Published in final edited form as:

Transl Stroke Res. 2012 September 1; 3(3): 390–396. doi:10.1007/s12975-012-0190-9.

Acute rosiglitazone treatment during reperfusion after hyperglycemic stroke is neuroprotective not vascular protective

Sara Morales Palomares, Ph.D., Julie G. Sweet, B.S., and Marilyn J. Cipolla, Ph.D.

Departments of Neurology, Obstetrics, Gynecology & Reproductive Sciences, and Pharmacology, University of Vermont College of Medicine

Abstract

Reperfusion therapy for ischemic stroke can cause secondary brain injury, especially under hyperglycemic (HG) conditions. Here we investigated the effect of acute treatment with rosiglitazone, a peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonist, prior to postischemic reperfusion, on stroke outcome during HG stroke. Male Wistar rats that were either normoglycemic (NG) or HG by STZ (50 mg/kg; for 5–6 days) underwent middle cerebral artery occlusion (MCAO) for 2 hours with 2 hours of reperfusion. Animals were treated i.v. with rosiglitazone (1mg/kg; n=16), rosiglitazone (1mg/kg) + the free radical scavenger Tempol (50mg/kg; n=10) or vehicle (n=16) ten minutes prior to reperfusion and infarct volume, edema formation and cerebral blood flow (CBF) were measured. Compared to NG, HG stroke significantly increased infarct volume from $5.2 \pm 3.0\%$ vs. $14.7 \pm 3.6\%$ ($p < 0.05$). Rosiglitazone prevented the increased infarct volume induced by HG that was only $6.9 \pm 2.0\%$ ($p < 0.05$ vs. HG) but did not have any effect on edema formation that was increased by 3.0% in both HG vehicle and rosiglitazone-treated ipsilateral vs. contralateral hemispheres ($p < 0.05$). Combined treatment of rosiglitazone + Tempol did not significantly change brain water content that remained 2.2% greater than contralateral ($p < 0.05$), but reversed the neuroprotective properties of rosiglitazone in HG MCAO animals such that infarct volume was $14.3 \pm 4.4\%$ ($p > 0.05$ vs. vehicle). The lack of an effect of combined treatment of rosiglitazone + Tempol may be due to a decrease in reperfusion CBF that was only 60% of baseline ($p < 0.01$) compared to 82% and 89% for HG vehicle and rosiglitazone treated animals ($p > 0.05$). In conclusion, acute rosiglitazone treatment prior reperfusion was neuroprotective but not vascular protective during HG stroke.

Keywords

Ischemic stroke; hyperglycemia; infarct volume; edema; cerebral blood flow; rosiglitazone

Introduction

Despite some progress in understanding the pathophysiology of ischemic stroke, the success in stroke treatment has largely failed. Reperfusion of the ischemic brain by rapid recanalization of an occluded vessel is currently the only effective therapy for ischemic stroke [1]. However, postischemic reperfusion is not entirely beneficial. The window of opportunity for improvement of stroke outcome is brief (<6 hours), after which reperfusion can result in cerebral injury [2]. Reperfusion injury is especially prevalent in hyperglycemic (HG) ischemic stroke [3], suggesting hyperglycemia may be an important factor in brain damage after reperfusion. Compared to normoglycemia, hyperglycemia significantly

worsens stroke outcome, including larger infarction, edema formation and a higher risk of mortality [4,5]. Hyperglycemia fundamentally exacerbates brain damage by altering vascular responses [6,7]. In addition, hyperglycemia induces pro-inflammatory, pro-thrombotic and pro-vasoconstrictive changes in the vasculature that impair autoregulation and postischemic reperfusion blood flow [6]. In the complexity of hyperglycemia's effects after ischemia/reperfusion (I/R), inflammation and oxidative stress appear to be important key mechanisms. Thus, reactive oxygen species (ROS) and pro-inflammatory factors found in HG stroke are higher than in normoglycemic conditions [8,9].

