Effects of astaxanthin on oxidative stress induced by Cu^{2+} in prostate cells^{*}

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Abstract: Astaxanthin (AST), a carotenoid molecule extensively found in marine organisms and increasingly used as a dietary supplement, has been reported to have beneficial effects against oxidative stress. In the current paper, the effects of AST on viability of prostate cells were investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay; cell apoptosis and intracellular reactive oxygen species (ROS) levels were determined by flow cytometry; the mitochondrial membrane potential (MMP) was measured by fluorospectrophotometer; and activities of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were evaluated by a detection kit. The results show that copper ion (Cu^{2+}) induced apoptosis, along with the accumulation of intracellular ROS and MDA, in both prostate cell lines (RWPE-1 and PC-3). AST treatments could decrease the MDA levels, increase MMP, and keep ROS stable in RWPE-1 cell line. An addition of AST decreased the SOD, GSH-Px, and CAT activities in PC-3 cell line treated with Cu^{2+} , but had a contrary reaction in RWPE-1 cell lines. In conclusion, AST could contribute to protecting RWPE-1 cells against Cu^{2+} -induced injuries but could cause damage to the antioxidant enzyme system in PC-3 cells.

Key words: Oxidative stress; PC-3; RWPE-1; Astaxanthin; Copper ion

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1 Introduction

Prostate cancer is the second most frequently diagnosed cancer and the second leading cause of cancer-related death in men; the incidence and mortality of this disease are high in both North America and Western Europe, and currently low, but increasing, in Asia. Considerable evidence indicates that both genetic and environmental factors are primarily involved in its evolution.

Copper ion (Cu^{2+}) is an essential trace element for human health. An imbalance in the metabolism of

Cu^{2+} could be an etiologic factor for prostate cancer development. It participates in a variety of important metabolic pathways in free radical forms, such as superoxide dismutase (SOD) scavenging intracellular free radicals and cytochrome oxidase transmitting respiratory chain electron. Low intracellular Cu^{2+} concentrations could influence the activities of these enzymes and the normal metabolisms of the cells. Interestingly, the redox properties of the metal also mediate its toxicity because uncontrolled production of reactive oxygen species (ROS) results in oxidative stress, which does not follow a correct antioxidant response and consequently damages the biological macromolecules such as nucleic acids, proteins, and lipids (Adler *et al.*, 1999; Auten and Davis, 2009; Maltepe and Saugstad, 2009; Linder, 2012). Under normal conditions, all processes involved in copper

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intake, distribution, utilization, and excretion are precisely regulated (Rosenzweig and O'Halloran, 2000; Kim *et al.*, 2008; de Feo *et al.*, 2009; Banci *et al.*, 2010; Festa and Thiele, 2011; Haas *et al.*, 2011).

Both exogenous and endogenous sources contributed to the formation of intracellular ROS (Winterbourn, 2008). Exogenous sources include radiation and environmental agents. Major endogenous sources of cellular ROS are microsomes, peroxisomes, and mitochondria. Other endogenous sources of ROS include enzymes such as xanthine oxidase, amino-acid oxidases, lipoxygenase, and cyclo-oxygenase. Superoxide release, as a result of the activity of the latter two enzymes, could be especially important in prostate cancer because of prostaglandin biosynthesis (Schewe, 2002). In addition, deregulated androgen signaling increases ROS in prostate cancer (Ripple *et al.*, 1997; Sun *et al.*, 2001; Tam *et al.*, 2003; Frohlich *et al.*, 2008; Basu *et al.*, 2009), which is consistent with the results of other studies that prostate cancer development is associated with oxidative stress (Paschos *et al.*, 2013).

Antioxidants, especially carotenoids, play an important role in the regulation of the oxidative process. They have strong antioxidant effects due to their double-bonded structures, allowing for their delocalization of impaired electrons. In recent years, the interests in astaxanthin (AST; 3,3'-dihydroxy- β - β -carotene-4,4'-dione) have been continuously growing. AST is a type of carotenoid, with antioxidant activity that is 100–1000 times greater than that of vitamin E. AST is commonly found in crustaceans such as shrimp and crab, as well as marine organisms such as salmon, krill, and algae (Barros *et al.*, 2014). As reported, dietary supplementation with AST has beneficial effects in the treatments of inflammation, cardiovascular disease, and oxidative damages, suggesting that AST is a functional food ingredient (Ohgami *et al.*, 2003; Pashkow *et al.*, 2008; Fassett and Coombes, 2009; Preuss *et al.*, 2009). However, there are no reports about the effect of AST on oxidative stress in prostate cell lines, especially in prostate epithelial (RWPE-1) and prostate cancer (PC-3) cell lines treated with Cu^{2+} .

