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Research Article

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Protective Effect of Quercetin in Blood during Ischemic Stroke is correlated with Declining Malondialdehyde and Iron Level but Enhancing Antioxidant Activity of Superoxide Dismutase

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Abstract Quercetin has been documented to display positive effect on anti-oxidation and anti-inflammation. Ischemic stroke may produce a large number of reactive oxygen species so as to elevate oxidative damage. Appropriate essential trace element of iron (Fe) and antioxidant of superoxide dismutase (SOD) are required for the brain for maintaining normal brain functions. This study was to explore whether protective effect of quercetin in blood during ischemic stroke is correlated with reducing oxidative damage by declining malondialdehyde (MDA), iron (Fe), but enhancing SOD activity. Rats were intraperitoneally administered with quercetin (20 mg/kg) once daily for 10 days before stroke surgery. The surgery of ischemic stroke was conducted by ligation of the right middle cerebral artery and the right common carotid artery for 1 hour. After completing the brain surgery, the fresh whole blood was collected via directly performing cardiac puncture and the serum sample was harvested for further biochemical analysis. Our results showed that ischemic stroke obviously enhances MDA and Fe level but declines SOD activity as compared to the control group. By contrast, pretreatment of rats with quercetin before ischemic stroke significantly reverses these effects relative to the ligation subject. Accordingly, our findings indicate that ischemic stroke obviously promotes oxidative damage. However, quercetin can significantly turnovers these adverse effects. We infer that quercetin seems can exert its powerful antioxidant potential to protect stroke patients to ameliorate ROS-conducted oxidative damage.

Keywords Ischemic stroke ·Quercetin · Malondialdehyde · Iron ·Superoxide dismutase

Introduction

It has been recognized that ischemic stroke is the major type of stroke clinically and is mainly results in disability and even mortality in older people [1-4]. Ischemic stroke may generate abundant reactive oxygen species (ROS) so as to elevate oxidative tension in the affected brain and body [1-2]. Moreover, the generated ROS results from ischemic stroke can promote deleterious lipid peroxidation, promoting further oxidative damage in living organisms [3-4].

Quercetin is a flavonoid compound and is mainly existed in vegetables and fruits such as red apple, red onion, cranberry, and blueberry [5-7]. Further, quercetin has been proposed displaying anti-oxidant andante-inflammatory property in diminishing toxic ROS in living organisms [8]. For these reasons, quercetin has been clinically applied to prevent some human diseases such as cardiovascular disorder, tumors as well as neurological disorders [9-10].

Essential trace element of iron (Fe) is needed for all cells and living organisms for their life [11-14]. Much attention has been paid on it because Fe is an integral part of the component of hemoglobin in erythrocytes [14]. However, accumulation or reduction of Fe level has been documented in responsible for the etiology of various human diseases [15-18].

Antioxidant of superoxide dismutase (SOD) is the major protective enzymes within the blood mainly avoiding ROS attack [19-21]. By contrast, reduction of which activity is thinkable to be attenuated the antioxidant efficacy so as to lead to an elevated oxidative stress [19-21]. The purpose of this study was to investigate whether protective effect of quercetin in blood during ischemic stroke is correlated with reducing oxidative damage by means of declining the concentration of MDA, Fe, but enhancing the SOD activity.

Materials and Methods

Animal and Serum Samples Preparation

Experimentally, forty male Sprague-Dawley rats, weighing from 220-270 g, were encompassed in this study. All rats were housed under controlled conditions in the animal room (22 ± 2°C, 50 ± 20% relative humidity, 12-h light-dark cycle). One week after caging, rats were randomly divided into four groups of 10 each as follows: Control (treated with normal saline); ligation (normal saline was administered before ligation of right middle cerebral artery (RMCA) plus right common carotid artery (RCCA) for 1 hour); quercetin (intraperitoneally injected rats with quercetin at dosage of 20 mg/kg once in a day for consecutive 10 days); and prevention (pretreatment of rats with quercetin at dosage of 20 mg/kg once in a day for 10 days followed by ligation of the RMCA plus the RCCA for 1 hour). On day 11, all rats were anesthetized and the fresh whole blood samples were collected via directly cardiac puncture. The obtained whole blood samples were centrifuged at 4°C for 10 min at 650 g, and the supernatants of the serum samples were harvested and ready for performing further biochemical analysis. Throughout the experiment, all animal used protocol that listed and mentioned above has been approved by the Institutional Animal Care and Use Committee (IACUC) of Central Taiwan University of Science and Technology.