Rosiglitazone, an agonist of the ligand-activated transcription factor peroxisome proliferator-activated receptor-gamma (PPAR- γ), has been shown to possess anti-inflammatory [10,11] and antioxidant [12,13] properties, including in the vasculature. Although chronic treatment of diabetic patients with rosiglitazone has increased risk of cardiovascular disease, including stroke [14], the use of rosiglitazone for acute treatment such as reperfusion injury appears to be beneficial. Experiments in vivo have shown a neuroprotective effect of rosiglitazone when animals are treated prior to or after the induction of focal or global cerebral ischemia [15]. However, if rosiglitazone is protective during reperfusion is not known, especially during HG stroke when oxidative stress is high. In the present study, we investigated if acute rosiglitazone treatment ten minutes prior to postischemic reperfusion would improve infarction and edema formation during HG stroke. We also investigated if combined treatment with rosiglitazone plus the antioxidant Tempol would provide greater protection during HG stroke.

Materials and Methods

Animal model of transient focal ischemia

All procedures were approved by the Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animals were male Wistar rats (~300 g) that were either normoglycemic (NG) or HG. Animals were made HG by a single intraperitoneal injection of streptozotocin (50 mg/kg) 5-6 days prior to middle cerebral artery occlusion (MCAO), as previously described [16]. Glucose was measured on the day of the surgery by a commercially available glucose monitor (Freestyle Lite, Abbott, Abbott Park, IL). Animals that had blood glucose levels of <280 mg/dL were excluded from the study (see Table 1).

Temporary filament occlusion of the middle cerebral artery (MCA) was used to induce I/R in both NG and HG animals, as previously described [16,18]. Animals were anesthetized with isoflurane in oxygen and ventilated to maintain blood gases within normal ranges (see Table 1). NG and HG animals were exposed to 2 hours of ischemia and 2 hours of reperfusion by suture removal. Cerebral blood flow (CBF) was continuously measured during MCAO and for 2 hours of reperfusion using laser Doppler flowmetry (Perimed Periflux 5010 Laser Doppler System), as previously described [16,18]. Briefly, after initial anesthesia but before midline incision for placement of the monofilament, a skin incision was made and the microtip of the laser-Doppler fiber-optic probe was affixed over a thinned area of skull posterior to the coronal suture and lateral to the sagittal suture over the MCA perfusion domain. The probe was left in place, and CBF was recorded during the experiment. Laser Doppler was used to measure changes in CBF from baseline, including both the decrease in CBF during occlusion and the extent of reperfusion after suture removal.

Experimental Protocols and Drug Treatments

In the first set of experiments, both NG and HG animals were infused intravenously via femoral catheter with the PPAR- γ agonist rosiglitazone (1 mg/kg, n=13) or vehicle (saline; n=13) 10 minutes prior to reperfusion, as previously described [17]. At the end of the reperfusion period, the animals were decapitated and the brains removed for measurement of acute infarction. Acute infarct volume was measured using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Briefly, brains were removed and sliced into 2 mm coronal sections. The brain slices were incubated in 2% TTC in 1 $\times$ phosphate buffered saline (PBS) for 30 minutes at 36.5°C to stain for infarction. The brain slices were subsequently fixed in 3.7% PBS-buffered formalin for 30 minutes at 4°C for imaging. Images were captured using a digital scanner and analyzed for acute injury volume with ImageJ software. The percent of infarcted brain tissue in the ipsilateral hemisphere was corrected for edema by calculating the percent infarct volume after the area of each contralateral brain section was subtracted from the ipsilateral.

In the second set of experiments, HG animals only (n=6) underwent MCAO for measurement of vasogenic edema using wet and dry weights. At the end of the reperfusion period, the animals were decapitated and the brain removed for measurement of water content, as previously described [16]. Briefly, ipsilateral and contralateral cerebral cortex (brainstem and cerebellum removed) were weighed wet then dried over-night at 90°C. The brain sections were then weighed dry, and the ratio of wet to dry weight for each hemisphere was used as a measure of edema.

In the third set of experiments, a combined treatment of rosiglitazone (1 mg/kg) + the free radical scavenger Tempol (50 mg/kg; n=10) was given intravenously 10 minutes prior to reperfusion and infarction and edema formation measured, as described above.

Drugs

TTC, formalin and Tempol were purchased from Sigma (St Louis, MO, USA). Rosiglitazone was purchased from Cayman Chemicals (Ann Arbor, MI).

