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Brief Communication

# PPAR gamma activation protects the brain against microvascular dysfunction in sepsis ☆

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ABSTRACT

Sepsis is a severe disorder characterized by systemic inflammatory responses in the presence of an infection and may progress to multiple organ dysfunction and death. Alterations in cerebral microcirculation fulfill a crucial role in the pathogenesis of severe sepsis, and include a decrease in capillary density and disturbances in leukocyte movement along capillaries. Nevertheless, the mechanisms involved in sepsis-associated cerebral microcirculatory alterations have so far not been defined. We investigated the effect of the peroxisome proliferator-activated receptor gamma (PPARγ) selective agonist rosiglitazone on leukocyte/endothelial cell interaction and functional capillary density in the brain in the cecal ligation and puncture (CLP) model of sepsis. Anti-inflammatory effects of rosiglitazone on the cerebral microcirculation were marked. Functional capillary density increased and leukocyte rolling and adhesion were decreased in animals submitted to CLP and treated with rosiglitazone. Our data provide evidence for involvement of PPARγ activation in leukocyte–endothelium interactions and alterations in capillary density. Improved cerebral perfusion in animals treated with rosiglitazone, suggests that PPARγ activation is protective against cerebral microvascular dysfunction in sepsis.



## Introduction

Sepsis is a systemic response to infection characterized by hemodynamic and metabolic derangement that may result in septic shock, multiple organ system failure, and death. Although antibiotic therapy may effectively treat an underlying infection, it is not sufficient to reverse the systemic inflammation and its consequences.

Microcirculatory dysfunction plays a pivotal role in the pathogenesis of severe sepsis. There is evidence for a decrease in functional capillary density, and an increase in the proportion of nonperfused or intermittently perfused capillaries (den Uil et al., 2008). Monitoring microcirculatory blood flow in sepsis has gained increasing attention not only as a prognostic parameter but also as a possible guideline for therapeutic maneuvers (Koh et al., 2010). Moreover, microcirculatory alterations nonresponsive to therapy predict a poor outcome in critically ill patients (Sakr et al., 2004).

Several *in vitro* and *in vivo* studies have demonstrated that pharmacological activation of peroxisome proliferator-activated receptor gamma (PPARγ) by natural or synthetic ligands, including thiazolidinediones (TZD), has anti-inflammatory effects (Haraguchi et al., 2008); (Zingarelli et al., 2003); (Zingarelli and Cook, 2005). PPARγ is a nuclear receptor expressed in various cells, including monocytes, macrophages, T cells, endothelial cells, and other cells involved in the progression of sepsis. PPARγ ligands have anti-inflammatory effects and improve outcomes in clinical conditions such as atherosclerosis, rheumatoid arthritis, allergy and sepsis (Szeles et al., 2007); (Zingarelli, 2005). In cerebral infection by *Staphylococcus aureus*, PPARγ agonist treatment attenuated inflammation and limited bacterial dissemination (Kielian et al., 2008). Based on this information, the purpose of this study was to investigate whether the PPARγ selective agonist rosiglitazone reduces the inflammatory response in the cecal ligation and puncture (CLP) model of sepsis, by modulating leukocyte/endothelial cell interaction and functional capillary density, thereby improving cerebral microcirculation.

## Materials and methods

### Experimental animals

Ten weeks old Male Swiss mice (Oswaldo Cruz Foundation breeding unit) weighing 20 to 25 g were used. The animals were kept at a constant temperature (25 °C) with the access to pellet diet and water in a room with a 12 h light/dark cycle. The protocol was approved in accordance with the ethical guidelines of the Institutional

*Abbreviations:* PPARγ, peroxisome proliferator-activated receptor gamma; CLP, cecal ligation and puncture; TZD, thiazolidinediones.

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*Surgical procedure and CLP model*

Sepsis was induced by CLP as previously described (Gomes et al., 2006). After the surgery all animals received 1 ml s.c. of sterile isotonic saline. Fifteen minutes after the CLP procedure, mice were divided into six random groups: sham + vehicle (DMSO, v/v, i.v.), sham + rosiglitazone (0.5 mg/kg, i.v.) CLP + vehicle (DMSO, v/v, i.v.), CLP + GW9662 (1 mg/kg, i.v.) and CLP + GW9662 (1 mg/kg, i.v.) + rosiglitazone (0.5 mg/kg, i.v.), 9 animals per group.

*Assessment of the severity of sepsis*

At 24 h after infection, mice were scored for severity of sepsis. In this assessment, higher scores reflect increased severity. Mice were scored based on the following variables: piloerection, curved trunk, alterations on gait, seizures, limb paralysis, coma, respiratory rate, skin color alterations, heart rate, lacrimation, palpebral closure, grip strength, limb, abdominal, body tone and body temperature alterations. Each animal received a total score between 1 and 11: 1–3 (mild sepsis); 3–7 (moderate sepsis) and 8–11 (severe sepsis) (Reis et al., unpublished data). In our CLP model, all animals that were ranked as moderate sepsis were used for intravital microscopy analysis. Animals with mild or severe sepsis were excluded from the experimental protocol.

