. The antidiabetic drugs thiazolidinediones (TZDs) including troglitazone, rosiglitazone, pioglitazone, and ciglitazone, used for the treatment of type 2 diabetes, are synthetic PPARγ agonists [7, 60]. They regulate the expression of genes that are involved in lipid metabolism and insulin action by activation of PPARγ. Furthermore, TZDs have beneficial anti-inflammatory properties that are widely used for treatment of patients with inflammatory diseases [7].

5. PPARγ and CD36

Macrophage and macrophage CD36 play a critical role in clearing PE and protecting host from high density of parasites during acute blood-stage malaria infection [28, 30, 61]. Therefore, upregulation of CD36 expression on macrophages may trigger parasite clearance and enhance survival of the host. CD36 can be upregulated by different molecules. Treatment macrophages with oxLDL and inflammatory cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), upregulate CD36 expression [62]. The mechanism of induction of CD36 by oxLDL and these cytokines is due to the ability to activate PPARγ [63]. In addition, both IL-13 and IL-4, which induce an alternative activation of macrophages [64], induce expression of CD36 by generation of natural ligands of PPARγ [5, 65]. The functional consequences of CD36 expression and PPARγ activation induced by IL-13 are to enhance phagocytosis of PE [65]. The role of PPARγ in CD36 expression is further confirmed by using macrophages lacking expression of PPARγ. By using macrophages differentiated from PPARγ-deficient embryonic stem cells, PPARγ is not essential for macrophage differentiation, but it is required for basal expression of CD36 [66] and necessary for the regulation of CD36 in response to PPARγ ligands in PPARγ-deficient stem cell-differentiated macrophages [67]. IL-13 up-regulates macrophage CD36 expression, and it is ineffective on CD36 induction on PPARγ-deficient macrophages compared to wild-type cells [65]. It is particularly interesting because IL-13 not only induces macrophage alternative activation [64] which could help control parasite burden while limiting associated inflammation and thus reducing host pathology, but also enhances CD36-dependent PE phagocytosis without TLR2 involvement [65].

Incubation of macrophages with PPAR-RXR agonists, including 15d-PGJ2, 9-*cis*-retinoic acid (9-*cis*-RA), and TZD increase CD36 expression on macrophages which associates with increased capacity for phagocytosis of PE [28]. This increase in phagocytosis of PE is accompanied by a decrease in parasite-induced TNF-α production. PPARγ agonist rosiglitazone enhances phagocytic clearance of PE and inhibits inflammatory responses to infection via inhibition of *pf* GPI-induced activation of the MAPK and NF-κB signaling pathways [8]. Furthermore, rosiglitazone reduces the parasitemia in a CD36-dependent manner in the *Plasmodium chabaudi chabaudi* hyperparasitemia model and improves the survival rate even when treatment is initiated as late as day 5 after infection [8]. These results indicate that specific upregulation of CD36 by these compounds may represent a novel means for modulating host clearance of PE and proinflammatory responses to *Plasmodium falciparum* [28]. Despite studies about PPARγ agonists that enhance CD36 expression and uptake PE, this treatment raises concerns that treating individual with PPARγ agonists could also enhance CD36 expression on endothelial cells and thus triggers the adherence of PE to endothelial cells in various blood vessels. Therefore, specific upregulation of CD36 expression on macrophages may represent a novel therapeutic strategy to treat malaria infections.

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Research Article

Protective Effects of Peroxisome Proliferator-Activated Receptor-α Agonist, Wy14643, on Hypoxia/Reoxygenation Injury in Primary Rat Hepatocytes

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This study investigates the effects and possible mechanism of an agonist of PPARα, Wy14643, on primary hepatocytes subjected to H/R injury in rats. H/R induced a significant increase ALT, AST, MDA in the culture medium and ROS in the hepatocytes. These effects were reversed by pretreatment with Wy14643 in the dose-dependent manner. The activity of SOD and the level of GSH in the hepatocytes were decreased after H/R, which were increased by Wy14643 pretreatment. Moreover, the mRNA expressions of PPARα significantly increased in H/R+Wy14643 groups when compared with that in H/R group. A PPARα agonist, Wy14643, exerts significant protective effect against H/R injury in primary hepatocytes via PPARα activation and attenuating oxidative stress.

1. Introduction

Ischemia/reperfusion (I/R) injury is a serious complication precipitated during short-term expansion of the invading parasitic pathogens, such as by *Entamoeba histolytica* as the infections lead to local tissue damage and hypoxia [1]. The condition has also been reported to occur in certain intracellular bacterial infections, for example, *Chlamydia* species. Mechanisms of I/R injury involve complex and multiple pathways, including the direct ischemic cellular damage as well as the cell injury due to the activation of inflammatory response after reperfusion [2]. A hepatic, in vitro hypoxia/reoxygenation (H/R) model can be use to study the pathophysiology of this injury [3]. Previous study showed that the generation of reactive oxygen species (ROS) is likely to be an important factor in H/R-induced cell damage. Thus, ROS are generated immediately after H/R and activate proapoptotic/inflammatory signalling in the cell or directly damage cell organelles. These include direct oxidation of cellular components and lipids (lipid peroxidation), activation of inflammatory gene transcription, and possible activation of the innate immune response [2, 3]. Administration of antioxidants such as glutathione could afford protection against I/R injury [4, 5]. Oxygen deprivation (hypoxia) during ischemia and subsequent reoxygenation upon reperfusion are thought to be the major factors contributing to ROS production and the subsequent cellular damage [6]. Hypoxia increases mitochondrial reactive oxygen species (ROS) generation at Complex III, and the predominant source of ROS by oxygen limitation originates from mitochondria [7]. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor related to retinoid, steroid, and thyroid hormone receptors. Peroxisome proliferator-activated receptor-α (PPARα) is one of the three subtypes of the nuclear receptor PPAR family [8]. The structure of PPARα consists of an aminoterminal region that allows for ligand-independent activation and constitutive activity on the receptor and is negatively regulated by phosphorylation and the carboxyl-terminal ligand binding domain. PPARα has a wide range of effects on metabolism, cellular proliferation and the immune response [9]. Besides its metabolic regulating effects, PPARα also exerts anti-inflammatory and antioxidant effects in different organs. As is well known that PPARα have anti-inflammatory properties [10], we proposed that it may have a similar effects on hepatic I/R injury. Previous studies suggest that PPARα agonists protect many

organs against I/R injury such as heart [11], kidney [12], and brain [13]. PPARα agonists can inhibit the expression of oxidative stress genes via a mechanism termed ligand-dependent repression by PPARα. It has been demonstrated that the anti-inflammatory effect of PPARα ligands is also dependent on the inhibition of functional NF-κB activation and AP-1 activation [14]. Furthermore, PPARα stimulation by Wy14643 induces expression and activation of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione (GSH), which protects hepatocytes against hepatic I/R injury mice model in vivo [15]. These beneficial effects of Wy14643 are possibly associated with enhancement of anti-oxidant and inhibition of inflammation response. Razeghi et al. have found a downregulation in expression of PPARα in a rat model of hypoxia [16]. However, there is no report about the effect of H/R on expression of hepatocytes PPARα-mRNA in vitro. In the present study, we determined whether PPARα activation by the selective agonist Wy14643 had a protective role in H/R injury of hepatocytes in vitro.

2. Materials and Methods

2.1. Animals. Male Sprague-Dawley rats (weighing 220–280 g) were used in these experiments. Temperature and relative humidity were kept at (22 ± 2)°C and (50 ± 5)%, respectively. All rats were obtained from the Center of Experimental Animals in Anhui Medical University. This project was approved by the Committee for Research and Animal Ethics of the Anhui Medical University. They were allowed free access to a commercial standard chow and water ad libitum before the experimental procedure began. All rats were acclimatized to our animal facility for at least 1 week before experiment to avoid stressful stimuli.

2.2. Hepatocyte Isolation and Culture. Rat hepatocytes were isolated and cultured as previously described [17]. Male Sprague-Dawley rat livers were minced after perfusion with 0.5 g/L collagenase IV (Sigma, USA) through the portal vein. Hepatocytes were separated from nonparenchymal cells by centrifugation at 50 g for 4 min at 4°C. The viability of the collected hepatocytes exceeded 90%, as determined by the trypan blue exclusion test. Hepatocytes were resuspended in William's E medium (Gibco, USA) containing 100 mL/L fetal calf serum, 27×10^{-6} mol/L $NaHCO_3$, 100×10^{-9} mol/L insulin, and 10×10^{-9} mol/L dexamethasone at pH 7.4. Hepatocytes were then plated on type I collagen—coated 24—multiwell plates and incubated overnight in an atmosphere of 95% air and 5% CO_2 at 37°C. Cells were studied according to the experimental protocols.

2.3. Hypoxia/Reoxygenation Treatments and Groups. H/R injury in vitro was performed as previously described [18]. Hepatocytes were isolated and maintained overnight at 37°C in a humidified incubator containing 95% air and 5% CO_2 (referred to as normoxic conditions). The next day, hypoxic conditions were attained by exposure to 95% N_2 and 5% CO_2 gas mixture in a humidified incubator for 4 h. Hypoxic exposure was confirmed in each experiment by measuring the ambient PO_2 of the gas above the monolayers. Reoxygenation of hypoxic cultures was achieved with normoxia conditions for another 10 h, whose ambient values should return to pre-hypoxic levels within 5 min. Six groups of culture hepatocytes (6 wells each) were separated as follows. (1) The control group was exposed to normoxic medium for 14 h. (2) The H/R injury group was exposed to hypoxic (4 h) and then reoxygenation (10 h) conditions as described above. (3) Model H/R hepatocytes treated with different doses of Wy14643 (10×10^{-6}, 30×10^{-6}, and 100×10^{-6} mol/L, resp.) (Pirinixic acid, CAS 50892-23-4, Cayman Chemical, USA). Different concentrations of Wy14643 were added to the culture medium 60 min before H/R course [15]. Wy14643 were prepared in 10% (v/v) DMSO (dimethyl sulfoxide) and 90% (v/v) William's E medium. The final DMSO concentration in cell cultures was 0.1% (this concentration affected neither cell viability nor hepatocytes damage). (4) DMSO group hepatocytes were pretreated for 60 min with 0.1% DMSO before H/R.

2.4. Mitochondria Isolation. The cell medium was collected, centrifuged at 450 g to remove cell debris. The hepatocytes mitochondrial fraction was prepared according to the method reported by Johnson and Lardy. The homogenate was centrifuged at 600 g for 10 min, and the supernatant was centrifuged for 5 min at 15,000 g to obtain the mitochondrial pellet which was washed with a medium and centrifuged for 5 min at 15,000 g.