Data calculations and statistical analysis

Results are presented as mean $\pm$ SEM. Acute infarct volume was corrected for brain edema and calculated as: $([\text{wet weight} - \text{dry weight}]/\text{wet weight}) \times 100\%$. Percent water content was compared between ipsilateral and contralateral sides of the brain using paired t-test. Reperfusion CBF was determined from laser Doppler units as a percent change from baseline CBF. Differences between individual groups were determined using one-way analysis of variance (ANOVA) with a posthoc Student-Newman-Keuls test for multiple comparisons, where appropriate. Differences were considered significant when $p < 0.05$.

Results

Effect of rosiglitazone on infarct volume

Hyperglycemia has been shown to worsen stroke outcome [4,5]. In the present study we compared infarct volume in normoglycemic (NG) or hyperglycemic (HG) animals that underwent MCAO for 2 hours and 2 hours of reperfusion. Figure 1 shows that infarct volume was significantly increased in HG animals, demonstrating a detrimental effect of hyperglycemia on infarct formation during stroke.

To test rosiglitazone as potential therapy for reperfusion injury we treated NG and HG animals that underwent 2 hours of MCAO and of 2 hours reperfusion with 1mg/kg rosiglitazone 10 minutes prior reperfusion. Figure 1 shows that acute rosiglitazone treatment

prevented the increase in infarct volume in HG but not in NG animals, suggesting a neuroprotective effect of rosiglitazone under HG conditions only.

Effect of rosiglitazone on brain water content

We have previously shown that hyperglycemia significantly increases water content in the ipsilateral brain after MCAO compared to NG animals [17]. To determine if acute rosiglitazone treatment, that was neuroprotective during HG stroke (Figure 1) could decrease blood-brain barrier (BBB) permeability and prevent edema formation a separate group of animals was treated with rosiglitazone prior to postischemic reperfusion and brain water content measured. Figure 2 shows that acute rosiglitazone treatment 10 minutes prior to reperfusion did not affect edema formation induced by HG stroke, suggesting that rosiglitazone is not vascular protective.

Effect of rosiglitazone and Tempol on infarct volume and brain water content

It is well known that oxidative stress is an important mechanism in the pathophysiology of HG stroke, especially during reperfusion [6,9]. Thus, to protect the vasculature in addition to neurons during stroke, we combined rosiglitazone treatment with the free radical scavenger Tempol (50 mg/kg). We found that treatment of HG MCAO animals with the combined treatment of rosiglitazone + Tempol 10 minutes prior reperfusion did not have a significant effect on the ipsilateral water content compared to HG vehicle (Figure 3a). Moreover, rosiglitazone + Tempol treatment prevented the neuroprotective properties of rosiglitazone alone and the acute injury volume was not different from vehicle treated animals (Figure 3b).

Effect of MCAO and rosiglitazone on reperfusion CBF

Because the effects of rosiglitazone or rosiglitazone + Tempol on stroke outcome can be due to changes in CBF and an effect on reperfusion blood flow, we compared CBF prior to and after MCAO in HG animals treated with rosiglitazone alone or the combination of rosiglitazone + Tempol. Figure 4 shows changes in relative CBF during 2 hours of MCAO and 2 hours of reperfusion. There was no difference in the depth of ischemia that was maintained for the duration of occlusion (2 hours). However, while reperfusion resulted in recanalization in all groups, it was significantly decreased in rosiglitazone + Tempol treated animals, suggesting combined treatment may worsen infarct volume by limiting reperfusion CBF.

Discussion

The present study investigated the effect of acute rosiglitazone treatment ten minutes prior to postischemic reperfusion on acute infarction and edema formation during HG stroke. We found that rosiglitazone prevented the increase in acute injury volume but not edema formation induced by hyperglycemia, suggesting that rosiglitazone is neuroprotective but not vascular protective under these conditions. Combined treatment of rosiglitazone plus Tempol also failed to protect the vasculature and reversed the neuroprotective effect of rosiglitazone alone, possibly by decreasing reperfusion CBF (Figure 4). Together these results suggest that while rosiglitazone may be neuroprotective in the acute setting after HG stroke it does not prevent edema formation. In addition, combined therapy with Tempol does not appear to be an effective approach to treating HG stroke.

