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Research Article

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Paraquat-induced Oxidative Injury in Erythrocytes is correlated with Decreased Magnesium Level and Reduced Antioxidant Activity

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Abstract Paraquat (PQ) is commonly used in controlling wild plants in agriculture and public environment. PQ ingestion in human mainly dies for hypoxia due to the enhanced oxidative stress. Magnesium and antioxidants play important role in erythrocytes. This study attempted to investigate whether PQ-induced oxidative injury in erythrocytes is involved in the alterations of antioxidants and magnesium. Rats were divided into four groups of 10 each and treated with a single, intraperitoneal injection of PQ at dosages of 0, 10, 20, or 40 mg/kg, respectively. Erythrocytes were harvested via directly cardiac puncture after 24 h post-injection. Experimental results showed that the malondialdehyde (MDA) level was increased in parallel with the PQ-treated dosage. In contrast, the magnesium(Mg) level and the enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) were opposite to PQ-treated dosage experimentally. Our findings clearly demonstrate that PQ-induced oxidative injury in erythrocytes is involved in the alterations of magnesium level and antioxidant activity.

Keywords Paraquat, Oxidative stress, Magnesium, Antioxidant, Malondialdehyde

Introduction

Paraquat (PQ) is extensively used to remove wild plants in agriculture and the public environment [1]. PQ brings substantial benefits to the farmers due to its cheaper price, fast action, as well as rapid deactivation once contacts with soils [2]. Along with the popular use of herbicide come growing concerns over the impact of PQ on human health [1-2]. Previous study has clearly demonstrated that PQ toxicity is involved in the overproduction of deleterious reactive oxygen species (ROS) [2]. In vivo study shows that massive ROS produces by PQ can spontaneously covalent binding to lipid membrane, protein, as well as nucleic acid, contributing further adverse effects such as lipid peroxidation, enzymatic damage, mutagenesis, and carcinogenesis [3-6].

Multiple lines of evidence have recently focused on exploring the relationship between the essential metal of magnesium (Mg)and pathogenesis of human diseases [7, 8]. Essential metal of Mg is indeed required for living organisms and serves a wide range of fundamental and beneficial functions such as a declining inflammatory effect, acting as a cofactor in more than 300 enzymatic reactions in human, facilitating energy metabolism in cells, and

maintaining cell membrane integrity [9]. In contrast, in vivo study has demonstrated that reduction or restriction of Mg supplementation can enhance PQ toxicity [9]. Furthermore, administration of ischemic patients with magnesium sulfate significantly provides excellent therapeutic efficacy [10].

Proper antioxidant activity is helpful for cells toeliminate harmful ROS andavoiding or preventing further oxidative attack [12-15]. Conversely, decrease of which activity may weaken ROS scavenging ability in cells [12-15]. PQ ingestion mainly dies for hypoxia due to the damageof the lungs, liver,and the kidney [1-2]. Erythrocyte is specifically considered a suitable in vivo experimental model for evaluating ROS toxicity due to their enriched poly-unsaturated fatty acids (PUFA) component [16]. This present experiment attempted to investigate whether PQ induced oxidative cellular injury is correlated with the alterations of antioxidant activity and magnesium levelin erythrocytes.

Materials and Methods

Experimental animal treatment and harvest of erythrocytes

Experimentally, forty male Sprague-Dawley rats, weighing from 220-270 g were encompassed in this work. All animals were purchased from the National Laboratory Animal Breeding and Research Center (Taiwan). The protocol of this present work was approved by the Institutional Animal Care and Use Committee (IACUC). All rats were kept in stainless-steel mesh cages, housed under controlled conditions (22 ± 2°C, 50 ± 20% relative humidity, 12-h light-dark cycle) with diet and water. One week after caging, rats were randomly divided into four groups of 10 each and injected intraperitoneally with a single dose of 0 (saline as control), 10, 20, or 40 mg/kg of PQ purchased from Sigma Chemical Co (St. Louis, MO, USA), respectively. Twenty-four hours after administration, whole blood samples were collected in heparin-containing anticoagulant tubes via directly cardiac puncture technique. The collected blood samples were immediately centrifuged at 4°C for 10 min at 650 g and the plasma was discarded. The erythrocytes were harvested after washed three times with ice-cold isotonic saline solution and centrifuged at 4°C for 15 min at 650 g.