In this paper, the effects of AST on Cu^{2+} -induced oxidative stress in prostate cells and prostate cancer cells are investigated.

2 Materials and methods

2.1 Materials

RWPE-1 and PC-3 cell lines were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Purified preparations of AST and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO, USA). RPMI 1640 was purchased from GIBCO (Grand Island, NY, USA), and Annexin V-fluorescein isothiocyanate (FITC), ROS, SOD, catalase (CAT), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) detection kits were obtained from Beyotime (Nantong, China). Other reagents used in our research were of analytical grade.

2.2 Cell culture

Both cell lines were cultured in RPMI 1640 supplemented with 10% (0.1 g/ml) fetal bovine serum and 100 U/ml penicillin-streptomycin. The cells were maintained at 37 °C and 5% CO_2 in a humid environment, and stock solutions of AST and CuSO_4 were prepared.

2.3 Determination of cell viability

Cells were harvested and counted, then seeded on a 96-well plate (1×10^5 PC-3 cells/well, 5×10^4 RWPE-1 cells/well) for 12 h. Different concentrations of CuSO_4 and AST were prepared with serial dilutions ranging from 100 to 180 $\mu\text{mol/L}$ and 0.001 to 10 $\mu\text{mol/L}$, respectively (Shen, 2014). AST was dissolved in dimethyl sulfoxide (DMSO) as a stock solution. For cell treatment, the AST was diluted in RPMI 1640 to obtain the final concentration, which was then added in sextuplicate to the cells (DMSO concentration in a dilution was $<0.01\%$). After a 24-h or 48-h incubation, 20 μl of 5 mg/ml MTT reagent was added to each well. The cells were further incubated for 4 h in the dark, before 150 μl of DMSO was added and fully vibrated. Absorbance was measured at 490 nm (A_{490}) using a microplate reader (Thermal Lab system, Finland). The relative cell viability (%) was calculated as (A_{490} of treated samples/ A_{490} of untreated samples) $\times 100\%$.

2.4 Annexin-V/PI double-staining and flow cytometry

Apoptosis was double-stained with Annexin V-FITC and propidium iodide (PI) according to the manufacturer's instructions (Annexin V-FITC apoptosis detection kit) (Saha *et al.*, 2003). Live cells were shown as Annexin V-negative and PI-negative (A^-/PI^- , B3). Since cells with sustained plasma membrane integrity did not take up the PI, cells stained with Annexin V but not with PI were considered to be in the early stages of apoptosis (A^+/PI^- , B4). In the late apoptosis stage or the necrosis secondary stage to apoptosis, the cell membrane lost its integrity, allowing staining of the cells with both Annexin V and PI (A^+/PI^+ , B2). Cells stained only with PI (A^-/PI^+ , B1) were considered necrotic. Briefly, RWPE-1 (5×10^4 cells/well) and PC-3 (1×10^5 cells/well) were seeded into six-well plates and treated with Cu^{2+} and AST for 48 h, washed with an ice-cold phosphate buffer solution (PBS), and subsequently stained with a 100- μ l incubation buffer containing 50 μ g/ml Annexin V-FITC and 50 μ g/ml PI at 37 °C in the dark for 15 min. Treated cells were immediately analyzed in a flow cytometer (FC500, Beckman Coulter, USA).

Intracellular ROS levels were measured using the cell permeable substrate 2',7'-dichlorofluorescein diacetate (DCFH-DA), a converted detectable fluorescent product (Rajeshkumar *et al.*, 2015). After 48 h incubation, the cells were washed with a cold PBS solution prior to adding 1 ml PBS containing 10 μ mol/L DCFH-DA (excitation: 488 nm; emission: 525 nm), and then continually incubated for 20 min in the dark at 37 °C. The treated cells were immediately washed and resuspended in PBS before ROS production was measured by using flow cytometry (FC500, Beckman Coulter, USA). The data were analyzed with CellQuest software.

