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Rosiglitazone treatment reduces hippocampal neuronal damage possibly through alleviating oxidative stress in chronic cerebral hypoperfusion

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ABSTRACT

Oxygen free radicals and lipid peroxidation may play significant roles in the progress of injury induced by chronic cerebral hypoperfusion of the central nervous system. Rosiglitazone, a well known activator of PPARγ, has neuroprotective properties in various animal models of acute central nervous system damage. In the present study, we evaluate the possible impact of rosiglitazone on chronic cerebral hypoperfused-rats in regard to the levels of oxidative stress, reduced glutathione, and hippocampal neuronal damage. Chronic cerebral hypoperfusion was generated by permanent ligation of both common carotid arteries of Wistar rats for one month. Animals in treatment group were given rosiglitazone orally at doses of 1.5, 3, or 6 mg/kg per day of the 1 month duration. The treatment significantly lowered the levels of both malondialdehyde and neuronal damage, while elevated the reduced glutathione level markedly. These findings suggest that the beneficial effect of rosiglitazone on hypoperfusion-induced hippocampal neuronal damage might be the result of inhibition of oxidative insult.

1. Introduction

One of the pathophysiological mechanisms of neural injury accounted for dementia is suggested to be a state of chronic cerebral hypoperfusion (CCH) (Farkas et al., 2004). During the course of hypoperfusion, neuropathological injury is particularly associated with oxidative stress, in which progressive neuronal insult is the consequence (Annahazi et al., 2007; Zhang et al., 2011; Kasparová et al., 2005). Moreover, in response to cerebral ischemia, microglia promptly synthesize and release proinflammatory cytokines, reactive oxygen species (ROS), and eicosanoids, some of which seem to be damaging at high concentration (Annahazi et al., 2007).

Both neurons and glia possess peroxisome proliferator-activated receptor γ (PPARγ). One of synthetic agonists for PPARγ, rosiglitazone, is neuroprotective against neurodegenerative and neurological disorders including ischemia (Breidert et al., 2002; Pedersen et al., 2006). In PPARγ-agonist mediated neuroprotection, decreased inflammation and reduced oxidative stress are suggested to be possible players (Yi et al., 2008; Sundararajan et al., 2005; Hong et al., 2011).

We have previously shown that rosiglitazone attenuated cognitive deficit induced by CCH of rats. Regarding the study, the maintenance of normal synaptic function and the reduction of glial cell activation might be related to the therapeutic effects of rosiglitazone (Sayan-Ozacmak et al., 2011). In the present study, we were in the search of finding out possible effect of different doses of rosiglitazone on the state of oxidative stress and hippocampal neuronal injury following CCH in rats.

2. Material and methods

2.1. Animals

Adult male Wistar rats (250–300 g) were used for the experiment and housed in individual cages in a room maintained at 25 ± 2 °C and 45–55% relative humidity with a 12 h light–dark cycle. They were allowed to have water and food freely. All experimental procedures regarding the animals were approved by the Ethics Committee of the Zonguldak Karaelmas University. Maximum care for humanely approach to animals was of primary purposes.

2.2. Surgery

The rats were anesthetized with intraperitoneal administration of ketamine (90 mg/kg) throughout the following surgical

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procedure. Through a midline cervical incision, both common carotid arteries were exposed and gently separated from carotid sheath and vagus nerve. In rats chosen randomly for ischemic groups, each artery was ligated with a 5/0 silk suture. As sham operated controls, the animals received the same operation without ligation. The rectal temperature was maintained at 37 °C during the surgery with the help of a heating lamp. After the operation, the animals were kept in cages with food and water *ad libitum*.

2.3. Experimental design

Fifty animals were randomly divided into groups of sham, ischemia, and ischemia treated with three different doses of rosiglitazone (Rosvel 4 mg, Sanovel Ilac San. Tic. A.S., Istanbul, Turkey). Each pill was carefully crushed followed by appropriate weighing of the powder for each animal. The powder of rosiglitazone was mixed with peanut butter and given orally at doses of 1.5, 3.0, and 6.0 mg/kg/day, beginning 7 days before the surgery and continuing through 30 days. Sham operated control animals were given only the peanut butter. The amounts chosen for the drug administration were based on those providing protective effects in the rat model of focal cerebral ischemia.

2.4. Biochemical analysis

Oxidant and antioxidant status of rat brain subjected to hypoperfusion was assessed by measuring the levels of lipid peroxidation and reduced glutathione (GSH). Lipid peroxidation was evaluated by measuring the level of malondialdehyde (MDA), a by-product of lipid peroxidation. By using a motor-driven pestle, tissue samples were homogenized in ice-cold trichloroacetic acid (TCA) by adding 10 ml of 10% TCA per g of tissue. After centrifugation, 750 μl supernatant was added to an equal volume of 0.67% thiobarbituric acid and heated to 100 °C for 15 min. The absorbance of the samples was then measured spectrophotometrically at 535 nm. For measuring the GSH content of the samples, to the 0.5 ml of supernatant obtained by using the same homogenization procedure as described above, 2 ml of 0.3 M Na_2HPO_4 solution was added. A 0.2 ml solution of dithiobisnitrobenzoate was added into the mixture, and the absorbance at 412 nm was measured immediately after vortexing.