Determination of the malondialdehyde (MDA) concentration in erythrocytes

Malondialdehyde (MDA) is a stable end-product of lipid peroxidation. The MDA concentration is commonly used as a biomarker for predicting the intensity of oxidative injury. The reagent of thiobarbituric acid (TBA) was purchased from E. Merck (Germany) and used for MDA analysis. In the present study, the MDA level was assayed according to the method of Sunderman et al [18]. Reagent of 1, 1, 3, 3-tetra ethoxy propane (TEP) was used as a standard solution in the reaction with thiobarbituric acid-reactive substance (TBARS). Briefly, 2 ml of each erythrocyte sample was used; the analytical principle of this method is based on the determination of the pink color that is produced by the interaction of TBA with the component of MDA. The MDA level was determined using a spectrophotometer (Thermo Scientific Multiskan Spectrum, USA) to measure absorption at a wavelength of 532 nm. Hemoglobin (Hb) assay kit (Cayman Chemical Company, USA) was used and the level was measured by means of spectrophotometric measurement of absorption at a wavelength of 540 nm. The MDA level was calculated and expressed in terms of nmol per g of hemoglobin (Hb).

Measurement of the magnesiumlevel in erythrocytes

For Mg level analysis, the collected erythrocyte samples were digested with ultra-pure grade solution of nitric acid. All containers used prior to the experiment were soaked with 50% nitric acid which was purchased from Merck (Germany), rinsed with ultra-pure deionized water collected from the Milli-Q system (Millipore, USA), and followed by drying in the oven at 50°C. Standard solution (1000 μg/ml) ofanalyzed Mg metal was purchased from Merck (Germany) and was dissolved in ultra-pure grade solution of nitric acid. Essential metal concentration of Mg was measured at the wavelength of 285.2 via the instrument of Savant AAZ graphite furnace atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd., Melbourne, Australia) with PAL4000 auto-sampler and longitudinal Zeeman Effect background correction system in the present work.

Determination of the enzyme activity of SOD, CAT, and GPX

In brief, the harvested erythrocyte samples were lysed in four times its volume of ice-cold water followed by centrifugation at 4°C for 15 min at 10000 g. After washing completely, the supernatants were collected for enzyme analysis. Experimentally, SOD activity was determined according to the method of the Cayman superoxide dismutase assay kit (Cayman Chemical Company, USA). In general, the tetrazolium salt was utilized for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. The CAT activity was measured according to the Cayman catalase assay kit (Cayman Chemical Company, USA). The analytical principle is based on the reaction of the enzyme with methanol in the presence of hydrogen peroxide. The formaldehyde produced is measured with the chromogen of 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole. Finally, detection principle of GPX is that the GPX can catalyze the oxidation of glutathione by cumenehydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted into a reduced form with concomitant oxidation of NADPH to $NADP^+$. The resulting decrease in the absorbance at the wavelength of 340 nm was measured. All enzyme activities were determined via spectrophotometer (Thermo Scientific Multiskan Spectrum, USA) and expressed in terms of U per g of Hb. All experimental results were expressed as mean ± S.D. and the obtained data were analyzed using the statistical tests of one-way analysis of variance (ANOVA). Once the analyzed data showed significant differences among the group means, each group was compared using the least significant difference (LSD) test. Statistical differences were significantly considered at a p-value of less than 0.05 in the present experiment.

