Antioxidant attenuation of ROS-involved cytotoxicity induced by Paraquat on HL-60 cells

Wen-Hua Zhang, Yang Yang, Chang-Jun Lin, Qin Wang

School of Life Sciences, Lanzhou University, Lanzhou, China; qwang@lzu.edu.cn

ABSTRACT

The cytotoxic effects of Paraquat, an herbicide refractory to treatment after intentional or accidental contact, were investigated on the human leukemia HL-60 cells. With the establishment of Paraquat injury model of HL-60 cells, trypan blue exclusion assaying was performed to have determined the effects of Paraquat-induced cytotoxicity on HL-60 cells in a concentration-dependent manner. Upon treatment with various concentrations of Paraquat, pronounced increase on the levels of intracellular production of $O_2^{\cdot-}$ and H_2O_2 was detected with employment of fluorescent probes. Indicative of the oxidative stress, levels of MDA and T-AOC were quantitated to have determined the causal role for Paraquat in subjecting HL-60 cells to oxidative damage. Based on this finding, effects of antioxidant enzymes including GSH, NAC, CAT and SOD on attenuating the Paraquat-induced oxidative damage on HL-60 cells were examined, aiming to identify the most effective antioxidant enzyme for alleviating the cytotoxicity induced by Paraquat. In conjunction with the determination of cytotoxicity exerted by all the antioxidant enzymes on HL-60 cells, GSH-with its least inherent cytotoxicity on HL-60 cells-was identified as a promising candidate ingredient for extenuating the Paraquat-induced cytotoxicity.

Keywords: Paraquat; HL-60 Cells; Oxidative Damage; Cytotoxicity; Antioxidant Enzymes

1. INTRODUCTION

As response to increasingly more environmentally-associated outbreaks of diseases, Paraquat (PQ) had already gained tremendous attention since 1966, following the determination of the toxicity of PQ for a number of animal species [1] and a report on two cases of accidental poisoning by PQ in human [2]. Herein, PQ is the trade name of the dichloride salt of the radical 1, 1'-dimethyl-4, 4'-dipyridyliuim, whose herbicidal property was reported in 1958. Since its toxicity is dosage-dependent, a wide spectrum of studies on PQ-induced toxicity had been conducted at various levels, aiming to standardize the safe dosage to minimize its toxicity to other untargeted organisms. Although an in vivo experiment upon the toxicity of PQ, which set up the rats and mouse as study objects and factored into the acute and chronic toxicity as well as teratogenicity and mutagenicity, was conducted to have claimed that PQ would be safe if used following the recommended use instruction, the hard-to-control manipulation of use in practice and differentiated susceptibility of animal species necessitate the investigations of underlying mechanism of PQ-induce cytotoxicity. Moreover, advanced techniques had already been employed to identify effective candidate molecules to reduce PQ-induced toxicity [3].

PQ is biologically active after it has been sprayed in the field. It's either strongly bound to soil particles or decomposed into a non-toxic form by soil bacterial and sunlight [4]. However, active form of PQ is highly toxic to humans and many cases of acute poisoning and death had been reported over the past few decades [5-9]. The most frequent routes of exposure to PQ, either accidentally or intentionally, in humans and animals are by ingestion or through direct skin contact. Regardless of the well-tuned administration of circulation and body-defense systems, PQ could be rapidly distributed in most tissues, with the highest concentration in the lungs and kidneys [10], where the compound accumulates and causes great damage in Clara cells and alveolar type I and II epithelial cells [11]. Mechanisms involving the generation of Reactive Oxygen Species (ROS) were implicated in the PQ-induced cell apoptosis [12-16]. Although the information on the possible mechanism of human carcinogenesis associated with PQ exposure is limited, several papers have suggested a possible link to Non-Hodgkin's Lymphoma by induction of chromosomal aberrations, gene mutations in human lymphocytes, and apoptosis in human B lymphocytes [17,18].

ROS is hypothesized to induce cell damage or initiate a cascade of signaling mechanisms that ultimately lead to cell adaptation, apoptosis or necrosis [19]. The mecha-

nisms of PQ toxicity include the formation of ROS mediated by cytochrome P_{450} reductases and subsequent damage of ROS to cellular macromolecules [20]. The dysfunction of pulmonary microvascular endothelial cell membrane and high oxidative potential in pulmonary microvascular endothelial cells, which resulted from the overproduction of hydrogen peroxide, had been well-documented to be involved in PQ-induced cytotoxicity [21,22]. Additionally, EPR techniques had been employed to have confirmed that PQ could have triggered the intracellular production of ROS during which the formation of oxygen radicals ($O_2^{\bullet-}$) and hydroxyl radical (OH.) involved the mechanism of hydrogen transfer [23]. As one of the PQ-induced toxicity modes, mechanism of ROS-induced neuronal damage by PQ had been proposed [24]. Thus, Cellular Reactive Oxygen Species metabolism has been the focus of intense interest regarding physiological activities, due in part to the evidence that over expression of antioxidant enzymes confers resistance to oxidative stress, improvement in disease and increased lifespan, respectively [25,26]. ROS including superoxide, hydrogen peroxide and hydroxyl radicals are commonly generated through cellular metabolism and their physiological balance is achieved through neutralization of cellular antioxidant enzymes, which include Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) [27,28]. Oxidative stress occurs as a consequence of the imbalance between the production of ROS and cellular antioxidant capacity. Antioxidant enzymes, which are biologically and functionally coupled with ROS, have been evolved to maintain the high-efficiency performance of cell functions. A putative illustration of how a cell responds at risk of oxidative damage had been proposed [29].

