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

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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 PPAR γ 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 PPAR γ 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 $56 \pm 7\%$; $p < 0.05$; $n = 6/\text{group}$). 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 PPAR γ antagonist) prevented all the above PPAR γ -mediated actions. Thus, PPAR γ activation confers neuroprotection after TBI by anti-inflammatory, anti-apoptotic and anti-oxidative mechanisms.

Keywords

Rosiglitazone; Brain trauma; Inflammation; Oxidative stress; Neuroprotection

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

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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 (PPAR γ) is a ligand-activated transcription factor of nuclear hormone receptor superfamily (Escher and Wahli, 2000). 15-d-Prostaglandin J2 is the natural ligand of PPAR γ , while several thiazolidinediones (TZDs) are potent synthetic agonists (Kapadia et al., 2008). As PPAR γ 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 PPAR γ expression

At 1 day following CCI injury, the mRNA expression of PPAR γ 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 PPAR γ mRNA (by 4.4 fold; $p < 0.05$) compared to sham (Fig. 1). Whereas, treatment with GW9662 (a PPAR γ antagonist; 4 mg/kg, *i.p.* at 5 min and 12 h) prevented the post-TBI induction of PPAR γ mRNA expression (Fig. 1).

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 $3.09 \pm 0.14 \text{ mm}^3$ ($n = 6$) 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 $37 \pm 6\%$; $p < 0.05$; $n = 6$ 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 PPAR γ 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 $31 \pm 8\%$; $p < 0.05$; $n = 6$) (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 $53 \pm 11\%$; $p < 0.05$; $n = 6$) 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-B₄ (macrophage/activated microglia marker) or ICAM1 (Figs. 5A and B). Whereas, mice subjected to CCI injury and treated with vehicle showed several GSI-B₄⁺ cells and ICAM1⁺ capillaries at 2 days after TBI (Figs. 5C and D). Rosiglitazone treatment significantly decreased both GSI-B₄ and ICAM1 immunostaining (Figs. 5E and F) compared to vehicle group. Treating mice with the PPAR γ antagonist GW9662 following TBI had no significant effect on either GSI-B₄ or ICAM1 immunostaining (Figs. 5G and H). At 1 day after CCI injury, mRNA levels of the pro-inflammatory genes IL6, MCP1 and ICAM1 were significantly lower in the rosiglitazone-treated group compared to vehicle group (Fig. 6A).

2.5. Rosiglitazone induced HSP and anti-oxidant gene expression in the injured brain

HSPs and anti-oxidant enzymes are known to promote neuroprotection. We presently observed that TBI significantly increased the expression of 3 HSPs (HSP70, HSP27 and HSP32/HO1) and 3 anti-oxidant enzymes (SOD1, SOD2 and catalase) at 1 day after injury in the vehicle-treated mice compared to sham control (Fig. 6B). Treating mice subjected to CCI injury with rosiglitazone led to a further significant enhancement of the expression of all these mRNAs in the injured cortex (Fig. 6B). Rosiglitazone treatment also significantly elevated the HSP27 and HO1 immunoreactive protein levels estimated with immunohistochemistry and Western blotting in the cortex at 1 day after CCI injury compared to vehicle treatment (Fig. 7).

3. Discussion

PPAR γ is a transcription factor of nuclear hormone receptor superfamily (Blanquart et al., 2003). The PPAR protein is a complex of several domains including a DNA-binding domain (DBD), a ligand binding domain (LBD) and a D-domain that links DBD and LBD (Escher and Wahli, 2000). In the absence of a ligand, the PPAR will be associated with a corepressor complex to trans-repress the target genes. Whereas, when a specific ligand binds to the LBD, the PPAR complex dimerizes with retinoic acid-X-receptor (RXR) leading to release of the corepressor (Yi et al., 2007). The PPAR:RXR heterodimer translocates to nucleus and binds to the promoters of several genes that contain a cis-acting element known as peroxisome-proliferator response element (PPRE) to either initiate or suppress the transcription of the target genes (Blanquart et al., 2003).