Analysis of the Malondialdehyde (MDA) Level in Serum

Two hundred µl of the obtained serum sample was pipetted into Pyrex tube contains 3 ml cold H_3PO_4 solution (1% w/v) followed by adding 1 ml of the TBA reagent into tube and boiled at 100 ℃ for 1 hour. Four ml of the butanol solution was pipetted into tube and centrifuged at 1600 g for 5 minutes. Finally, the supernatant was collected and the MDA level was assayed using spectrophotometry (U-1900, Hitachi, Japan) at the wavelength of 532 nm. The reagent of 1,1,3,3-tetraethoxypropane was used as a standard solution in reaction with thiobarbituric acid (TBA) reactive substance. Basically, analyzing principle of this method is based on determining the pink color which is produced by the interaction of TBA with MDA.

Determination of the Superoxide Dismutase (SOD) Activity in Serum

For analyzing the activity of superoxide dismutase, the Cayman's superoxide dismutase assay kit (Cayman Chemical Company, USA) was used in the present experiment. The tetrazolium salt was applied to measure the superoxide radical which is generated by Xanthineoxidase and hypoxanthine. Experimentally, the SOD activity in serum was detected by means of the instrument of spectrophotometer (Thermo Scientific Multiskan Spectrum, USA).

Determination of the Iron (Fe) Concentration in Serum

Twenty µl of the obtained serum sample was used for the determination of the Fe level. In brief, all containers which were used were completely soaked with the concentration of 50% nitric acid, rinsed with ultrapure water and

followed by drying in an oven at the temperature of 50℃for later use.The standard solution of Fe metal was dissolved in the concentration of 0.1 mol/L nitric acid solution purchased from Merck, Germany. The concentration of Fe metal was measured by Savant AA Z graphite furnace atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd., Melbourne, Australia) with PAL4000 auto-sampler and longitudinal Zeeman Effect background correction experimentally.

Statistical Analysis

All the achieved data were expressed as mean ± S.D. The experimental values were analyzed using the statistical method of Kruskal-Wallis one-way analysis of variance (ANOVA). Once the analyzed data showed significant differences among the groups, each group was compared using the Fisher's Least Significant Difference (FLSD) test. The statistical differences were significantly considered at a *P*-value of less than 0.05 in the present experiment. a: $P < 0.05$, *vs*. control group; b: $P < 0.05$, *vs*. ligation subject.

Results

The Malondialdehyde (MDA) Levels in Serum

In this current research, the value of the MDA in the group of control, ligation, quercetin, and prevention was 15.65 ±2.91, 19.57 ± 3.10, 11.25 ± 0.72, and 15.59 ± 0.69nmol/g protein, respectively (Fig 1). Relative to the control group, the MDA level was prominently higher in the ischemic rats. On the other hand, the MDA level was significantly lower in the prevention group ($P< 0.01$) as compared to the ligation subject.