Results

Malondialdehyde (MDA) level in erythrocytes

Determination of the MDA level is widely used as a biomarker of oxidative injury that results from the lipid peroxidation process. As shown in Figure 1, the MDA value in PQ-treated dosages of 0, 10, 20, and 40 mg/Kg was 0.96±0.05,1.18 ± 0.05, 1.50 ± 0.06, and 1.88 ± 0.08 μmol/g Hb, respectively. The MDA level was significantly (*P < 0.05)* elevated in paralleled with PQ-injected dosage.

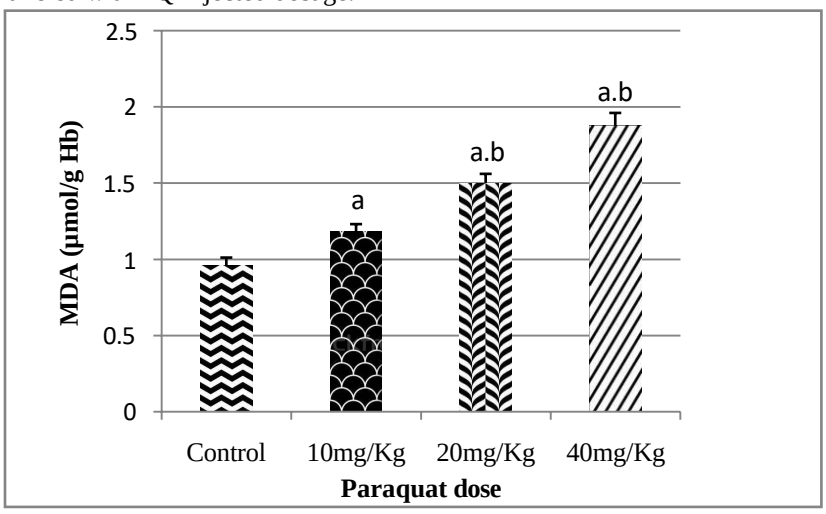

Figure 1: Profiles of the MDA level in erythrocytes

Data were expressed as mean ± S.D. (N=40). The statistical method of One-way ANOVA followed by Least Significant Difference test was used. a: Significant difference ($p < 0.05$) from control subjects. b: Significant difference ($p < 0.05$) from 10mg/kg of the paraquat-treated group.

Magnesium concentration in erythrocytes

The Mg concentration in the control and the PQ-treated dosage of 10, 20, and 40 mg/Kg was 2.62 ± 0.07, 2.42 ± 0.09, 2.22 ± 0.06, and 1.77 ± 0.05 μg/g Hb, respectively (Figure 2). Obviously, PQ administration significantly (*P < 0.05)* diminished the essential metal level of Mg in erythrocytes in a dose-dependent manner in the present study.

