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Microdialysis combined blood sampling technique for the determination of rosiglitazone and glucose in brain and blood of gerbils subjected to cerebral ischemia

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ABSTRACT

Rosiglitazone is a potent synthetic peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist which improves glucose control in the plasma and reduces ischemic brain injury. However, the pharmacokinetics of rosiglitazone in the brain is still unclear. In this study, a method using liquid chromatography–mass spectrometry coupled with microdialysis and an auto-blood sampling system was developed to determine rosiglitazone and glucose concentration in the brain and blood of gerbils subjected to treatment with rosiglitazone (3.0 mg kg^{-1}, i.p.). The results showed the limit of detection was 0.04 μg L^{-1} and the correlation coefficient was 0.9997 for the determination of rosiglitazone in the brain. The mean parameters, maximum drug concentration (C_{max}) and the area under the concentration–time curve from time zero to time infinity (AUC_{inf}), following rosiglitazone administration were 1.06 ± 0.28 μg L^{-1} and 296.82 ± 44.67 μg min L^{-1}, respectively. The time to peak concentration (C_{max} or T_{max}) of rosiglitazone occurred at 105 ± 17.10 min, and the mean elimination half-life ($t_{1/2}$) from brain was 190.81 ± 85.18 min after administration of rosiglitazone. The brain glucose levels decreased to 71% of the basal levels in the rosiglitazone-treated group when compared with those in the control ($p < 0.01$). Treatment with rosiglitazone decreased blood glucose levels to 80% at 1 h after pretreatment of rosiglitazone ($p < 0.05$). In addition, pretreatment with rosiglitazone significantly reduced the cerebral infarct volume compared with that of the control group. These findings suggest that this method may be useful for simultaneous and continuous determination of rosiglitazone and glucose concentrations in brain and plasma. Rosiglitazone was effective at penetrating the blood–brain barrier as evidenced by the rapid appearance of rosiglitazone in the brain, and rosiglitazone may contribute to a reduction in the extent of injuries related to cerebral ischemic stroke via its hypoglycemic effect.

1. Introduction

Rosiglitazone, an aminopyridyl thiazolidinedione, is a very potent synthetic peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist and an effective antidiabetic agent. The PPAR-γ is a nuclear membrane-associated transcription factor [1]. A member of the nuclear hormone receptor superfamily, PPAR-γ was originally reported to be highly expressed in adipocytes and to play an important role in their differentiation, as well as in lipid biosynthesis and glucose homeostasis [2]. It exerts its glucose-lowering effects by increasing insulin sensitivity in liver and peripheral tissues [3,4]. Recently, rosiglitazone has been shown to decrease neuroinflammation in rats after stress [5]. The strong association between increased glycemia and increased cardiovascular complications has generated a great deal research on prevention of diabetes. Diabetic hyperglycemia has also been shown to be a strong risk factor related to the development of cerebrovascular complications (i.e., stroke). The neuroprotective effects of rosiglitazone were proven to reduce cardiovascular risk by increasing insulin sensitivity in peripheral blood [6] and to reduce infarct volume with a time window of efficacy of 2 h after cerebral ischemia [7].

Rosiglitazone is highly protein-bound to serum proteins (99.8%), and it is primarily eliminated via metabolism in the liver by

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cytochrome P450 isoenzyme 2C8 [8]. Following oral administration, rosiglitazone is rapidly absorbed (T_{max} is approximately 1 h), and the elimination half-life ($t_{1/2}$) is approximately 3–5 h [9]. Although the pharmacokinetics of rosiglitazone in the blood have been studied, its pharmacokinetics in the central nervous system (CNS) is not clear. Drug pharmacokinetic profiles in blood and brain have been analyzed from a theoretical perspective and compared with experimental data obtained using microdialysis and other techniques. For many drugs, unbound brain concentrations are much lower than the corresponding plasma concentrations, but equilibrium between brain and plasma is very rapid and $t_{1/2}$ in brain is similar to that in plasma [10].

