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The effect of ellagic acid on photodynamic therapy in leukemia cells

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Abstract. Ellagic acid (EA) is a naturally phenolic acid presented in different foods. It has a variety of biological activities including antioxidant, anti-inflammatory, anti-microbiological and anti-cancer properties. On account of its antioxidant activity, EA might protect cancer cells from free radical damage in photodynamic therapy (PDT) during which reactive oxygen species (ROS) production was stimulated leading to irreversible tumor cell injury. In this study, the influence of EA on K562 cells in 5-aminolevulinic acid (ALA)-based PDT is demonstrated. Cell apoptosis was assayed by flow cytometry. Oxidative damage induced by PDT was investigated by measurement of malondialdehyde (MDA). Comet assay was used to evaluate the potential genotoxic effect induced by PDT on the cells. The results showed that EA supplementation alone did not affect the lipid peroxidation, DNA damage and apoptosis in K562 cells. It increases the lipid peroxidation, DNA damage, apoptosis and decreases the survival rate in K562 cells induced by ALA-PDT. The singlet oxygen quencher sodium azide suppresses apoptosis, lipid peroxidation and DNA damage induced by EA in PDT. In conclusion, EA consumption during PDT did not decrease the effectiveness of cancer therapy on malignant cells. The effect of antioxidants on PDT maybe was determined by its sensitization ability to singlet oxygen.

Key words: Ellagic acid — Reactive oxygen species — Photodynamic therapy — Sodium azide — Leukemia K562

Introduction

Photodynamic therapy (PDT) is an approved treatment for several types of cancer as well as for age-related macular degeneration (Martijn et al. 2006). Clinical trials continue to expand the role of PDT in cancer and in the treatment of localized microbial infections, as reviewed in (Brown et al. 2000; Agostinis et al. 2011). PDT includes loading of the target cells with a photosensitizer and subsequent illumination with visible light (Ion 2010). When the oxygen is present, the combination of light and photosensitizer causes generation of reactive oxygen species (ROS), in particular singlet oxygen ($^1O^2$), superoxide anion and hydroxyl radical (Glasauer and Chandel 2014), resulting in target cell death either through necrosis or apoptosis (Acedo et al. 2014).

The initial photochemical processes leading to cell death may follow two principal pathways: The photosensitizer molecule absorbs a light photon and becomes an excited triplet state (Castano et al. 2006; Garland et al. 2009). The excited photosensitizer molecule can then transfer energy to molecular oxygen to produce singlet oxygen (type II reaction) or suffer electron transfer (type I reaction) to form superoxide radical anion and/or hydroxyl radicals (Mroz et al. 2010; Ogilby et al. 2010). Both pathways can occur simultaneously and the ratio between them depends on the photosensitizer and the nature of the substrate. However, direct and indirect evidence supports a prevalent role for $^1O^2$ in the molecular processes initiated by PDT (Niedre et al. 2002; Agostinis et al. 2011). 5-Aminolevulinic acid (ALA) is the precursor of photosensitizer protoporphyrin IX (PPIX) in the biosynthetic pathway of heme. Following the exogenous administration of ALA, tumors selectively accumulate PPIX,

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which under suitable irradiation generates either cytotoxic species leading to cellular damage. Therefore, ALA is widely used as a topical drug in PDT (Wu et al. 2003; Zhang and Zhang 2004).

Consumption of fruits and vegetables is known to lower the risk of several diseases. Such health benefits are mainly attributed to the content of antioxidant compounds most notably polyphenols including gallic and ellagic acids, as reviewed in (Crozier et al. 2009). Ellagic acid (EA) is a naturally polyphenolic compound found in different fruits and nuts like pomegranate, red raspberry, strawberry, blue berry and walnut. It has a variety of biological activities including antioxidant (Rehman et al. 2012), anti-inflammatory (Rogerio et al. 2008), anti-microbiological (Zambuchini et al. 2008) and anti-cancer (Oidovsambuu et al. 2013; Zhao et al. 2013) properties. The anti-cancer properties of EA include induction of cell cycle arrest and apoptosis (Larrosa et al. 2006). The molecular mechanisms responsible for these effects remain largely unknown, but its potent scavenging action on ROS might be involved (Rehman et al. 2012).

