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BRAIN RESEARCH

Research Report

PPARγ agonist rosiglitazone is neuroprotective after traumatic brain injury via anti-inflammatory and anti-oxidative mechanisms

Jae-Hyuk Yi[a], *Seung-Won Park*[a,b], *Nathaniel Brooks*[a], *Bradley T. Lang*[a], *Raghu Vemuganti*[a,*]

[a]*Department of Neurological Surgery, University of Wisconsin, K4/8 Mail code CSC 8660, 600 Highland Avenue, Madison, WI, 53792, USA*
[b]*Department of Neurological Surgery (SP), Chung-Ang University, Seoul, Republic of Korea*

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ABSTRACT

Peroxisome proliferator-activated receptor (PPAR)-γ is a ligand-activated transcription factor of nuclear hormone receptor superfamily. Thiazolidinedione rosiglitazone is a potent agonist of PPARγ which was shown to induce neuroprotection in animal models of focal ischemia and spinal cord injury. We currently evaluated the therapeutic potential of rosiglitazone (6 mg/kg at 5 min, 6 h and 24 h; *i.p.*) following controlled cortical impact (CCI)-induced traumatic brain injury (TBI) in adult mice. CCI injury increased the cortical PPARγ mRNA levels which were further elevated by rosiglitazone treatment. In addition, rosiglitazone treatment significantly decreased the cortical lesion volume measured at 7 days compared to vehicle treatment (by 56±7%; $p<0.05$; $n=6$/group). Following TBI, the spared cortex of the rosiglitazone group showed significantly less numbers of GSI-B4$^+$ activated microglia/macrophages and ICAM1$^+$ capillaries, and curtailed induction of pro-inflammatory genes IL6, MCP1 and ICAM1 compared to vehicle group. Rosiglitazone-treated mice also showed significantly less number of TUNEL$^+$ apoptotic neurons and curtailed induction of caspase-3 and Bax, compared to vehicle control. In addition, rosiglitazone significantly enhanced the post-TBI expression of the neuroprotective chaperones HSP27, HSP70 and HSP32/HO1, and the anti-oxidant enzymes catalase, Cu/Zn-SOD and Mn-SOD, compared to vehicle. Treatment with GW9662 (a specific PPARγ antagonist) prevented all the above PPARγ-mediated actions. Thus, PPARγ activation confers neuroprotection after TBI by anti-inflammatory, anti-apoptotic and anti-oxidative mechanisms.

1. Introduction

Traumatic brain injury (TBI) is a major disabling condition in young adults all over the world. Several pathological events including inflammation, apoptosis, oxidative stress and excitotoxicity during the acute stage after an injury are known to precipitate the neuronal death and neurological dysfunction (Raghupathi, 2004; Jennings et al., 2008). Hence, therapeutic compounds that can target multiple pathophysiological mechanisms can be extremely useful in preventing post-TBI neuronal death. Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-activated transcription factor of

* *Corresponding author.*
E-mail address: Vemugant@neurosurg.wisc.edu (R. Vemuganti).

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nuclear hormone receptor superfamily (Escher and Wahli, 2000). 15-d-Prostaglandin J2 is the natural ligand of PPARγ, while several thiazolidinediones (TZDs) are potent synthetic agonists (Kapadia et al., 2008). As PPARγ plays a significant role in glucose and lipid homeostasis, 2 TZDs rosiglitazone and pioglitazone are currently approved by the United States Food and Drug Administration (FDA) for type-2 diabetes treatment (Sood et al., 2000).

Recent studies showed that both rosiglitazone and pioglitazone are extremely neuroprotective in animal models of acute CNS insults including focal ischemia, spinal cord injury (SCI) and surgical trauma (Sundararajan et al., 2005; Zhao et al., 2005, 2006; Pereira et al., 2006; Collino et al., 2006; Tureyen et al., 2007; Park et al., 2007; McTigue et al., 2007; Hyong et al., 2008). Their efficacy was also shown in animal models of chronic CNS injuries like Parkinson's disease, Amyotrophic lateral sclerosis and Alzheimer's disease (Breidert et al., 2002; Schütz et al., 2005; Pedersen et al., 2006). We currently evaluated the therapeutic potential of rosiglitazone in minimizing secondary lesion expansion, apoptotic markers, infiltration of macrophages/activation of microglia, and the expression of inflammatory, anti-oxidant and heat-shock protein (HSP) genes in adult mice subjected to controlled cortical impact (CCI) injury.

2. Results

2.1. TBI-induced cortical PPARγ expression

At 1 day following CCI injury, the mRNA expression of PPARγ was observed to be upregulated (by 2.1 fold; $p<0.05$) in the cortical tissue surrounding the injury epicenter compared to sham control (Fig. 1). Rosiglitazone treatment following CCI injury (6 mg/kg, *i.p.* at 5 min, 6 h and 12 h) resulted in a significantly higher induction of PPARγ mRNA (by 4.4 fold; $p<0.05$) compared to sham (Fig. 1). Whereas, treatment with GW9662 (a PPARγ antagonist; 4 mg/kg, *i.p.* at 5 min and 12 h) prevented the post-TBI induction of PPARγ mRNA expression (Fig. 1).