Microdialysis technique allows sampling of the unbound portion of the drug in the fluid space from which the sample is taken and causes minimal perturbation to the physiological processes [11]. Microdialysis techniques for continuous sampling and analysis of neurochemical substances in the extracellular fluid of the brain provide useful information on metabolic pathways [12]. The aim of this study was to investigate the pharmacokinetics of rosiglitazone in the brain by using a microdialysis-liquid chromatography–mass spectrometry system (LC–MS/MS) after intraperitoneal administrations of rosiglitazone to gerbils. A secondary aim of the present study was to develop an auto-blood sampling system (DR-II) that could be coupled with a microdialysis analyzer (CMA/600) to continously measure blood glucose concentrations after treatment with rosiglitazone.

2. Material and methods

2.1. Animals

Adult male gerbils (n = 42, 65–75 g) were obtained from the Laboratory Animal Center of Taichung Veterans General Hospital (TCVGH). Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of TCVGH (#La-92090). All animals were housed at a temperature of 25 °C and in a light-controlled room (12:12 h light–dark cycle) with standard rat chow and water ad libitum. The gerbils were separated into three experiments. In the first experiment, gerbils (n = 12) were subjected to cerebral microdialysis study; in the second experiment, gerbils (n = 12) were subjected to blood sampling in the pharmacokinetic study; and in the third experiment, gerbils (n = 18) were subjected to cerebral ischemic model. All gerbils were randomly divided into two groups: group 1 (control group) was treated with saline and group 2 (rosiglitazone group) was treated with rosiglitazone (3.0 mg kg^{-1}, i.p.).

2.2. Reagents and chemicals

Rosiglitazone maleate standard (74.3% of rosiglitazone free base) was purchased from GlaxoSmithKline (SB Pharmaco Puerto Rico Inc., Cidra, Puerto Rico). Acetonitrile and ethanol were HPLC-grade and purchased from Merck (Darmstadt, Germany). Analytical grade formic acid and ammonium formate were also obtained from Merck. A stock solution of rosiglitazone at 3.0 mg mL^{-1} was prepared in 1 mL 99.9% ethanol. The stock solution was then further diluted in 3 mL saline to yield the appropriate working solutions (0.75 mg mL^{-1}) with ethanol. All concentrations are expressed in terms of pure base. Rosiglitazone concentrations of calibration curves of 0.1, 0.5, 1, 10, 20 and 50 μg L^{-1} were prepared by serial dilution of stock solution with ethanol.

2.3. Microdialysis and detection of cerebral blood flow

In this experiment, gerbils (n = 12) were anesthetized with 4% isoflurane in induction followed by a maintenance dose (1–2%) and the body temperature was maintained at 37 °C throughout the experiment with a heating pad (CMA/150). The gerbils were placed on the stereotaxic instrument (David Kopf Instruments, Tujunga, CA) with the nose bar positioned 4.0 mm below the horizontal line. A midline incision was made to expose the skull and a microdialysis MAB 6 probe (0.6 mm outer diameter with 15 kDa cut-off PES membrane, Stockholm, Sweden) was inserted into the right cortex with coordinates: AP + 0 mm, ML + 5.0 mm, DV − 5.0 mm from the Bregma. The microdialysis probe was perfused with Ringer's solution (147 mM Na^+; 2.2 mM Ca^{2+}; 4 mM K^+, pH 7.0) at a rate of 2 μL min^{-1} using a CMA/100 microinfusion pump, and dialysates were collected every 15 min in a CMA/140 fraction collector. Following a post-surgical stabilization of the dialysate levels (approximately 2 h), drug-free samples were collected into the CMA/140 fraction collector (acting as the baseline collection) and rosiglitazone/saline was then administered. Dialysates were assayed for rosiglitazone concentrations by a LC–MS/MS [13] system and glucose concentrations by a microdialysis analyzer. Changes in cortex cerebral blood flow (CBF) were also evaluated in these gerbils using the optical fiber probe (0.8 mm in diameter) of a laser Doppler blood flow monitor (MBF 3D, Moor Instruments, Axminster, UK). The flow signal was averaged with a 5-s time constant, and the signal was recorded continuously on an x–y recorder.