2.5 Measurement of mitochondrial membrane potential

In order to measure the mitochondrial membrane potential (MMP), the cell suspension was incubated with 10 μ mol/L Rh123 (excitation: 488 nm; emission: 534 nm) for 10 min in the dark at 37 °C, then immediately washed and resuspended in PBS for three separate times. The fluorescence was analyzed via a fluorospectrophotometer (Cary Eclipse, Varian, USA) (Brawek *et al.*, 2010).

2.6 Measurements of MDA, SOD, CAT, and GSH-Px

RWPE-1 and PC-3 cells were seeded in 6-well plates at a density of 5×10^4 and 1×10^5 cells/well, respectively. Subsequently, the cells were treated with Cu^{2+} and AST. After a 48-h incubation, cells were collected for measurements of MDA, SOD, CAT and GSH-Px using a detection kit according to the manufacturer's instructions. The results were corrected for their protein levels based on the reference manual for a BCA protein assay kit (Changsha Auragene Bioscience, Hunan, China).

2.7 Statistical analysis

All data were reported as the mean $\pm$ standard deviation (SD). Comparison between groups was made by one-way analysis of variance (ANOVA), using SPSS software (Version 21.0). Each experiment was carried out in triplicate. A *P*-value of <0.05 is considered statistically significant.

3 Results

3.1 Effects of AST and Cu^{2+} on viabilities of RWPE-1 and PC-3 cells

To investigate the cytotoxicities of AST and Cu^{2+} on the viabilities of RWPE-1 and PC-3 cells, concentration- and time-dependent experiments were designed and carried out (Fig. 1). Cells were incubated with AST and Cu^{2+} of increasing initial concentrations (0.01 to 10 μ mol/L and 100 to 180 μ mol/L, respectively) for either 24 or 48 h and the cell viability was determined by performing MTT assay. AST induced a significant increase of PC-3 cells. Treatment with higher concentrations of AST (more than 0.01 μ mol/L) for 48 h resulted in significant increases of RWPE-1 cells. Supplementation of Cu^{2+} to PC-3 cells resulted in an increase of cell viability after 24 h, while the growth was significantly suppressed after 48 h. For RWPE-1 cells, Cu^{2+} treatment did not lead to significant decreases after 24 h. Moreover, it was shown that Cu^{2+} treatment at low concentrations could promote cell growth (up to 129.8% for the viability) after 48 h, while 180 μ mol/L Cu^{2+} treatment inhibited the growth of cells to 70.6%. Eventually, the effects of different concentrations of AST (0.001, 0.1, and 10 μ mol/L as low dose (LD), middle dose

Fig. 1 Effects of AST and Cu²⁺ on cell viabilities of RWPE-1 and PC-3 cells

AST promoted PC-3 cell proliferation in 24 and 48 h. AST promoted RWPE-1 cell proliferation in 48 h while inhibited cell proliferation in high concentrations in 24 h. Cu²⁺ increased cell viability of PC-3 cells in 24 h, while suppressed cell viability in 48 h. Cu²⁺ treatment did not decreased viability of RWPE-1 cells in 24 h. Cu²⁺ promoted RWPE-1 cell growth in low concentrations, but inhibited RWPE-1 cell growth in high concentrations in 48 h. Data are expressed as mean±SD (*n*=3)

(MD), and high dose (HD), respectively) on the oxidative damage of 120 μmol/L Cu²⁺-treated prostate cells were studied.

3.2 Effects of AST on apoptosis of RWPE-1 and PC-3 cells in the presence of Cu²⁺

To further confirm effects of AST on apoptosis in both cells induced by Cu²⁺, Annexin V-FITC/PI double staining was performed. Representative dot plot diagrams and specific percentages, obtained through flow cytometry of the RWPE-1 and PC-3 cells treated with different concentrations of AST for 48 h, were shown in Figs. 2 and 3 and Table 1. In the non-apoptotic, viable control cells, Annexin V-FITC-negative and PI-negative staining was found in B3.

