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Rosiglitazone, a PPAR gamma agonist, Attenuates Inflammation After Surgical Brain Injury in Rodents

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Abstract

Introduction—Surgical brain injury (SBI) is unavoidable during many neurosurgical procedures. This inevitable brain injury can result in postoperative complications including brain edema, blood-brain barrier disruption (BBB) and cell death in susceptible areas. Rosiglitazone (RSG), a PPAR- γ agonist, has been shown to reduce inflammation and provide neuroprotection in experimental models of ischemia and intracerebral hemorrhage. This study was designed to evaluate the neuroprotective effects of RSG in a rodent model of SBI.

Methods—65 adult male Sprague-Dawley rats were randomly divided into sham, vehicle and treatment groups. RSG was administered intraperitoneally in two dosages (1mg/kg/dose, 6mg/kg/dose) 30 minutes before surgery, and 30 minutes and 4 hours after surgery. Animals were euthanized 24 hrs following neurological evaluation to assess brain edema and BBB permeability by IgG staining. Inflammation was examined using myeloperoxidase (MPO) assay and double-labeling fluorescent immunohistochemical analysis of IL-1 β and TNF- α .

Results—Localized brain edema was observed in tissue surrounding the surgical injury. This brain edema was significantly higher in rats subjected to SBI than sham animals. Increased IgG staining was present in affected brain tissue; however, RSG reduced neither IgG staining nor brain edema. RSG also did not improve neurological status observed after SBI. RSG, however, significantly attenuated MPO activity and qualitatively decreased IL-1 β and TNF- α expression compared to vehicle-treated group.

Conclusion—SBI causes increased brain edema, BBB disruption and inflammation localized along the periphery of the site of surgical resection. RSG attenuated inflammatory changes, however, did not improve brain edema, BBB disruption and neurological outcomes after SBI.

Keywords

Rosiglitazone; surgical brain injury; inflammation; brain edema; blood brain barrier; myeloperoxidase

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1. Introduction

Neurosurgical operations can result in inevitable brain injury due to surgical trauma, retractor stretch, intraoperative hemorrhage and electrocautery damage (Andrews and Muto, 1992; Solaroglu et al., 2004; Jadhav et al., 2007a). This surgical brain injury (SBI) is unavoidable (Deletis and Sala, 2001) and results in brain edema, disruption of blood brain barrier (BBB), oxidative stress, and cell death in the vulnerable functional tissue along the periphery of surgical resection (Matchett et al., 2006; Jadhav et al., 2007a; Lo et al., 2007). SBI not only has medical significance but also has medicolegal implications due to the practice of 'defensive medicine' which results in excess expenditure to the tune of \$ 70–126 billion a year ("Addressing the New Health Care Crisis," U.S. Department of Health and Human Services, March 2003).

Rosiglitazone (RSG), a peroxisome proliferating activating receptor- γ (PPAR- γ) agonist, is shown to be neuroprotective in focal cerebral ischemia and intracerebral hemorrhage (Allahtavakoli et al., 2006; Chen et al., 2006; Luo et al., 2006; Pereira et al., 2006; Zhao et al., 2007). RSG is a constituent of the thiazolidinediones nuclear hormone receptor superfamily and is known for its anti-inflammatory actions via activation of PPAR- γ . The activation of PPAR- γ leads to the inhibition of the inflammatory NF κ B pathway (Luo et al., 2006; Tureyen et al., 2007). Thus, RSG attenuates the expression of pro-inflammatory genes and cytokine production by regulating ligand activation of transcription factors (Luo et al., 2006; Chen et al., 2006).

Inflammation is a key component of brain injuries resulting from different etiologies such as trauma, ischemia, neurodegeneration, and excitotoxicity (Esiri M, 2007; Wang et al., 2007; Williams et al., 2007). Acute inflammation involves the activation of a range of cells including neutrophils and microglia, in addition to inflammatory mediators such as cytokines and chemokines (Wang et al., 2007), which can contribute to disruption of BBB leading to brain edema as well as neuronal damage (Stamatovic et al., 2006). Thus, inflammatory processes can exacerbate brain injury and worsen the neurological outcomes (Luo et al., 2006). In the present study we hypothesized that SBI causes inflammation in susceptible brain tissue and RSG provides neuroprotection via its anti-inflammatory actions.