2.5. Measurement of Intracellular ROS Generation. 2,7-Dichlorofluorescein diacetate (DCFH-DA) was used as indicatior of intracellular formation of ROS as described previously [19]. DCFH-DA is cell-permeant probe that enters the cell followed by cleavage of the diacetate molecules by cellular esterases. The probe becomes fluorescent when it is oxidized in cells by ROS. In brief, hepatocytes were plated onto 24-well plates at a density of 60,000 cells/well. 24 h after plating, cells were washed twice with PBS and subsequently incubated in PBS+ containing 10 μM DCHFH-DA (Invitrogen, Calif, USA) for 45 min at dark. Subsequently, cells were rinsed with PBS, and 500 μL of fresh Earle's salt solution was added to each well. Fluorescence was measured using prewarmed SpectraMax (Molecular Devices, Calif, USA) spectrofluorometer, with excitation wavelengths of 485/535 nm for 20 min. The slope of the linear part of the graph was used to calculate the rate of increases in fluorescence. As a positive control, H_2O_2 was added just before placing the plate into the plate reader. The increase in fluorescence by ROS production was expressed as % of control.

2.6. Detection of Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Malondialdehyde (MDA) Level in Culture Medium. The Lipid peroxidation in cultured hepatocyte was determined by detecting the level of MDA, which is the end product of lipid peroxidation; in the liver mitochondria was determined by measuring the level of the thiobarbituric acid-reactive substances spectrophotometrically at 532 nm according to the method reported by Buege and

Aust. ALT and AST, markers of hepatocellular injury, were measured using commercial available kit. These assay kits were purchased from the Jiancheng Bioengineering Institute (Jiancheng, China) [20].

2.7. Measurement of Glutathione (GSH) Levels and Activity of Supeoxide Dismutase (SOD) in the Hepatocytes. GSH content was measured by the Owens and Belcher method, which was determined using the following procedure. The isolated mitochondria were suspended in a buffer (pH 7.5) containing 0.1 mol/L sodium phosphate and 5 mmol/L EDTA. After precipitating with 15% (w/v) sulfosalicylic acid containing 5 mmol/L EDTA, the total glutathione level was measured spectrophotometrically at a wavelength of 412 nm using yeast glutathione reductase and 5,50-dithio-bis(2-nitrobenzoic acid), as described by Tietze. SOD activity was measured through the inhibition of nitroblue tetrazolium (NBT) reduction by O_2 generated by the xanthine oxidase system. The content of GSH and activity of SOD were measured using commercial kits (Jiancheng, China) [21].

2.8. Ultrastructure Assessment of Rat Hepatocytes. Hepatocytes fixed in 10 g/L Glutaral, dehydrated, dried and surface gilded according to standard procedures. Electron microscope was used to assess the degree of hepatic damage (JEM-1010, JEOL, Japan).

2.9. Measurement of PPARα mRNA Levels by Real-Time Polymerase Chain Reaction (Real Time PCR). Total RNA was extracted from cultured hepatocytes as recently described. Total RNA was extracted using the Trizol reagent (Invitrogen, USA), the value A at 260/280 nm was detected, and the concentration of RNA was calculated. cDNA was synthesized according to the manufacturer's instruction for the reverse transcription kit (Promega, USA), then semiquantitative real-time polymerase chain reaction analysis using SYBR Green PCR Master Mix (Invitrogen, USA). The cDNAs were quantified with an ABI StepOne sequence detection system (Applied Biosystems, Calif, USA). Primers were synthesized by Sangon (China). The rat, specific primer (sense and antisense primers) for PPARα was sense: 3′-GTGGCTGCTATAATTTGCTGTG-5′, antisense: 5′-GAAGGTGTCATCTGGATGGGT-3′. The primer β-action was sense: 5′-TGGAATCCTGTGGCATTCCATCCATGAAAC-3′, antisense: 5′-AGGCTATCCCAGGCTTTGC-3′. Real-time PCR was performed in a 25 μL of reaction containing 12.5 μL of 2X SYBR Green Supermix, 200 nM primers and cDNA. The cycles for PCR were as follows: 95°C for 20 s, 54°C for 7 min, 40 cycles of 95°C for 20 s, 54°C for 30 s, and 72°C for 30 s. The fold changes in gene expression of PPARα was calculated using the comparative C_t (cross-threshold) method. Briefly the C_t of the housekeeping gene β-action was subtracted from the C_t of PPARα to get ΔC_t. The ΔC_t value of control sample was the subtracted ΔC_t of the rest of the treatments to get the $\Delta\Delta C_t$ value. Fold differences compared to control sample are obtained by calculating $2^{-\Delta\Delta C_t}$ for each treatment group.

2.10. Statistical Analyses. All data were expressed as mean ± SD. The statistical significance of differences between groups was analyzed using the one-way analysis of variance (ANOVA) and methods of LSD with the SPSS11.5 for windows XP statistical software package. The P values less than 0.05 was considered statistically significant.

3. Results

3.1. Pretreatment with the PPARα Agonist Wy14643 Decreases Hepatocytes Damage Induced by H/R Injury. ALT and AST levels in the medium of hepatocytes cultured under normal condition and H/R stress were shown in Figures 1(a) and 1(b), respectively. ALT and AST level increased after H/R (ALT, $P = 0.006$; AST, $P = 0.0032$). The increase in the medium ALT as well as AST activity induced by hepatic H/R was significantly attenuated by pretreatment Wy14643 (100×10^{-6}, 30×10^{-6}, 10×10^{-6} mol/L) in a dose-dependent manner. (ALT, $P = 0.004$; $P = 0.095$; $P = 0.041$. AST, $P = 0.001$; $P = 0.0062$; $P = 0.0071$.) DMSO group was not considered statistically when compared with H/R group ($P = 0.08$) the results demonstrated Wy14643 has the dose-dependent protective effects on hepatic injury.

3.2. Pretreatment with the PPARα Agonist Wy14643 Increases Antioxidant Enzymes Induced by H/R Injury. The MDA level increased after H/R and maintained at a considerably high concentration during the period of cultivation ($P = 0.008$). Wy14643-pretreated group (100×10^{-6}, 30×10^{-6}, 10×10^{-6} mol/L) exhibited a decrease in the content of MDA compared with H/R group ($P = 0.0025$; $P = 0.0094$; $P = 0.056$) (Figure 1(c)). SOD and GSH were presented in Figure 2(a) and Figure 2(b). In H/R group, both these enzymes activities were significantly lower when compared with control group (SOD, $P = 0.0003$; GSH, $P = 0.0005$). But in the Wy14643-pretreated group (100×10^{-6}, 30×10^{-6}, 10×10^{-6} mol/L), the antioxidant activities were markedly higher when compared with the H/R group (SOD, $P = 0.002$; $P = 0.059$; $P = 0.076$. GSH, $P = 0.0017$; $P = 0.0075$; $P = 0.0098$). DMSO group was not considered statistically when compared with H/R group ($P = 0.085$).

3.3. Pretreatment with the PPARα Agonist Wy14643 Decreases ROS Induced by H/R Injury. ROS levels in the hepatocytes cultured under normal condition and H/R stress were shown Figure 3. ROS level increased after H/R (ROS, $P = 0.0032$), The increase in the hepatocytes induced by hepatic H/R was significantly attenuated by pretreatment of Wy14643 (100×10^{-6}, 30×10^{-6}, 10×10^{-6} mol/L) in a dose dependent manner. ($P = 0.0025$; $P = 0.0070$; $P = 0.0090$). DMSO group was not considered statistically when compared with H/R group ($P = 0.08$); the results demonstrated Wy14643 has the dose-dependent decrease ROS on hepatic injury.

3.4. PPARα Agonist Wy14643 Upregulates Hypoxia-/Reoxygenation-Induced PPARα-mRNA Expression in Hepatocytes. PPARα-mRNA expression was assessed in the absence and presence of Wy14643 during inducing hepatocytes by H/R.

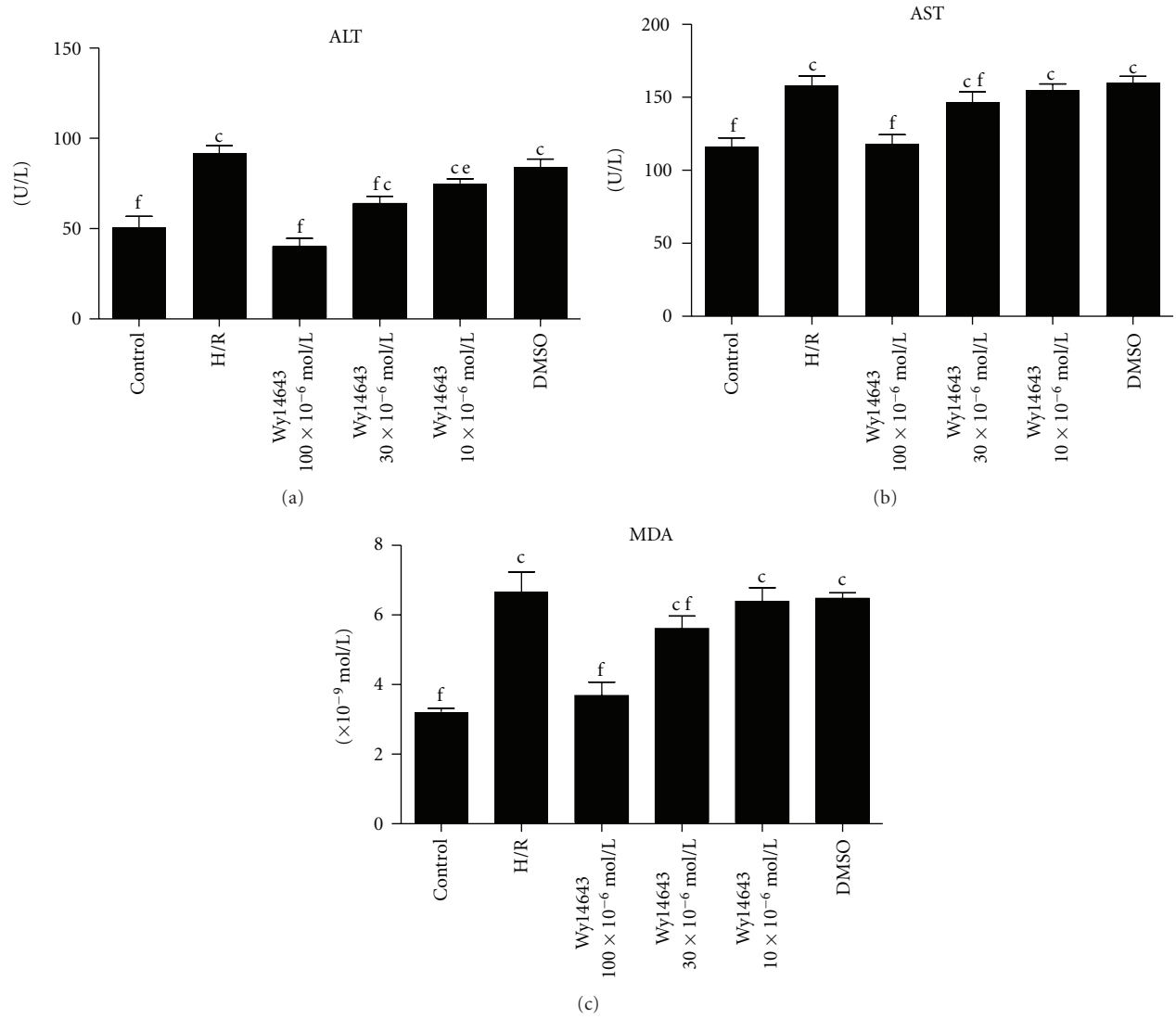

Figure 1: (a) ALT in different groups (Mean ± SD, $n = 6$). After 4 h of hepatocellular hypoxia and 10 h of reoxygenation. The levels of ALT in hepatocytes medium were determined. $^{c}P < 0.01$ versus control group; $^{e}P < 0.05$, $^{f}P < 0.01$ versus H/R group. (b) AST in different groups (Mean ± SD, $n = 6$). After 4 h of hepatocellular hypoxia and 10 h of reoxygenation. The levels of AST in hepatocytes medium were determined. $^{c}P < 0.01$ versus control group; $^{f}P < 0.01$ versus H/R group. (c) MDA in different groups (Mean ± SD, $n = 6$). After 4 h of hepatocellular hypoxia and 10 h of reoxygenation. The levels of MDA in hepatocytes determined. $^{c}P < 0.01$ versus control group; $^{f}P < 0.01$ versus H/R group.

