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The role of rutin in focal cerebral ischemia: oxidative stress factors and rotarod test

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Abstract

Ischemic stroke is caused by the interruption of cerebral blood flow that leads to brain damage with long-term sensorimotor deficits. Free radical induced neural damage is concerned in cerebral ischemia–reperfusion (IR) injury and antioxidants are reported to have neuroprotective activity. The present study was designed to assess the neuroprotective role of rutin (vitamin P), and its probable mechanism.

The middle cerebral artery occlusion (MCAO) was induced for 2 h and reperfused for 22 h in male Wistar rats. The administration of rutin (50 mg/kg, IP) was done once daily for 14 days before MCAO. Then, the brain MDA, total thiol levels and motor coordination (Rotarod test) were assessed.

MDA levels of brain were non-significantly increased in MCAO group compared to control group. The thiol level of brain was significantly decreased in MCAO group compared to control group ($p<0.05$). The thiol level increased in MCAO+rutin and it was identical to control group ($p<0.05$). The rotarod test was shown that time stands rates in MCAO group decreased significantly compared to control group ($p<0.05$). It was increased in MCAO+rutin group compared to MCAO group ($p<0.05$).

The improvement of behavior test and endogenous antioxidant enzymatic activities, indicated that rutin have neuroprotective role. Thus, rutin treatment may represent a novel approach in lowering the risk or improving the function of ischemia–reperfusion brain injury-related disorders.

Keywords: Rutin, Oxidative Stress, Rat, Cerebral Ischemia.

Introduction

Stroke is one of the principal causes of death and disability worldwide. Ischemic stroke constitutes 85% of all stroke cases. No effective treatment has been established to prevent damage to the brain (1). Ischemic hypoxic brain injury often causes irreversible brain damage. The cascade of events leading to neuronal injury and death in ischemia includes the release of cytokines and free radicals, and induction of inflammation, apoptosis, and excitotoxicity (2, 3). Reperfusion of ischemic areas could exacerbate ischemic brain damage through the generation of reactive oxygen species. The lack of effective and widely applicable pharmacological treatments for ischemic stroke patients may explain a growing interest in traditional medicines (4).

The brain is very sensitive to damage caused by oxidative stress, due to its rapid oxidative metabolic activity, low antioxidative capacity, and poor neural cell recovery ability; therefore, it is safe to state that oxidative stress is an important factor in acute ischemic stroke (5). Reactive oxygen species and other toxic free radicals, released by inflammatory cells, are identified to contribute to functional disruption and neuronal death (6,7).

Rutin (quercetin-3-rhamnosyl glucoside) is a flavonoid glycoside found in buckwheat which is richly present in vegetables, fruits, tea, wine and herbs (8, 9). It is a powerful phenolic antioxidant that has diverse pharmacological properties, including antioxidant, anticancer, and anti-inflammatory properties (10).

Several reports have established that rutin scavenges super-oxide radicals, maintains the levels of biological antioxidants, increases antioxidant enzymatic activity in vitro, reduces lipid peroxidation and cytokine production, and prevents cognitive impairment following injuries, such as hypoxia/ischemia and CNS injuries in rat models (11, 12). Especially, administration of rutin before transient cerebral ischemia or at the onset of reperfusion has shown a reduction of ischemic neural apoptosis by increasing endogenous antioxidant enzymatic activities in experimental animals (13). Thus, this study was conducted to determine the preventive effects of rutin on oxidative stress factors, functional neurologic and movement activity in a rat stroke model.

Materials and Methods

1. Animals

Male Wister rats weighing 200–250 g were used in the present study purchased from Mashhad university sciences and kept under standard housing conditions at a temperature between 20°C and 25°C, with a 12 h light–dark cycle and a relative humidity of 50%. All animals had free access to food and water. Rutin was injected (IP) to animals for 14 days before ischemia. Then they were randomly separated into three groups (n=7); control, middle cerebral artery occlusion (MCAO), MCAO + Rutin.