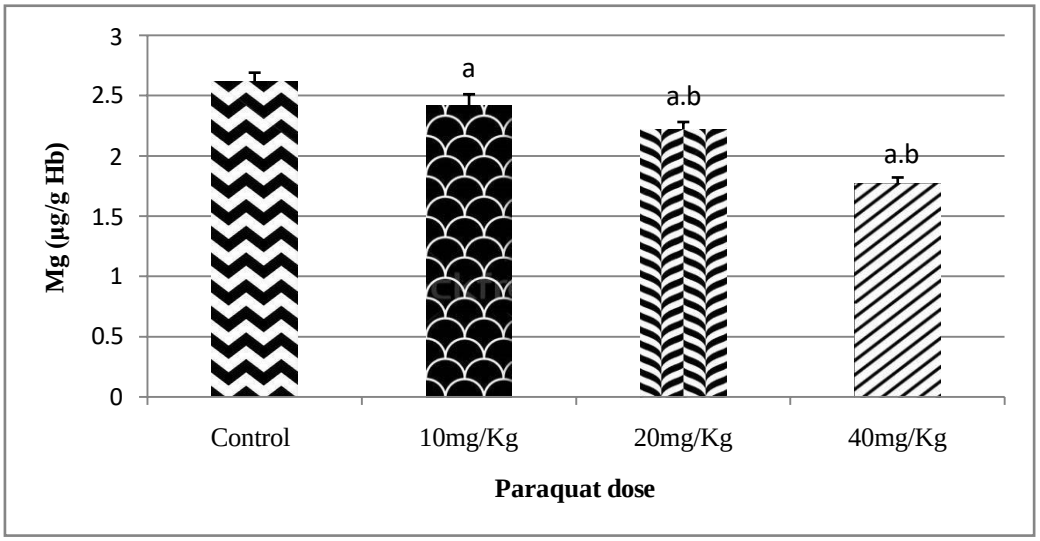

Figure 2: Profiles of Mg level in erythrocytes. Data were expressed as mean ± S.D. (N=40).

A statistical method of One-way ANOVA followed by Least Significant Difference test was used. a Significant difference ($p < 0.05$) from control subjects. b Significant difference ($p < 0.05$) from 10mg/kg of the paraquat-treated group.

Antioxidant activity of SOD, CAT, and GPX in erythrocytes

Figure 3 showed that the value of enzyme activity of SOD in PQ-treated dosages of 0, 10, 20, and 40 mg/Kg was 924 ± 72, 864 ± 87, 796 ± 95, and 663 ± 71 U/g Hb, respectively. CAT activity in the control and the PQ-administrated dosage of 10, 20, as well as 40 mg/Kg was 170 ± 18, 154 ± 16, 133 ± 15, and 104 ± 9 U/g Hb, respectively(Figure 4). Finally, the GPX activity in PQ-treated dosages of 0, 10, 20, and 40 mg/Kg was 32.6 ± 2.7, 29.4 ± 3.6, 24.8 ± 2.9, and20.9 ± 2.9, respectively (Figure 5). Obviously, antioxidant activities of SOD, CAT, as well as GPX were opposite to PQ-treated dosage. Data indicated that PQ administration markedly ($P < 0.05$) declined the enzyme activity of SOD, CAT, as well as GPX in erythrocytes.

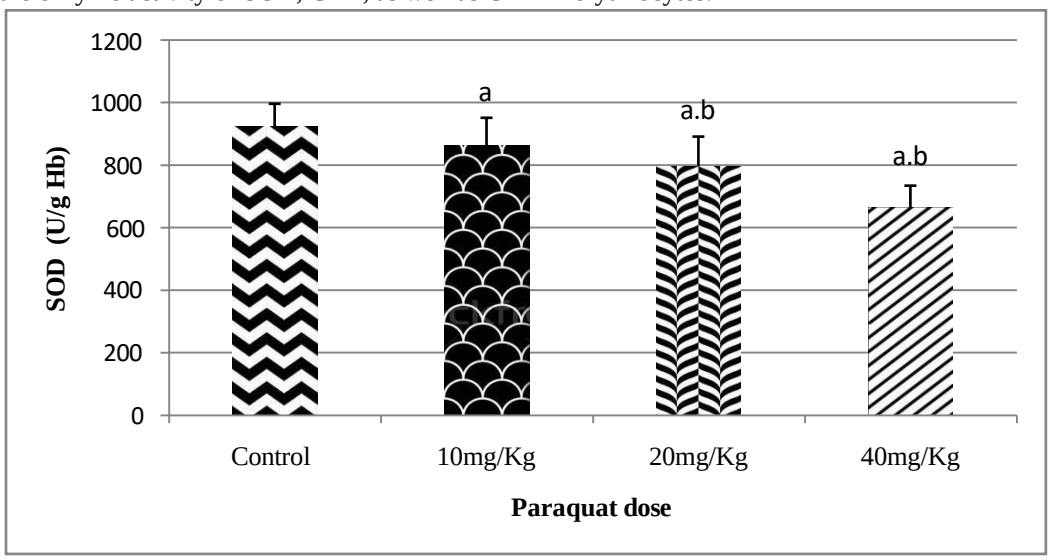

Figure 3: Profiles of SOD activity in erythrocytes. Data were expressed as mean ± S.D. (N=40).