Quantitative analysis showed that the mRNA expressions of PPARα in the H/R group was significantly decreased when compared with the control group ($P = 0.005$). However, when comparing the Wy14643 group (100×10^{-6}, 30×10^{-6}, 10×10^{-6} mol/L) with the H/R group, mRNA expressions of PPARα were increased after the addition of Wy14643 ($P = 0.0018$; $P = 0.0073$; $P = 0.0098$) (Figure 4). DMSO group was not considered statistically when compared with H/R group ($P = 0.065$).

3.5. Ultrastructure Alterations of Hepatocytes. The ultrastructure of hepatocytes was normal in the control group (Figure 5(a)). Compared with the control group, H/R group was markedly damaged characterized by mitochondrial crista destruction markedly decreased and nucleus structure destruction (Figure 5(b)). In Figure 5(c), Wy14643 group (100×10^{-6} mol/L), almost normal appearance of mitochondrion and nucleus structure. In Figure 5(d), Wy14643 group (30×10^{-6} mol/L), Mitochondrion swelled mildly and normal nucleus structure. In Figure 5(e), Wy14643 group (10×10^{-6} mol/L), Mitochondrion swelled moderatedly, with mitochondrial crista interruption and nucleus structure destruction. In Figure 5(f) (DMSO group), mitochondrion swellen significantly and nucleus structure destruction.

4. Discussion

This study provides compelling evidence that the selective PPARα agonist Wy14643 protects the hepatocytes from transient H/R injury. An increase in the cell medium's ALT

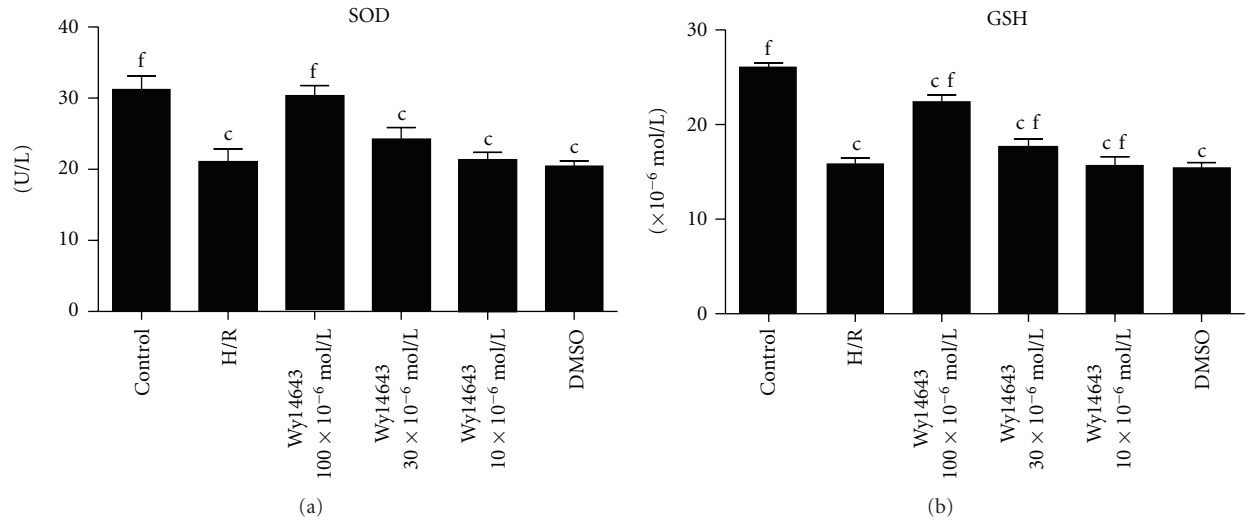

Figure 2: (a) SOD in different groups (Mean ± SD, $n = 6$). After 4 h of hepatocellular hypoxia and 10 h of reoxygenation. The levels of SOD in hepatocytes determined. $^{c}P < 0.01$ versus control group; $^{f}P < 0.01$ versus H/R group. (b) GSH in different groups (Mean ± SD, $n = 6$). After 4 h of hepatocellular hypoxia and 10 h of reoxygenation. The content of GSH in hepatocytes mitochondria fractions was determined. $^{c}P < 0.01$ versus control group; $^{f}P < 0.01$ versus H/R group.

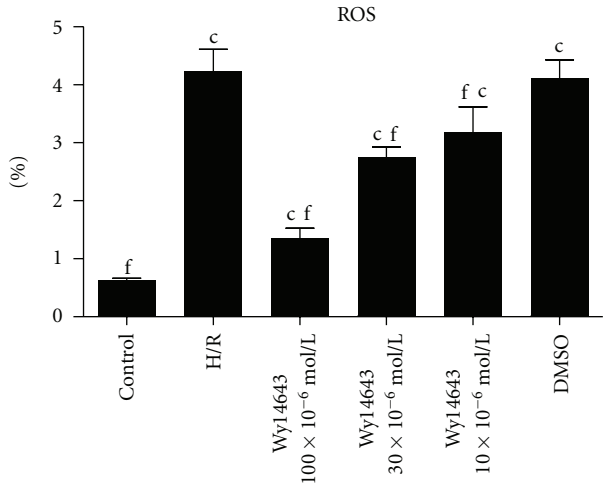

Figure 3: The content of ROS in different groups was determined by DCFH-DA (Mean ± SD, $n = 6$). After 4 h of hepatocellular hypoxia and 10 h of reoxygenation. $^{c}P < 0.01$ versus control group; $^{f}P < 0.01$ versus H/R group.

and AST levels has been usually used as an effective indicator of impaired hepatocytes with H/R. The protective effect of Wy14643 demonstrated herein by reducing ALT and AST levels is associated with an inhibition of oxidative stress and upregulation of hepatocytes PPARα-mRNA expression. Previous studies provided evidence that Wy14643 protected the rat liver from hepatic I/R injury [10]. In our study, we found that pretreatment of Wy14643 resulted in a marked reduction of ALT and AST levels with dose-dependent manner in the cell medium compared with the control group, as well as we demonstrated a significant down regulation in PPARα-mRNA after H/R, and this downregulation was significantly attenuated by Wy14643.

The precise sequence of events leading to hepatic I/R is still a matter of debate. However, two major culprits have been identified including uncontrolled oxidative stress and unfettered inflammation, whose effects culminate in the necrotic cell death of hepatocytes, the hallmark of severe hepatic I/R [22]. Therapies aimed at curbing inflammation and blocking oxidative necrosis of hepatocytes in the setting of hepatic I/R have been heavily explored. The precise sequence of events leading to hepatic I/R has not been completely elucidated. As is well known H/R has been shown to stimulate a burst of ROS of mitochondrial and extra mitochondrial origin; as reported, ROS was generated by cytosol and mitochondria, in different cell types cultured under 1.5–5% O_2 [23, 24]. Although hypoxia stimulates ROS generation and the contribution of mitochondria to this process may vary depending on whether reoxygenation follows the hypoxic phase, it has been reported that Wy14643 uncouples mitochondrial oxidative phosphorylation in isolated hepatic mitochondria [25]. MDA is the end product of lipid peroxidation considered as a sensitive index to assess lipid peroxidation [26]. SOD, an oxygen radical scavenger which converts superoxide anion radicals present in the upper stream of reactive oxygen metabolism cascade and protects cells against damage. The hepatic MDA levels significantly increased, indicating the presence of enhanced lipid peroxidation due to H/R injury [27], whereas the level of SOD declined, demonstrating the depletion of antioxidant pool in hepatocytes H/R. In this study we showed that Wy14643 significantly decreased MDA levels in the hepatocytes when compared with the H/R group. In addition, high levels of SOD were observed in the Wy14643-pretreated groups. According to these findings, Wy14643 has protective effects against the oxidative stress induced hepatocytes injury. This finding is in agreement with other reports showing that Wy14643 enhances expression of antioxidant enzymes such

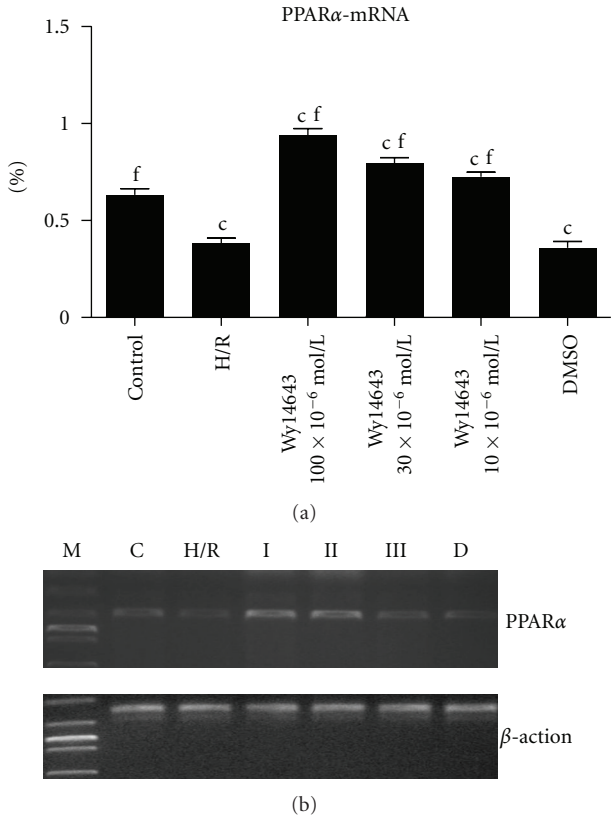

Figure 4: After 4 h of hepatocellular hypoxia and 10 h of reoxygenation. Effect of hypoxia and reoxygenation on PPARα-mRNA expression in hepatocytes. (Mean ± SD, $n = 6$) $^{c}P < 0.01$ versus control group; $^{e}P < 0.05$, $^{f}P < 0.01$ versus H/R group. (C: control group; H/R: hypoxia/reoxygenation group; I: Wy14643 group 100 $\times 10^{-6}$ mol/L; II: Wy14643 group 30 $\times 10^{-6}$ mol/L; III: Wy14643 group 10 $\times 10^{-6}$ mol/L; D: DMSO group).

as SOD and catalase in the rat liver [28]. Our data suggests that the mechanism underlying the protective action of Wy14643 against hepatocellular H/R is the direct ROS scavenging effect.