2.4. Liquid chromatography/mass spectrometry (LC–MS/MS) system

A Dynamax ProStar 210 liquid chromatograph system (Thermo Finnigan, San Jose, CA, USA) consisting of a binary pump was used. Chromatographic separation was achieved by using an XTerra RP18 analytical column (25 cm × 4.6 mm, 5 μm) (Waters, Milford, MA, USA) with a mobile phase of acetonitrile–10 mM ammonium formate, pH 5.0 (80:20, v/v). The analytical column was preceded in-line by a precolumn filter of 3 mm frit (Supelco, Bellefonte, PA, USA). The flow rate was 0.5 mL min^{-1}, and the run time was 15 min. The injection volume was 5 μL and the entire column effluent was directed into the mass spectrometer. Under these conditions, rosiglitazone gave a retention time of 7.8 min and eluted with no detectable interfering compounds. A Varian 1200L triple quadrupole LC-MS (Varian, Inc., Walnut Creek, CA, USA) equipped with atmospheric pressure chemical ionization (APCI). Tuning and calibration of the mass analyzer was performed by infusing poly (propylene glycol) calibration solution (Varian, Inc.) at 10 μL min^{-1} and monitoring five mass-to-charge ratios in the range of 59–1138 u. The data acquisition software Varian MS workstation, version 6.2, was used to control the LC–MS/MS system and to perform analyses. The parameters of the ionization efficiency were optimized with regard to maximum signal intensity of the protonated precursor molecule $[M+H]^+$ by using flow injections of 5 μL samples (0.1 mg L^{-1}). Optimal operating conditions of the APCI interface in positive mode were obtained as follows, vaporizer temperature, 500 °C; drying gas (nitrogen, 99.99% purity) temperature, 50 °C; housing temperature, 50 °C; corona discharge current, 8 μA; capillary voltage, 75 V; shield voltage, 600 V; quadrupole 0 offset voltage, −2 V; lens voltage, −2 V; quadrupole 1 offset voltage, −1.1 V; quadrupole 1 guide voltage, −6 V; quadrupole 2 offset voltage, −15 V; quadrupole 3 offset voltage, −10 V; and nebulizing gas, dry gas and auxiliary gas (nitrogen, 99.99% purity) pressures, 30, 10 and 5 psi, respectively. The MS/MS reaction selected to monitor rosiglitazone was the transition from m/z 358, the $[M+H]^+$ ion, to a product ion at m/z 135. The protonated molecules were isolated and collision-activated dissociation with argon as the collisional gas at a pressure of 0.7 mTorr and collisional energy of 24 eV. Samples were analyzed in selected reaction monitoring (SRM) positive ionization mode. During SRM analysis mode, mass peak width was 1.0

mass unit at half height for both Q1 and Q3, the scan time was 1 s, and 5 μscans were averaged for each spectrum.

2.5. Analysis of glucose concentration

The microdialysis analyzer (CMA/600, Carnegie Medicin, Stockholm, Sweden) was employed for the determination of glucose concentrations. Glucose is oxidized by glucose oxidase. Peroxidase catalyses the reaction between the hydrogen peroxidase formed, phenol, and 4-amino-antipyrine to form the red–violet colored quinoneimine, which is detected at 546 nm [14].

2.6. Pharmacokinetic study and auto-blood sampling by DR-II

In the pharmacokinetic study, gerbils ($n = 12$) were anesthetized with administered urethane (1.5 g kg^{-1}, i.p.) and the body temperature was maintained at 37 °C throughout the experiment with a heating pad (CMA/150). Blood samples were automatically collected from the gerbils via an implanted catheter in the jugular vein using a computer-aided auto-blood sampling system (DR-II, Eicon, Kyoto, Japan). Blood samples (50 μL) were collected every 15 min. In order to preserve glucose, 5 μL of 1 M NaF solution was added to the blood sample and then centrifuged at 1000 g for 10 min at 4 °C. Aliquots of the supernatant (plasma sample) were assayed for glucose concentrations by a microdialysis analyzer.