2.5. Histopathological evaluation

Following decapitation, the brains of five rats chosen randomly for each group were isolated and fixed in 37% of formalin followed by processing in acetone, alcohol, and formalin solution series for total of 14 h. Each sample was then embedded in paraffin. Sections, which were 4 μm thick, were cut and stained with both hematoxylin–eosin (H&E) and cresyl violet for morphological analysis. In addition, hippocampal CA1 neurons were counted at four high power fields chosen randomly for each sample by using oculometric analysis in order to assess whether or not neuronal loss is quantified. The investigator who performed these measurements was unaware of the experimental design.

2.6. Data analysis

Each data point represents mean ± S.E.M. (n = 10 for each group in all experiments). For statistical evaluation, SPSS 11.0 statistical software package program was used (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was applied for statistical comparison of groups, followed by analysis with post hoc Tukey test to determine differences between the groups. Probability values of 0.05 or less were considered statistically meaningful.

3. Results

Approximately 1.66-fold increase in MDA content was measured in ischemia group (43.44 ± 2.86 nmol/g tissue), a significant difference ($P < 0.001$) compared to that in sham group (26.21 ± 0.95 nmol/g tissue) (Fig. 1). Administration of rosiglitazone at 1.5 mg/kg/day had no any effect on MDA content (47.33 ± 2.44 nmol/g tissue). Average MDA contents of the last two treatment groups (29.12 ± 1.11 and 29.83 ± 1.77 nmol/g tissue, respectively) were significantly different from that of the ischemia group ($P < 0.001$). On the other hand, comparing with the sham group, mean MDA contents of both groups were statistically indifferent ($P > 0.05$).

GSH content of the tissues in ischemia group was approximately 50% of that in sham control group (0.14 ± 0.005 vs. 0.28 ± 0.03 mmol/g tissue), a statistically meaningful reduction ($P = 0.001$) (Fig. 1). Mean GSH contents for ischemia and rosiglitazone treatments with 1.5 (0.2 ± 0,02 mmol/g tissue) and 6 mg/kg/day (0.22 ± 0.01 mmol/g tissue) were indistinguishable ($P > 0.05$). The treatment with 3 mg/kg, however, caused a remarkable and statistically significant increase in the GSH content (0.25 ± 0.01 mmol/g tissue, approximately 90% of that in sham group).

Fig. 1 also shows the representative images of hippocampal and cortical neurons stained with cresyl violet. Normal appearance of hippocampal pyramidal neurons is evident in CA1 area of sham animals. In ischemia control group, pyramidal neurons, surrounded by some intact cells, were damaged and shrinked. Their nucleus were pyknotic with indistinct nucleoli and eosinophilic cytoplasm. In rosiglitazone-treated group with 1.5 mg/kg/day, small populations of ischemic neurons were scattered among intact neurons in the CA1 subregion. The treatment with rosiglitazone at concentrations of both 3 and 6 mg/kg/day provided histologic appearances very similar to that observed in sham control group in respect to the number of intact cells (Table 1) as well as morphologic characteristics of the cells. Evaluating the cerebral cortex, normal cortical neurons with eosinophilic cytoplasm and nuclei with prominent nucleoli were obvious in sham control group, while such ischemic changes as pyknotic nucleus, basophilic cytoplasm, and nuclear collapse were marked in ischemia control group beside a significant reduction in intact cell number (Table 1). The tissues treated with 1.5 mg/kg/day of rosiglitazone have significantly less number of damaged neurons than that counted in ischemia control group. Another words, neuronal loss was much less than that observed in ischemic tissues. Intact neurons evaluated in tissue sections of 3 mg/kg/day of rosiglitazone treatment were very similar to those in sham operated control group. In rosiglitazone-treated group with 6 mg/kg/day, minimal neuronal loss were observed.

4. Discussion

CCH is regarded as a particular player in the development of memory dysfunction in such neurological diseases as Alzheimer's disease (AD) and vascular dementia (Peng et al., 2007; Annahazi et al., 2007). Creating its experimental animal model requires permanent occlusion of the common carotid arteries (2VO), causing eventually neuronal damage and microglial activation (Annahazi et al., 2007; Zhang et al., 2011). A variety of underlying mechanisms, including oxidative stress as well, has been postulated to be associated with neuronal damage and microglial activation (Annahazi et al., 2007; Kasparová et al., 2005). In the present study, the level of MDA content in hypoperfused-animals was significantly increased, while that of GSH was markedly decreased in comparison with those in sham operated animals. These findings suggested that CCH could end up with an increased generation of ROS, leading to the neuronal damage.