2. Results

Brain water content was not attenuated with Rosiglitazone treatment

Brain water content in the frontal ipsilateral lobe (susceptible to SBI) was significantly higher in all animals subjected to SBI as compared with sham surgery group. However, there was no significant difference in brain water content between vehicle treated group and groups treated with either doses of RSG (1mg/kg, 6 mg/kg) (Figure 1). Other areas of the brain were also evaluated, however, there were no significant differences observed in brain water content between sham surgery, vehicle treated and RSG treated groups (contralateral frontal lobe data in figure 1, other brain regions not shown) similar to previous reports (Jadhav et al., 2007a).

Rosiglitazone did not reduce blood brain barrier permeability

The breakdown of the BBB was qualitatively determined by IgG staining similar to previous reports (Jadhav et al., 2007b, Yamaguchi et al., 2007). The frontal contralateral lobe which served as control did not show any IgG staining (brown staining). In contrast, in the frontal ipsilateral lobe, IgG serum proteins were increasingly present surrounding the site of resection (Figure 2). Higher magnification indicated that the extravasation of IgG was more around the micro vasculature (Figure 2). However, RSG treatment (6mg/kg) did not show any qualitative difference in IgG staining thus, indicating that it did not decrease BBB permeability after SBI.

Inflammation was reduced with Rosiglitazone treatment

Myeloperoxidase (MPO), a marker for the infiltration of neutrophils, was assayed for quantitative indication of the presence of inflammation in the ipsilateral frontal lobes from different groups. MPO observed at high optical density (460nm) signifies an increased presence of inflammation. Vehicle treated groups had a significantly higher MPO (6.85 ± 1.35 U/g) compared to sham surgery groups (0.22 ± 0.03 U/g). In RSG (1 mg/kg) treated groups, there was a significant decrease in MPO (2.15 ± 0.82 U/g) compared with vehicle treated groups demonstrating its anti-inflammatory properties (Figure 3A).

Immunohistochemical analysis of well known inflammatory markers TNF- α and IL-1 β indicated that these markers were increased in the neurons (NeuN, neuronal marker) in the affected brain tissue in the vehicle treated rats. However, in animals treated with RSG (6mg/kg), there appeared to be decreased levels of both inflammatory markers in the affected brain tissue, further illustrating that treatment with RSG decreased inflammation (Figure 3B).

Neurological evaluation

A 21 point sensorimotor scoring was performed by a blinded observer to evaluate the neurological outcomes similar to previous reports (Lo et al., 2007). As expected, SBI decreased the neurological score in the vehicle treated animals as compared to the sham surgery group. However, RSG group did not show any significant difference compared to the vehicle treated group.

3. Discussion

The present study shows for the first time that inflammatory changes occur in the affected brain tissue after SBI. We demonstrated that RSG, a PPAR- γ agonist reduces inflammation however does not provide neuroprotection after SBI in rodents. Previous studies have elucidated that oxidative stress, disruption of BBB and brain edema are critical in the pathophysiology of SBI (Jadhav et al., 2007; Matchett et al., 2006; Lo et al., 2007). BBB disruption and brain edema are common complications after SBI which can lead to deteriorating neurological outcomes (Fasano and Penna 1992; Manninen et al. 1999; Tommasino 1992). Inflammation has been reported to play a role in the development of vasogenic brain edema (Zhang et al., 2006) which occurs as a result of BBB disruption. Furthermore, our previous study also showed that matrix metalloproteinases which play an important role in inflammation are increasingly activated after SBI (Yamaguchi et al., 2007). Our present results indicated that inflammation was mitigated with RSG treatment, which is consistent with numerous experimental studies; validating anti-inflammatory effects of RSG and its neuroprotective potential in cerebral ischemia and intracerebral hemorrhage (Allahtavakoli et al., 2006; Chen et al., 2006; Luo et al., 2006; Park et al., 2007; Pereira et al., 2006; Zhao et al., 2007). The neuronal cells were co-localized with inflammatory markers and we also observed significant neutrophil infiltration after SBI (MPO assay). These changes were reversed after RSG treatment. The involvement of other inflammatory cells such as microglia (Kelly et al., 2007; Byrnes and Faden, 2007; Bye et al., 2007) needs to be further elucidated in SBI.