2.7. Cerebral ischemia model

In order to determine whether the neuroprotective effects of rosiglitazone, gerbils were divided into the control (saline, $n = 9$) and rosiglitazone (3.0 mg kg^{-1}, i.p., $n = 9$) groups. Saline or rosiglitazone were given 6 h prior to cerebral ischemia. Each gerbil was anesthetized with urethane (1.5 g kg^{-1}, i.p.) and its body temperature was maintained at 37 °C with a heating pad (CMA/150). A midline neck incision was made and the right carotid artery was exposed and separated from the vago-sympathetic trunk. The right carotid artery was loosely encircled with a 4-0 suture for later occlusion. The gerbil's head was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the nose bar positioned 4.0 mm below the horizontal line. Following a midline incision, the skull was partially removed to expose the right middle cerebral artery (MCA). The MCA was loosely encircled with an 8-0 suture for later occlusion. A focal cerebral ischemia was induced by occlusion of the right common carotid artery (CCA) and the right MCA (CCA + MCA) for 60 min, followed by an additional 3 h of reperfusion. Twenty-three hours after cerebral ischemia, each gerbil was anesthetized and perfused with 2% isotonic heparinized saline. The brain was removed and sliced into five 2-mm-thick coronal sections for 2,3,5-triphenyltetrazolium chloride (TTC staining) as described by Bederson et al. [15]. After 20 min, brain slices were placed in 10% buffered formalin in the dark and refrigerated until photographed. Slices were projected and traced by the computer software "Optimas" (version 6.2, Media Cybernetics, Silver Spring, MD). Infarct volumes were quantified by weighing the traced infarct area to the ipsilateral hemisphere and normalized by the contralateral hemisphere.

2.8. Statistical analysis

All data were analyzed by Mann–Whitney U test or Wilcoxon signed-ranks test. Non parametric Mann–Whitney U tests were used in Figs. 2–5 for statistical analysis between groups. Non parametric Wilcoxon signed-ranks test was used for statistical analysis in various time points of the same group. Differences were considered statistically significant at $p < 0.05$. All data are expressed as mean ± SEM.

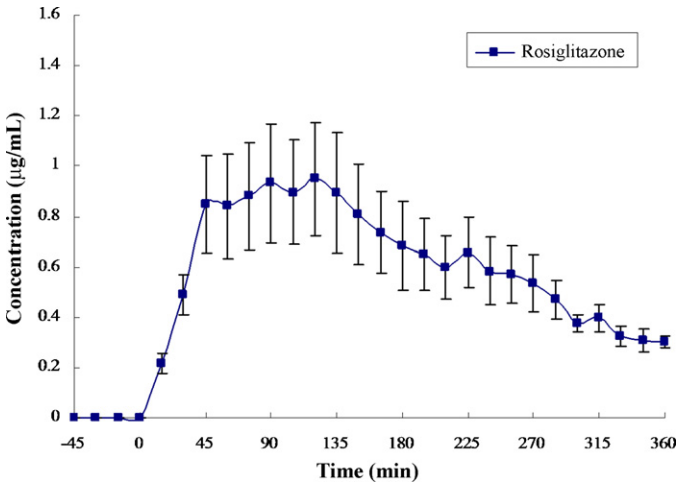

Fig. 1. Time profile of brain concentration of rosiglitazone in gerbils receiving an intraperitoneal injection of 3 mg kg^{-1} rosiglitazone. Data are presented as the mean ± SEM ($n = 6$).

3. Results and discussion

To assess the applicability of the present assay for use in pharmacokinetic studies involving rosiglitazone administration, the assay was used to determine the brain concentration of rosiglitazone in male gerbils that had received intraperitoneal injection of rosiglitazone at a dose of 3 mg kg^{-1}. The results showed the limit of detection was 0.04 μg L^{-1} and the correlation coefficient was 0.9997 for the determination of rosiglitazone in gerbil brains. The temporal profile for the rosiglitazone concentrations in the brain is shown in Fig. 1. The mean concentration of rosiglitazone in the brain increased during the first 15 min and reached a plateau at about 90–120 min. The concentration of rosiglitazone was readily measurable, suggesting that the current assay is adequate for determining the pharmacokinetic characteristics of rosiglitazone at a dose of 3 mg kg^{-1}. The calculated pharmacokinetic parameters, including the time to reach peak concentration (T_{max}), maximum drug concentration (C_{max}), the mean elimination half-life ($t_{1/2}$) and the area under the concentration–time curve from time zero to time infinity (AUC_{inf}) are listed in Table 1.