Of various TZDs which are potent exogenous agonists of PPAR γ , rosiglitazone and pioglitazone were shown to be well-tolerated with few side effects in humans (Sood et al., 2000). As they control blood glucose levels by stimulating PPAR γ , these 2 TZDs are FDA-approved for type-2 diabetes treatment (Sood et al., 2000). Of these two, the affinity of rosiglitazone to PPAR γ was shown to be 10 times higher than pioglitazone (Escher and Wahli, 2000). Recent studies showed that both these TZDs decrease the contusion volume, microglial activation, astrogliosis and myelin loss leading to better preservation of motor neurons with less pain following SCI in adult rodents (Park et al., 2007; McTigue et al., 2007). Mechanistically TZD treatment was observed to curtail post-SCI expression of many chemokines, cytokines, adhesion molecules and pro-inflammatory transcription factors while

promoting the expression of neuroprotective genes (Park et al., 2007). Our laboratory and other groups also showed that treatment with rosiglitazone or pioglitazone significantly decreases the infarct volume as well as neurological dysfunction in rodent models of focal ischemia by preventing inflammation and oxidative stress (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).

TBI leads to extensive secondary neuronal death (Onyszchuk et al., 2008) which will be mediated by the synergistic action of many pathophysiologic mechanisms including inflammation, glutamate excitotoxicity, apoptosis, ionic imbalance, edema and oxidative stress during the acute period after an injury. Hence, combination therapies with drugs that can target more than one mechanism are beneficial. In addition, compounds that can target multiple pathways simultaneously can be more effective. Recent studies showed that PPAR γ agonists are such compounds that induce neuroprotection by targeting several down-stream destructive mechanisms which precipitate brain damage following an acute CNS insult (Park et al., 2007; Tureyen et al., 2007).

Our results showed that rosiglitazone treatment significantly reduced lesion volume compared to vehicle treatment. Previous studies by Luo et al. (2006) following focal ischemia showed that rosiglitazone is effective at 3–12 mg/kg concentration given *i.p.*, with an optimal dose of 6 mg/kg. A recent study from our laboratory showed that multiple doses of rosiglitazone given during the first day after SCI are extremely beneficial (Park et al., 2007). Our studies also showed that this dosing regimen doesn't result in any mortality or overt symptoms such as altered grooming or feeding behavior (Park et al., 2007; Tureyen et al., 2007). Based on these earlier studies, we presently used a dose of 6 mg/kg given *i.p.* at 5 min, 6 h and 24 h after TBI with an aim to prevent inflammation and oxidative stress which are at peak during the first day after an injury. In concurrence with the earlier studies this dosing regimen induced no observable side effects in adult mice. Interestingly, animals treated with the PPAR γ antagonist GW9662 showed significantly greater lesion volume compared to vehicle group suggesting that PPAR γ activation by its endogenous ligands is an essential neuroprotective event which can be enhanced by providing exogenous agonists. Indeed, a similar result has been reported in ischemia (Victor et al., 2006).

Reduced inflammation following TBI might have contributed to the neuroprotection induced by rosiglitazone. Following TBI, rosiglitazone-treated mice showed significantly less number of GSI-B $_4^+$ cells in the injured cortex, suggesting a curtailed microglial activation and macrophage infiltration. Activated microglia and macrophages release a host of neurotoxic substances including oxygen free radicals that are extremely toxic to neurons. ICAM1 expression on cerebral capillaries which promotes macrophage infiltration after brain injury (Knobloch and Faden, 2002) was also observed to be less in the rosiglitazone group compared to vehicle group. Post-TBI induction of the pro-inflammatory cytokine IL6 and chemokine MCP1 mRNA expression was also curtailed by rosiglitazone treatment. This is in support of the previous studies that indicated the anti-inflammatory potential of TZDs following cerebral ischemia and SCI (Sundararajan et al., 2005; Zhao et al., 2005, 2006; Collino et al., 2006; Pereira et al., 2006; Tureyen et al., 2007; Park et al., 2007). Recent studies showed that rosiglitazone treatment curtails inflammatory gene expression at 6 h after SCI when the secondary neuronal damage was still evolving indicating prevention of inflammation as a potential cause rather than an effect of rosiglitazone action (Park et al., 2007). We presently observed that rosiglitazone treatment significantly decreased the induction of inflammatory genes after TBI as well, but as the gene expression was estimated at 1 day after TBI, we cannot rule out the possibility that this might be an effect rather than a cause. The reduction in the lesion volume observed in the rosiglitazone-treated animals might be in part due to decreased apoptosis in the injured cerebral cortex. We observed that rosiglitazone treatment significantly decreased the number of TUNEL $^+$ neurons in the cortical area adjacent to the injury core. In

addition, post-TBI mRNA induction of the pro-apoptotic genes caspase-3 and Bax was significantly lower in the rosiglitazone group compared to vehicle group.