The anti-inflammatory actions of RSG, however, did not have any beneficial effects on brain edema after SBI. One explanation may be that RSG is not specific in targeting edema, particularly vasogenic edema. The formation of vasogenic edema is characterized by an increased permeability of the BBB (Xi et al., 2002). Yet treatment with Rosiglitazone did not appear to preserve the BBB, as shown with the IgG staining. The anti-inflammatory effects of RSG were not sufficient to reduce brain edema and preserve BBB integrity possibly also due to the severity of the injury. SBI showed an increase of approximately 2% in brain water content in the affected brain tissue resulting in localized brain edema.

Another possibility for ineffectiveness of RSG may be the early time course for evaluation of brain edema in this study. The time course for this study was based on our previous study, which indicates that brain edema peaks at 24 hours after SBI which also correlates with the clinical scenario (Jadhav et al, 2007). However, inflammation and its deleterious outcomes may be a delayed effect after SBI. Future studies can be designed to address whether anti-inflammatory treatment with RSG or other pharmacological agents will reduce brain edema and BBB disruption at later time points after SBI. However, in our opinion, a delayed therapeutic potential may not be clinically viable in SBI wherein the common and major complications such as BBB disruption and brain edema are likely to result in first 24 hrs after neurosurgical procedures.

SBI caused inflammatory changes in susceptible brain tissue along with localized brain edema and disruption of BBB and deterioration in neurological outcome. RSG, a PPAR- γ agonist did not reduce brain edema and BBB disruption or improve the neurological status; however it attenuated inflammation in the affected brain tissue.

4. Experimental Procedures

All experimental procedures were evaluated and approved by Loma Linda University Institutional Committee for Animal Care and Handling (IACUC).

Surgical Brain Injury

65 adult male Sprague Dawley rats (350–485g) were used for SBI modeling as previously described (Jadhav et al., 2007b, Yamaguchi et al., 2007). Briefly, animals were anesthetized with 3% isoflurane (in mixture of 70% medical air, 30% oxygen) and positioned in a stereotactic frame (Benchmark) under a surgical operating microscope. A midline incision was made through the skin and connective tissue layer to expose the frontal region of the cranial bone. A microdrill was used to fashion a square cranial window (5mm each side) 2mm lateral and 1mm posterior to the sagittal and coronal planes through the bregma respectively. The dura was reflected to expose the brain. Two incisions were made using a flat blade (width = 1.5mm and length = 6mm) along the sagittal and coronal planes of the 5 mm $\times$ 5 mm window plane leading away from the bregma and the excised brain was then lifted and removed as previously described (Jadhav et al., 2007b, Matchett et al., 2006, Yamaguchi et al., 2007). Bleeding was controlled through intraoperative packing and saline irrigation. Once hemostasis was achieved, the dura and bone were restored to their original positions and skin was sutured using a 3-0 silk on reverse cutting needle (Ethicon). All vital signs were monitored throughout the surgery. Animals' body temperature was controlled and maintained between 35–37° celsius indicated by rectal probe. Sham surgery included only a craniotomy without dural incisions. Postoperative monitoring included maintenance of body temperature and post-operative fluids (saline). All animals were sacrificed 24 hours after surgery.

Animal Groups

Four groups, namely sham, vehicle treated and RSG treated (lower and higher dose) were studied. RSG (AvandiaTM, GlaxoSmithKline, USA) was used in two concentrations, 1mg/kg and 6mg/kg, based on previous studies (Luo et al, 2006, Pereira et al, 2006). The drug was dissolved in vehicle (10% DMSO for 1mg/kg, 25% DMSO for higher dosage of 6mg/kg) while vehicle treated animals received 25% DMSO. All treatments were administered intraperitoneally 30 minutes prior to surgery, and 30 minutes and 4 hours post-surgery.