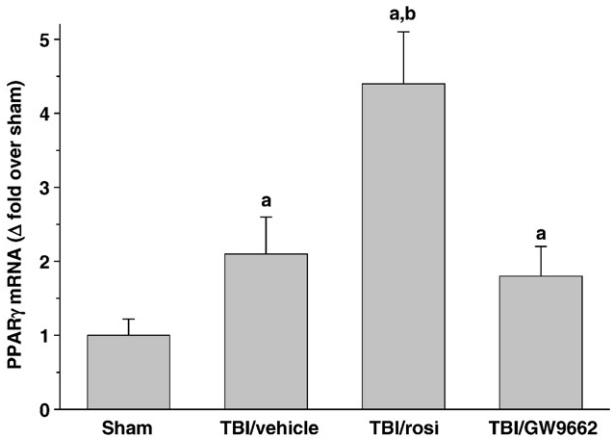

Fig. 1 – At 1 day after CCI injury, the PPARγ mRNA expression analyzed with real-time PCR increased significantly in the cortex surrounding the injury compared to sham. Treating with rosiglitazone, but not with PPARγ antagonist GW9662 led to a further significant increase in PPARγ mRNA levels. CCI injury and/or treatment with rosiglitazone or GW9662 had no significant effect on the expression of the house-keeping control 18S rRNA (data not shown). The bars represent mean ± SD of $n=4$ in each case. Statistics: $^{a}p<0.05$ compared to sham and $^{b}p<0.05$ compared to vehicle/TBI group (One way ANOVA followed by Tukey–Kramer multiple comparisons test).

2.2. Rosiglitazone treatment decreased the cortical contusion volume following TBI

A moderate grade CCI injury in adult mice resulted in significant cortical neuronal damage and TUNEL staining by 1 day and a cavitation by 7 days in the ipsilateral cortex. The injury size was observed to be similar at 1 day and 7 days after CCI injury (Fig. 2). At 7 days after CCI injury, the cortical contusion volume in the vehicle treated control group was observed to be 3.09 ± 0.14 mm^3 ($n=6$) that spanned 0 to 2.4 mm from Bregma (Fig. 3). Treatment with rosiglitazone (*i.p.*; 6 mg/kg at 5 min, 12 h and 24 h) resulted in a significantly smaller contusion volume compared to vehicle control (by $37\pm6\%$; $p<0.05$; $n=6$ per group). Rosiglitazone treatment led to a similar degree of protection at 1 day after CCI injury as well (data not shown). On the other hand, preventing post-TBI PPARγ activation by treating mice with GW9662 (4 mg/kg, *i.p.*; at 5 min, 12 h and 24 h) resulted in a significantly larger contusion volume compared to vehicle control (by $31\pm8\%$; $p<0.05$; $n=6$) (Fig. 3).

2.3. Rosiglitazone curtailed post-TBI apoptosis

The ipsilateral cortex of mice subjected to CCI injury and treated with vehicle showed many TUNEL$^+$ cells at 2 days of survival (Figs. 4A and D). Rosiglitazone-treatment significantly reduced the number of TUNEL$^+$ cells (by $53\pm11\%$; $p<0.05$; $n=6$) compared to the vehicle (Figs. 4B and D). Whereas, the number of TUNEL$^+$ cells in the mice treated with GW9662 showed no significant difference from the vehicle group (Figs. 4C and D). The TUNEL$^+$ cells were counted in the area adjacent to the injury epicenter (Fig. 4E shaded area). Many TUNEL$^+$ cells were observed to be positive for the neuronal marker NeuN (Fig. 4F, arrows), but rarely for the astroglial marker GFAP (Fig. 4G). Rosiglitazone treatment also significantly curtailed the induction of pro-apoptotic genes caspase-3 and Bax compared to vehicle treatment at 1 day after CCI injury (Fig. 6A).

2.4. Rosiglitazone decreased inflammation after CCI injury

Induction of ICAM1 expression on endothelial cells, infiltration of macrophages and activation of microglia are hallmarks of cerebral inflammation. Cerebral cortex of sham-operated mice showed no appreciable staining for either GSI-B_4 (macrophage/activated microglia marker) or ICAM1 (Figs. 5A and B). Whereas, mice subjected to CCI injury and treated with vehicle showed several GSI-B_4^+ cells and ICAM1$^+$ capillaries at 2 days after TBI (Figs. 5C and D). Rosiglitazone treatment significantly decreased both GSI-B_4 and ICAM1 immunostaining (Figs. 5E and F) compared to vehicle group. Treating mice