The Cu²⁺ had a greater effect on the apoptosis of PC-3 cells because the percentage of cells in the late apoptosis stage or the necrosis secondary stage to apoptosis (A⁺/PI⁺, B2) increased significantly in the presence of Cu²⁺, which increased the dot numbers of RWPE-1 and PC-3 in B2 from 0.01% to 15.47% and 21.50%, respectively. However, similar results were obtained in AST treatment groups and there were no significant differences when compared with the Cu²⁺ treatment group. The population of cells progressed to advanced apoptosis because Cu²⁺ could induce cell apoptosis progression and the AST treatment seemed to have no clear indication of protective effects on apoptosis for the RWPE-1 or PC-3 cells.

Fig. 2 Effects of AST on cell apoptosis in Cu^{2+} -treated RWPE-1 cells

RWPE-1-CK: control group; RWPE-1- Cu^{2+} : RWPE-1 cells treated with $120 \mu\text{mol/L}$ Cu^{2+} ; RWPE-1- $10 \mu\text{mol/L}$: RWPE-1 cells treated with $10 \mu\text{mol/L}$ AST and $120 \mu\text{mol/L}$ Cu^{2+} ; RWPE-1- $0.1 \mu\text{mol/L}$: RWPE-1 cells treated with $0.1 \mu\text{mol/L}$ AST and $120 \mu\text{mol/L}$ Cu^{2+} ; RWPE-1- $0.001 \mu\text{mol/L}$: RWPE-1 cells treated with $0.001 \mu\text{mol/L}$ AST and $120 \mu\text{mol/L}$ Cu^{2+}

Fig. 3 Effects of AST on cell apoptosis in Cu^{2+} -treated PC-3 cells

PC-3-CK: control group; PC-3- Cu^{2+} : PC-3 cells treated with $120 \mu\text{mol/L}$ Cu^{2+} ; PC-3- $10 \mu\text{mol/L}$: PC-3 cells treated with $10 \mu\text{mol/L}$ AST and $120 \mu\text{mol/L}$ Cu^{2+} ; PC-3- $0.1 \mu\text{mol/L}$: PC-3 cells treated with $0.1 \mu\text{mol/L}$ AST and $120 \mu\text{mol/L}$ Cu^{2+} ; PC-3- $0.001 \mu\text{mol/L}$: PC-3 cells treated with $0.001 \mu\text{mol/L}$ AST and $120 \mu\text{mol/L}$ Cu^{2+}

Table 1 Effects of AST on the apoptosis of the RWPE-1 and PC-3 cells treated with Cu²⁺

Treatment	B1 (%)		B2 (%)		B3 (%)		B4 (%)	
	RWPE-1	PC-3	RWPE-1	PC-3	RWPE-1	PC-3	RWPE-1	PC-3
CK	0.02±0.01 ^c	0.03±0.01 ^c	0.01±0.01 ^b	0.01±0.00 ^b	99.97±0.01 ^a	99.96±0.01 ^a	0.00±0.00 ^c	0.00±0.00 ^b
Cu ²⁺	0.72±0.04 ^a	0.23±0.01 ^a	15.47±0.33 ^a	21.50±0.42 ^a	80.98±0.60 ^b	75.64±0.55 ^b	2.82±0.31 ^b	2.64±0.15 ^a
HD	0.51±0.10 ^b	0.15±0.01 ^b	16.28±1.12 ^a	21.63±2.14 ^a	79.35±1.43 ^b	75.57±2.11 ^b	3.85±0.35 ^a	2.65±0.16 ^a
MD	0.43±0.08 ^b	0.23±0.05 ^a	14.32±0.63 ^a	21.02±0.59 ^a	80.74±0.83 ^b	76.64±0.69 ^b	4.50±0.22 ^a	2.11±0.30 ^a
LD	0.51±0.04 ^b	0.23±0.03 ^a	16.28±0.22 ^a	23.38±1.75 ^a	79.15±0.35 ^b	74.24±1.84 ^b	4.06±0.19 ^a	2.15±0.11 ^a

Results were presented as the mean±SD (*n*=3). Different lowercase letters in the same column indicated significant differences, *P*<0.05. CK: control group; Cu²⁺: 120 μmol/L Cu²⁺ group; HD: 10 μmol/L AST+120 μmol/L Cu²⁺ group; MD: 0.1 μmol/L AST+120 μmol/L Cu²⁺ group; LD: 0.001 μmol/L AST+120 μmol/L Cu²⁺ group. B1: necrotic cells; B2: late apoptotic cells; B3: live cells; B4: early apoptotic cells