Under the chromatographic conditions described, excellent separation and detection of rosiglitazone was achieved in brain samples of gerbils [13]. The correlation coefficient was higher than 0.999 (r^2 value = 0.9997), indicating good linearity of this assay. Moreover, few or no observed peaks interfered with the analyses in the mass ion chromatograms of brain dialysates (data not shown). Microdialysis sampling, as an in vivo technique, makes possible the investigation of biochemical events in the extracellular fluid of any tissue [10]. In this study, microdialysis coupled with LC–MS/MS technique was shown to be a powerful tool for central pharmacokinetic studies. The microdialysis technique also provides information about the protein-unbound fraction of the rosiglitazone and may minimize the degradation of rosiglitazone in the brain [16].

Table 1
Pharmacokinetic parameters of rosiglitazone following intraperitoneum injections of rosiglitazone at a dose of 3 mg kg^{-1} in gerbils ($n = 6$).

Pharmacokinetic parameters	Mean ± SEM
T_{max} (min)	105 ± 17.10
C_{max} (μg L^{-1})	1.06 ± 0.28
$t_{1/2}$ (min)	190.81 ± 85.18
AUC_{inf} (μg min L^{-1})	296.82 ± 44.67

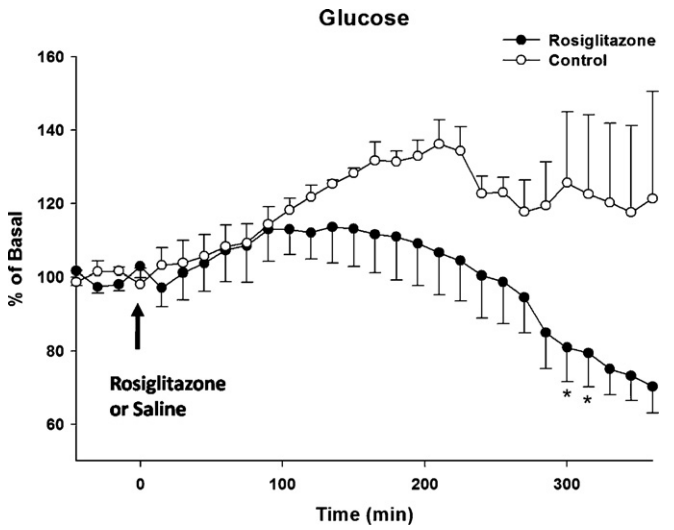

Fig. 2. Time profiles of the effect of rosiglitazone on the changes in glucose levels in the gerbil cortex. Data are presented as mean ± SEM ($n = 6$). $^{*}p < 0.05$ compared with the control group.

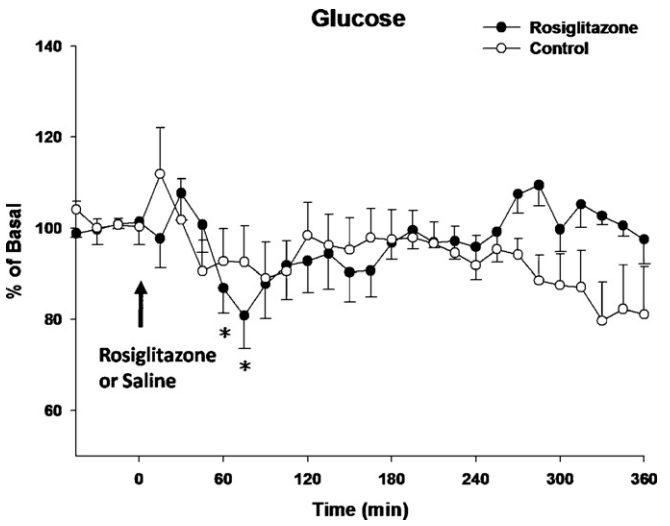

Fig. 3. Time profiles of the effect of rosiglitazone on the changes in glucose levels in the gerbil plasma. Data are presented as mean ± SEM ($n = 6$). $^{*}p < 0.05$ compared with the control group.