The mechanism of PDT exerted on tumor cell killing is the production of ROS. These oxidative actions may be modified by the phytochemicals present in food. Although studies involving use of antioxidants during cancer therapy are promising, research on this topic is still scarce and controversial (González et al. 2005, 2006; Ozben 2007). Studies indicate that supplementation with dietary antioxidants may improve the efficacy of radiation therapy by increasing tumor response and decreasing some of its toxicity on normal cells (Prasad et al. 2001). The other suggests that dietary antioxidants should not be used during radiation therapy, because they would protect cancer cells against radiation damage (Salganik 2001). Each of these is based on different conceptual frameworks that are derived from results obtained from specific experimental designs, and thus, each may be correct within its parameters.

Ellagic acid has antioxidant properties and it was popular in diet and medicine (Türk et al. 2010). It should be noticed that any antioxidant found to reduce toxicity of tumor therapy on healthy tissue has the potential to decrease effectiveness of cancer therapy on malignant cells. To assess whether EA interferes with ALA-PDT treatment, the present study investigated the antioxidant activity of EA and demonstrated the influence of EA on PDT.

Material and Methods

Chemical reagents

5-aminolevulinic acids, L-glutamine, trypan blue and EA were purchased from Sigma Co., Ltd. (USA), RPMI-1640 medium was from Gibco Co., Ltd. (USA). Dimethyl sulfoxides (DMSO), penicillin and streptomycin were obtained from Solarbio (China). Newborn calf serum (NCS) was from Sijiqing (China). Annexin V-FITC-PI Apoptosis Detection Kit was obtained from centre-Bio Co., Ltd. (China). Other chemicals used in the present study were of analytical grade.

Equipments

Apoptosis was analyzed on a four-color fluorescence capability FACScalibur flow cytometer (BD Biosciences, USA). The fluorescence spectra were measured with the 970CRT fluorophotometer (Shanghai SANCO Instrument Co., Ltd, China). The fluorescence images of comet assay were examined with a fluorescence microscope (Olympus, Japan). The radiation source was a xenon lamp (USHIO, Japan).

Cell culture

K562 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) newborn calf serum, 1% (v/v) L-glutamine and 100 units/ml antibiotics (penicillin and streptomycin) at 37°C in 5% CO^2 humidified incubator.

EA treatment

EA was dissolved in 100% DMSO and stored at –20°C. For the cell growth assay, cells were seeded into six-well plates at a density of 1×10^5 cells/ml and were treated with EA or with DMSO only (as control) in triplicates. The final concentration of DMSO was kept at less than 0.05%. For the dose-dependent experiment, cells were treated with EA for 24 h, respectively. For the time-dependent experiment, cells were treated with EA and detected at 12, 24, 36 and 48 h.

Photodynamic treatment

K562 cells in the exponential phase of growth were harvested and suspended in RPMI 1640 medium at a density of 1×10^5 cells/ml. Cells were incubated for 4 h with 1 mM ALA at 37°C. Then, cells were seeded in six-well plates and illuminated with a light intensity of 350 mW/cm^2 and a light doses of 105 J/cm^2 at 37°C. K562 cells were incubated with EA for 20 min at 37°C before ALA-PDT irradiation. The radiation source is a xenon lamp emitting wavelengths over the range 400–800 nm, which encompassed the activation wavelengths for PPIX (420–800 nm).

Cell viability assay

K562 cells were seeded into 6-well plates at a density of 1×10^5 cells/ml incubated at 37°C, 5% CO^2. After experiments, cells

were stained by 0.2% trypan blue solution and monitored on a hemacytometer by a light microscopy. The percent of cell survival was calculated as follows:

% Survival = (survival /control) ×100 %

Determination of MDA

The analysis of malondialdehyde (MDA) as a marker of lipid peroxidation end products was carried out according to the previous report of Jentzsch et al. (1996). To assay the lipid peroxidation, 2 ml of cells suspensions (3×10^5 cells/ml) were centrifuged at 1000 rpm for 3 min, resuspended in 2 ml Hank's, and mixed with 240 µl 10% SDS (sodium dodecyl sulphate) at room temperature. After 20 min, the reaction mixture was deproteinized with 1.2 ml 10% trichloroacetic acid for 10 min. Then, 1 ml homogenate was centrifuged at 4000 rpm for 10 min, the lysate was resuspended in 4 ml distilled water in a tube, and 1 ml of 0.45% thiobarbituric acid was added. This tube was boiled for 1.2 h and cooled for 2–3 min at room temperature. Then 2.5 ml n-butanol was added and centrifuged at 3000 rpm for 15 min. The supernatant was obtained and the fluorescence intensity at 546 nm was determined using a fluorophotometer excited at 530 nm. The standards of MDA were prepared by acid hydrolysis of 1,1,3,3-tetramethoxypropane. A calibration curve was used to calculate MDA concentration, and results were expressed in nanograms of MDA *per* 10^6 cells.