Brain Water Content

Animals were placed under deep anesthesia and sacrificed 24 hours post-surgery. Brains were removed under ice and sectioned into the following six regions: frontal ipsilateral, frontal

contralateral, parietal ipsilateral, parietal contralateral, cerebellum and brain stem. Sectioned brains were weighed immediately (wet weight) and placed in an oven set at 104° celsius for 48 hours to obtain the dry weight. Brain water content was calculated using the following equation: Percent brain water content=((Wet weight)-(Dry weight)/ (Wet weight)) × 100 (Xi et al., 2002).

Blood Brain Barrier Permeability by Immunoglobulin G (IgG) Staining

According to methods previously described (Richmon et al, 1998) perfused brains were sectioned and incubated with goat anti-rat IgG biotin-conjugated antibody (Santa Cruz Biotechnology, CA) overnight. Protocol using the Avidin-biotin complex (ABC) kit was followed accordingly for staining.

Immunohistochemical Staining

Animals were sacrificed at 24 hours under deep anesthesia. Brains were perfused with 200 ml of PBS (Sigma-Aldrich Corp, MO) followed by 100 ml of 4% phosphate buffered formalin (Fisher Scientific Inc). Brain tissue was then fixed with sucrose and formalin and stored at 4° celsius. Frozen brains were sectioned and immunofluorescence studies followed standard protocols. (Shimamura et al, 2006). Antibodies used for immunofluorescence were mouse anti NeuN (Chemicon, MAB377), goat anti TNF- α (Santa Cruz Biotechnology, CA), rabbit anti IL-1 β (Santa Cruz Biotechnology, CA). Photomicrographs of sections were viewed and analyzed using an Olympus BX51 fluorescent microscope and MagnaFire SP Version 2.1B software.

Myeloperoxidase (MPO) Assay

MPO assay was used to determine inflammatory changes in the affected brain tissue. All animals were deeply anesthetized and sacrificed at 24 hours. As previously described perfused brains were sectioned into frontal ipsilateral, frontal contralateral, parietal ipsilateral, and parietal contralateral and then frozen at -80° celsius (Lo et al., 2007). However, solely the frontal ipsilateral section was used to measure MPO activity. Frontal ipsilateral brain tissue was thawed and homogenized with 1ml 50mmol/L Tris-HCl. Tissue was then homogenized with 3ml sodium buffer, divided into three 1.5 ml (Eppendorf) tubes, centrifuged at 4C, max G, for 30 minutes. The supernatant was discarded; pellet was re-suspended in 0.3ml of 0.5% hexadecyltrimethylammonium bromide (HTAB) in potassium phosphate buffer for 2 minutes. The brain tissue was then frozen in liquid nitrogen and then thawed at 25C. Sonication of brain tissue for 10 seconds at 25° celsius, freezing of brain tissue and thawing at 25° celsius were performed three times. Thereafter, samples were incubated at 4° celsius for 15 min and centrifuged at 12500 g for 15 minutes. Three tubes of supernatant (0.9ml) were collected and mixed with the same volume of 0.005% o-dianisidine dihydrochloride and hydrogen peroxide solution. Absorbance was measured spectrophotometrically at 460 nm at three time points, every 4 minutes. 50 mmol/L sodium phosphate was used as the blank solution. MPO was calculated by taking the average of the three timepoints using the following equation: Absorbance (time point) / (weight of brain tissue sample(g)).

Neurological Evaluation

Neurological outcomes were assessed by a blinded observer just before euthanization at 24 hrs using a 21 point sensorimotor scoring system as per previous reports (Lo et al., 2007). Briefly, the scoring system consisted of seven tests with scores of 1–3 for each test. These seven tests included: (i) spontaneous activity, (ii) symmetry in the movement of four limbs, (iii) forepaw outstretching, (iv) lateral turning, (v) climbing, (vi) body proprioception, and (vii) response to vibrissae touch. The score given to each rat at the completion of the evaluation was the

summation of all seven individual test scores. The minimum neurological score was 3 and the maximum was 21.