The results indicated that rosiglitazone rapidly (within 15 min) entered the extracellular fluid of brain cortex following intraperitoneal administration. The concentration of rosiglitazone in the gerbil brain continued to increase from 15 to 120 min after drug administration, peaking at 105 min, suggesting continuing entry of rosiglitazone into the brain. This study demonstrated that rosiglitazone can effectively and rapidly penetrate the blood–brain barrier (BBB) as a previous study [17]. Previous data [9] demonstrated that the time to reach C_{max} (T_{max}) and the mean elimination $t_{1/2}$ for rosiglitazone from the blood were 1.1 h and about 3–4 h, respectively, after oral administration. The $t_{1/2}$ of elimination of rosiglitazone from the brain is consistent with that of the blood. The data suggest that the metabolic rate of rosiglitazone and its duration of effect in the brain were similar in the blood. However, the T_{max} for rosiglitazone in this study was about 1.5 times higher than in the blood, which may be because the absorption of rosiglitazone differs between blood and brain, or because different routes of drug administration were applied (oral vs. i.p.)[18]. Rosiglitazone showed an appreciable capacity to penetrate the blood–brain barrier supports the claim that the drug exerts it pharmacodynamic effect on the CNS.

The time profiles of rosiglitazone and its effect on changes in glucose levels in the brain in the saline-treated and rosiglitazone-treated groups are shown in Fig. 2. Treatment with rosiglitazone significantly decreased glucose levels to 71% of the baseline when compared with that of the control group ($p < 0.01$). However, the marked decrease was not observed in the control group, while glucose levels remained at their basal levels and increased to 136% of the baseline at the 3–5 h time point. The effect of rosiglitazone on changes in plasma glucose levels is shown in Fig. 3. The plasma glucose levels decreased to about 80% of the basal ($p < 0.05$) within 1 h after rosiglitazone was administered, and returned to the basal levels after 1 h.

There were no significant differences in cerebral blood flow between the rosiglitazone and saline-treated groups (Fig. 4). All animals had infarction in the cortex and caudate-putamen. Mean total infarct sizes in the control and rosiglitazone groups were $79 \pm 7\,mm^3$ ($15.3 \pm 1.2\%$) and $45 \pm 13\,mm^3$ ($8.1 \pm 2.5\%$), respectively, as shown in Fig. 5. Pretreatment with rosiglitazone significantly reduced infarct sizes by 43% ($p < 0.01$) when compared with those of the control group.

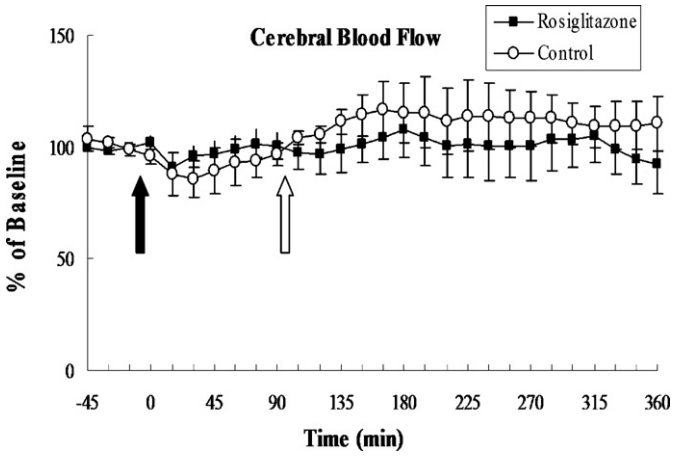

Fig. 4. Cerebral blood flow was recorded by a laser Doppler during the microdialysis sampling experiment. Dark arrow indicates gerbils were given injections of saline and rosiglitazone. Blank arrow indicates the time to peak concentration (T_{max}) after rosiglitazone administration. Data are presented as mean ± SEM ($n = 6$).

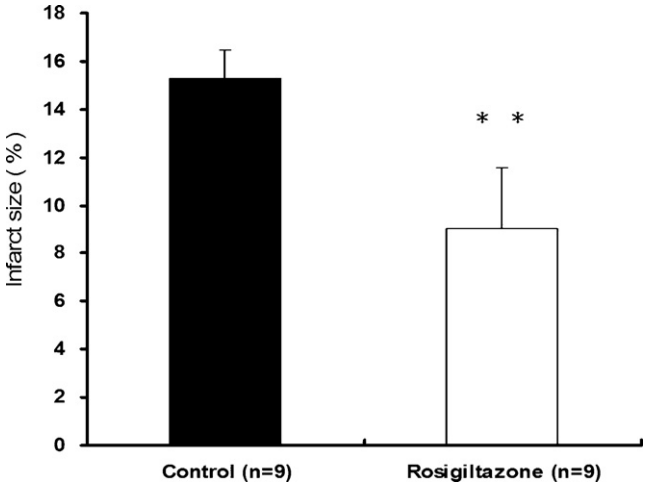

Fig. 5. Effects of rosiglitazone on the infarct size which was determined by TTC staining in gerbils subjected to 1 h MCA + CCA-occlusion followed by reperfusion for 23 h. Pretreatment of rosiglitazone (3 mg kg^{-1}, $n = 9$) was given 6 h prior to the MCA + CCA-occlusion. Data are expressed as mean ± SEM. $^{**}p < 0.01$ for infarct size vs. the control group ($n = 9$) as determined by Mann–Whitney U test.