*Intravital microscopy in mouse brain*

The animals were anesthetized by intraperitoneal injection of a mixture of 10 mg/kg xylazine and 75 mg/kg ketamine hydrochloride, tracheostomized and artificially ventilated (Hugo Basile) with room air. The jugular vein was cannulated to allow injection of fluorescent tracers. Arterial pressure and heart rate were monitored with a catheter placed in the right carotid artery connected to a quartz transducer, which in turn was connected to an automatic acquisition system hemodynamic data (Biopac Systems, Santa Barbara, CA). Core temperature was monitored with a rectal probe, and body temperature was maintained at 37 °C with a homeothermic blanket system (Harvard Apparatus, Boston, Massachusetts).

The anesthetized animals were immobilized in a stereotaxic frame, the left parietal bone was exposed by a midline skin incision, a craniotomy was performed with high-speed drill, and the *dura mater* was incised and everted to expose the cerebral pial microcirculation (Carvalho-Tavares et al., 2000). The cranial window was suffused with artificial cerebrospinal fluid (in mmol/l: NaCl 132, KCl 2.95, CaCl2 1.71, MgCl2 0.64, NaHCO3 24.6 dextrose 3.71 and urea 6.7 at 37 °C, pH 7.35).

The animals were then placed under an upright fixed-stage intravital microscope with a mercury lamp (Olympus BX51/WI, USA) coupled to a CCD digital video camera system (Optronics, Goleta, California). Olympus objectives 10× and 20× were used to produce a total magnification of 100× and 200×, respectively at the monitor.

*Assessment of capillary density, leukocyte rolling and adhesion*

After intravenous administration of 0.1 ml of 5% FITC-labeled dextran, microscopic images of the cerebral microcirculation were acquired by Archimed 3.7.0 software for online counting of the capillaries using Saisam software (Microvision, France). Functional capillary density, considered as the total number of spontaneously perfused capillaries (vessels with diameters less than 10 μm) per square mm of surface area (1 $mm^2$), was determined by counting each capillary branch over a period of 4 min, as described previously in detail (Sabino et al., 2008). For labeling circulating leukocytes, animals received intravenous administration of rhodamine 6G (0.3 mg/kg body weight) and fluorescence associated with leukocytes was visualized by epi-illumination. Five randomly selected venular segments, 30 to 100 μm in diameter and 100 μm long, were observed for 30 s in each preparation examined for leukocyte recruitment and the mean was calculated. Leukocyte–endothelial interactions were evaluated by determining the number of leukocytes adhered to the venular wall for 30 s. Rolling leukocytes were defined as crossing the 100 μm venular segment at a speed below the circulating red blood cells, and were expressed as number of cells/min.

*Cerebral myeloperoxidase (MPO) activity*

The neutrophil infiltration in brain was measured indirectly by quantifying MPO enzyme activity. Briefly, brain extracts were homogenized (50 mg/ml) in 0.5% hexadecyltrimethylammonium bromide and centrifuged at 4000 rpm for 15 min at 4 °C. MPO activity in the supernatant was measured spectrophotometrically as the change in optical density at 460 nm and 37 °C, using tetramethylbenzidine (1.6 mM) and $H_2O_2$ (0.5 mM) as the substrate.

*Statistical analysis*

All data were analyzed by ANOVA with Newman–Keuls post hoc test. Data are reported as means ± SEM. $P$ value < 0.05 was considered significant.

## Results

*Rosigitazone treatment improves the clinical condition of septic mice*

We developed a clinical score to assess the severity of sepsis in our CLP model. This clinical score takes into consideration several alterations in appearance; physiology and behavior that are associated with severe sepsis in mice (see Materials and methods section). We observed that the severity score decreased from 6.0 ± 0.4 to 3.3 ± 0.2 ($P$<0.0001) in rosiglitazone-treated septic animals, even though treated animals were still ranked as moderate sepsis.

*Rosiglitazone decreases leukocyte–endothelial interaction and increases functional capillary density in mice brain microvasculature during experimental murine sepsis*

Figs. 1A and B show that rolling and adhesion of leukocytes in septic animals were increased when compared to the control group. Rosiglitazone treatment decreased leukocyte rolling and adhesion in septic mice and treatment with the specific antagonist GW9662 abolished the protective effects of rosiglitazone with respect to both rolling and adhesion (Figs. 1A and B). Panel 1 illustrates the increased leukocyte–endothelial interaction in the cerebral microcirculation of CLP mice (Panel 1B) as compared to either sham (data not illustrated, but shown in Fig. 1) or sham mice treated with rosiglitazone (Panel 1A). As we observed in Figs. 1A and B, rosiglitazone diminished leukocyte–endothelial interaction in septic animals (Panel 1C) and GW 9662 treatment effectively impaired rosiglitazone effect (Panel 1D).

Table 1 shows mean arterial pressure and heart rate of septic mice. We did not observe significant alterations in haemodynamic parameters in the control CLP group or in the rosiglitazone treated group. Mice subjected to CLP had a significant decrease in the number of spontaneously perfused capillaries when compared to the control group. Treatment with rosiglitazone reversed capillary rarefaction when compared to the CLP group that did not receive rosiglitazone. The protective effect of rosiglitazone was completely abolished in the presence of the PPARγ antagonist GW9662, whereas GW9662 alone had no impact on the brain capillary density of septic mice (Fig. 1C).