Statistical Analysis

All data was analyzed using ANOVA to account for differences between groups and expressed as mean $\pm$ standard error using Sigma Stat software, version 3.0.1. Data was found to be significant at $p < 0.05$.

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Figure 1. Effect of RSG on Brain Water Content

The figure shows quantified data representing brain water content (brain edema) in the contralateral and ipsilateral frontal lobes of the brain at 24 hrs after SBI. There is no significant difference in the brain water content between the sham surgery, vehicle treated and RSG treated groups (1mg/kg and 6mg/kg) in the contralateral frontal lobe. The ipsilateral frontal lobes show significantly higher brain water content in the vehicle and RSG treated groups (1mg/kg and 6mg/kg) as compared to the sham surgery group. However, there is no significant difference between the RSG treated groups and vehicle treated groups. $p < 0.05$, (*) denotes significant difference compared to sham surgery group. 'n' number is as follows: sham surgery = 8, SBI + vehicle = 7, SBI + RSG 1mg/kg = 4, SBI + RSG 6mg/kg = 8.

Figure 2. BBB Permeability by IgG Staining

The figure depicts IgG staining (brown color) in the vehicle treated (fig A and C) and RSG (6mg/kg, fig B and D) treated rats at 24 hrs after SBI. There is IgG extravasation in the affected brain tissue after SBI in the ipsilateral frontal lobe. The contralateral frontal lobe (marked by asterisk) does not show any staining. There appears to be no qualitative difference in the IgG staining between the vehicle treated and RSG treated groups (demarcated by dotted line). Similar findings were seen at higher magnification (figs C and D, ROI marked by boxes in figs A and B) which show extravasated IgG protein surrounding the microvasculature (depicted by arrows). The scale bars denote 1mm in panels A and B and 100μm in panels C and D. The figure is representative of data from 3 animals per group.

Figure 3. Effect of RSG on Inflammation

Figure 3A shows the effect of RSG (1mg/kg) on myeloperoxidase (MPO) activity at 24 hrs after SBI in the ipsilateral frontal lobe. There is increased MPO activity after SBI in the vehicle treated group as compared to the sham surgery. This increased MPO activity indicative of inflammation was attenuated by RSG treatment (1mg/kg). $p < 0.05$, (*) denotes significant

difference compared to sham surgery group and (#) denotes significant difference compared to vehicle treated group. Number of animals is as follows: sham surgery = 4, SBI + vehicle = 6 and SBI + RSG = 4. Figure 3B shows double fluorescent immunohistochemical representative pictographs depicting inflammatory markers IL-1 β and TNF α (green color, FITC) co-stained with NeuN, a marker for neuronal cells (red color, Texas Red) in ipsilateral frontal lobe at 24 hrs after SBI. The region of interest (ROI) from the sections used for fluorescent immunostaining was obtained from the ipsilateral frontal lobe, more precisely from the edge of the resection as depicted in figures 2A and 2B for vehicle and RSG treated groups respectively. The distribution of immunoreactivities in the vehicle treated groups (panels A–C and G–I) suggests that neurons express inflammatory mediators IL-1 β and TNF α after SBI. Qualitatively, these markers are attenuated in the RSG treated (6mg/kg) group (panels D–F and J–L). The merged images (panels C, F, I and L) show magnified images of the cells in the insets. Arrows depict the merged immunoreactivities of the inflammatory markers and Neun. The scale bar denotes 100 μ m. The figures are representative of data from 3 animals per group.

Figure 4. Neurological Outcomes

The figure shows quantified data of sensorimotor neurological evaluation at 24 hrs after SBI in different groups. The graph shows that both vehicle treated and RSG treated animals showed significantly greater neurological deficits as compared to sham surgery animals. However, there was no significant difference between the vehicle treated and RSG treated animals. $p < 0.05$, (*) denotes significant difference compared to sham surgery group. The number of animals is as follows: sham surgery = 9, SBI + Vehicle = 11 and SBI + RSG 6mg/kg = 4.