Flow cytometry

Cell apoptosis was assayed by using Annexin V-FITC-PI apoptosis detection kit. Cells were washed twice with cold PBS (0°C) and resuspended in binding buffer at 1×10^6 cells/ml. First, 400 µl cells solution was incubated with 10 µl of 20 µg/ml Annexin V–FITC for 15 min at 4–8°C in the dark, then incubated with 10 µl of 50 µg/ml PI for another 5 min at 4–8°C in the dark, and finally analyzed by flow cytometry at room temperature.

Comet assay

DNA damage was quantified by the comet assay as described previously (Singh et al. 1988). Cell solution at 3×10^5 cells/ml in cool PBS (0°C) mixed with same volume of 1% low melting point agarose in PBS was pipetted onto glass slides on ice precoated with 2% normal melting point agarose. After 10 min, the slides were incubated in lysis buffer (200 mM NaOH, 2.5 M NaCl, 100 mM EDTA-$Na^2.2H^2O$ and 1% (w/v) N-lauroyl sarcosine (sodium salt), pH 12.5–13.0) at 0°C for 1 h. Slides were then washed and incubated in alkaline unwinding buffer (300 mM NaOH and 1 mM EDTA-$Na^2.2H^2O$, pH 12.5–13.0) for 20 min. Following electrophoresis for 20 min at 6.28 V in unwinding buffer, nuclei were stained with 0.02% (v/v) ethidium bromide for 20 min at room temperature. Digital images of 30 cells were randomly captured for analysis by Casp-2.2 analysis software. "% DNA in tail" was calculated as the extent of DNA damage.

Statistical analysis

Data were processed using EXCEL analysis software (Microsoft Co., Washington, USA) and expressed as means ± SD. Differences between the groups were assessed by the two-tailed Student's t-test for unpaired samples. Results were considered significantly different when $p < 0.05$ was obtained.

Results

Influence of EA on cell survival

To analyze the effect of EA on K562 cell growth/proliferation, we treated K562 cells cultures with different concentrations of EA (0–100 µM) for 48 h. As showed in Fig. 1, EA inhibited cell proliferation in a time-dependent manner. After 48 h incubation, the viable cells in the culture treated with EA were reduced comparing with those in control ($p < 0.05$). While the concentration of EA was 10 µM, the lowest concentration used in this study, also have antiproliferative activity ($p < 0.05$). Cell apoptosis was examined by flow cytometry using

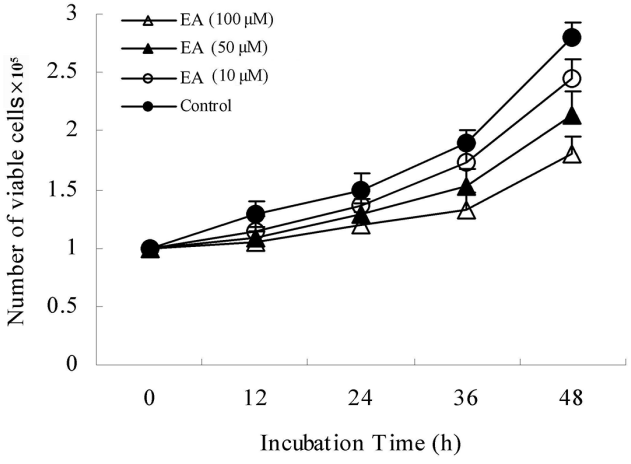

Figure 1. Ellagic acid (EA) inhibits K562 cells growth. Equal amounts of inoculants (1.0×10^5 viable cells) were seeded in the cultures containing different concentration of EA (0–100 µM). Results represent means ± SD of three independent experiments.