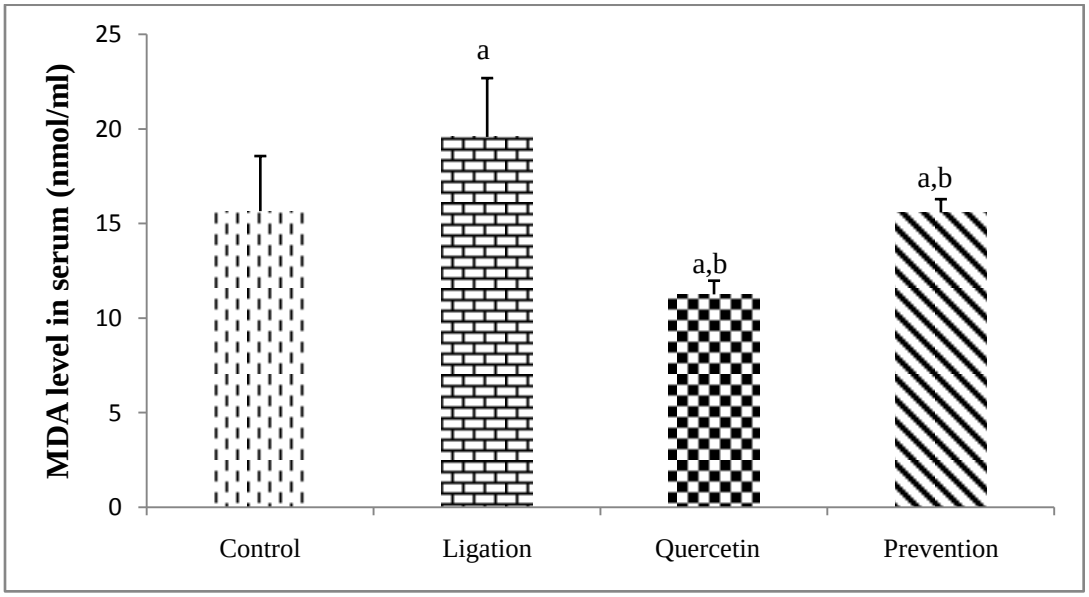

Figure 1: Profiles of MDA level in serum. Data were expressed as mean ± S.D. The statistical method of One-way ANOVA followed by Least Significant Difference was used. a: Significant difference ($p<0.05$) from control subjects. b: Significant difference ($p<0.05$) from prevention group.

The Iron (Fe) Concentrations in Serum

The Fe level in the control, ligation, quercetin, and prevention groups was 30.15 ± 3.46, 39.58 ± 0.87, 17.23 ± 2.66, and 26.52 ± 5.73ng/g, respectively. We observed that the Fe level was significantly higher in the ischemic subject as compared to the control group ($P < 0.01$). In contrast, the Fe level was markedly lower in prevention group ($P< 0.01$) as compared to the ligation subject as listed in figure 2.

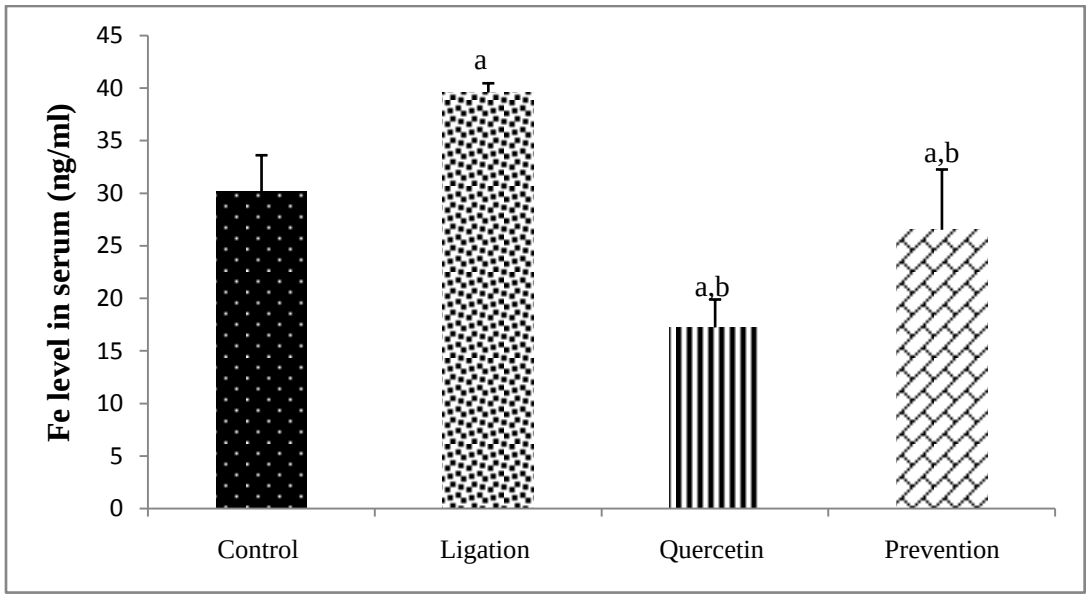

Figure 2: Profiles of Fe level in serum. Data were expressed as mean ± S.D. The statistical method of One-way ANOVA followed by Least Significant Difference was used. a: Significant difference ($p < 0.05$) from control subjects. b: Significant difference ($p < 0.05$) from prevention group.