GSH is a major cellular antioxidant which is found mainly in cytosol where it is synthesized from its constituent amino acids and in mitochondria where it plays a key protective role against oxidant-induced cell death [29]. Because of its antioxidant function, hypoxia would be expected to reduce intracellular GSH stores. The present study examined for the first time the impact of mitochondria GSH depletion on the survival of hepatocytes which were pretreated by Wy14643 during exposure to hypoxia. Previous studies reported the decrease of hepatocellular GSH stores by hypoxia. This study addressed the role of GSH, particularly in mitochondria, on the susceptibility of hepatocytes to hypoxia-induced oxidative stress. Consistent with the burst of ROS generation, it has been shown that hypoxia depletes GSH stores [30, 31]. In agreement with these findings we showed GSH depletion in both cytosol and mitochondrial compartments compared with H/R. Here, we found that Wy14643 increased the content of GSH in the hepatocytes which were induced by H/R. Our result showed that Wy14643 significantly increased the production of GSH compared with the H/R group.

In the present study, we found that H/R stress lead to decreases of PPARα-mRNA in hepatocytes. A variety of stimuli, including hypobaric hypoxia, are also capable of inducing a switch in substrate use associated with downregulation of PPARα-regulated genes [32, 33]. Wy14643 attenuates the increase in PARP (poly-ADP-ribose polymerase) activity caused by splanchnic artery occlusion (SAO) shock, ROS produce strand breaks in DNA, which trigger energy-consuming DNA repair mechanism and activate the nuclear enzyme PARP. PARP plays an important role in ischemia/reperfusion injury. PARP activation results in the depletion of its substrate NAD^+ and also in a reduction in the rate of glycolysis. As NAD^+ functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD^+ depletion leads to a rapid fall in intracellular ATP rapidly followed by cellular dysfunction and death—the PARP Suicide Hypothesis. Huss et al. [33] found that hypoxia deactivates PPARα by reducing the availability of its obligate partner RXR (retinoid X receptor). Mitochondrial oxidative phosphorylation is inhibited, and ATP generation is reduced, which aggravates hepatocellular anoxic injury. Hence, PPARα may improve the energy supplement of hepatocytes meanwhile enhancing the anti-ischemia ability of hepatocytes. Previous data showed a rat cardiac model of hypoxia and have found a downregulation in its expression of PPARα [34]. These results show, similar to our study, that the expression of PPARα-mRNA in hepatocytes which were exposed to H/R was reduction. After pretreatment of Wy14643, the expression of PPARα-mRNA increased; meanwhile, the damage of hepatocytes was relieving. Thus, it is conceivable; the expression of PPARα during inflammation and hypoxia may serve as a counter regulator of ROS production.

In summary, PPARα agonist Wy14643 decreased the injury degree with the hepatocytes H/R injury model, which is associated with modulating the expression of PPARα. Moreover, Wy14643 also protected the hepatocytes against oxidative stress. These findings are particularly interesting because they demonstrate that a regulatory factor PPARα expressed in liver parenchymal cells, but not in Kupffer cells, may have significant impact on the hepatic inflammatory response. From a clinical standpoint, most of the new knowledge that we have gathered on the multiple "hepatoprotective" functions of PPARα including protection from oxidative necrosis is both conceptually important and directly relevant to clinical problems associated with liver transplantation and liver disease. However, before the clinical therapeutic application of this agent, further investigations should be performed. The effects of Wy14643 on prolonged H/R injury or the effects on the chronic phase should be studied.

Authors Contributions

K. Chen and Y.-H. Li contributed equally to this work, K. Chen, Y.-H. Li, S.-Q. Xu, S.-H. Hu, and L. Zhang designed research, K. Chen, S.-H. Hu, and S.-Q. Xu performed

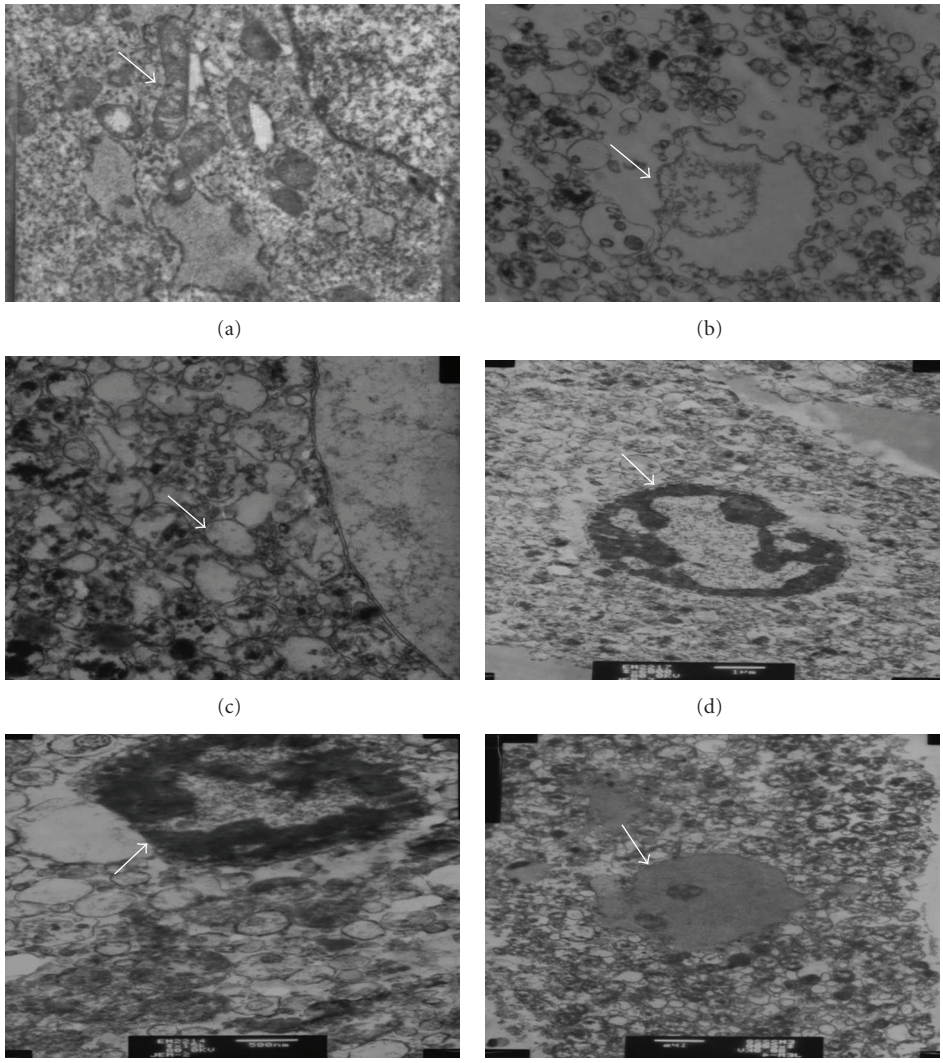

Figure 5: Ultrastructure alterations of hepatocytes (a) Control group, Nomal appearance of mitochondrion, nucleus struct. (X = 12 000) (b) H/R group, Mitochondrial crista destruction and nucleus structure destruction. (X = 12 000) (c) Wy14643 100×10^{-6} mol/L group, Nomal appearance of mitochondrion and nucleus structure almost. (X = 12 000) (d) Wy14643 30×10^{-6} mol/L group, Mitochondrion swellen mildly and nucleus structure are nomal nearly. (X = 12 000) (e) Wy14643 10×10^{-6} mol/L group, Mitochondrion swellen significantly, vacuolar degeneration and mitochondrial crista destruction, nucleus structure destruction. (X = 12 000) (f) DMSO group, Mitochondrion swellen significantly, vacuolar degeneration and mitochondrial crista destruction, nucleus structure destruction. (X = 12 000).

research, K. Chen, Y.-H. Li, and L. Zhang analyzed data, and K. Chen, and Y.-H. Li wrote the paper.

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Review Article

The Case for the Use of PPARγ Agonists as an Adjunctive Therapy for Cerebral Malaria

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Cerebral malaria is a severe complication of *Plasmodium falciparum* infection associated with high mortality even when highly effective antiparasitic therapy is used. Adjunctive therapies that modify the pathophysiological processes caused by malaria are a possible way to improve outcome. This review focuses on the utility of PPARγ agonists as an adjunctive therapy for the treatment of cerebral malaria. The current knowledge of PPARγ agonist use in malaria is summarized. Findings from experimental CNS injury and disease models that demonstrate the potential for PPARγ agonists as an adjunctive therapy for cerebral malaria are also discussed.

1. Introduction

Few diseases have the global health and economic impact of malaria [1]. In 2009, an estimated 225 million people were infected with malaria and close to a million people succumbed to their infection [2]. Malaria is caused by apicomplexan parasites belonging to the genus *Plasmodium*. Five species infect humans, *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and most recently, *P. knowlesi* [3]. The majority of morbidity and mortality is caused by *P. falciparum* infection, with the highest burden born by children and pregnant women. In the absence of prompt and effective treatment, *P. falciparum* infection can progress quickly, rapidly becoming severe and fatal. The rise in drug-resistant parasites complicates the administration of effective treatment.

Severe malaria has multiple manifestations that can occur singly or in combination. They include hyperparasitemia, high fever, haemoglobinuria, acute renal failure, acute pulmonary edema, metabolic acidosis and respiratory distress, hypoglycemia, anemia, and cerebral malaria, which is characterized by coma and convulsions. Cerebral malaria has the highest mortality rate of all the severe complications and is associated with long-term cognitive and neurological deficits in surviving children [4–6].

Intravenous artesunate is now the standard of care for severe malaria in both adults and children following the landmark SEAQUAMAT and AQUAMAT trials that demonstrated the superiority of artesunate over quinine in adults and in children [7, 8]. However, even with the improved efficacy of artesunate, fatality rates remained high, 15% in adults and 10.9% in children. Adjunctive therapies, defined as therapies administered in combination with antiparasitic drugs that modify pathophysiological processes caused by malaria, have been pursued as a way to improve the outcome of severe malaria. Adjunctive therapies may also help extend the efficacy of antiparasitic drugs, an important consideration given the emergence of artemisinin resistance [9, 10]. Several adjunctive therapeutic strategies have been tested in *P. falciparum* cerebral and severe malaria so far, unfortunately with-out much success (see [11] for a recent review). A number of adjunctive therapies (including nitric oxide, arginine, erythropoietin, levamisole) have demonstrated encouraging results in experimental models of cerebral malaria or in clinical trials in uncomplicated malaria and are awaiting evaluation in severe malaria [11].