For repeated blood samples from small rodents, a number of techniques have been developed. Among these methods, three widely applied techniques are amputation of the tail-tip, tail incision, and retro-orbital puncture [19]. However, the above assays are associated with stressful behavioral responses and require anesthesia. In addition, these techniques may also affect subsequent sampling of blood during a short period of time. A simple method for the implantation of silicone cannula into the rat jugular vein was developed. These procedures were much less stressful to the animal than the above-mentioned conventional methods [14]. The implanted cannula can be used to either administer drugs or collect blood samples with less stress to the animals. In addition, a computer program was used to ensure that a blood volume of 50 μL was collected automatically every 15 min during treatment with the drug. During the sampling procedures, 50 μL of saline was also delivered to compensate for the blood volume withdrawn from the animal.

Evidence has emerged which showed that subjects with T2DM have a two- to five fold increased risk for stroke compared with those without diabetes [20], particularly for nonhemorrhagic stroke [21]. Risk factors for increased risk of stroke in the T2DM are myriad and include elevated blood pressure, aberrations in lipid metabolism, abnormalities in fibrinolysis, age, smoking, and hyperglycemia [6,16,22–24]. Several studies indicated that acute elevation in glucose stores result in aggravation of brain tissue acidosis [25], and hyperglycemic subjects showed aggravated ischemia brain lesions due to raised blood glucose concentrations [26]. Rosiglitazone is a highly selective and potent agonist for the PPAR-γ which is involved in the control of glucose transport, production, and utilization. The rosiglitazone pretreatment decreased glucose concentration by about 20% within 1 h in the gerbil brain. Therefore, the glucose-lowering effect of rosiglitazone in the brain may also play a role in preventing T2DM-induced cerebrovascular complications. Moreover, an increase in serum glucose may increase the extent of damage during ischemia. However, insulin-induced hypoglycemia has been shown to be neuroprotective [27,28]. In animals with hypoglycemia, decreased lactic acid production may be neuroprotective [29]. The mechanism regarding the neuroprotective effect of rosiglitazone in brain damage may be related to its capacity to induce hypoglycemia; rosiglitazone-induced hypoglycemia may therefore be beneficial.

In addition, to rule out the possibility the rosiglitazone might alter CBF, laser Doppler flowmetry was used to monitor cortex CBF in both controls and rosiglitazone-treated gerbils. The data obtained from the rosiglitazone group indicates that cerebral blood flow remained in the baseline, meaning that sudden appearances of rosiglitazone in the brain may not affect the brain circulation. It has been showed that PPAR-γ is expressed in all cell types in the CNS [30]. Neuroprotection against focal ischemic brain injury by rosiglitazone was demonstrated, indicating that rosiglitazone has neuroprotective effects that are least partially mediated via anti-inflammatory actions after cerebral ischemia [5,7]. These findings also suggest rosiglitazone may reduce risk related to cerebrovascular complications (i.e., stroke). In this study, microdialysis combined with an auto-blood sampling technique to determine dynamic changes in rosiglitazone and glucose concentrations in the brain and blood of gerbils was investigated.

4. Conclusion

In summary, these results indicated that protein-unbound rosiglitazone can effectively transport into the brain. The microdialysis sampling coupled with the LC–MS/MS system was a robust assay with high-sensitivity for determining the pharmacokinetic properties of rosiglitazone in gerbil brain and it provided pharmacokinetic data which demonstrated the likely mechanism by which rosiglitazone exerts its effects. The treatment with rosiglitazone exerted a glucose-lowering effect in non-diabetic gerbil brains, but not in peripheral blood vessels. The glucose-lowering effect of rosiglitazone may be beneficial for preventing acute stroke in T2DM.

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