Here, based on the establishment of in vitro PQ injury model of HL-60 cells, we have studied the ROS-involved cytotoxicity induced by PQ in HL-60 cells through measuring the contents and time-resolved changes of malondialdehyde (MDA), total anti-oxidation competence (T-AOC), $O_2^{\bullet-}$ and H_2O_2. A concentration- and time-dependent inhibition of cell growth has also been quantified to determine whether the release of $O_2^{\bullet-}$ and H_2O_2 into cytosol could potentially induce the cytotovicity on HL-60 cells. In addition, we have explored that whether the treatment with antioxidant enzymes could attenuate the PQ-induced cytotoxicity on HL-60 cells. Inspired by the PQ-detoxication effects of multiple emulsions [30], we hope the GSH could be developed as an ingredient in attenuating the PQ-induced toxicity.

2. MATERIALS AND METHODS

2.1. Cell Line and Culture Medium

Human Promyelocytic Leukemia HL-60 cell line was obtained from the cell library of Institute of Cancer Molecular biology and Drug Screening at Lanzhou Univer-

sity. RPMI-1640 cell culture medium was purchased from Gibco (Santa Clara, USA). Mycoplasma-free Neonatal Bovine Serum was purchased from Hangzhou Sijiqing Biological Engineering Materials.

2.2. Reagents, Assay Kits and Other Materials

Paraquat was purchased from Sigmal-Aldrich. Assay kits of MDA and T-AOC were purchased from Promega (Madison, WI, USA). Culture dishes and 24-well plates were purchased from Hangzhou Sijiqing Biological Engineering Materials (Hangzhou, China). 2', 7'-Dichlorofluorescein diacetate (DCF-DA) and Dihydroethidium (DHE) probes were purchased from Molecular Probes (Eugene, OR, USA). Other common experiment materials were of analytically pure.

2.3. Culture of Human Promyelocytic Leukemia HL-60 Cells and Analysis of Cells Proliferation by Trypan Blue Exclusion

HL-60 cells were grown in RPMI medium supplemented with 2g/L NaHCO3, 100U/ml penicillin, 100μg/ml streptomycin and 10% fetal calf serum in a humidified atmosphere of 95% air and 5% CO_2. Cultures were initiated at a density of 104/ml at 37℃ and grown exponentially to about 106/ml in 72 hours. Under the hypothesis that the imbalance between oxidative stress and antioxidant capacity could be restored if the antioxidant enzymes are replenished subsequently to the oxidative damage [31], cells were treated with various concentrations of PQ and desired concentrations of antioxidant enzymes for assigned time to determine both the PQ-induced cytotoxicity and antioxidant-mediated cytoprotection. To perform the trypan blue exclusion, cells were washed and harvested with sterile PBS. The cell suspension was mixed at the ratio of 4:1 with 0.4% trypan blue solution and incubated for 5 min. The viable cells were counted in a hemocytometer with an inverted- phase contrast microscope (Olympus IX81, Japan).

2.4. Detection of Intracellular Production of $O_2^{\bullet-}$ and H_2O_2 by Inverted Fluorescent Microscope

For assessment of intracellular ROS produced by treatment with PQ in the HL-60 cells, the DCF-DA and DHE assay were performed [32,33]. The production of H_2O_2 was measured with a non-polar compound 2', 7'-dichlorofluorescein diacetate (DCF-DA) that readily enter the cells, where it is cleaved to form non-fluorescent 2', 7'-dichlorofluorescein (DCFH) by endogenous esterases. DCFH reacts with H_2O_2 to produce a fluorescent compound 2', 7'-dichlorofluorescein, which is trapped inside the cells and indicates the intracellular level of H_2O_2 [34,35]. To detect the intracellular production of $O_2^{\bullet-}$, DHE probe was used where DHE is oxidized to

oxoethidium by superoxide anions [36]. After treatment with PQ, HL-60 cells were incubated with DCF-DA and DHE for 1 hour, respectively. For quantification of fluorescence intensities of DCF-DA and DHE the glass slides were placed in an inverted fluorescent microscope (Olympus, Japan) to take the images with excitation wavelength at 485nm and emission wavelength at 525nm and 610 nm for DCF-DA and DHE, respectively.