This review will focus on the utility of PPARγ agonists as an adjunctive therapy for the treatment of cerebral malaria. The current knowledge of PPARγ agonist use in malaria will be summarized. We will also summarize data on additional

mechanisms of action attributed to PPARγ agonists that may be of benefit in cerebral malaria.

2. The Pathogenesis of Cerebral Malaria

Cerebral malaria is a severe complication of *P. falciparum* infection. It occurs in nonimmune individuals, with the greatest burden born by children in sub-Saharan Africa. Although the parasite is a key player in the development of cerebral malaria, hyperparasitemia does not necessarily correlate with disease severity, and cerebral pathology can develop even with the use of effective antiparasitic therapy. It has long been recognized that the host immune response plays an important role in mediating pathology in malaria, and this has fueled the search for effective immunomodulatory adjunctive therapies.

Sequestration of parasitized erythrocytes (PEs) in the microvasculature of the brain (and other organs), resulting in vascular occlusion and local tissue hypoxia and ischemia, is the hallmark feature of cerebral malaria [12]. Sequestration of PEs occurs via receptor-ligand interactions, with parasite-derived ligands expressed on the surface of PEs (a major one being *P. falciparum* erythrocyte membrane protein-1 or Pfemp-1) binding to receptors expressed on microvascular endothelial cells. Postmortem, in vitro, and genetic studies support that ICAM-1 is the major sequestration receptor for PEs in the brain, while the scavenger receptor CD36 is the major receptor outside the brain [13–21].

Parasites produce a variety of bioactive molecules that can elicit innate immune responses in the host [22]. An excessive inflammatory response with elevated levels of pro-inflammatory cytokines, especially TNF, is a major contributor to cerebral malaria pathology [23]. TNF, produced by activated endothelium and recruited leukocytes, can upregulate cell adhesion molecules, including ICAM-1, and exacerbate PE sequestration. Higher levels of TNF have been observed in cerebral malaria and correlated with mortality [24–26], and genetic predisposition to overproduce TNF in response to infection has been associated with susceptibility to cerebral malaria [27, 28]. Elevated levels of TNF are also seen in the cerebral spinal fluid (CSF) of infected children and correlated with encephalopathy [29]. Interestingly, CSF TNF levels did not correlate with serum levels, implying independent cerebral generation of TNF. Elevated levels of additional inflammatory mediators including IFNγ, IL-6, IL-1β, IL-1ra, IL-10, MIP-1α and MIP-1β, MCP-1, and IP-10 have been observed in cerebral malaria patients [26, 30–34].

Parasite sequestration and inflammation can lead to endothelial activation and dysfunction. Activated endothelium can lead to monocyte and platelet recruitment further impeding vessel flow and contributing to tissue hypoxia and ischemia [35]. Widespread endothelial activation (including increased ICAM-1 expression and the disruption of cell-junction proteins) has been observed in postmortem studies of cerebral malaria patients [36], and markers of endothelial activation and dysfunction such as soluble ICAM-1, von Willebrand factor, and angiopoietin-2 are elevated in cerebral malaria [37–39]. Low nitric oxide (NO) bioavailability (potentially due to quenching by cell-free hemoglobin released during hemolysis) contributes to the development of endothelial dysfunction in malaria infection [40, 41].

Sequestration, inflammation, and endothelial dysfunction can lead to a breakdown of the blood-brain barrier (BBB). Hemorrhages are common autopsy findings in cerebral malaria [12, 42, 43], as are focal disruptions of the BBB [44]. The activation of perivascular macrophages and axonal damage observed in cerebral malaria may be the result of cytokines, parasite antigens, and plasma proteins crossing the BBB, in addition to local hypoxic and inflammatory conditions [36, 45, 46].

Metabolic perturbations are also common in children with cerebral malaria and may contribute to pathology. Vascular obstruction leading to hypoxia, or TNF-induced cytopathic hypoxia, have been proposed as possible causes [47–49].

Recent investigations using fluorescein angiography and fundoscopy have permitted a view of the brain microvasculature in living patients with cerebral malaria, by imaging the retina (the only part of the central nervous system (CNS) vasculature that is available for direct observation). Pediatric cerebral malaria patients had evidence of PE sequestration and thrombi (containing both fibrin and platelets) in their vasculature that were associated with perfusion abnormalities and areas of ischemia and tissue damage (retinal whitening). Focal disruptions of the BBB were observed most often, but not always, in association with hemorrhages [44, 50, 51]. Postmortem analysis revealed axonal damage not only in areas of hemorrhage but also in areas of vascular occlusion by sequestered parasites and/or fibrin-platelet thrombi [51].

Cerebral malaria is a complex disorder that is as yet not fully understood. Multiple processes likely contribute to its development including peripheral and CNS inflammation, PE sequestration, vascular endothelial activation, prothrombotic activation, blood flow obstruction, tissue hypoxia and ischemia, metabolic changes, and BBB dysfunction, leading to neurodegeneration. These processes can contribute to the seizures and coma seen in cerebral malaria patients and the neurologic and cognitive deficits which persist in a portion of cerebral malaria survivors [4, 29]. The activation of PPARγ appears to play an important role in recovery in several models of CNS injury and disease, by limiting inflammation and cytotoxicity and promoting reparative mechanisms. These mechanisms may also be protective in the context of cerebral malaria as well. Interestingly, PPARγ was one of only two genes in a malaria-resistance locus identified using a genome-wide analysis of inbred mouse lines [52], supporting a protective role for PPARγ in malaria.

3. PPARγ and Its Agonists

PPARγ is a member of the family of nuclear hormone receptors which function as ligand-activated transcription factors [53]. PPARγ endogenous ligands include oxidized fatty acids and prostanoids, and synthetic ligands include the thiazolidinedione (TZD) class of antidiabetic drugs (e.g., rosiglitazone and pioglitazone). Upon ligand activation, PPARγ

heterodimerizes with the retinoid X receptor (RXR), a nuclear receptor for 9-cis-retinoic acid. The ligand-bound PPARγ-RXR heterodimer regulates gene transcription by binding to conserved DNA sequences called PPRE (PPAR response elements) on target genes. PPARγ can also regulate other transcription factors, through nongenomic transrepression, where the inhibition of transcription occurs by preventing the dissociation of corepressors or by sequestering the coactivators necessary for the binding of the transcription factor to DNA [54].

Originally characterized in adipocytes as a regulator of lipid and glucose metabolism, current evidence indicates that PPARγ is present in most cell types (including immune cells, endothelial cells, and neurons) and mediates multiple functions in both physiological and pathological conditions [55, 56].

PPARγ agonists have been extensively studied in many inflammatory settings, in vitro, in animal models, and in humans, and in most cases they have demonstrated anti-inflammatory properties [57]. These anti-inflammatory properties are early events (observed prior to any metabolic effects) and occur even with low-dose administration of the agonists [58]. PPARγ agonists can inhibit proinflammatory responses from a variety of cells including macrophages, dendritic cells, T cells, endothelial cells, vascular smooth muscle cells, microglia, and astrocytes [59–74]. The anti-inflammatory properties of the agonists are mediated by the transrepression effects of activated PPARγ on transcription factors including activator protein-1 (AP-1), signal transducers and activators of transcription 1 (STAT-1), nuclear factor kB (NF-κB), and nuclear factor of activated T cells (NFAT). PPARγ agonists can also suppress inflammation by PPARγ-independent mechanisms, for example, the suppression of JAK-STAT-dependent inflammatory responses in activated microglia and astrocytes via the induction of members of the suppressor of cytokine signaling (SOCS) family [75, 76].

Data have also been accruing on the neuroprotective properties of PPARγ agonists in models of CNS injury, ischemic stroke, and diseases of the CNS including multiple sclerosis, ALS, and Parkinson's disease [77–80]. These data suggest that PPARγ may be involved in coordinating cellular responses to CNS injury and disease. The potential benefit of the anti-inflammatory and neuroprotective properties of PPARγ agonists in cerebral malaria will be discussed below.

4. Generation of Endogenous PPARγ Ligands in Malaria Infection

Plasmodium falciparum may itself activate PPARγ, perhaps as part of a strategy aimed at enhancing symbiotic survival between the parasite and the host. Hemozoin, a pigment produced by *Plasmodium* to detoxify free heme generated by the degradation of haemoglobin [81], can produce large amounts of hydroxyl-fatty acids, including 15-hydroxyecosatetraenoic acid (15-HETE), 13-hydroxyoctadecadienoic acid (13-HODE), and 4-hydroxynonenal (4-HNE) by heme-catalyzed lipoperoxidation [82]. 15-HETE and 13-HODE are specific ligands of PPARγ, and 4-HNE is an inducer of PPARγ [83]. Hemozoin-mediated immunosuppressive effects on myeloid cell functions including phagocytosis, inflammatory responses, oxidative burst, and dendritic cell differentiation and maturation have been reported [84–89]. Hemozoin was able to induce the upregulation of PPARγ mRNA, while the inhibition of PPARγ reversed some of the hemozoin-mediated effects, suggesting that the immunomodulatory effects of hemozoin may be, at least partly, mediated by PPARγ activation [90].

5. The Use of PPARγ Agonists in Malaria: What We Know So Far

The use of PPARγ agonists to modulate immune responses to malaria was initially motivated by reports demonstrating that PPARγ regulates CD36 transcription and that PPARγ agonists have anti-inflammatory properties [61, 63].

At that time, the scavenger receptor CD36 was revealed to be a major, noninflammatory, phagocytic receptor for non-opsonised mature-stage PEs [91]. It was speculated that CD36-mediated phagocytosis of PEs represented an innate immune mechanism for controlling the parasite burden in nonimmune individuals (who are most at risk of developing severe disease) [91–94]. Later, CD36-mediated phagocytosis of ring-stage PEs and stage I and IIa gametocytes was also reported [95, 96]. The importance of CD36-mediated innate control of acute blood-stage malaria was demonstrated in vivo, in a murine model of hyperparasitemia (*P. chabaudi* AS infection) [97]. In this model, mice deficient in CD36 had higher parasitemia levels and higher mortality compared to CD36-sufficient mice [97].

Various PPARγ agonists including the natural ligands 15d-PGJ2 and 9-cis-retinoic acid (which binds RXR to activate the PPARγ-RXR heterodimer), and the synthetic TZDs, ciglitazone, troglitazone, and rosiglitazone, were shown to upregulate the CD36 expression on monocytes and enhance the CD36-mediated phagocytosis of PEs [92, 95, 96, 98]. And unlike Fc-mediated phagocytosis, CD36-mediated uptake of PEs occurred in a noninflammatory manner that was not associated with release of TNF or IL-6 [91, 99]. This process appeared similar to the CD36-mediated clearance of apoptotic cells, which is also non-inflammatory, but did not appear to involve cooperation with integrins [91, 100, 101]. PPARγ agonists also dramatically upregulated the uptake of ring-stage PEs and gametocytes [95, 96]. These findings where extended in vivo using the mouse model of hyperparasitemia. Mice receiving rosiglitazone had lower parasitemia compared to controls [102]. This reduction in parasitemia was CD36 dependent, as it was not observed in mice deficient in CD36.