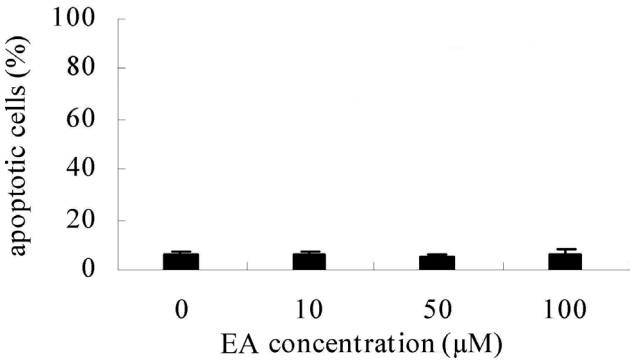

Figure 2. Effects of EA on apoptotic. The data were assessed 24 h incubation. Results represent means ± SD of three independent experiments.

Annexin V-FITC-PI Apoptosis Detection Kit. The result indicates that EA (0–100 μM) could not induce apoptosis in K562 cells 24 h after incubation (Fig. 2)

Influence of EA on cell survival in ALA-PDT treatment

To investigate the effect of EA on PDT we measured cell survival and cell apoptosis after EA and PDT treatment. As showed in Fig. 3, Incubation cell with light alone or ALA alone in the dark did not decrease cell viability. After cell with ALA and light, cell survival was significantly decreased. Approximately 35% cells survived in the ALA-PDT. In comparison with untreated cells (control) the increase in apoptosis (approximately 20%) was observed after ALA-PDT treatment.

When cells were exposed to EA and PDT, a dose-dependent decrease in cell survival was observed compared to cells exposed to PDT alone (Fig. 3, $p < 0.05$). Cell apoptosis were examined by flow cytometry. In comparison with untreated cells (control), the increase in apoptosis (22.87%) was observed after ALA-PDT treatment. EA enhanced the apoptosis induced by ALA-PDT in a dose-dependent manner (Fig. 3). When EA supplemented together with sodium azide (NaN^3, 100 μM) under the same condition, EA could not enhanced the apoptosis in K562 cells induced by ALA-PDT (Fig. 4, $p > 0.05$).

Influence of EA on lipid peroxidation and DNA damage in ALA-PDT treatment

In parallel with measurement of the influence of EA and PDT on cell survival, we examined the lipid peroxidation and DNA damage after treatments**.** Result was assessed 24 h after ALA-PDT irradiation. MDA produced was measured to evaluate the lipid peroxidation. MDA amount was determined by measuring the change of fluorescence intensity by the fluorophotometry. DNA damage was quantified with the comet assay. "% DNA in tail" was calculated as the extent of DNA damage. K562 cells were incubated with EA for 20 min at 37°C before ALA-PDT irradiation. EA supplementation alone did not affect the MDA concentration

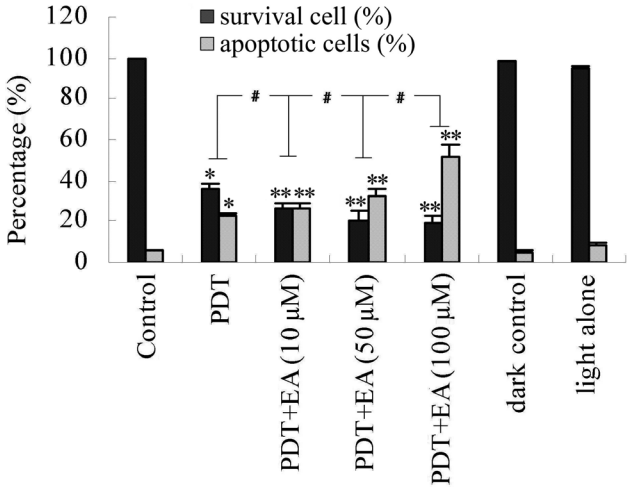

Figure 3. Effects of EA on cell survival and apoptotic 24 h after ALA-PDT irradiation. K562 cells were incubated 20 min prior ALA-PDT irradiation with different concentrations of EA (0–100 μM). Results represent means ± SD of three independent experiments. # $p < 0.05$; * $p < 0.05$ *vs.* control; ** $p < 0.05$ *vs.* ALA-PDT.