3.3 Effects of AST on ROS production in RWPE-1 and PC-3 cells treated with Cu²⁺

The cells were stained with DCFH-DA to examine a level of ROS before a fluorospectrophotometer was applied. The data in Fig. 4 show that the ROS level in PC-3 cells treated with 120 μmol/L Cu²⁺ was higher than that in the untreated groups. The addition of AST significantly impaired Cu²⁺-induced ROS production. The HD-treated groups showed better scavenge ability resulting in a decrease of ROS production as compared with the control groups. In contrast, Cu²⁺ induced a significant decrease of ROS accumulation in RWPE-1 cells, implying that Cu²⁺ treatment can induce greater damage to PC-3 cells.

3.4 Effects of AST on MMP levels in RWPE-1 and PC-3 cells treated with Cu²⁺

In order to characterize the changes in the mitochondrial events induced by Cu²⁺ treatment, we observed the collapse of MMP in both cells with Rh123. When the MMP level decreased, the mitochondrial cell membrane was partially destroyed, so that Rh123 could be released into a matrix, causing the enhancement of the fluorescence intensity. As shown in Fig. 5, the addition of different concentrations of AST (LD, MD, and HD) on RWPE-1 cells reversely reduced MMP; therefore, the fluorescence intensity decreased from 143.0% to 79.6%, 67.0%, and 26.0% of the level in the control group (100%), respectively. Moreover, the addition of AST resulted in MMP reduction in a concentration-dependent manner in PC-3 cells induced by Cu²⁺ treatment. These results suggest that AST protected RWPE-1

Fig. 4 Effects of AST on ROS production in Cu²⁺-treated RWPE-1 and PC-3 cells

Values are expressed as mean±SD (*n*=3). ** *P*<0.01 compared with control; ## *P*<0.01 compared with Cu²⁺ group

Fig. 5 Effects of AST on MMP in Cu²⁺-treated RWPE-1 and PC-3 cells

Values are expressed as mean±SD (*n*=3). ** *P*<0.01 compared with control; ## *P*<0.01 compared with Cu²⁺ group

cells against apoptosis induced by Cu^{2+} by reversing the down-regulation of MMP.

3.5 Effects of AST on MDA level and SOD activity in RWPE-1 and PC-3 cells treated with Cu^{2+}

ROS overproduction could interfere with the intracellular redox homeostasis leading to oxidative stress. To determine whether AST was able to release cell oxidative stress induced by Cu^{2+} , the intracellular MDA levels were determined after treatment as described above. As shown in Fig. 6, exposure of both kinds of cells to Cu^{2+} resulted in significant increases in the intracellular MDA levels, but the MDA levels in PC-3 cells were lower. However, an addition of LD AST effectively reduced the intracellular MDA generation in RWPE-1 cells, whereas MD and HD AST treatments resulted in a significant increase of MDA in PC-3 cells, indicating that AST accelerated oxidative stress induced by Cu^{2+} in PC-3 cells.

SOD is an important enzyme that is crucial for the prevention of diseases correlated to oxidative stress. As shown in Fig. 6, the treatment with Cu^{2+} resulted in a significant decrease of SOD activity in RWPE-1 and PC-3 cells to 57.1% and 53.8%, respectively. In contrast, HD treatment of AST significantly increased SOD activity, as compared with the control group, while LD treatment resulted in a significant decrease of SOD activity in PC-3 cells, indicating that a low concentration of AST may decrease its ability to scavenge ROS in PC-3 cells.