HSPs act as chaperones to prevent aggregation and denaturation of proteins. In addition, HSPs are known to decrease glutamate release, oxidative stress and apoptosis and hence can confer neuroprotection after acute brain injuries (Kelty et al., 2002; Mehlen et al., 1996). CCI injury led to increased expression of cortical HSP70, HSP27 and HO1 mRNA levels which were further enhanced by rosiglitazone treatment. Rosiglitazone also enhanced the protein expression of HSP27 and HO1 after TBI. In addition, rosiglitazone treatment led to a significant induction of anti-oxidant enzymes SOD1, SOD2 and catalase in the injured brain which indicates an increased capability to handle free radicals and other oxidants leading to better neuronal survival.

In conclusion, our study showed that TBI led to increased expression of PPAR γ and treatment with PPAR γ agonist rosiglitazone induces significant neuroprotection. Several mechanisms including prevention of inflammation, apoptosis and oxidative stress in addition to induction of HSPs might have synergistically contributed to the beneficial actions of rosiglitazone after TBI. We also showed that PPAR γ antagonist GW9662 treatment worsens the outcome after TBI indicating a direct action of rosiglitazone mediated by stimulating PPAR γ . While this is the first study showing the efficacy of PPAR γ stimulation after TBI, a previous study showed the beneficial effects of PPAR α agonist fenofibrate following lateral fluid-percussion-induced TBI in adult rats (Besson et al., 2005). It might be of therapeutic interest to develop potent dual agonists that can induce both the isoforms of PPAR.

4. Experimental procedures

4.1. Controlled cortical impact injury and drug administration

TBI was induced in adult male C57BL/6 mice (22–25 g) with a CCI device as described earlier (Rao et al., 2001). All surgical procedures were conducted and animals were cared for according to the animal welfare guidelines (1985 Principles of the Guide for the Care and Use of Laboratory Animals, U.S. Department of Health and Human Services Publ. 85–23) and were approved by the animal care committee of the University of Wisconsin-Madison. In brief, a moderate grade injury was induced through a craniectomy (2 mm in diameter, 1 mm lateral and 1 mm caudal to Bregma) at a velocity of 3 m/s and 1 mm deformation under isoflurane anesthesia. The exposed cortex was covered with Surgicel, the wound was closed with sutures and 0.5% bupivacaine (100 μ l) was injected along the incision to provide local anesthesia. During the surgery and recovery from anesthesia, body temperature was maintained at 37–38 °C with a heating pad and a lamp. After recovering from anesthesia, mice were returned to their cages with free access to food and water. To cohorts of mice, rosiglitazone (6 mg/kg; Cayman Chemicals, Ann Arbor, MI) or GW9662 (4 mg/kg; PPAR γ antagonist; Sigma Chemicals, St Louis, MO) or vehicle (4% DMSO) was injected *i.p.* at 5 min, 6 h and 24 h after TBI.

4.2. Determination of lesion volume

Lesion volume was measured at 7 days after TBI as described earlier (Dempsey and Rao, 2003). In brief, an animal was perfused transcardially with buffered paraformaldehyde, the brain was post-fixed, cryoprotected and sectioned (coronal; 40 μ m thick at an interval of 480 μ m). 6 serial sections from each brain covering the cortical lesion (from 0 to –3.0 mm from Bregma as per the Mouse Brain Atlas of Paxinos and Watson) were stained with Cresyl violet and scanned using the NIH Image Program (written by Wayne Rasband; <http://rsb.info.nih.gov/ij>). The volume of the lesion was computed by the numeric integration of data from the serial sections in respect to sectional interval.

4.3. Immunohistochemistry

Parallel sets of 40 μ m coronal sections from each mouse were immunostained with antibodies against ICAM1 (1:1000; goat anti-ICAM1; R&D systems inc., MN, USA), HO1 (1:2000; rabbit anti-HO1; Stressgen, BC, Canada) and HSP27 (1:1000; rabbit anti-HSP27; Stressgen, BC, Canada) using the standard procedures. In brief, sections were rinsed in 0.1 M Tris-buffered saline with 0.1% Triton-X100 (TBS-T; 3×5 min), incubated in 1% H_2O_2 for 10 min and washed in TBS-T (3×5 min). The sections were incubated in 10% normal donkey serum in TBST for 1 h and at 4 °C overnight in the desired antibodies. The sections were washed in 1% donkey serum in TBS-T (3×5 min) and incubated in biotinylated donkey anti-rabbit or anti-goat secondary antibody (1:1000; Santa Cruz, CA, USA) for 1 h. Sections incubated in the absence of primary or secondary antibodies served as negative controls. For GSI-B₄ staining, sections were washed in 0.1 M PBS (3×10 min), incubated with biotinylated GSI-B₄ (1:1000; Sigma Chemical Co.) in PBS for 2 h, incubated for 1 h in streptavidin-HRP (1:300, Dako, Carpinteria, CA, USA) and washed in PBS (3×5 min). GSI-B₄-reactive cells were visualized using DAB as chromogen as described earlier (Kapadia et al., 2006).