A statistical method of One-way ANOVA followed by Least Significant Difference test was used. a: Significant difference ($p < 0.05$) from control subjects. B: Significant difference ($p < 0.05$) from 10mg/kg of the paraquat-treated group.

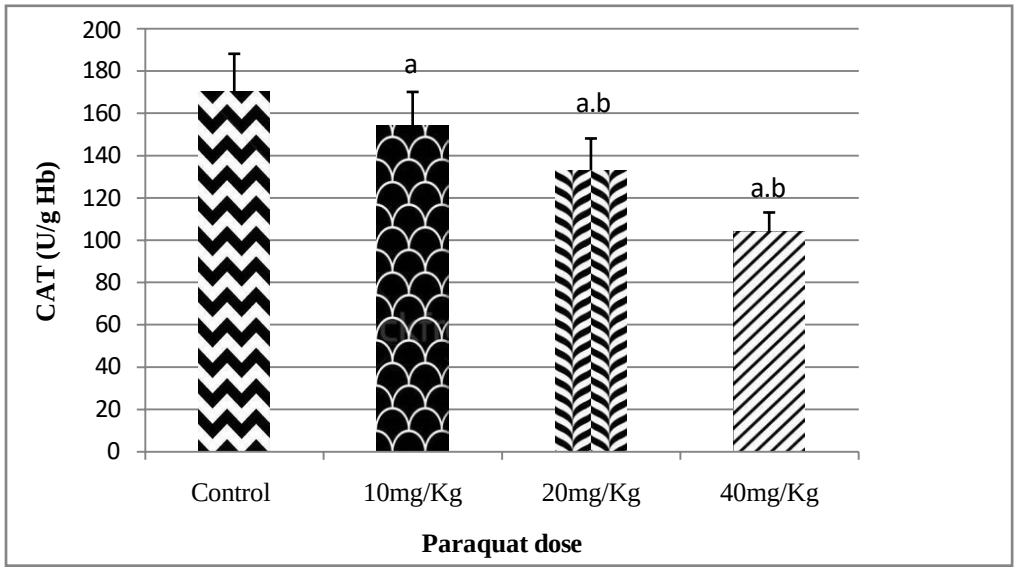

Figure 4: Profiles of CATactivity in erythrocytes. Data were expressed as mean ± S.D. (N=40).

A statistical method of One-way ANOVA followed by Least Significant Difference test was used. a: Significant difference ($p<0.05$) from control subjects. b: Significant difference ($p<0.05$) from 10mg/kg of the paraquat-treated group.