2.5. Flow Cytometric Quantification of $O_2^{\bullet-}$ and H_2O_2

Intracellular production of H_2O_2 and $O_2^{\bullet-}$ was measured using DCF-DA and DHE as probes. HL-60 cells treated with PQ for 12 hours were washed and resuspended in PBS containing 20μM DCF-DA and 20μM DHE, respectively. After 1 hour incubation in the dark at 37℃, the extracellular fluorescence was removed by washing with PBS for three times. Then cells were suspended with PBS to the concentration of 106/ml. The intracellular contents of H_2O_2 and $O_2^{\bullet-}$ were determined by flow cytometry with excitation wavelength at 485nm and emission wavelength at 525nm and 610 nm for DCF-DA and DHE, respectively.

2.6. Determination of the Levels of MDA and T-AOC

The increase in malondialdehyde (MDA) level upon treatment with PQ indicates oxidative damages to major cellular components, whereas the reduction in the level of T-AOC occurs as a response to the oxidative stress [27,28]. The levels of MDA and T-AOC were measured by MDA assay kit [37] and T-AOC assay kit, respectively. Well- grown HL-60 cells were treated with various concentrations of PQ for 12 hours. After incubation, cells were washed twice with PBS, harvested and sonicated following the instructions of the kits. The levels of MDA and T-AOC were determined with a DU 640 spectriphotometer (Beckman instrument Inc, CA, USA). The expression of MDA and T-AOC contents were converted and presented as the following, respectively:

$$\text{MDA content} = \left(\frac{At - Ato}{Ak - Ako} \times 10nmol/ml \right) \Big/ Ct$$

At: Absorbance of sample tube;
At_0: Absorbance of blank sample tube;
Ak: Absorbance of standard tube;
At_0: Absorbance of blank sample tube;
Ct: content of sample protein.

$$\text{T} - \text{AOC content} = \left(n \frac{At - Ato}{0.01} / 30 \right) \Big/ Ct$$

At: Absorbance of sample tube;
At_0: Absorbance of blank sample tube;
n: dilution factor;
Ct: content of sample protein;
The reaction duration was 30 min.

2.7. Statistical Analysis

All experiments were performed independently more than 4-6 times and data are presented as mean ± standard ($\bar{\chi} \pm SD$) deviation unless otherwise indicated. Statistical significance was determined between untreated groups and treated groups at each time point, with the one-way analysis of variance (ANOVA) and the t-test. A value of p<0.05 was considered to be significant.

3. RESULTS

3.1. Analysis of PQ-Induced Toxicity on HL-60 Cells

3.1.1. Antiproliferative Effects of PQ

To examine the cytotoxicity of PQ, HL-60 cells were treated with various concentrations of PQ for 48 hours and cell viability was assayed by trypan blue exclusion (**Figure 1**). PQ exerted a significant effect on the proliferation of HL-60 cells in a time-dependent manner during which no significant changes were observed until 16 hours while pronounced effects on inhibiting the cells proliferation occurred after 16 hours (**Figure 1(a)**); PQ at concentration below 5μM exerted insignificant effects on the proliferation of HL-60 cells while the proliferation of HL-60 cells was arrested upon treatment with PQ at concentration above 100μM. At concentrations ranging from 5μM to 100μM, PQ decreased HL-60 cells viability in a concentration-dependent manner (**Figure 1(b)**).
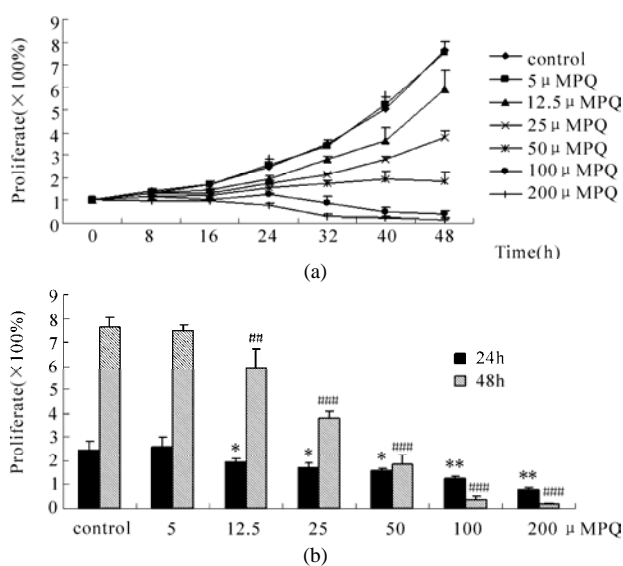

(a)

(b)

Figure 1. Profile of antiproliferative effects of PQ on HL-60 cells. Cells in culture dishes were treated with various concentrations of PQ for 48 hours and cells viability was measured by trypan blue exclusion in ranges of both 24 hours and 48 hours. (a) Profile of time-course proliferation of HL-60 cells by treatment with the various concentrations of PQ. (b) Comparison of antiproliferative effects of each concentration of PQ. **p<0.01 Vs24 h control and ## p<0.01，### p<0.001 Vs 48h control.