4.4. TUNEL staining and fluorescence double labeling

In situ detection of apoptotic cells was performed using TUNEL staining with the *In Situ* Cell Death Detection Kit (Roche Molecular Biochemicals, Indianapolis, IN, USA) as described earlier (Kapadia et al., 2006). Briefly, sections were permeabilized with 1% Proteinase K (in 50 mM Tris/5 mM EDTA buffer) for 15 min, rinsed with PBS and incubated in TUNEL reaction mixture for 1 h at 37 °C. Samples were rinsed in PBS (3×5 min), coverslipped using Antifade solution (Molecular Probes, Invitrogen) and observed under the fluorescence microscope (Nikon TE2000, Nikon USA). The apoptotic cells (FITC green-stained) were counted in the peri-injury area of 3 ipsilateral cortical sections of each mouse. For double labeling to identify the apoptotic cell type, TUNEL stained sections were washed in TBS (3×5 min) and incubated with rabbit anti-GFAP (1:2000; Dako, Carpinteria, CA, USA) or mouse anti-NeuN (1:500, Chemicon, Temecula, CA, USA). The sections were washed in TBS and incubated for 1 h with secondary antibodies (1:200; Alexa Fluor 594 donkey anti-rabbit or anti-mouse, Molecular Probes, Eugene, OR, USA).

4.5. Real-time PCR analysis

Gene expression analysis was conducted as described earlier (Tureyen et al., 2008). The following transcripts were estimated using the primers designed with Primer Express Software (Applied Biosystems USA) based on the mouse GenBank numbers given in parenthesis: PPAR γ (NM_011146), IL6 (NM_031168), MCP1 (AF065933), ICAM1 (BC008626), HSP27 (NM_013560), HSP70 (NM_010479), HO1 (NM_010442), SOD1 (NM_011434), SOD2 (NM_013671), catalase (NM_009804), caspase-3 (NM_009810), Bax (NM_007527) and 18S rRNA (M11188). In brief, cohorts of mice subjected to CCI injury or sham operation and treated with vehicle or rosiglitazone were killed at 1 day of survival ($n = 4/\text{group}$). Total RNA was extracted from the ipsilateral cortex of each mouse using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). 1 μ g of RNA from each sample was reverse transcribed with oligo (dT)₁₅ and random hexamer primers using M-MuLV reverse transcriptase (Life Technologies, Rockville, MD, USA). 10 ng of cDNA of each sample and gene-specific primers were added to SYBR Green PCR Master Mix (SYBR Green I Dye, AmpliTaq DNA polymerase, dNTPs with dUTP and optimal buffer components; Applied Biosystems) and subjected to PCR amplification in a Perkin-Elmer TaqMan 5700 Sequence Detection System (1 cycle at 50 °C for 2 min, 1 cycle at 95 °C for 10 min, and 40 cycles at 95 °C for 15 s and 1 cycle at 60 °C for 1 min). PCR reactions were conducted in duplicate. The amplified transcripts were quantified with the comparative C_T method using 18S rRNA as the internal control as described earlier (Vemuganti et al., 2006).

4.6. Western blotting

Protein levels of Hsp27 and HSP32/HO1 in brain tissue were estimated as described earlier (Satriotomo et al., 2006; Dhodda et al. 2004). Whole ipsilateral cortex from the mice treated with vehicle, rosiglitazone and GW9662 after CCI injury was obtained at 1 day of recovery ($n = 4/\text{group}$). The tissue was homogenized in ice cold-lysis buffer (50 mM Tris, 150 mM NaCl, 1% Triton and 1% SDS) and protease inhibitors (1 mM PMSF, 10 $\mu\text{g}/\text{mL}$ leupeptin, 10 $\mu\text{g}/\text{mL}$ pepstatin, 10 $\mu\text{g}/\text{mL}$ aprotinin and 1 mM EDTA; pH 7.4). The homogenate was centrifuged at 15,000 g for 10 min at 4 °C, and the supernatant (30 μg protein equivalent) from each sample was electro-phoresed on Bio-Rad Criterion precast polyacrylamide gels, transferred to polyvinylidene difluoride membranes and probed with polyclonal anti-HSP27 (1:1000), polyclonal anti-HO1 (1: 1000) and monoclonal anti- β -actin antibodies (1:10,000; Sigma Chemical Co.) followed by HRP-conjugated anti-rabbit or anti-mouse IgG (1:2000). The protein bands recognized by antibodies were detected by enhanced chemiluminescence according to the manufacturer's instructions (Pierce, Rockford, IL, USA).