2. Drugs and chemicals

Rutin was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3. MCAO method

Animals were anesthetized with ketamine/xylazine (150:10 mg/kg) mixture. The external carotid artery was isolated and coagulated, a monofilament nylon suture (diameter of approximately 0.3 mm) with a round trip was inserted into the internal carotid artery through the external carotid artery stump, occluding the middle cerebral artery for 2 hours. After occlusion, the animals were reanesthetized and the filament was withdrawn to restore blood flow. Body temperature was regulated at 37 °C with a temperature control system. After 22 hours, animals were killed, brain and serum blood were extracted (figure 1).

4. Biochemistry

4.1. MDA

Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were also measured. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a pink colored complex which has peak absorbance at 535 nm. 2 mL of TBA/TCA (trichloroacetic acid)/HCL (hydrochloric acid) reagent was added to 1 mL of homogenate and the solution was heated in a water bath for 40 min. After cooling, the solution was centrifuged at 1000 g for 10 min. The absorbance was measured at 535 nm. The MDA concentration was calculated as follows: (m)= Absorbance/(1.65 × 105) (14).

4.2. Thiol

Total SH groups were measured using DTNB (2, 2-dithiodibenzoic acid) as reagent. This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm. Briefly, 1 mL Tris-EDTA (ethylenediaminetetraacetic acid) buffer (pH = 8.6) was added to 50 μL brain homogenate in 1 mL cuvettes and sample absorbance was read at 412 nm against Tri s-EDTA buffer al one (A).Then 20μL DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min (at laboratory temperature) the sample absorbance was read again ($A21$). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation (14):

Total thiol concentration (mM) = ($A2–A1–B$) ×1.070.05× 13.6.

4.3. Behavioral tests: rotarod test

Animals were tested on the rotarod at a speed of 8 rpm. Animals 3 days before ischemia and 24 hours after ischemia were tested with rotarod. The animal was placed on the rotating drum and the time spent to reach the criterion of remaining on the rotarod for 2 min without falling down was recorded. The animal was tested for a maximum of 5 min and, if after this period it was unable to reach the criterion, a score of 5 min was recorded.

5. Statistical analysis

Values are reported as mean ±SEM. Statistical analysis was performed with SPSS software version 13. Statistic calculations were carried out with one way analysis of variance (ANOVA) for multiple pair wise comparisons of groups. A significant difference was defined as $p<0.05$.

Results

MDA: Figure 2 illustrated that MDA levels of brain was non-significantly increased in MCAO group (2.38± 0.31 mg/dl) compared to control group (1.88±0.49 mg/dl). Following treatment with Rutin, the brain MDA levels were changed to 1.8±0.09 mg/dl and it wasn't significant.

Thiol: At the end of our study, the total thiol level of brain was significantly decreased in MCAO group (3.54± 6.32 mg/dl) compared to control group (22.87± 8.81 mg/dl) ($p<0.05$). The total thiol level was increased following treatment with rutin (20.17±7.21 mg/dl), so it was similar to control group ($p<0.05$) (figure 3).

Rotarod test: Figure 4 showed that time stands rates on rotarod in MCAO group (36.6±3.19) decreased significantly compared to control group (120±1.2) ($p<0.05$). In group MCAO+rutin (72±20.34), the time stands on rotarod significantly increased compared to MCAO group ($p<0.05$).

Discussion

Ischemia and reperfusion cause brain injury via different pathways. Some studies demonstrate that ROS are elevated during cerebral ischemia and reperfusion, which plays a major role in the pathophysiology of ischemic stroke or cerebral ischemia-reperfusion related injury (15, 16). In order to investigate the mechanism of protection induced by rutin against the ischemic cerebral injury, antioxidant defenses including MDA and Thiol in the injured brain tissue of rats were assessed.

In the first section of our findings, brain MDA levels don't have any significant change following MCAO and rutin treatment. This is clearly in contrast with other researches which show a marked increase in MDA levels. Annapurna et al. reported that rutin (10 mg/kg) administered 10 min before reperfusion resulted in significant reduction of infarct size, MDA, and MPO levels and significant increase in superoxide dismutase (SOD) and catalase (CAT) levels (16). This controversy may be due to involvement of multiple pathways in global cerebral ischemia-reperfusion injury.