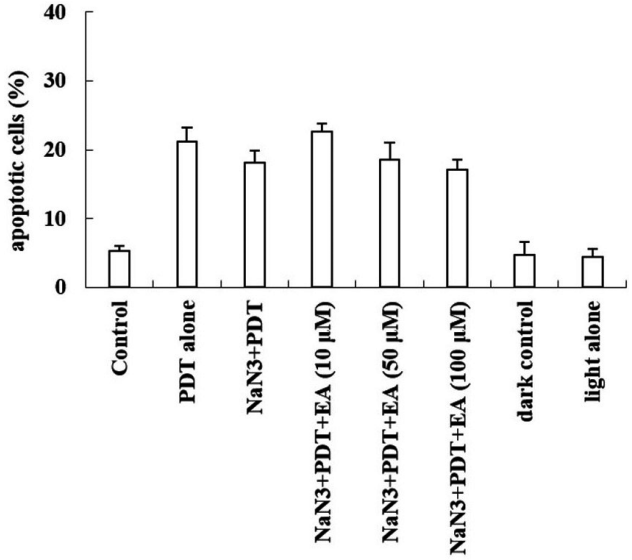

Figure 4. Effects of NaN^3 combined with EA on apoptotic in ALA-PDT treatment. K562 cells were incubated 20 min prior ALA-PDT irradiation with different concentrations of EA (0–100 μM) and NaN^3. Results represent means ± SD of three independent experiments.

and percentage DNA in tail compared to control cells as showed in Figs. 5A and 6A ($p > 0.05$). Significant increase in the amounts of MDA and level of DNA damage was found in K562 cells after ALA-PDT treatment compared with control cells or cells treated with ALA dark (dark control) or light alone (Figs. 5B and 6B, $p < 0.05$). EA (0–100 μM) enhanced the lipid peroxidation and DNA damage in K562 cells induced by ALA-PDT ($p < 0.05$). With regard to the effect of EA supplemented together with sodium azide on PDT-induced DNA damage and lipid peroxidation in K562 cells, DNA strand breakage and MDA amount in only PDT together with sodium azide group and supplemented EA group was not significantly different (Figs. 7 and 8, $p > 0.05$).

Discussion

Dietary antioxidants, such as vitamin E, ascorbic acid, and polyphenols, have been shown to have protective effects against a number of diseases in humans, including cancer and heart disease, as well as to be able to counteract the deleterious effects of many carcinogens. The beneficial effects of these compounds are typically credited to their antioxidant activities, although inhibition of cellular mediators of cell death, including protein kinases and eicosanoids has also been postulated (Gopalakrishna and Gandimeda 2002).

It has been suggested that EA biological effects may be partially due to its high free-radical scavenging activity (Rehman et al. 2012). EA is a phenolic compound that has been shown previously to have potent anticarcinogenic/anti-mutagenic properties, in addition to its strong antioxidant activity (Pari and Sivasankari 2008). EA has hydrogen atoms available for abstraction and can remain stable through delocalization of electrons across the conjugated ring and side chains. Also, these hydrogen atoms may form hydrogen bonds reducing free radicals affinity to the DNA. Several studies indicated that EA had a scavenging action against both oxygen and hydroxyl radicals, and inhibited

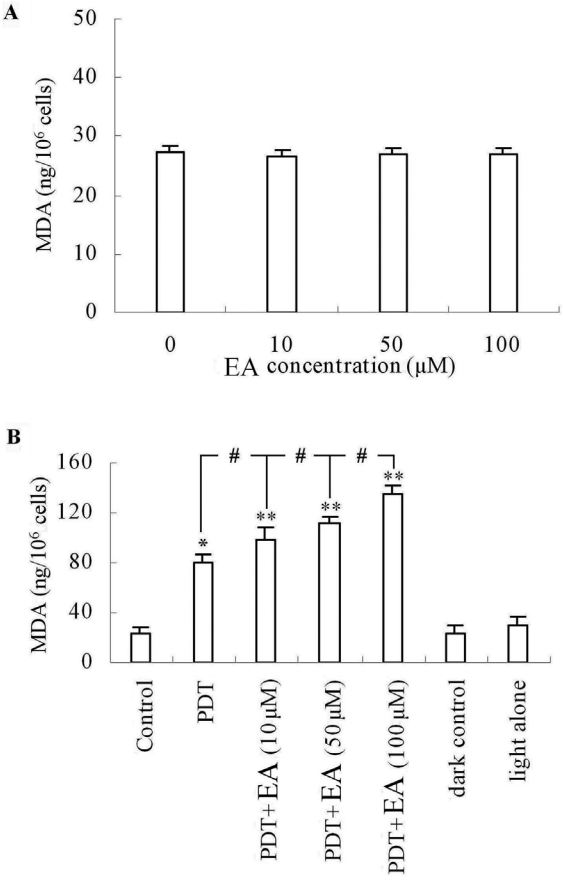

Figure 5. Influence of EA on lipid peroxidation in K562 cells with (**A**) and without (**B**) PDT treatment (24 h incubation). Results represent means ± SD of three independent experiments. # $p < 0.05$; * $p < 0.05$ *vs.* control; ** $p < 0.05$ *vs.* ALA-PDT.