4.7. Statistical analysis

The data are expressed as mean $\pm$ SD. Comparisons among groups were performed by one-way analysis of variance (ANOVA) followed by Tukey—Kramer multiple comparisons post-hoc test.

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Fig. 1.

At 1 day after CCI injury, the PPAR γ mRNA expression analyzed with real-time PCR increased significantly in the cortex surrounding the injury compared to sham. Treating with rosiglitazone, but not with PPAR γ antagonist GW9662 led to a further significant increase in PPAR γ 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 $\pm$ 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).

Fig. 2. Representative Cresyl Violet stained brain sections (A to E) from mice subjected to sham (A), and CCI injury killed at 1 h (B), 5 h (C), 1 day (D) and 7 days (E). Panels a, b, c and d shows the magnified boxed areas of A, B, C and D, respectively. Panels e, f, g and h shows TUNEL staining in the boxed areas of A, B, C and D, respectively.

Fig. 3.

At 7 days after CCI injury, adult mice showed a cortical contusion that spanned from 0 to ~3.2 mm from Bregma. Rosiglitazone treatment significantly decreased and GW9662 treatment significantly increased the cortical contusion volume. The top panel shows serial sections from representative mice of each group stained with Cresyl Violet. The lesion was outlined in black. Six mice were used per group. 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).

Fig. 4. PPAR γ agonist rosiglitazone treatment decreases TUNEL⁺ cells in the injured cerebral cortex at 2 days after TBI. Panels A to C show representative images of TUNEL⁺ cells in vehicle (A), rosiglitazone (B), and GW9662 (C) treated mice. Panel D shows the number of TUNEL⁺ cells counted in the shaded cortical area shown in panel E. Bars represent mean $\pm$ SD of $n = 6$ per group. Statistics: * $p < 0.05$ compared to vehicle control by ANOVA followed by Neuman—Keul's multiple comparisons post-test. Many TUNEL⁺ cells showed co-labeling with the neuronal marker NeuN (F), but not with the astroglial marker GFAP (G).

Fig. 5. Rosiglitazone significantly prevented the macrophage infiltration/microglial activation and ICAM1 induction after CCI injury. The panels shows GSI-B4 stained (A, C, E and G) and ICAM1 immunostained (B, D, F and H) sections from representative mice sacrificed after sham-operation (A and B) and at 2 days after TBI and treated with either vehicle (C and D) or rosiglitazone (E and F) or GW9662 (G and H). Similar pattern of staining was observed with n of 4 mice per group.

Fig. 6. Real-time PCR analysis showed a significant upregulation of pro-inflammatory (IL6, MCP1 and ICAM1) and pro-apoptotic (caspase-3 and Bax) gene expression in the cerebral cortex at 1 day after CCI injury compared to sham (A). Rosiglitazone treatment significantly curtailed the post-TBI induction of all these transcripts (A). Gene expression of neuroprotective genes including HSPs (HSP27, HSP70 and HSP32/HO1) and anti-oxidant enzymes (SOD1, SOD2 and catalase) was also significantly upregulated in the cortex at 1 day after CCI injury (B). All these transcripts showed a further increased expression in the rosiglitazone treated group compared to sham or vehicle/TBI groups (B). The bars represent mean $\pm$ SD of $n = 4$ per group.

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 post-test).

Fig. 7.

Rosiglitazone treatment significantly increased the post-TBI induction of HSP27 and HO1 protein expression in the cerebral cortex. The panels show representative sections immunostained for HO1 (A to D) and HSP27 (E to H) from the mice sacrificed after sham-operation (A and E), and 2 days after TBI and treated with either vehicle (B and F) or rosiglitazone (C and G) or GW9662 (D and H). A similar pattern of staining was observed with n of 4 mice per group. Panels I and J shows representative immunoblots of HO1 (I) and HSP27 (J) prepared with the cortical tissue of cohorts of mice subjected to TBI and treated with vehicle, rosiglitazone and GW9662. The house-keeping control β -actin levels were not altered by TBI or any treatment (I and J). The bars in I and J are mean $\pm$ SD of $n = 4$ per group. Statistics: * $p < 0.05$ compared to the vehicle/TBI group by ANOVA followed by Tukey—Kramer multiple comparisons post-test.