3.6 Effects of AST on GSH-Px and CAT in RWPE-1 and PC-3 cells treated with Cu^{2+}

GSH-Px enzyme, one of the most important antioxidant enzymes in the human body, functions as a detoxification of hydrogen peroxide. As shown in Fig. 6, the treatment with Cu^{2+} resulted in a significant decrease of GSH-Px activity in RWPE-1 and PC-3 cells,

Fig. 6 Effects of AST on the oxidative enzyme system in Cu^{2+} -treated RWPE-1 and PC-3 cells
Values are expressed as mean $\pm$ SD ($n=3$). ** $P < 0.01$ compared with control; # $P < 0.05$, ## $P < 0.01$ compared with Cu^{2+} group

only reaching to 54.2% and 71.8%, respectively, of the level of the control group; the addition of AST significantly increased GSH-Px activity in RWPE-1 cells and displayed concentration-dependent decreases in both cells, as compared with the cells only treated with Cu^{2+} . However, after AST treatment, the GSH-Px activity in PC-3 cells was lower than that after only Cu^{2+} treatment in all groups. In summary, AST could increase the activity of GSH-Px in RWPE-1 cells treated with Cu^{2+} , resulting in oxidative injury.

CAT enzyme could remove hydrogen peroxide in vivo to protect the cells; thus, it is one of the key enzymes in the antioxidant enzyme system. As shown in Fig. 6, treatment with Cu^{2+} still resulted in a significant decrease of CAT in both types of cells, and the CAT level in RWPE-1 cells was significantly higher than that in PC-3 cells in all the treated groups. The addition of AST did not reverse CAT reduction in PC-3 induced by Cu^{2+} , while the LD concentration of AST increased CAT level significantly in RWPE-1 cells.

4 Discussion

The intrinsic balance could be interfered by several environmental stresses. ROS are among the most potent and omnipresent threats faced by any living organism. Intracellular accumulation of ROS such as superoxide anion, hydrogen peroxide, singlet oxygen, hydroxyl radical, and peroxy radical, usually results from toxic reactions or normal metabolic processes. These oxidative modified products might interfere with several functions in cancer cells, such as cell proliferations and genetic mutations. Inherent oxidative stress is a potent factor of angiogenesis and is considered to be involved in pathophysiology of cancers (Kuroki *et al.*, 1996). ROS, also termed oxidants, were common by-products of the standard aerobic cellular metabolism, and were continuously generated in cells and synchronously scavenged by an array of antioxidant mechanisms (Dröge, 2002). Oxidative stress results from an imbalance between ROS production and the cell antioxidant defense capability, and causes reactions among those oxidative molecules and lipids, proteins and DNA (Montezano and Touyz, 2012; Ambati *et al.*, 2014). Oxidative stress had been clarified as an interference factor in the

prooxidant-antioxidant balance, resulting in potential cell damage (Sies, 1985). In our results, Cu^{2+} accelerated ROS production in prostate cancer cells. We determined that prostate cancer cells could be more sensitive to oxidative stress induced by Cu^{2+} .

AST is an orange-pinkish carotenoid extensively found in marine organisms, which have various biological activities such as antioxidant, anti-cancer, and anti-inflammatory activities (Preuss *et al.*, 2009; Yang *et al.*, 2013; Kimura *et al.*, 2014). Some results showed that AST protected neuronal cells against oxidative damage, and thus it has been a potent candidate for brain food (Liu and Osawa, 2009), and additionally could protect PC12 cells against glucose toxicity (Zhang *et al.*, 2015). On the other hand, Cu^{2+} could lead to various cell injuries by inducing oxidative stress and mitochondrial dysfunction (Murphy and Taiz, 1997; Mira *et al.*, 2002; Ma *et al.*, 2014). Cu^{2+} -induced production of hydrogen peroxide and hydroxyl radicals has been directly correlated with the damage to proteins and lipids (Wang *et al.*, 2015). However, there have been no reports about the effects of AST on oxidative stress induced by Cu^{2+} in prostate cells. As an excellent anti-oxidant, AST suppressed ROS production in prostate cancer cells. Also, AST increased the ROS of normal prostate cells treated with Cu^{2+} to the same level as the control. Our results confirmed that AST could regulate oxidant status in prostate cells.

ROS initiated autocatalytic lipid peroxidation, and generated various potential genotoxic breakdown products, including alkoxyl radicals, peroxy radicals, and aldehydes such as MDA. MDA has been used for many years as a convenient biomarker for lipid peroxidation because of its facile reaction with thiobarbituric acid to form an intensely colored chromogen (Rodríguez-Sureda *et al.*, 2015). In this study, Cu^{2+} induced apoptosis along with the accumulation of intracellular ROS and increased MDA levels in both cells. In turn, AST treatment could reduce ROS and MMP accumulation and increase their MDA levels. According to our results, AST could have protective effects against Cu^{2+} -induced oxidative damage.