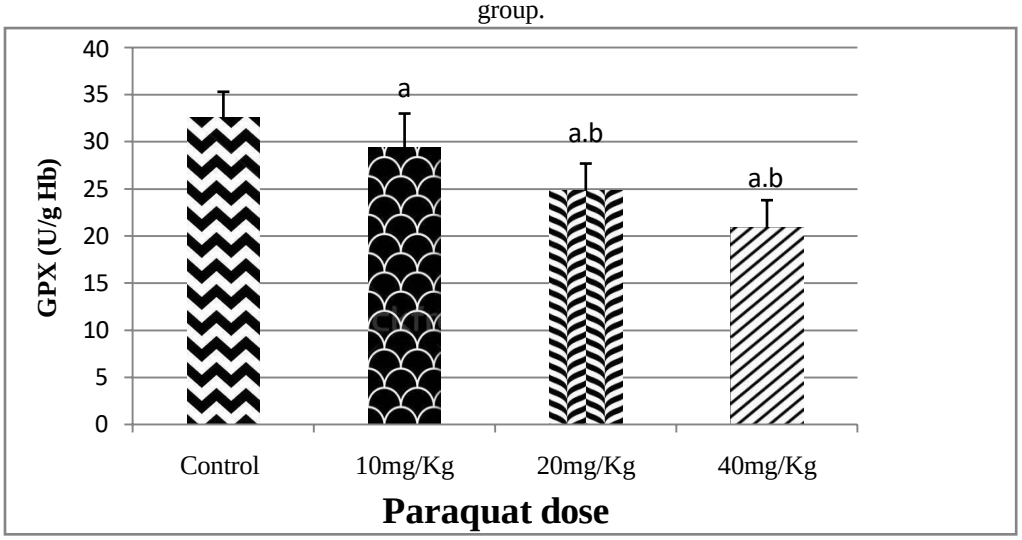

Figure 5: Profiles of GPXactivity in erythrocytes. Data were expressed as mean ± S.D. (N=40).

A statistical method of One-way ANOVA followed by Least Significant Difference test was used. a: Significant difference ($p<0.05$) from control subjects. b: Significant difference ($p<0.05$) from 10mg/kg of the paraquat-treated group.

Discussion

Non-selective contact herbicide of PQ is commonly used in the agriculture and the public environment due to its enormous benefits including fast action, cheaper price, as well as rapid deactivation once contacts with soils [1-2]. So far, there is still no effective therapeutic strategy that has been achieved in treating PQ intoxication cases at all. In this regard, no matter occupational, suicidal, or accidental ingestion, PQ poisoning indeed worldwide continues to pose a major public health concern. Overproduction of the toxic ROS including superoxide radicals, hydrogen peroxide, as well as hydroxyl radical is implicated with PQ toxicity [2-3]. Previous research has suggested that PQ-

induced inflammatory effect is involved in the generation of toxic ROS as well as the production of inflammatory cytokine [4]. Furthermore, recent investigations have clearly demonstrated that a variety of human disorders such as cerebral ischemia, pesticide intoxication, cancer, diabetes mellitus, birth defect, renal disease, cardiovascular disorders, as well as Parkinson's disease are correlated with massive ROS generation [17-21]. Clinical and animal study has evidenced that the most amounts and the sensitive affected cell in the bloodstream after PQ ingestion is the erythrocytes [1-3]. The major biological function of erythrocytes is to bind with the oxygen and transports it into the whole body cells, tissues, as well as organs. Literature has revealed the fact that the massive generated ROS after PQ poisoning is capable of actively covalent binding with the component of poly-unsaturated fatty acid (PUFA) in cells [17-18]. Because of its relative enrichment of the component of PUFA in erythrocytes, further deleterious ROS-mediated lipid peroxidation can not only damage to the erythrocytes but also results in the interruption of oxygen binding. Under this circumstance, the occurrence of severe whole-body hypoxia occurs [16, 19-21]. Our experimental results showed that the MDA concentration, the commonly used biomarker of oxidative damage, was increased in parallel with PQ-administered dosage as listed in Figure 1. Apparently, PQ-induced adversely oxidative damage in erythrocytes is positively correlated with PQ-treated dosage, and the trend of our experimental finding was in accordance with the preceding study [3, 20-22].