3.1.2. Changes of MDA and T-AOC Levels Induced by PQ

To determine whether contents of MDA and T-AOC inside HL-60 cells undergo appreciable fluctuations upon treatment with PQ, MDA kit and T-AOC kit were performed to assay the Oxidative stress-initiated physio-chemical variations of HL-60 cells. After treatment with various concentrations of PQ for 24 hours, the MDA level had increased. In particular, 100μM PQ elevated MDA level by 85.1% while 12.5μM PQ exerted insignificant effect on the changes of MDA level, whereas 25μM and 50μM PQs increased the MDA levels by 37.2% and 48.1%, respectively (**Figure 2**). Yet, upon treatment by 12.5μM, 25μM, 50μM and 100μM PQs, the level of T-AOC decreased by 62.8%, 77.7%, 87.6% and 98.8%, respectively (**Figure 3**).

3.1.3. Effects of PQ on the Intracellular Levels of $O_2^{\bullet-}$ and H_2O_2

For assessment of intracellular production of $O_2^{\bullet-}$ and H_2O_2 upon treatment with PQ, cells well-grown on 24 well plates were assayed by the DHE and DCF-DA assay kits. HL-60 cells were treated by 50μM PQ for 12 hours. Images were taken by fluorescent microscope after incubating cell samples with DHE and DCF-DA for 1 hour, respectively. In the DHE group, red fluorescence were detected in 50μM PQ-treated HL-60 cells (**Figure 4(d)**) while no significant fluorescence signals were detected on control ones (**Figure 4(b)**), which indicated that the treatment with PQ induced the overproduction of $O_2^{\bullet-}$. In the DCF-DA group, the absence of detectable green fluorescence on the control group (**Figure 5(b)**), which was detected on the 50μM PQ-treated HL-60 cells (**Figure 5(d)**), confirmed that the treatment with 50μM PQ triggered the intracellular production of H_2O_2. In addition, the viability of 50μM PQ-treated HL-60 cells decreased compared with the control group, whereas their morphology remained physiologically undamaged (**Figure 5(d)**).

Quantifications of $O_2^{\bullet-}$ and H_2O_2 were determined by flow cytometry. HL-60 cells were treated by various concentrations of PQ for 12 hours until confluent. In parallel with control group, PQ-treated HL-60 cells were hybridized with DHE and DCF-DA probes separately
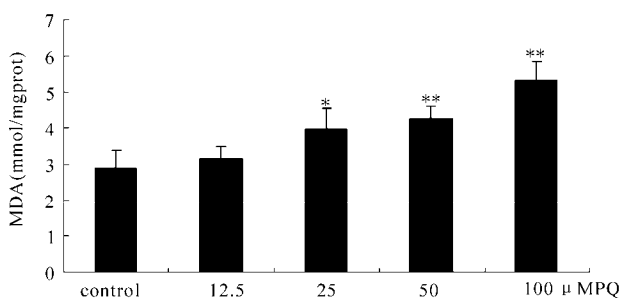
Figure 2. Intracellular MDA levels of HL-60 cells increased by treatment with various concentrations of PQ for 24 hours. *p<0.05, **p<0.01 Vs the control.
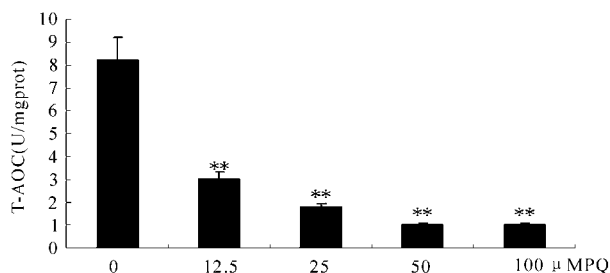
Figure 3. Decrease in T-AOC levels of HL-60 cells upon treatment by various concentrations of PQ for 24 hours. The levels of T-AOC was decreased substantially by 100μM PQ by 100μM PQ by 98.8% while 12.5μM, 25μM, 50μM reduced the T-AOC levels by 62.8%, 77.7%, 87.6%, respectively. **p<0.01 Vs the control.

for 1 hour and the fluorescence intensity was measured. Contents of $O_2^{\bullet-}$ and H_2O_2 produced by treatment of PQ were presented and compared (**Figures 6** and **7**). In the DHE group, levels of $O_2^{\bullet-}$ increased drastically in a concentration-independent manner. In the DCF-DA group, levels of H_2O_2 elevated substantially. From 12.5μM, 25μM to 50μM, the levels of H_2O_2 increased in a concentration-dependent manner and dropped slightly at the point of 100μm.