Reperfusion of the ischemic tissue is important to salvage brain tissue and decrease morbidity and mortality due to stroke. However, reperfusion itself also result in a cascade of adverse reactions that paradoxically injure tissue, a phenomenon termed “reperfusion injury”. Inflammation and oxidative stress represent important mechanisms in the

pathogenesis of reperfusion injury [6,20,21]. The restored blood flow reintroduces oxygen that provides an abundant substrate to ROS [6], as well as blood-borne leukocytes that secrete cytokines and also release ROS promoting further brain injury [20,21]. Reperfusion injury is exacerbated in HG ischemic stroke [3], possibly by increasing ROS and inflammatory factors [8,9]. The use of rosiglitazone in focal or global cerebral ischemia has been investigated previously. In these previous studies, treatment was given prior to or after ischemic stroke and under normoglycemic conditions [11,15]. This study is the first evidence demonstrating the neuroprotective effects of rosiglitazone during reperfusion in HG stroke animals. A limitation of this study is that we have not determined the mechanism by which rosiglitazone is neuroprotective. However, because rosiglitazone possess anti-inflammatory [10,11] and antioxidant [12,13] properties, it is possible that rosiglitazone improved stroke outcome by affecting inflammatory and oxidative stress processes. In fact, it has been shown that PPAR- γ agonists decrease neutrophil accumulation and pro-inflammatory proteins such as tumor necrosis factor- α , interleukin (IL)-1 β and IL-6 in a focal ischemic brain model [22] and attenuated ischemia-induced ROS by increasing superoxide dismutase/catalase and decreasing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase levels [23]. It is worth noting that we measured infarction after 2 hours of reperfusion, an acute time period. Thus, it is unclear what the final infarct may evolve into and we cannot exclude that the neuroprotection seen with rosiglitazone was only temporary.

Rosiglitazone did not reduce brain edema in HG stroke animals, suggesting that it was not vascular protective since vasogenic edema results from increased BBB permeability. Diabetic patients treated with rosiglitazone can develop fluid retention and edema [24]. Although the underlying mechanisms are unclear it has been proposed that rosiglitazone-induced edema can be a result of increased vascular permeability by a direct effect on endothelial barrier function [25], by activating protein kinase C (PKC) [26] and/or by increasing expression of permeability agents such as vascular endothelial growth factor (VEGF) [26,27]. These effects of rosiglitazone on vascular permeability may explain why rosiglitazone was not vascular protective in HG stroke animals. In contrast, Xiong *et al* have shown that the effect of rosiglitazone in brain swelling is dose-dependent [28]. While 0.5 mg/kg had no effect, 2 and 5 mg/kg of rosiglitazone administered immediately and 2 hours after MCAO significantly reduced brain swelling [28]. Thus, it is also possible that the concentration of rosiglitazone used in these experiments (1 mg/kg) may not have been high enough to be effective for vascular protection.

Because oxidative stress is high during cerebral I/R especially under HG conditions [9], we also investigated if combined treatment with rosiglitazone plus the antioxidant Tempol would provide greater protection during HG stroke. We used a dose of Tempol of 50 mg/kg which was somewhat higher than others have shown to be neuroprotective during MCAO under normoglycemic conditions, i.e., 20 mg/kg [29]. This dose was used because of the high oxidative stress associated with HG stroke. However, administration of rosiglitazone + Tempol prior reperfusion was not vascular protective. Moreover, combined treatment reversed the neuroprotective properties of rosiglitazone alone. Although we have not investigated the mechanism by which rosiglitazone + Tempol prevented neuroprotection, since rosiglitazone + Tempol significantly decreased CBF during reperfusion it is possible that the decrease in reperfusion could increase infarction. In addition, it has been shown that under certain conditions, Tempol has the potential to increase oxidant stress in cells and tissues including vascular smooth muscle and endothelial cells [30,31]. Thus, we cannot exclude a pro-oxidant effect of Tempol that can increase oxidative stress and worsen brain infarct in our experimental conditions. Although it is possible that the dose of Tempol used was too high in the present study, the concentration used was not all that much higher than has been shown to be protective during stroke under normoglycemic conditions. Thus, it is possible that the high oxidative stress associated with postischemic reperfusion under HG

conditions renders Tempol ineffective or pro-oxidant, worsening outcome either through enhanced ROS and/or decreased reperfusion CBF.