These data are consistent with the reported ability of PPARγ activation to polarize macrophages towards an alternatively activated phenotype [57]. Alternatively activated macrophages have reduced expression of proinflammatory cytokines, enhanced expression of anti-inflammatory cytokines, in particular IL-10, and enhanced expression of pattern-recognition receptors, including CD36. They have been implicated in pathogen sequestration, wound healing,

and phagocytosis of apoptotic cells. In the context of malaria, alternatively activated macrophages could help control parasite burden while limiting associated inflammation, thus reducing host pathology [103].

Although reducing the parasite burden by enhancing phagocytic clearance of parasites (especially ring-stage PEs) will undoubtedly be beneficial to the outcome of infection and may be a contributing mechanism to the genetic resistance offered by hemoglobinopathies (sickle cell and both α-thalassemia and β-thalassemia) and glucose-6-phosphate dehydrogenase and pyruvate kinase deficiencies [104–106], in the context of cerebral malaria, parasitemia levels are not correlated with disease severity. Rather, inflammation, and especially TNF levels seem to correlate with disease severity, encephalopathy, and death [23, 29]. Thus, the anti-inflammatory properties of PPARγ agonists may be their most important quality when it comes to the treatment of cerebral malaria.

Human monocytes and murine macrophages treated with PPARγ agonists generate significantly less TNF in response to malaria-related inflammatory stimuli including parasite lysates and *P. falciparum* glycosylphosphatidyl inositol (GPI), a malaria toxin that interacts with TLR2 [98, 107, 108]. This was associated with the inhibition of NF-κB and MAPK signaling [102]. PPARγ is known to inhibit the NF-κB signaling [54], and a PPARγ-mediated inhibitory effect on MAPK signaling has recently been described [109]. However, whether the anti-inflammatory effects of the agonists were related to PPARγ activation was not directly examined.

The effects of PPARγ agonists in vivo have been tested in a mouse model of experimental cerebral malaria (*P. berghei* ANKA). Cerebral pathology in this model is the result of an uncontrolled proinflammatory response to infection [47, 110]. Infected mice treated with rosiglitazone had a more balanced inflammatory response, with reduced plasma levels of TNF, a reduced TNF to TGFβ ratio, and higher IL-10 levels ([102], and unpublished results by Serghides et al.). Mice receiving rosiglitazone were also protected from developing signs of cerebral pathology and had significantly improved survival rates. This was evident even when rosiglitazone was administered as late as 5 days postinfection, just prior to the initiation of cerebral pathology [102]. The effects of rosiglitazone treatment on endothelial dysfunction and cerebral pathology in this model are currently under investigation in our lab.

Given the encouraging data in the mouse models, a phase I/IIa randomized double-blind placebo-controlled trial was undertaken to test the safety, tolerability, and efficacy of rosiglitazone adjunctive therapy in 140 Thai adults with uncomplicated *falciparum* malaria [111]. Rosiglitazone (4mg twice daily for 4 days) was administered as an adjunctive therapy in combination with atovaquone-proguanil and was found to be safe and well tolerated. Patients receiving rosiglitazone had significantly reduced 50% and 90% parasite clearance times, with the mean 90% parasite clearance time being reduced by 25% in the rosiglitazone group (from 40.4 h in placebo to 30.9 h in the rosiglitazone group). It is tempting to speculate that improved parasite clearance was due to enhanced CD36-mediated clearance, but direct evidence is lacking. However, these findings do corroborate the effects of rosiglitazone on parasitemia observed in the mouse model, a process that was CD36 dependent [102]. A nonstatistically significant trend towards greater fever clearance at 4 hours posttreatment was observed in those receiving rosiglitazone (43% afebrile in the rosiglitazone group compared to 27% afebrile in the placebo group, $P = .073$). Patients receiving rosiglitazone also had significantly lower levels of IL-6 and MCP-1 and trended towards significantly lower levels of TNF at 24 and 48 hours posttreatment [111]. Both the fever reduction and the lower levels of proinflammatory biomarkers suggest that treatment with rosiglitazone was associated with anti-inflammatory effects that were obvious early during the course of therapy in these patients.

The findings in the rosiglitazone trial share some similarities to those of a randomized trial of vitamin A supplementation in children from Papua New Guinea [112]. 9-cis-retinoic acid is a metabolite of vitamin A and an agonist of PPARγ (via RXR ligation), and like rosiglitazone, has been shown to enhance CD36-mediated PE uptake and reduce malaria-induced TNF production in vitro [113]. Children supplemented with vitamin A had lower parasitemia levels and fewer febrile episodes than did children in the control group, although both groups had the same rate of infection [112], suggesting a common mechanism of enhanced innate clearance of PEs and reduced inflammation.

6. Lessons from the Use of PPARγ Agonists in Neuroinflammatory and Neurodegenerative Diseases

Data on the anti-inflammatory and neuroprotective properties of PPARγ agonists in models of neuroinflammatory and neurodegenerative disease states may give us an insight into how PPARγ agonist could function in cerebral malaria [77–80].

Relevant to cerebral malaria pathology, PPARγ is expressed not only in immune cells and in peripheral organs, but also in the CNS (microglia, astrocytes, perivascular macrophages, oligodendrocytes, and neurons) and in human brain microvascular endothelial cells [114–116]. Further, PPARγ agonists such as rosiglitazone and pioglitazone can cross the BBB [117], and thus, can exert their effects not only peripherally but also directly on the CNS.

As mentioned above cerebral malaria is an inflammatory disease [23, 49]. Proinflammatory cytokines, especially TNF, initiate an inflammatory cascade that leads to endothelial activation, cell adhesion molecule upregulation, enhanced PE, leukocyte- and platelet-endothelial adhesion, endothelial dysfunction, and BBB breakdown [47]. Perivascular macrophages, astrocytes, and microglia are also activated in cerebral malaria and can produce inflammatory mediators leading to neuronal damage [118]. Several anti-inflammatory properties of relevance to cerebral malaria pathology have been ascribed to PPARγ agonists. PPARγ agonists have been shown to inhibit the following: the expression of inflammatory mediators, such as TNF, IL-6, IL-1b, and COX-2, from activated monocytes and microglial

[74, 119]; the release of chemokines including MCP-1, MIP-1a, and MIP-1b; the expression of chemokine receptors on leukocytes; the inflammation-induced upregulation of cell adhesion molecules on vascular endothelium, including ICAM-1 [120, 121]; the recruitment of leukocytes to injured sites [74, 122]; the release of matrix metalloproteinases (which degrade the extracellular matrix and contribute to BBB dysfunction) from macrophages and glial cells [123, 124]. In the context of cerebral malaria, these activities could result in less proinflammatory cytokines peripherally and in the CNS, a reduction in PE adhesion and leukocyte recruitment in the brain, and protection of the BBB integrity.

Malaria is associated not only with inflammation, but also with oxidative stress, conditions that together can lead to increased cytotoxicity. Elevated levels of TNF in addition to oxidants such as superoxide and free heme can lead to neuronal damage [125, 126]. TNF, superoxide, and free heme (caused by hemolysis) are all elevated in cerebral malaria and may contribute to the neuronal damage detected in brains of cerebral malaria patients [43, 45, 46, 127]. In addition to their anti-inflammatory properties, PPARγ agonists also have antioxidant properties. PPARγ agonists enhance the endothelial and neuronal expression and activity of superoxide dismutase-1 (SOD-1) and catalase (both of them have functional PPREs in their promoter) [128–132]. SOD-1 and catalase detoxify superoxide by catalyzing its conversion into water and oxygen. PPARγ can also suppress superoxide generation by decreasing the expression of components of the NAD(P)H oxidase complex [129, 130, 133]. Rosiglitazone-induced reduction in NAD(P)H oxidase activity has been detected in models of hypertension and diabetes [128, 134]. Heme oxygenase-1 (HO-1) also contains a PPRE in its promoter and can be upregulated by PPARγ activation [135]. HO-1 is induced during conditions of oxidative stress and catalyses the breakdown of heme into biliverdin, iron, and CO. CO is anti-inflammatory and can inhibit TNF while inducing IL-10 release [136]. HO-1 induction protects astrocytes from heme-mediated oxidative injury, and astrocytes deficient in HO-1 are much more susceptible to cell death [137]. HO-1 and CO have been shown to be protective in experimental cerebral malaria and were associated with reduced inflammation, protection of the BBB, and enhanced survival [138].

Oxidative stress can also result in decreased NO bioavailability, via scavenging by cell-free hemoglobin and/or superoxide-mediated formation of the toxic peroxynitrite [139]. Low NO bioavailability has been associated with disease severity, while NO supplementation improves disease outcome in human and experimental cerebral malaria ([40, 41, 140–143], submitted by Serghides et al.). By enhancing cell-free hemoglobin detoxification (via HO-1 upregulation) and by reducing the levels of reactive oxygen species (via SOD-1 and catalase upregulation), PPARγ agonist activity may enhance NO bioavailability [144]. A trial in diabetic patients is currently underway examining whether pioglitazone will improve NO bioavailability (clinicaltrials.gov ID NCT00770367).

An additional neuroprotective property of PPARγ agonists is their ability to regulate the expression of the glutamate receptor GLT1/EAAT2 (GLT1/EAAT2 has six putative PPREs in its promoter region) [145]. Glutamate is the major excitatory neurotransmitter in the mammalian CNS, but high amounts of glutamate released in the intersynaptic spaces can cause neurodegeneration and excitotoxic neuronal death. Glutamate plays an important role in many CNS pathologic conditions including ischemia, trauma, and neurodegenerative disorders [146]. Glutamate levels have not been measured in humans but were shown to be elevated in the CSF and in the cerebral cortex of mice with experimental cerebral malaria, suggesting that glutamate toxicity may occur in cerebral malaria. In these mice, glutamate levels correlated with the development of cerebral symptoms [147, 148]. The mechanism for maintaining low extracellular glutamate levels is astrocytic uptake via glutamate transporters including GLT1/EAAT2, which is responsible for the removal of up to 90% of extracellular glutamate. PPARγ agonists increased astrocytic expression of GLT1/EAAT2 mRNA and protein in vitro [145] and protected astrocytes and neurons from glutamate-induced cell death [145, 149, 150]. In rats, rosiglitazone prevented the stress-induced decrease in synaptosomal glutamate uptake, by enhancing glial expression of GLT1/EAAT2 [151].