3.2. Protective Effects of Antioxidant Enzymes on the PQ-Induced Cytotoxicity of HL-60 Cells

3.2.1. The Protective Effects of Antioxidant Enzymes on the PQ-Induced Antiproliferation of HL-60 Cells

Treated with 50μM PQ for 12 hours, HL-60 cells were subsequently incubated with GSH, NAC, SOD, CAT and GSH-inhibitor BSO for 48 hours during which the proliferation of cells was examined by Trypan blue exclusion every 8 hours. Within either 24 hours or 48 hours, GSH, NAC, SOD and CAT improved the proliferation of PQ-treated Hl-60 cells (**Figure 8(a)**) where the combination of BSO with GSH had substantially compromised the potency of GSH in enhancing the proliferation of cells. In particular, the protective effects of antioxidant enzymes performed better in 48 hours than in 24 hours (**Figures 8(a)** and **8(b)**). Among the four antioxidant enzymes, GSH, NAC and CAT demonstrated relatively potent effects on enhancing the proliferation of PQ- treated cells while the effect conferred by SOD wasn't significant. To screen the most efficient antioxidant enzyme for protecting HL-60 cells against PQ toxicity, cytotoxicity of these oxidant enzymes on HL-60 cells was also examined by treatment with 50μM PQ, 100μM GST, 200μM NAC, 200U/ml SOD, 400U/ml CAT and 50μM BSO, respectively (**Figure 9**). Therefore, GST was identified to have exerted least cytotoxicity on HL-60 cells while NAC and CAT were moderately effective in extenuating the PQ-induced cytotoxcity, factoring into their inherent minor toxicities on HL-60 cells.
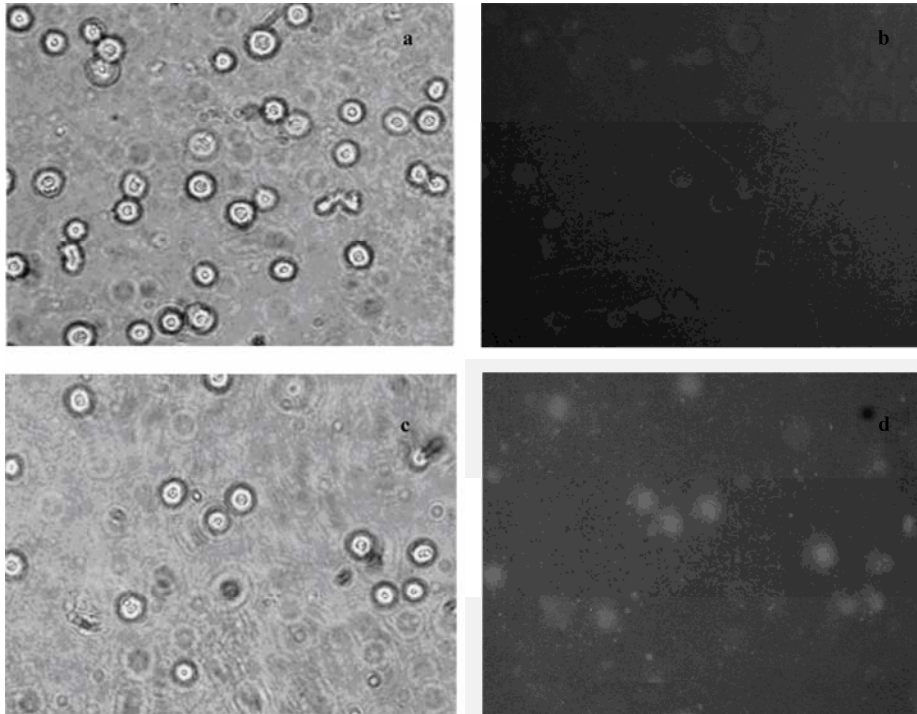
Figure 4. Determination of the intracellular production of O2$^{\bullet-}$ by fluorescent inverted microscope. (a) and (b) were HL-60 cells, whereas (c) and (d) were 50μM PQ-treated HL-60 cells. (a) and (c) were taken under visible light whereas (b) and (d) were taken fluorescent light with excitation wavelength as 485 nm and emission wavelength as 610 nm.
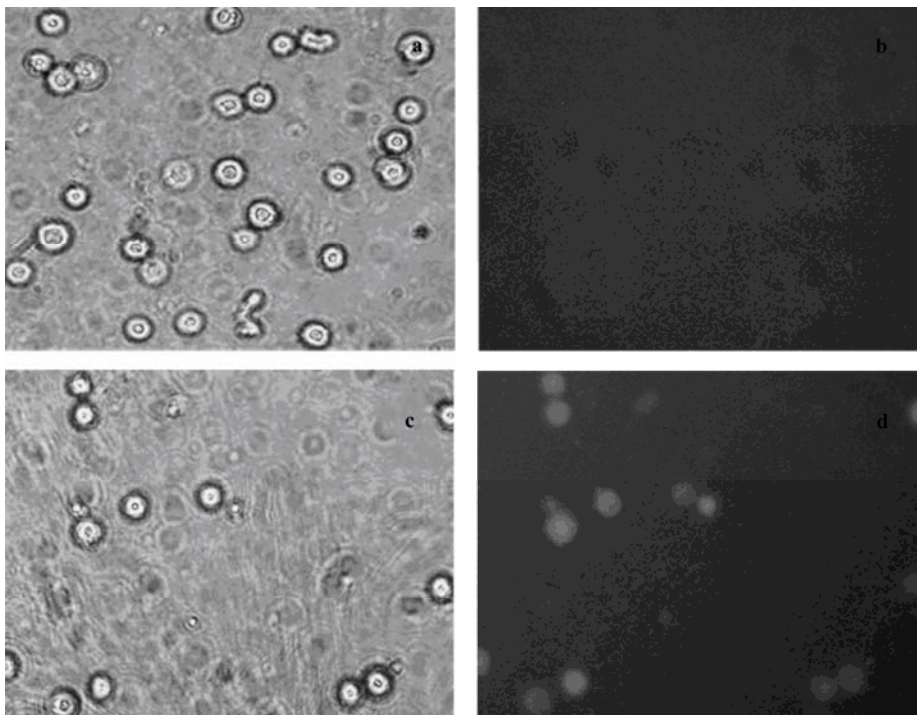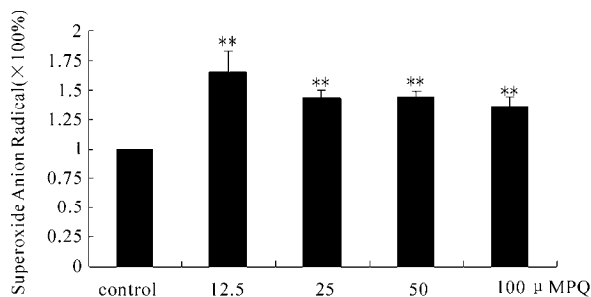