The Superoxide Dismutase (SOD) Activity in Serum

The SOD activity in the serum which was detected from the group of control, ligation, quercetin, and prevention was 0.0356 ± 0.001, 0.0327 ± 0.001, 0.0384 ± 0.001, and 0.0374 ± 0.01 U/ml, respectively. Compared to the control group, the SOD activity was obviously lower in the ischemic rats. Conversely, On the other hand, the SOD activity was significantly higher in the prevention group ($P < 0.01$) as compared to the ligation subject.

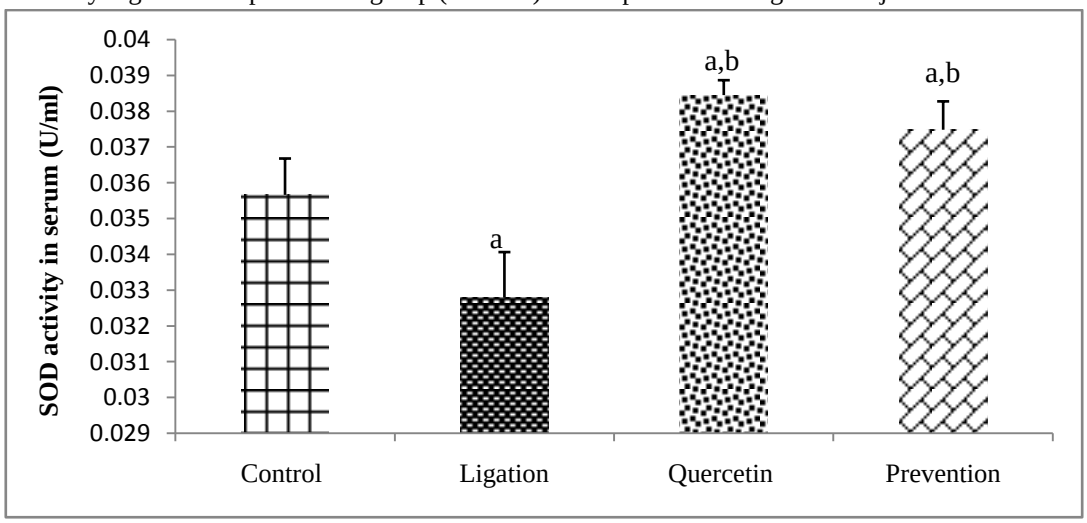

Figure 3: Profiles of SOD activity in serum. Data were expressed as mean ± S.D. The statistical method of One-way ANOVA followed by Least Significant Difference was used. a: Significant difference ($p < 0.05$) from control subjects. b: Significant difference ($p < 0.05$) from prevention group.

Discussion

Patients with ischemic stroke mainly suffer from dis-convenience due to the body paralysis [22]. In fact, the etiology of ischemic stroke is owing to the obstacle of the blood is unable to flow into the ischemic brain. Under this

situation, a variety and numerous ROS such as superoxide radicals, hydrogen peroxide as well as hydroxyl radicals are generated [22]. Given this fact, impairment of metabolism, less energy supplementationresults in cytokine releaseand eventually, inflammationand brain cells apoptosis and death occur in the ischemic brain [23].