Collectively these data support a neuroprotective role for PPARγ agonists via the attenuation of inflammation, oxidative stress, and cytotoxicity [152]. Such protective effects have been observed with PPARγ agonist use in models of ischemic and hemorrhagic stroke [153–158], and in models of CNS disease including Alzheimer's disease, multiple sclerosis (MS), amyotrophic lateral sclerosis, and Parkinson's disease [152]. In the ischemic models, PPARγ agonist use was associated with reduced brain injury and with improved neurological outcomes [124, 154, 159–162]. In the CNS disease models, PPARγ agonists attenuated neuron loss, prevented motor dysfunction, improved motor performance, and reversed memory decline [163–166]. Supporting data from human trials also exist. In a pilot study in Alzheimer's patients, rosiglitazone administration improved cognitive function [167, 168]. In a small placebo-controlled trial of pioglitazone use in patients with relapsing MS, gray matter atrophy and lesion burden, as assessed by MRI, were reduced in the pioglitazone group [169]. Diabetic patients receiving pioglitazone or rosiglitazone had improved functional recovery after stroke compared to patients not taking TZDs [170]. Clinical trials are underway testing the efficacy of TZDs in Alzheimer's (phase III), ALS (phase I/II), and Friedreich's ataxia (pilot).

7. Are PPARγ Agonists Promising Candidates for Adjunctive Therapy in Cerebral Malaria?

PPARγ activation may enhance the tolerance of the host to malaria infection by immunoregulatory mechanisms (modulation of the inflammatory response to infection), and by mechanisms that render tissues more resistant to inflammatory damage. Such immunomodulatory effects are likely to be protective in the context of cerebral malaria. However,

whether PPARγ activation following the onset of cerebral malaria (once the inflammatory cascade has begun) will be protective is an open question. Other immunomodulatory therapies tested in cerebral malaria in the past (e.g., anti-TNF antibodies, dexamethasone) have failed [11]. That PPARγ activation impacts several pathways and may have not only neuroprotective but also neuroregenerative effects improves the likelihood of efficacy. However, it is unknown whether the regenerative effects seen with long-term PPARγ agonist use in chronic CNS disease will also be obvious with a short treatment course, as would be administered in cerebral malaria.

Rosiglitazone (4 mg twice daily for 4 days administered in combination with atovaquone-proguanil) was found to be safe and well tolerated in uncomplicated malaria. Mean serum glucose, alanine aminotransferase, and aspartate aminotransferase levels did not differ between patients receiving placebo and those receiving rosiglitazone [111]. In addition, there were no differences observed in the incidences of adverse events including headache, myalgia, weakness, nausea, vomiting, diarrhea, or palpitations between the two groups [111]. TZDs are antidiabetic drugs, and so a concern would be the possible exacerbation of the hypoglycemia commonly seen in severe malaria; however, rosiglitazone and other TZDs function as insulin sensitizers and are generally not known to cause hypoglycemia, and as mentioned above, rosiglitazone did not cause hypoglycemia in patients with uncomplicated malaria [111]. Rosiglitazone may also worsen edema by increasing fluid retention, but clinically significant fluid retention tends to occur only with long-term use [171]. Increased risk of myocardial infarction and hepatotoxicity are risk factors associated with rosiglitazone use, but again these are complications associated with long-term use [172]. Finally, it is worth considering whether PPARγ agonists could have an impact on the acquisition of adaptive immunity to malaria via modulatory effects on dendritic cells, T cells, and B cells [57].

The existing data on the use of PPARγ agonists in malaria are encouraging, with rosiglitazone being safe, well tolerated, and efficacious in uncomplicated malaria patients. Given the anti-inflammatory, neuroprotective, and neuroregenerative properties reported for PPARγ agonists in models of CNS injury, ischemic stroke, and diseases of the CNS, we can hypothesize that PPARγ activation in cerebral malaria may lead to improved outcome and possibly less long-term cognitive and neurological deficits. However, a randomized double-blind placebo-controlled trial in patients with cerebral malaria will be required to determine if these hypotheses are correct.

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Review Article

A Role for Peroxisome Proliferator-Activated Receptors in the Immunopathology of Schistosomiasis?

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Peroxisome proliferator-activated receptors (PPARs) have been demonstrated to have a role in immune regulation. In general, they are anti-inflammatory and promote Th2 type responses, and they are associated with the alternative activation of macrophages. Interestingly, helminth infections, such as the schistosome blood flukes that cause schistosomiasis, are characterised by a Th2 response and the accumulation of alternative activated macrophages. This would suggest that at some level, PPARs could have a role in the modulation of the immune response in schistosomiasis. This paper discusses possible areas where PPARs could have a role in this disease.

1. Introduction

The peroxisome proliferator-activated receptors (PPARs) are a group within the 48 transcription factors of the nuclear hormone receptor family involved in lipid metabolism and inflammation [1]. To be transcriptionally active, they require hetrodimerisation with the retinoid X receptor (RXR) to which the resulting heterodimers bind with peroxisome proliferator-response elements (PPREs) on DNA after activation by a ligand-to-modulate transcription [2]. The PPREs are located at the 5′ end of the target gene and consist of a repeat sequence—AGGTCA—separated by one nucleotide [3]. The binding to the PPRE is orientated with PPAR at the 5′ end and RXR towards the 3′ end [2]. For transcriptional control to occur, the PPAR/RXR heterodimers have to interact with coactivators or suppressors for stimulation or inhibition of target-gene expression, respectively [2]. The PPARs can also block transcription of other genes by interacting with other transcription factors by non-genomic transrepression, whereby they inhibit transcription by preventing dissociation of corepressors or sequester co-activators needed for binding of the transcription factor to the DNA [4]. There are 3 isoforms of the PPAR receptors, PPARα, PPARβ/δ, and PPARγ [5]. PPARα is expressed in the liver, brown fat, heart, and skeletal muscle which have high levels of fatty acid catabolism, while PPARγ is expressed in adipose tissue, the colon, and in macrophages, it is the major regulator in adipocyte differentiation and is a determinant in insulin sensitivity [3]. PPARβ/δ is ubiquitously expressed and is thought to have a role in metabolic disorders [3]. Polyunsaturated fatty acids and eicosanoids act as natural ligands for these receptors; however, synthetic ligands exist such as fibrates that target PPARα and the thiazolidinediones that target PPARγ [3].

PPARS have been demonstrated to be important in a number of different disease states such as metabolic disorders [3], inflammation [6], malaria [7], Chagas disease [8], and leishmaniasis [9]. Recent studies have revealed a role for PPARs in the control of the immune response. In general, they are anti-inflammatory [10], promote the development of alternatively activated macrophages (AAMΦ) [11], and are Th2 biasing [12]. Helminth worms have an incredible ability to modulate the host immune response and, in general, promote a Th2-biased environment that commonly involves

the generation of AAMΦs [13], as occurs during schistosome infection. The fact that this parasite and other helminths induce Th2 biasing, with accumulation of AAMΦs, suggests that at some level, PPARs could be involved. This paper will explore the current state of knowledge in this area, focusing on the role of PPARs in the immunopathology of schistosomiasis and their potential as novel therapeutic targets.

2. Immune Regulation in Schistosomiasis

Schistosomiasis is a major health problem responsible for significant morbidity and mortality worldwide. It is estimated that approximately 200 million people are infected, causing severe disease in 20 million people [14]. The disease is caused by infection with the trematode worms, the schistosomes, of which *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium* are the most important in regards to human disease [15]. Pathology is associated with the host's immune response to the eggs which results in a granulomatous reaction [16]. Helminth parasites are able to modulate the host immune response, allowing them to have good longevity within the mammalian host. Helminth infections are noted for polarising the immune response more towards a Th2 response characterised by interleukin (IL)-4, 5, and 13, large amounts of IgE, and by $CD4^+$ T cells [17]. In schistosomiasis, the immune response is characterised by a switch from an early proinflammatory Th1 response to a Th2 response to eggs released by the female worm [18]. The Th2 response has the characteristics described above and is associated with AAMΦs ([19–21]). Cytokines such as INF-γ, IL-2, IL-12, and TNF-α are associated with the early Th1 response and are repressed during this switch [18]. Helminth parasites can achieve this modulation the by release of soluble factors which can interact with host immune cells [13]. There is good evidence for this in schistosome infections in which both live or dead eggs injected into naive mice rapidly induce a Th2 response [20]. Egg-derived products have been observed to drive the switch from the Th1 response to the Th2 response. Examples of this are the IL-4-inducing principal of *S. mansoni* eggs (IPSEs) that is secreted from the egg subshell into the surrounding granuloma area and has been demonstrated to induce human basophils to produce IL-4 and IL-13 [22]. Similarly, the glycoprotein, omega-1 that is secreted by *S. mansoni* eggs and present in secreted egg antigen (SEA), has been observed to drive human monocyte-derived dendritic cells towards Th2 polarisation and to generate Th2 responses *in vivo* in mice [23]. Egg-derived glycoconjugates, α3-fucosyltransferase, and core g 2-xylosyltransferase have been used with dendritic cells to produce a Th2 response in the murine model of disease caused by *S. mansoni* [24].

3. Immune Regulation by PPARs

It is believed that PPARs may be important in the regulation of the immune response, a role supported by the fact that PPARs have been described in monocytes, macrophages, neutrophils, peripheral blood lymphocytes, T cells, B cells, natural killer cells, dendritic cells, eosinophils, and mast cells [1]. Supporting this, ligands of PPARs have been shown to have a therapeutic role in several models of inflammatory and autoimmune diseases [6]. PPARγ agonists have been demonstrated to have anti-inflammatory effects in renal injury [25], murine carotid atherosclerosis [26], and in oxidative stress induced in a human diploid fibroblast model of aging [27]. PPARβ/δ agonists have additionally been demonstrated to have a protective role in a murine model of autoimmune encephalomyelitis [28]. Further, PPARγ agonists have been observed to inhibit the production of TNF-α in human monocytes [10].

Part of the anti-inflammatory mode of action of PPARs is due to the fact they can interact with transcription factors involved in inflammation such as NF-κB, activator protein-1 (AP-1), and signal transducers and activators of transcription (STAT) at a transcriptional level ([29, 30]). In the case of NF-κB and AP-1, PPARα has been shown to interact directly with p65, c-Jun, and CBP, thereby interfering with their transcriptional capacity [29], while the PPARγ agonist, 15d-PGJ2, inhibits STAT signalling indirectly [30]. Interference of these pathways results in the downregulation of the Th1 proinflammatory cytokines TNF-α, IL-1, -6, and 12 ([29, 30]).