There were several enzyme systems (SOD, CAT, and GSH-Px) that catalyzed reactions clearing free radicals and ROS. These form the body's endogenous defense mechanisms to protect against cell damages induced by free radicals. First, SOD, a

major cytoplasmic antioxidant enzyme, catalyzed the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxides which, in turn, were converted by either GSH-Px or CAT to water and oxygen, thus providing a combined enzymatic action against oxygen toxicity. The imbalance of SOD to GSH-Px and CAT resulted in the accumulation of H_2O_2 which might participate in the Fenton's reaction and consequently lead to the formation of noxious hydroxyl radicals (de Haan *et al.*, 1996). The present study investigated the antioxidant enzyme expression in long-term cultures of prostate cells. Considering the results, Cu^{2+} could significantly reduce SOD, GSH-Px, and CAT in both cells as shown in Fig. 6. The addition of AST decreased the SOD, GSH-Px, and CAT activities in PC-3 cells induced by Cu^{2+} , while they were up-regulated in RWPE-1 cells. It is speculated that AST could be able to protect RWPE-1 cells against Cu^{2+} -induced damage on the cell antioxidant enzyme system, and to a certain extent, promote enzyme activity.

5 Conclusions

Our data clearly demonstrated that AST has a potential ability to inhibit Cu^{2+} -induced oxidative stress in RWPE-1 cells. The underlying pathway was associated with the properties of AST to scavenge ROS, to restore MMP, and to increase antioxidant enzyme activity. However, AST induced the damage of the antioxidant system induced by Cu^{2+} in PC-3 cells, specifically through its regulation on the levels of CAT, GSH-Px, and MMP. This study therefore provided insight into the protective effects of AST on Cu^{2+} -induced oxidative stress. The molecular mechanism of AST against Cu^{2+} -induced oxidative stress still needs further exploration. Importantly, in vivo investigation is also required to assess whether AST could be a potential therapeutic agent for releasing Cu^{2+} -induced toxicity in prostate cancer.

Compliance with ethics guidelines

Hong-zhou MENG, Xiao-feng NI, Hai-ning YU, Shan-shan WANG, and Sheng-rong SHEN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题目: 虾青素对铜离子诱导的前列腺细胞氧化损伤的影响
目的: 研究虾青素对铜离子诱导的前列腺细胞氧化损伤的影响, 并探索其作用机制。

创新点: 首次研究虾青素对铜离子诱导的前列腺细胞及前列腺癌细胞氧化损伤的影响, 并比较其对两种细胞作用的差异。

方法: MTT 法测定铜离子与虾青素对前列腺细胞(RWPE-1)和前列腺癌细胞(PC-3)生长的影响; 采用细胞流式仪测定虾青素对铜离子诱导的RWPE-1和PC-3细胞凋亡的影响; 荧光分光光度法测定了虾青素对铜离子诱导的活性氧自由基(ROS)产生的影响; 采用罗丹明 123 (Rh123) 染色检测虾青素对铜离子诱导的细胞线粒体膜电位(MMP)变化的影响; 采用试剂盒测定了虾青素对铜离子存在下丙二醛(MDA)含量、超氧化物歧化酶(SOD)、过氧化氢酶(CAT)及谷胱甘肽过氧化物酶(GSH-Px)活性变化的影响。

结论: 结果表明, 铜离子能诱导 RWPE-1 和 PC-3 细胞凋亡, 并伴随细胞内 ROS 和 MDA 含量升高; 虾青素处理可显著降低 RWPE-1 细胞中 MDA 含量, 升高线粒体膜电位, 并保持 ROS 含量稳定; 虾青素处理可降低 PC-3 细胞中 SOD、GSH-Px 和 CAT 的活性, 而对 RWPE-1 细胞则作用相反。因此, 虾青素处理能有效降低铜离子对 RWPE-1 细胞引起的损伤, 而通过降低抗氧化酶活性加剧铜离子对 PC-3 细胞的损伤。

关键词: 氧化损伤; PC-3 细胞; RWPE-1 细胞; 虾青素; 铜离子