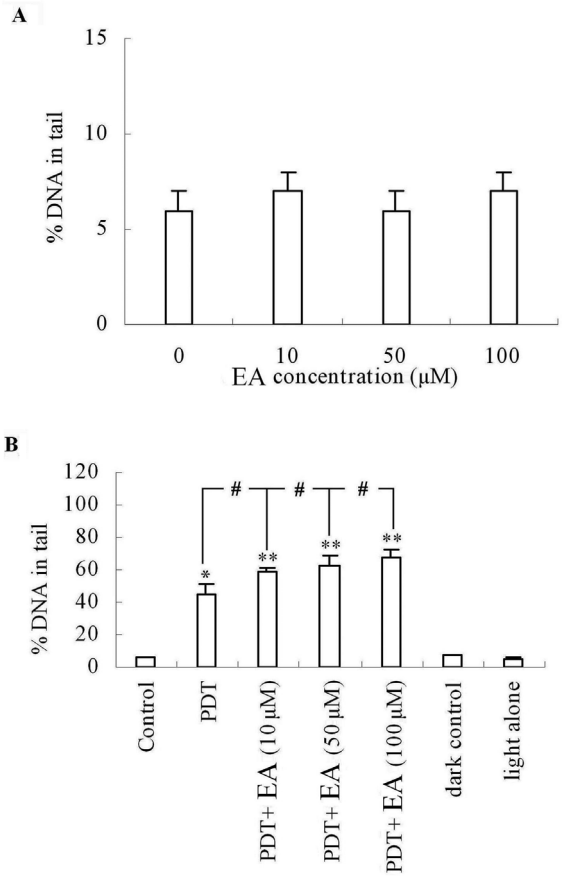

Figure 6. Influence of EA on DNA damage in K562 cells with (**A**) and without (**B**) PDT treatment (24 h incubation). Results represent means ± SD of three independent experiments. # $p < 0.05$; * $p < 0.05$ *vs.* control; ** $p < 0.05$ *vs.* ALA-PDT.

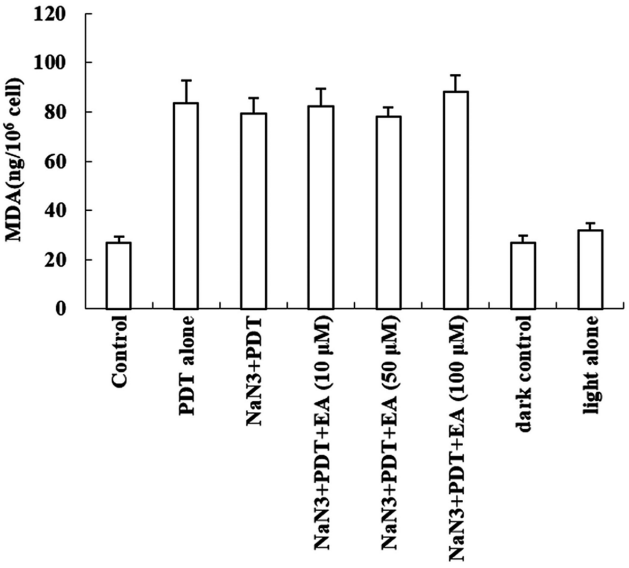

Figure 7. Effects of NaN^3 combined with EA on lipid peroxidation in PDT treatment. K562 cells were incubated 20 min prior ALA-PDT irradiation with different concentrations of EA (0–100 μM) and NaN^3. Results represent means ± SD of three independent experiments.