Notably, PPARs have been demonstrated to result in upregulation of Th-2 responses and downregulation of Th-1 responses. An agonist of PPARα, gemfibrozil, results in increased number of GATA3 positive T cells in the spleens of donor mice as well as the stimulation of its expression and DNA-binding activity resulting in IL-4 production [12]. In the same study, gemfibrozil was additionally observed to inhibit the expression and DNA-binding activity of T-bet, causing a decrease in INF-γ production. IL-4 can interact with PPARγ indirectly and directly in macrophages [31]. PPARγ expression is both directly and indirectly upregulated by IL-4. IL-4 will induce target-gene expression by increasing PPARγ expression and by increasing the production of PPARγ ligands via 15-lipoxygenase, which results in lipoperoxidation products such as linoleic acid (HODE) or arachidonic acid (HETE) [31]. Schistosomes and other helminths could potentially interact with PPARs through these pathways. They could do this indirectly via IL-4 and IL-13 as both cytokines can activate PPARγ resulting in the suppression of the proinflammatory response and activation of AAMΦs which favour the establishment of a chronic parasite infection [1]. Schistosomes could potentially interact with PPARs via hemozoin. Schistosomes produce hemozoin as a product from feeding on mammalian host red blood cells; its structure is identical to malarial hemozoin [1]. It is composed of a complex mixture of neutral lipids and polyunsaturated lipids from which lipoperoxidation products HETE and HODE acid, which are natural ligands for PPAR [1], are derived (Figure 1). Carter et al. [32] showed that macrophages that have previously phagocytosed schistosomal-derived hemozoin have a reduced ability to produce iNOS in response to LPS.

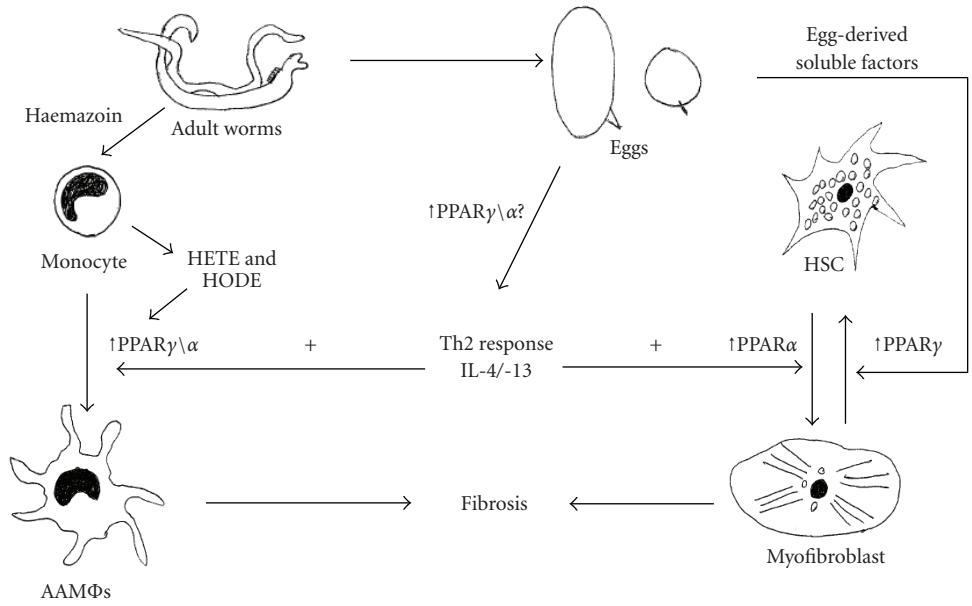

Figure 1: Summary of possible interactions in schistosomiasis with PPARs. This figure displays the possible pathways in which PPARs could be involved in schistosomiasis. PPARs could be involved in immune regulation, as they are associated in generation of a Th2 response. PPAR$\beta\gamma/\alpha$ both cause downregulation of Th1 cytokines and promote IL-4/-13 production. PPARs have a role in the alternate activation of macrophages where PPARγ/α have been demonstrated to be essential for this process. In schistosomiasis, AAMΦs have a protective effect and a role in Th2 biasing. Schistosomes could interact with this activation indirectly via induction of IL-4/-13 production and directly by the breakdown products of hemozoin, which can interact with PPARγ/α. In terms of pathology, the PPARs could interact with the transdifferentiation process of HSCs into fibrogenic myofibroblasts. They could limit this process by inhibiting transdifferentiation associated with increased PPARγ, whereas PPARα would be associated with generation of the fibrogenic myofibroblast.

4. PPARs and Alternatively Activated Macrophages

Macrophages have multiple roles with regards to the host immune response. They have a role in early detection of invading pathogens, both as antigen presenting cells (APCs) that initiate a host response and as effecter cells that can act to kill the invading pathogen [33]. Macrophages used to be classified as either activated or deactivated, but in recent years, this has changed to classically activated macrophages (CAMΦ) and AAMΦs [33]. CAMΦs are induced by INF-γ, TNF-α, and LPS and produce proinflammatory cytokines such as IL-1β, IL-12, IL-23, and TNF-α and the chemokines CXCL-9, -10, -11, and -16 [33]. AAMΦs are associated with production of IL-10 and are induced by IL-4 and IL-13 [33]. This classification has been expanded in recent years with proinflammatory macrophages being termed M1, while anti-inflammatory macrophages are termed M2. The M2 macrophages have been divided into different subsets, whereby AAMΦs are classified as M2a cells which are defined by low expression levels of IL-12 [34], or are M2b cells, which release high levels of IL-10 on activation by immune complexes, and M2c cells which are induced by IL-10 and are believed to be more similar to CAMΦ [33]. One of the main differences distinguishing AAMΦs from CAMΦs is in how they metabolise L-arginine. CAMΦ metabolise L-arginine into NO via iNOS, while AAMΦ metabolise L-arginine into urea and L-ornithine via arginase-1 (arg-1) [35].

AAMΦ have been associated with many helminth infections with many different roles attributed to them. In infection with the nematode *Brugia malayi*, AAMΦs are associated with Th-2 biasing [36, 37], while in infection with *Heligmosomoides polygyrus*, AAMΦs have a role in parasite clearance and host protection [38]. In cestode infections, AAMΦs have been associated with downregulation of the immune response in *Echinococcus multilocularis* [39] and Th-2 biasing in *Taenia crassiceps* infection as well as favouring parasite survival [40]. In *Schistosoma* infection, they have a role in Th-2 biasing as well as in downregulation of the Th-1 response and mediate immunopathology promoting host protection, but, at the same time, they promote progressive pathology due to granuloma formation ([21, 41]). Overall, their role seems to be host protective by causing downregulation of overaggressive inflammatory reactions, but they are also protective for the parasite, forming part of the immunomodulation strategy needed for successful colonisation of the host.

PPARs could potentially have a role in helminth infections by regulating AAMΦs. There are a number of studies demonstrating PPARγ as essential for AAMΦ activation and maturation in other disease states such as metabolic syndrome and leishmaniasis ([9, 11]). In metabolic syndrome, it has been shown with macrophage-specific PPAR-γ knockout mice that PPARγ is essential for AAMΦ maturation resulting in the mice developing diet-induced obesity, insulin resistance, and glucose intolerance [11]. Additionally, PPARγ and

PPARδ agonists have been observed to mediate arginase-1 expression in macrophages, and this expression is blocked in macrophages from PPARγ- and PPARδ-deficient mice [11]. Interactions between IL-4/13 and PPARs have been extensively studied in leishmaniasis in which PPARs promote AAMΦ-mediated susceptibility to the disease by stimulating intracellular amastigote growth in infected macrophages [11]. This is due to the lack of NO production in resultant AAMΦ which is essential for amastigote killing.

5. DO PPARs Modulate Host Pathology?

The hepatic stellate cell (HSC) is located within the liver sinusoid in the space of Disse where it is responsible for storage of vitamin A and the maintenance of a low-density matrix between the liver endothelium cells and the hepatocytes [42]. Maintenance of this matrix is important as it allows solutes in the plasma to reach the hepatocytes unimpeded, allowing the liver to function correctly [43]. In response to insult or injury to the liver, HSCs can undergo a process of transdifferentiation from the quiescent vitamin A-storing cell to a myofibroblast responsible for the accumulation of scar tissue within the space of Disse [44]. This has highlighted parallels between this cell type and that of the adipocyte which can undergo a similar process [45]. Adipocytes differentiate from a fibroblast-like preadipocyte and become lipid laden associated with the expression of PPARγ [46]. Quiescent HSCs express PPARγ which upon transdifferentiation into a myofibroblast-like cell lose their ability to store lipid droplets as the expression and activity of PPARγ decrease [47]. This has suggested a role for PPARγ agonists in the treatment of fibrosis. PPARγ agonists have been demonstrated to cause reversion of the myofibroblast back into a quiescent HSC ([45, 47]).

Recent studies have implicated a role for HSCs in the pathogenesis of schistosomiasis [48]. Activated HSCs have been observed in the murine model of disease and at the end stage of human disease with *S. japonicum* [49] and human disease with *S. mansoni* [50]. Notably, the PPARγ agonist rosiglitazone has been demonstrated to prevent fibrosis in *S. japonicum* infection of mice [51]. In this study, mice cotreated with the antischistosome drug praziquantel and rosiglitazone induced reduced expression of collagen 1 and 3, α smooth muscle actin (a marker for myofibroblasts), inflammation, increased expression of PPARγ, reduced NF-κB-binding activity, and reduced TNF-α levels [51]. In a recent study, Anthony et al. [52] showed that eggs of *S. mansoni* could downregulate fibrogenesis in the human HSC cell line, LX-2, causing regression from the activated myofibroblast to the quiescent HSC. This downmodulation was associated with increased expression of PPARγ at the gene level as well as with the accumulation of lipid droplets within the cytoplasm of HSCs. At the granuloma level, fibrosis first occurs towards the periphery of the granuloma site, and it was postulated in this study that antigens from the egg may inhibit fibrosis in close proximity to the egg as it is not until the egg is killed and destroyed that fibrosis occurs throughout the granuloma area. However, PPARγ could act as a double-edged sword, as it would be involved in alternative activation of macrophages at the granuloma site, which in turn can be responsible for collagen production by the production of arginase-1 which promotes the production of proline. Additionally, the Th2 response is profibrogenic and high levels of IL-13 are associated with fibrosis in schistosomiasis [53].

6. Conclusions

Schistosomiasis is characterised by a switch from an early Th1 response to a Th2 response and accumulation of AAMΦs in response to eggs released by the schistosome worms. PPARs have been demonstrated to cause downregulation of proinflammatory Th1 cytokines while simultaneously upregulating Th2 responses. They have additionally been shown to be essential in the alternative activation of macrophages. This suggests that PPARs may play a role in the regulation of the host response to schistosome antigens. Additionally, it has been demonstrated that *S. mansoni* eggs cause downregulation of fibrogenesis in the human-derived HSC cell line, LX2, a response associated with increased expression of PPARγ, and accumulation of lipid droplets within the cell's cytoplasm. Rosiglitazone, a PPARγ ligand, has been additionally been demonstrated to reduce pathology associated with *S. japonicum* infection in mice. The possible interactions with schistosomiasis and PPARs are summarised in Figure 1. Further studies of the role of PPARs in this disease and those caused by other helminth infections are, therefore, warranted and may help in the identification of new antipathology drug and vaccine targets for schistosomiasis and other important diseases caused by the parasitic helminths.

Conflict of Interests

The authors declare that they have no Conflict of interests.

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