Figure 5. Determination of the intracellular production of H_2O_2 by fluorescent inverted microscope. (a) and (b) were HL-60 cells, whereas (c) and (d) were 50μM-treated HL-60 cells. (a) and (c) were taken under visible light whereas (b) and (d) were taken fluorescent light with excitation wavelength as 485 nm and emission wavelength as 525 nm.

Figure 6. Quantification of $O_2^{\cdot-}$ upon treatment with desired concentrations of PQ. Control was presented as the basal level of $O_2^{\cdot-}$ of the HL-60 cells. **p<0.01 Vs the control.
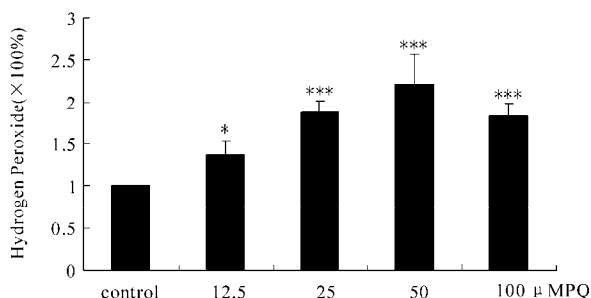

Figure 7. Quantification of H_2O_2 upon treatment with desired concentrations of PQ. Control was presented as the basal level of H_2O_2 of the HL-60 cells. *p<0.05 and ***p<0.001 Vs the control.
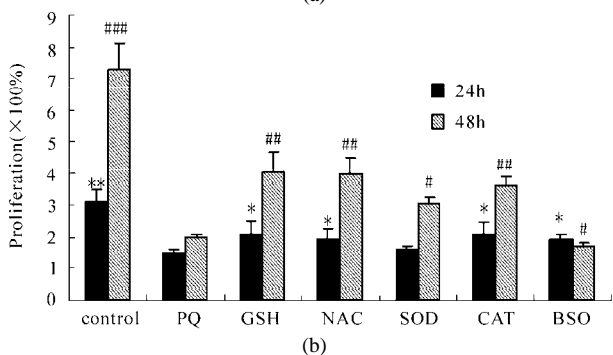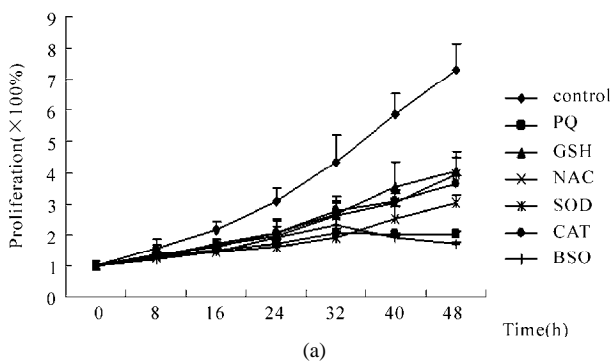

(a)

(b)

Figure 8. Effects of various antioxidant enzymes on the enhancing the proliferation of PQ-treated HL-60 cells. (a) Time-course profile of proliferation of HL-60 cells upon treatment with 50μM PQ, 100μM GST, 200μM NAC, 200 U/ml SOD, and 400 U/ml CAT and 25μM BSO, respectively. (b) Cytoprotective effects of 100μM GST, 200μM NAC, 200 U/ml SOD, and 400 U/ml CAT and 25Mm BSO examined on a range of both 24 hours and 48 hours.