In another part of our study, the thiol activity was decreased in MCAO group which is completely consistent with other studies (16-18). Decrease in total thiol activity during ischemia and reperfusion may be due to the attack oxygen free radicals and interaction of enzymes with peroxidation products, which can affect on the site activity (17,18). Another reason for reduction of activity can be attributed to the reduction in pH, i.e. acidosis. Ischemia renders the cells to undergo anaerobic metabolism, thereby, producing lactic acid and acidosis. Enzymes that are pH sensitive will, therefore, be easily affected. Thus, significant alteration in the antioxidant enzyme activities during cerebral ischemia and reperfusion may be responsible for more neurodegeneration than ischemia (19). In consistent with our finding, Khan et al reported a significantly depleted activity of antioxidant enzymes, glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) and content of glutathione (GSH) in MCAO group which were protected significantly in MCAO group pretreated with rutin. Conversely, the elevated level of thiobarbituric acid reactive species (TBARS), H_2O_2 and protein carbonyl (PC) in MCAO group was attenuated significantly in rutin-pretreated group when compared with MCAO group (13).

Rutin treatment significantly reduced the elevated tissue MDA levels. The beneficial effects of these flavonoids are attributed to their antioxidant and anti-inflammatory properties. The evidence from other study (16) clearly indicates that besides the peripheral organs, these bioflavonoids may also help to prevent tissue damage from oxidative stress in the brain. Also, Pu et al declared rutin (50 mg/kg x 2) improved spatial memory impairment in the 8-arm radial maze task and neuronal death in the hippocampal CA1 area (19).

Rutin treatment after MCAO significantly improved behavioral test (motor rotarod test) following stroke. This is in line with the apparent improvement of lipid peroxidation and thiol activity observed in brain of rats supplemented with this plant extract. Functional studies of impairment following ischemic injury often rely on assignment of the animal's performance to a subjective rating scale (e.g., postural reflex test) (20-21). The rotarod test is a well-established procedure for testing balance and coordination aspects of motor performance in rats. Recent studies have indicated that the accelerating rotarod task is a sensitive index for the assessment of motor impairment induced by traumatic brain injury, dopaminergic lesion, or ischemia in the rat. We chose to investigate the effects of rutin using an accelerating

rotarod because it represents a test agent that has been reliably shown to exhibit neuroprotection by numerous investigators (22-24).

Conclusion: rutin treatment may represent a novel approach in lowering the risk or improving the function of ischemia–reperfusion brain injury-related disorders. And it may have a predictive value for clinical efficacy with novel neuroprotective agents.

Acknowledgment: this study was done in Bojnurd University of Medical Sciences, Iran.

Conflict of interest: the authors declare that there are no conflicts of interest.

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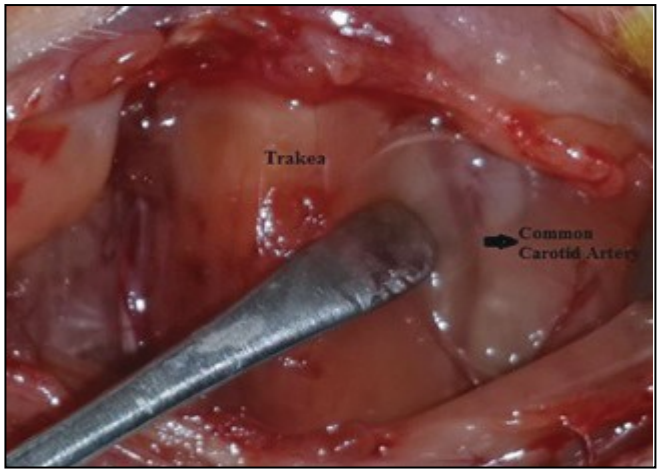

Figure 1: MCAO method: the common carotid artery is exposed.