Multiple lines of evidence have interested in exploring the relationship between the disturbance of essential metal levels and the etiology of human diseases [7-13]. Essential metal of Mg is required for the erythrocytes for their normal biological functions. Previous investigation has evidenced that Mg is known as the second most abundant intracellular cation [11]. It has been well documented that Mg possesses profound beneficial functions for living organisms such as maintaining cell membrane integrity, attenuating lipid peroxidation, declining inflammatory effect, acting as a cofactor in more than 300 enzymatic reactions, as well as facilitating energy metabolism especially for the glucose in erythrocytes [9, 10, 12]. Animal experiment has suggested that PQ-induced oxidative lung injury can be markedly improved by treating the rats with magnesium isoglycyrrhizinate [8]. Another clinical study concerning the beneficial effect of Mg has proposed that intravenous administration of acute stroke patients with magnesium sulfate significantly ameliorates brain damage [10]. Similar investigation has proposed that supplementation of fetal mice with magnesium sulfate not only can decline inflammatory effect but also can mitigate the MDA concentration due to the attenuated lipid peroxidation effect in the brain [23-24]. Furthermore, former in vivo study has indicated that administration of gerbils with magnesium sulfate can effectively preserve the energy of glucose as well as decline the concentration of excited toxic glutamate levels on the ischemic brain [25]. Our previous investigation has evidenced that a decreased Mg concentration was found in the ischemic brain cortex of gerbils during the period of focal cerebral ischemia and reperfusion [12]. Additionally, it has been reported previously that restriction of the dietary Mg significantly enhances PQ toxicity in rats [9]. On the other hand, a variety of preceding experimental evidence have clearly proved the fact that supplementation of rats with Mg effectively mitigates the MDA concentration due to the attenuated lipid peroxidation effect. Conversely, reduced Mg level not only can exacerbate the oxidative injury but also can enhance PQ toxicity in the animal model [9, 12, 25]. Our present result showed that an inverse correlation was found between PQ-treated dosage and Mg concentration in erythrocytes as listed in Figure 2. Accordingly, the interpretation of our present finding is that PQ exposure significantly results in the reduction of the Mg level in a dose-dependent manner. As mentioned above, reduction in the Mg concentration exactly aggravates the oxidative injury in erythrocytes. Given the fact, further harmful lipid peroxidation effect elevated.

It has been well-recognized that generation of excessive ROS in cells can result in a variety of cellular damages. In order to avoid this situations, the intracellular oxidative balance is strictly controlled. Oxidative stress is basically recognized as an imbalance between the formation of ROS and its elimination. There are three major antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), as well as glutathione peroxidase (GPX) mainly existed in erythrocytes [12-15]. Among them, SOD catalyzes the formation of toxic forms of superoxide radicals into oxygen and hydrogen peroxide; CAT is found primarily in erythrocytes and it converts toxic hydrogen peroxide into water and oxygen and finally, the GPX enzyme converts hydrogen peroxide into the non-toxic substances, water, and oxygen [26-27]. Erythrocytes are sensitive to ROS attack due to lack of nucleus and less self-repair

mechanism. Our experimental results showed that antioxidant activity of SOD, CAT, as well as GPX was opposite to PQ-administrated dosage. Previous in vivo study has revealed that cells expose to higher PQ dosage can significantly inhibit the SOD activity [28-29]. In contrast, other investigation has indicated that treatment of rats with a single low dose of 1.5 or 7.5 mg/kg of PQ can elevate the SOD activity [30]. Accordingly, it seems likely that lower PQ treatment dosage can stimulate antioxidant activity and conversely, higher PQ exposure can result in a decreased enzyme activity and this effect is regarded as enzyme inactivation. Previous in vivo investigation has indicated that antioxidant enzyme of CAT is enriched for in erythrocytes to eliminate the toxicity of hydrogen peroxide [12-14]. Our present results showed that an inverse association was found in CAT activity and PQ-treated dosage as listed in Figure 4. Similarly, our experimental observation showed that the GPX activity in erythrocytes was opposite to PQ-treated dosage as listed in Figure 5. Former study has proposed that higher PQ exposure can reduce the GPX activity due to the enzyme inactivation effect [12-16, 30]. Based on our present results, it seems likely to state our finding here that due to the enzyme inactivation, antioxidant activity of SOD, CAT, as well as GPX was significantly declined in erythrocytes in a dose-dependent pattern.

Conclusions

Appropriate magnesium level and antioxidant activity are indeed required for the erythrocytes not only in maintaining their cellular functions but also in eliminating oxidative stress. In contrast, decrease of both levels is capable of weakening the protective ability for erythrocytes. Taking all experimental results together, the interpretation of our experimental findings is that PQ-induced oxidative cellular injury in erythrocytes is closely associated with declined magnesium level and reduced antioxidant activity.

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