Quercetin is a natural flavonoid substancemainly existed in vegetables and fruits. In addition to this, study reports that quercetin displays a variety of beneficial effects to human health such as anti-oxidation, anti-inflammation, and anti-apoptosis[5-7].In fact, experiment has proven that quercetin can attenuate ROS-mediated lipid peroxidation due to its anti-oxidative property [8-10]. Former study from ratexperimentindicatesthe positive effect of quercetin against traumatic brain injury-induced anti-apoptotic and anti-oxidative roles [24]. Meanwhile, former research indicates that quercetin reduces liver thioredoxin-interacting protein to alleviate inflammation and lipid accumulation in diabetic rats[25]. Furthermore, anti-inflammatory research reports that quercetin exerts positive effects on suppressing lipopolysaccharide-induced oxidative and inflammatory responses, altering lipid peroxidation products, and enhancing the adaptive stress pathways in BV-2 microglial cells [26]. Finally, other cellular model reveals that quercetin exerts antioxidant efficacy to prevent A549 cells from ROS attack [27]. As already mentioned, it is clear to note that quercetin exactly possesses anti-inflammatory and anti-oxidative abilities to reduce ROS-mediated oxidative damage in living organisms. In the present study, the MDA level was significantly increased in the ligation group as compared to the control subject as listed in figure 1. It has been evidenced that a large amount of the generated ROS resulted from ischemic stroke can spontaneously react with the macromolecules of the components of poly-unsaturated fatty acid (PUFA) in cells so as to induce an elevated MDA level [8-10]. Because of this, enhanced MDA level not only representative higher oxidative tension but also directly reflects the intensity of cellular injury. It is therefore conceivable to manifest our experimental finding here that ischemic stroke may elevate ROS in the brain tissues; the generated ROS seems to release from the ischemic brain tissues by the bloodstream. As a result, elevated lipid peroxidation effect, as reflected by an increased MDA levelwas observed in blood.In contrast, pretreatment of animal with quercetin prior to the event of ischemic stroke canprominently attenuate ROS-mediated MDA level. As mentioned previously, this is the reason why quercetin can exert its anti-oxidative property to diminish ROS-induced lipid peroxidation, and our finding is consistence with the former investigation.

Essential trace element of iron (Fe) is essentialforall cells and living organisms [11-14]. However, excess or deficiency of which concentration has been documented to be harmful to the cells and is involved in some human diseases [15-18].Indeed, Fe accumulationcan actively react with the ischemic stroke-mediatedsuperoxide radicals via the Fenton reaction so as to generatemore toxic hydroxyl radicals [16-18]. In addition, accumulatedFe level has been reported in associated with an increased MDA level and implicated with a variety of human disorders such as hepatic disease, cerebral ischemia, viral infection, depression, anxiety, and fearfulness [16-18]. This present finding indicates that ischemic stroke can lead to an increased Fe level. In contrast, pretreatment of animal with quercetin prior to ischemic insult significantly reduces Fe level within the blood flow. Thereby, we propose that quercetin can indeed diminish the Fe level in blood. However, the precise mechanism of how Fe level is declined is needed to be further explored.

Antioxidant activity is crucial for cells to scavenge ROS and diminishes oxidative injury [19-21]. Ischemic stroke may generate ROS and these ROS can react with the PUFA component. Thereby, proper antioxidant is useful to detoxify ROS. Superoxide dismutase (SOD)possesses powerful ability to catalyze the reaction by detoxifying the superoxide radicals [19-21]. In this study, the SOD activity was significantly decreased ($P<0.01$) in the ligation subject as compared to the control group. As mentioned before, ischemic lesion can result in a decreased SOD activity. Here we suppose that generation of superoxide radical results from ischemic stroke is detoxified by SOD activity and this may be the reason why SOD activity is declined in the ischemic situation. In contrast, pretreatment of rats with quercetin prior to ischemia significantly enhances SOD activity in blood. Former investigation has demonstrated that quercetin can stimulate antioxidant expression and may be display medicinal effect[28].Our experimental finding is in agreement with the former study and suggests that quercetin can stimulate SOD expression. As a result, the SOD activity was increased in the prevention subject relative to the ischemic rats in the present experiment.

Conclusion

We propose that ischemic stroke not only disrupts the equilibrium between pro-oxidant and antioxidant reactions but also changes trace element levels in blood. Based on our current finding, it is crucial to note that quercetin is advantageous to reduce oxidative damage. In addition, it seems possible that quercetin can exert its powerful antioxidant potential to protect stroke patients to ameliorate ROS-conducted oxidative damage.

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