3.2.2. Effects of Antioxidative Enzymes on Regulating Levels of MDA and T-AOC

HL-60 cells grown to confluent were treated with 50μM PQ for 12 hours. Levels of MDA were measured after incubating PQ-treated HL-60 cells with 100μM GST, 200μM NAC, 200U/ml SOD, 400U/ml CAT for 12 hours. Compared with the control group, GST greatly decreased the level of MDA by 25.1% whereas NAC, CAT and SOD also reduced the level of MDA by 18.5%, 14.9%, 4.4%, respectively (**Figure 10**). Similarly, after treatment with 100μM GST, 200μM NAC, 400U/ml CAT and 200U/ml SOD for 12 hours, the level of T-AOC had been elevated significantly. Based on the ground level set by the 50μM PQ, GSH, NAC, CAT and SOD increased the level of T-AOC by 183.3%, 89.8%, 101.3% and 114.2%, respectively (**Figure 11**).
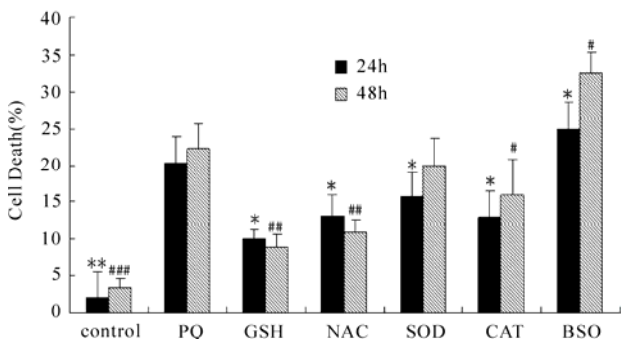

Figure 9. Cytotoxical effects of antioxidant enzymes on HL-60 cells. With HL-60 cells as control group, cytotoxicity indicated by the proliferation of cells was examined by treatment with 50μM PQ, 100μM GST, 200μM NAC, 200U/ml SOD, 400U/ml CAT and 50μM BSO. *p<0.05 and **p<0.01 Vs PQ for 24 hours incubation; #p<0.05, ##p<0.01 and ###p<0.001Vs PQ for 48 hours incubation.
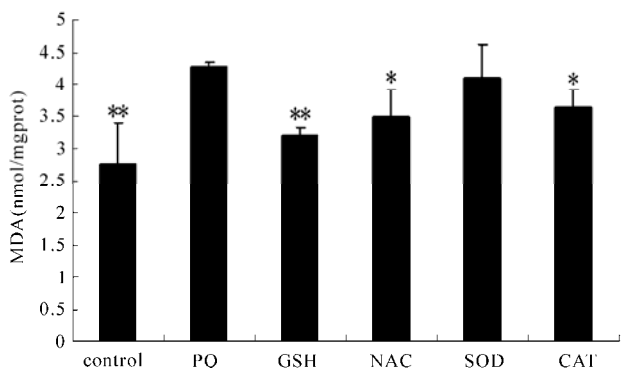

Figure 10. Modulation of MDA levels by treatment with antioxidant enzymes. With 50μM PQ-treated HL-60 cells as control and the intracellular MDA level of HL-60 cells as basal level, MDA level was reduced upon treatment with 100μM GST, 200μM NAC, 200U/ml SOD and 400U/ml CAT by 25.1%, 18.5%, 14.9%, 4.4%, respectively. *p<0.05 and **p<0.01 Vs 50μM PQ.
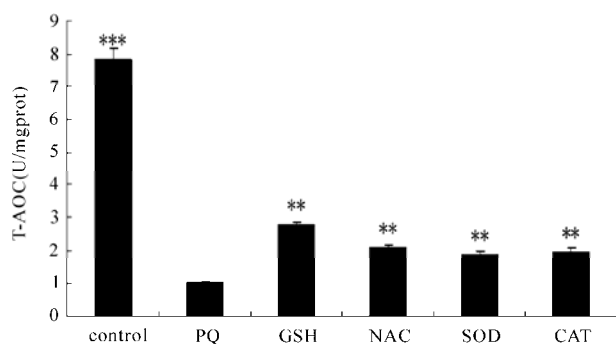
Figure 11. Increase of T-AOC levels upon treatment with antioxidant enzymes. With 50μM PQ-treated HL-60 cells as control and the intracellular T-AOC level of HL-60 cells as basal level, T-AOC levels were elevated by treatment with 100μM GST, 200μM NAC, 200U/ml SOD and 400U/ml CAT by 183.3%, 114.2%, 89.8% and 101.3%, respectively. *p<0.05 and **p<0.01 Vs 50μM PQ.