In conclusion, we have shown that acute rosiglitazone treatment during reperfusion is neuroprotective but not vascular protective in HG ischemic stroke. While protection of the neurons from hypoxic/ischemic injury is undoubtedly important, stroke is a vascular disorder affecting not only neurons, but other cell types including vascular cells. Thus, further investigations are needed to find a successful stroke therapy that protects not only neurons but also the vasculature.

Acknowledgments

We gratefully acknowledge the support of the National Institute of Neurological Disorders and Stroke (NINDS) grants RO1 NS043316 and National Heart, Lung and Blood Institute (NHLBI) grant PO1 HL095488 and the Totman Medical Research Trust.

References

1. Nakashima T, Minematsu K. Prospects of thrombolytic therapy for acute ischemic stroke. *Brain Nerve*. 2009; 61:1003–12. [PubMed: 19803399]
2. Hossmann KA. Pathophysiology and therapy of experimental stroke. *Cell Mol Neurobiol*. 2006; 26:1057–83. [PubMed: 16710759]
3. Quast MJ, Wei J, Huang NC, Brunder DG, Sell SL, Gonzalez JM, et al. Perfusion deficit parallels exacerbation of cerebral ischemia/reperfusion injury in hyperglycemic rats. *J Cereb Blood Flow Metab*. 1997; 17:553–9. [PubMed: 9183293]
4. Pulsinelli WA, Waldman S, Rawlinson D, Plum F. Moderate hyperglycemia augments ischemic brain damage: a neuropathologic study in the rat. *Neurology*. 1982; 32:1239–46. [PubMed: 6890157]
5. Capes SE, Hunt D, Malmberg K, Pathak P, Gerstein HC. Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview. *Stroke*. 2001; 32:2426–32. [PubMed: 11588337]
6. Martini SR, Kent TA. Hyperglycemia in acute ischemic stroke: a vascular perspective. *J Cereb Blood Flow Metab*. 2007; 27:435–51. [PubMed: 16804552]
7. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001; 414:813–20. [PubMed: 11742414]
8. Lin B, Ginsberg MD, Busto R, Li L. Hyperglycemia triggers massive neutrophil deposition in brain following transient ischemia in rats. *Neurosci Lett*. 2000; 278:1–4. [PubMed: 10643786]
9. Bemeur C, Ste-Marie L, Montgomery J. Increased oxidative stress during hyperglycemic cerebral ischemia. *Neurochem Int*. 2007; 50:890–904. [PubMed: 17433500]
10. Martens FM, Visseren FL, Lemay J, de Koning EJ, Rabelink TJ. Metabolic and additional vascular effects of thiazolidinediones. *Drugs*. 2002; 62:1463–80. [PubMed: 12093315]
11. Sundararajan S, Landreth GE. Antiinflammatory properties of PPARgamma agonists following ischemia. *Drug News Perspect*. 2004; 17:229–36. [PubMed: 15334171]
12. Hwang J, Kleinhenz DJ, Lassegue B, Griendling KK, Dikalov S, Hart CM. Peroxisome proliferator-activated receptor-gamma ligands regulate endothelial membrane superoxide production. *Am J Physiol Cell Physiol*. 2005; 288:C899–905. [PubMed: 15590897]
13. Bagi Z, Koller A, Kaley G. PPARgamma activation, by reducing oxidative stress, increases NO bioavailability in coronary arterioles of mice with Type 2 diabetes. *Am J Physiol Heart Circ Physiol*. 2004; 286:H742–8. [PubMed: 14551045]
14. Graham DJ, Ouellet-Hellstrom R, MacCurdy TE, Ali F, Sholley C, Worrall C, et al. Risk of acute myocardial infarction, stroke, heart failure, and death in elderly Medicare patients treated with rosiglitazone or pioglitazone. *JAMA*. 2010; 304:411–8. [PubMed: 20584880]
15. White AT, Murphy AN. Administration of thiazolidinediones for neuroprotection in ischemic stroke: a pre-clinical systematic review. *J Neurochem*. 2010; 115:845–53. [PubMed: 20964688]