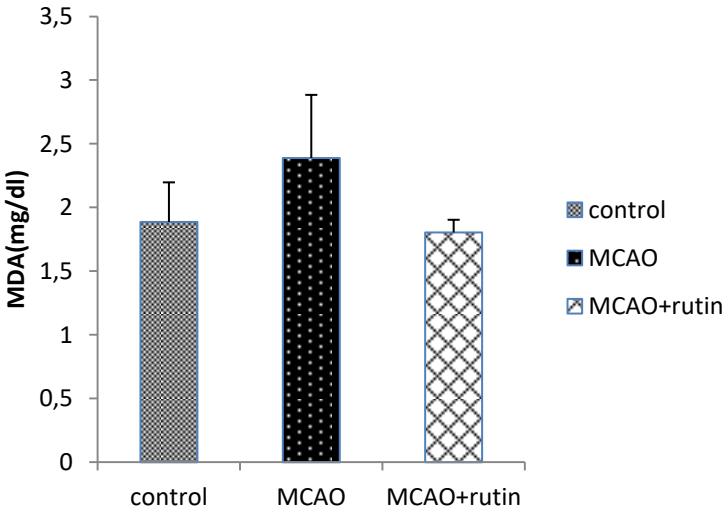

Figure 2. The MDA levels in three groups, control, MCAO: Middle cerebral artery occlusion and MCAO+rutin: Middle cerebral artery occlusion and intraperitoneal injection of Rutin. Values are expressed as mean ±SEM.

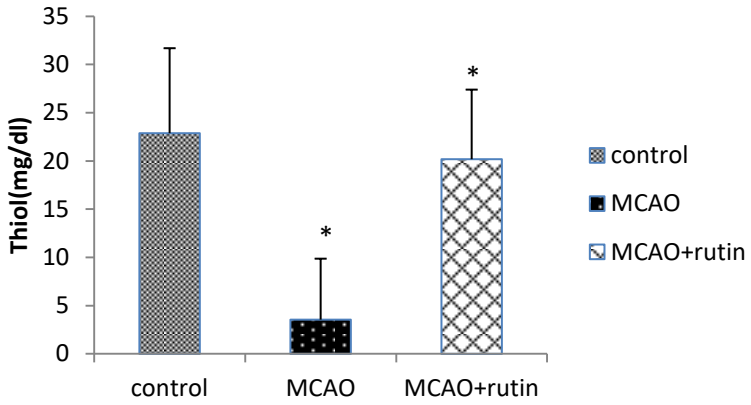

Figure 3. The thiol levels in three groups, control, MCAO: Middle cerebral artery occlusion and MCAO+rutin: Middle cerebral artery occlusion and intraperitoneal injection of Rutin. Values are expressed as mean ±SEM. *p < 0.05(* vs control and MCAO groups, respectively)

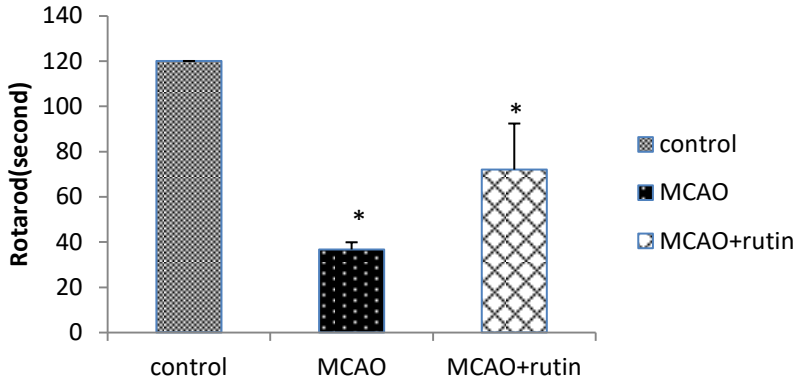

Figure 4. Rotarod test in three groups, control, MCAO: Middle cerebral artery occlusion and MCAO+rutin: Middle cerebral artery occlusion and intraperitoneal injection of Rutin. Values are expressed as mean ±SEM. *p < 0.05(* vs control and MCAO groups, respectively)