4. DISCUSSION

PQ has been well-documented to be able to cause a range of progressive and irreversible diseases to animal species and humans which are refractory to treatment [38-42]. As it used worldwide in agriculture, the toxicities of PQ on its untargeted organism are supposed to be well-administered. A wide spectrum of investigations pivoting on the treatment of PQ-induced diseases on humans have been conducting to either quantify the safe dosage [43,44], or to clinically monitor the long term evolution of PQ-caused disease [45]. Although its pathological mechanism remains mainly elusive, the contribution of oxidative stress in the pathogenic process has been evidenced [32]. However, the inadequacy to elucidate the oxidative stress-initiated pathway at the tissue level with different animal models necessitates relevant studies of that pathway at the cellular level. Toward this end, the PQ-induced injury model of HL60-cells was established to study the ROS-mediated cytotoxicity on HL-60 cells.

Antiproliferative effects of PQ on HL-60 cells and proliferation-enhancing effects of antioxidant enzymes on PQ-treated HL-60 cells were examined by Trypan blue exclusion assaying, respectively. The threshold of PQ concentration with physiological relevance was determined with finding that PQ concentration below 5μM conferred no pronounced effect on the cell growth and proliferation while PQ concentration above 200μM led directly to the death of all HL-60 cells. With concentration ranging from 12.5μM to 100μM, the antiproliferative effect of PQ was observed to be concentration-dependent, which was in agreement with a recent finding that production of hydrogen peroxide may contribute to the induction of apoptosis on HL-60 cells [46]. Subsequently, 50μM PQ was experimentally chosen to treat HL-60 cells for studying other physiochemical relevance.

Indicative of the ROS-induced damage to cellular components and ability of scavenging overproduced amounts of ROS, MDA and T-AOC were examined quantitatively to determine the cytotoxicity of PQ on HL-60 cells, respectively. Relatively high concentrations of PQ (50-100μM) caused an appreciable increase in the MDA level while the T-AOC level dropped accordingly in a concentration-dependent manner. It is generally consistent with the finding that various concentrations of PQ increased peroxidation in PC12 cells [24]. Thus, the involvement of imbalance between the oxidative competence and antioxidant capacity was determined in the PQ-induced cytotoxicity. Furthermore, the intracellular production of $O_2^{\bullet-}$ and H_2O_2 was confirmed by fluorescent inverted microscope through hybridizing cell samples with DHE and DCF-DA probes and quantified by employment of flow cytometric analysis. Determination of the intracellular production by treatment with various concentrations of PQ suggested that production of $O_2^{\bullet-}$ wasn't sensitive to the concentrations of PQ, whereas the intracellular production of H_2O_2 was in a concentration-dependent manner. That finding was in part consistent with the report that PQ (0.01-1mM) induced the intracellular burst of ROS in rat cortical neurons [47].

In our study, effects of GSH, NAC, SOD and CAT on extenuating PQ-induced cytotoxicity on HL-60 cells were further examined, following the treatment with 50μM PQ. In explicit agreement with the finding that levels of antioxidant enzymes increased in inverse proportion to the levels of ROS [48,49]. Among 100μM GST, 200μM NAC, 400U/ml CAT and 200U/ml SOD which were experimentally administered to examine their potency, 100μM GSH was determined to reduce the MDA level by 25.1% and increase the T-AOC level significantly by 183.3%. 200μM NAC functioned well with its performance on reducing MDA level and enhancing T-AOC level by 18.5% and 89.8%, respectively. As a parallel, BSO, which was the inhibitor of GSH, was also used to exclude the false positive results of the efficacy conferred by antioxidant enzymes. Hopefully, 100μM GSH was identified as the most effective antioxidant enzymes for attenuating the PQ-induced cytotoxicity, in combination with examining the inherent cytotoxicity of all antioxidant enzymes on the HL-60 cells.

5. CONCLUSIONS

In this study, a causal role can be determined for the involvement of oxidative damage in the PQ-induced cytotoxic effects on HL-60 cells. Upon treatment with various concentrations of PQ, the compromised capacity of HL-60 cells to scavenge the intracellular overproduced $O_2^{\bullet-}$ and H_2O_2 was responsible for the cytotoxicity of HL-60 cells on grounds that the levels of $O_2^{\bullet-}$, H_2O_2, MDA, T-AOC were altered unfavorably in the physiological level. Fur-

thermore, effects of antioxidant enzymes on attenuating the PQ-induced cytotoxicity of HL-60 cells were also examined to have identified the GSH as the most effective one for extenuating the PQ-induced cytotoxicity. However, whether it could be used as an ingredient in developing PQ safener remains to be settled. Moreover, further characterizing the underpinning mechanisms involved in PQ cytotoxicity might contribute substantially to a comprehensive understanding of how environmental toxicants including pesticides and herbicides lead to the increasingly more frequent outbreaks of a plethora of environment-associated diseases.

6. ACKNOWLEDGEMENTS

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