16. Cipolla MJ, Godfrey JA. Effect of Hyperglycemia on Brain Penetrating Arterioles and Cerebral Blood Flow Before and After Ischemia/Reperfusion. *Transl Stroke Res.* 2010; 1:127–34. [PubMed: 20563316]
17. Cipolla MJ, Huang Q, Sweet JG. Inhibition of Protein Kinase C{beta} Reverses Increased Blood-Brain Barrier Permeability During Hyperglycemic Stroke and Prevents Edema Formation In Vivo. *Stroke.* 2011; 42:3252–7. [PubMed: 21852606]
18. Cipolla MJ, Lessov N, Hammer ES, Curry AB. Threshold duration of ischemia for myogenic tone in middle cerebral arteries: effect on vascular smooth muscle actin. *Stroke.* 2001; 32:1658–64. [PubMed: 11441216]
19. Shimakura A, Kamanaka Y, Ikeda Y, Kondo K, Suzuki Y, Umemura K. Neutrophil elastase inhibition reduces cerebral ischemic damage in the middle cerebral artery occlusion. *Brain Res.* 2000; 858:55–60. [PubMed: 10700596]
20. Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. *J Transl Med.* 2009; 7:97. [PubMed: 19919699]
21. Wang CX, Shuaib A. Involvement of inflammatory cytokines in central nervous system injury. *Prog Neurobiol.* 2002; 67:161–72. [PubMed: 12126659]
22. Luo Y, Yin W, Signore AP, Zhang F, Hong Z, Wang S, et al. Neuroprotection against focal ischemic brain injury by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. *J Neurochem.* 2006; 97:435–48. [PubMed: 16539667]
23. Fong WH, Tsai HD, Chen YC, Wu JS, Lin TN. Anti-apoptotic actions of PPAR-gamma against ischemic stroke. *Mol Neurobiol.* 2010; 41:180–6. [PubMed: 20127524]
24. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, et al. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. *Circulation.* 2003; 108:2941–8. [PubMed: 14662691]
25. Idris I, Gray S, Donnelly R. Rosiglitazone and pulmonary oedema: an acute dose-dependent effect on human endothelial cell permeability. *Diabetologia.* 2003; 46:288–90. [PubMed: 12627329]
26. Sotiropoulos KB, Clermont A, Yasuda Y, Rask-Madsen C, Mastumoto M, Takahashi J, et al. Adipose-specific effect of rosiglitazone on vascular permeability and protein kinase C activation: novel mechanism for PPARgamma agonist's effects on edema and weight gain. *FASEB J.* 2006; 20:1203–5. [PubMed: 16672634]
27. Yamakawa K, Hosoi M, Koyama H, Tanaka S, Fukumoto S, Morii H, et al. Peroxisome proliferator-activated receptor-gamma agonists increase vascular endothelial growth factor expression in human vascular smooth muscle cells. *Biochem Biophys Res Commun.* 2000; 271:571–4. [PubMed: 10814503]
28. Xiong N, Sun F, Zhao H, Xiang J. Effect of Rosiglitazone Maleate on inflammation following cerebral ischemia/reperfusion in rats. *J Huazhong Univ Sci Technolog Med Sci.* 2007; 27:295–8. [PubMed: 17641846]
29. Kwon TH, Chao DL, Malloy K, Sun D, Alessandri B, Bullock MR. Tempol, a novel stable nitroxide, reduces brain damage and free radical production, after acute subdural hematoma in the rat. *J Neurotrauma.* 2003 Apr; 20(4):337–45. [PubMed: 12866813]
30. Alpert E, Altman H, Totary H, Gruzman A, Barnea D, Barash V, et al. 4-Hydroxy tempol-induced impairment of mitochondrial function and augmentation of glucose transport in vascular endothelial and smooth muscle cells. *Biochem Pharmacol.* 2004; 67:1985–95. [PubMed: 15130774]
31. May JM, Qu ZC, Juliao S, Cobb CE. Ascorbic acid decreases oxidant stress in endothelial cells caused by the nitroxide tempol. *Free Radic Res.* 2005; 39:195–202. [PubMed: 15763967]

Figure 1. Effect of rosiglitazone on acute injury volume during MCAO

Graph showing percent injury volume in normoglycemic (NG) and hyperglycemic (HG) rats that underwent 2 hours of ischemia and 2 hours of reperfusion. Animals were treated 10 minutes prior reperfusion with rosiglitazone (Rosi) or vehicle. Hyperglycemia (gray bars) significantly increased infarct volume compared to normoglycemia (black bars). Acute rosiglitazone treatment prevented the increase in injury volume in HG MCAO, suggesting a neuroprotective effect of rosiglitazone under HG conditions. * $p < 0.05$ versus NG and ^ $p < 0.05$ versus HG vehicle.

Figure 2. Effect of rosiglitazone on brain edema during hyperglycemic (HG) MCAO

Graph showing brain water content in ipsilateral and contralateral hemispheres from HG animals that underwent 2 hours of ischemia with 2 hours of reperfusion and treated acutely with either rosiglitazone (Rosi) or vehicle. Rosiglitazone did not affect the increased water content in the ipsilateral hemisphere during HG MCAO, suggesting is not effective in vascular protection. * $p < 0.01$ versus contralateral.

Figure 3. Effect of rosiglitazone + Tempol on acute injury volume and edema formation during MCAO

(a) Graph showing brain water content in ipsilateral and contralateral hemispheres from HG animals that underwent 2 hours of ischemia with 2 hours of reperfusion and treated acutely with a combination of rosiglitazone + Tempol or vehicle 10 minutes prior reperfusion. Rosiglitazone + Tempol did not affect the increase in the ipsilateral water content induced by HG MCAO, suggesting it was not vascular protective. * $p < 0.01$ versus contralateral. (b) Graph showing acute injury volume in HG MCAO animals treated 10 minutes prior reperfusion with rosiglitazone + Tempol or vehicle. Combined treatment was not able to prevent the increased in infarct volume in response to HG MCAO, suggesting that rosiglitazone + Tempol treatment was not neuroprotective.

Figure 4. Effect of rosiglitazone and Tempol on CBF during hyperglycemic (HG) MCAO
Changes in relative cerebral blood flow (rCBF) during 2 hours of MCAO (MCAo) and 2 hours of reperfusion (MCAr) using laser Doppler flowmetry and expressed as laser Doppler units (LDU). Shown are animals treated 10 minutes prior to reperfusion with rosiglitazone alone, rosiglitazone + Tempol or vehicle. All treatment groups had similar ischemia during filament occlusion, however, rosiglitazone + Tempol treatment 10 minutes prior reperfusion significantly decreased reperfusion CBF. ** $p < 0.01$ versus baseline.

Table 1
Physiological characteristics of hyperglycemic (HG) animals that underwent middle cerebral artery occlusion (MCAO)

	HG MCAO Vehicle (n=12)	HG MCAO Rosiglitazone (n=12)	HG MCAO Rosiglitazone + Tempol (n=12)
Blood glucose (mg/dL)	281 ± 11	333 ± 37	333 ± 19
Weight (g)	277 ± 9	302 ± 10	276 ± 3
CBF % drop vs. basal	-77 ± 4	-73 ± 2	-75 ± 3
CBF % recovery vs. basal	-20 ± 8	-13 ± 10	-35 ± 9
Blood Gases Start			
Arterial pH	7.40 ± 0.01	7.40 ± 0.01	7.39 ± 0.01
Arterial PCO ₂ (mmHg)	43.1 ± 2.8	40.6 ± 1.4	42.1 ± 2.3
Arterial PO ₂ (mmHg)	413 ± 14	421 ± 16	412 ± 23
Blood Gases Middle			
Arterial pH	7.38 ± 0.01	7.38 ± 0.01	7.39 ± 0.01
Arterial PCO ₂ (mmHg)	49.3 ± 2.0	49.6 ± 1.2	46.9 ± 0.8
Arterial PO ₂ (mmHg)	333 ± 20	346 ± 17	330 ± 17
Blood Gases End			
Arterial pH	7.36 ± 0.01	7.37 ± 0.01	7.39 ± 0.01
Arterial PCO ₂ (mmHg)	51.8 ± 2.0	50.6 ± 1.6	46.8 ± 0.4
Arterial PO ₂ (mmHg)	342 ± 26	362 ± 24	304 ± 26