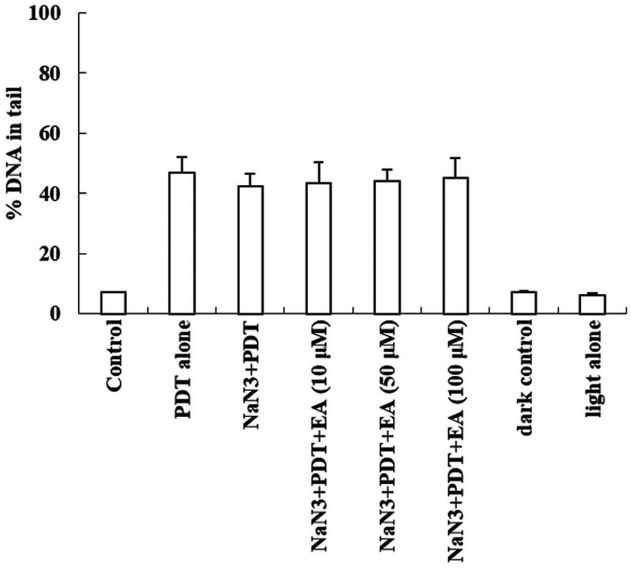

Figure 8. Effects of NaN^3 combined with EA on DNA damage in PDT treatment. K562 cells were incubated 20 min prior ALA-PDT irradiation with different concentrations of EA (0–100 μM) and NaN^3. Results represent means ± SD of three independent experiments.

lipid peroxidation and 8-OhdG formations *in vitro* and *in vivo* (Laranjinh et al. 1996; Vattem and Shetty 2003). Such findings implied that EA may decrease the effectiveness of cancer therapy which produces ROS to kill malignant cells. The present study focuses on the effect of EA on ROS-mediated ALA-PDT in K562 cells. The cell line K562 which is derived from the patient with chronic myelogenous leukaemia (CML) is commonly employed as the "*in vitro*" model of the blast phase of this disease.

It is apparent that high numbers of cancer patients are using antioxidants with or without the knowledge of their oncologist. The use of antioxidants during cancer therapy is currently a debated topic because of some contradictory findings (Akbas et al. 2006; Al-Sherbini et al. 2009; Sun et al. 2009). Some data indicate that antioxidants can protect healthy cells and tissues from the damage of free radical without affecting treatment efficacy (Akbas et al. 2006). On the other hand, other researches suggested that antioxidant decrease the effectiveness of cancer therapy on malignant cells (Richardson et al. 2000; Seifried et al. 2003). Combining these results, further research on antioxidants and chemotherapy are now warranted. This research mainly discusses the influence of EA on PDT.

Only a few studies have been published to date concerning antioxidants in photodynamic treatment. The role of antioxidants in PDT is only marginally examined. Frank et al. discovers that increasing intracellular concentrations of vitamin C contribute to the resistance of cultured cancer cells to prooxidant treatment modalities as ALA-PDT (Frank et al. 2006). Al-Sherbini et al. (2009) found that high concentrations of vitamin E enhance the PDT action against HeLa cervical cancer cell line. Rubio and colleagues (Rubio et al. 2014) demonstrates that in PDT-treated cells a p38MAPK-regulated pathway coordinates the p62/NBR1mediated clearance of cytosolic aggregates and mitigates PDT-induced proteotoxicity. Peter Ferenc and colleagues (Ferenc et al. 2010) demonstrated that pre-treatment with tyrosine kinase inhibitor genistein may significantly improve the effectiveness of PDT with hypericin in MCF-7 and MDA-MB-231 breast cancer cells. Our previous study (Zhang et al. 2012) found the soyabean isoflavones genistein and daidzein did not decrease the effectiveness of cancer therapy on malignant cells.

Ellagic acid is one of the most interesting substances with proapoptotic and antioxidant action that determines apoptosis, down regulation of IGF-II, activates p21 (waf1/Cip1) a cyclin-dependent kinase inhibitor able to arrest the cell cycle at the G1, and prevents destruction of p-53 gene by cancer cells. Some studies have reported that EA induces cell cycle arrest and/or apoptosis in several cancer cell lines (Losso et al. 2004; Mertens et al. 2004; Seeram et al. 2005). Larrosa et al. (2006) found a time- and dose-dependent inhibition of proliferation in cultures of the human colon cancer cell line Caco-2 after treatment with 1, 10 or 30 μM EA. Li et al. (2005) have reported that EA's antiproliferative effect was associated with cell-cycle: cells increased in the G^0/G^1-phase and decreased in the G^2/M-phase in human

bladder cancer T24 cells after treatment with 5, 10, 25 or 50 μM EA. As showed in Fig. 1, after 48 h of incubation, the viable cells in the cultures treated with EA were reduced when compared with those in the untreated cell cultures, but it was not reduced when compared with the amounts of inoculants (1.0×10^5 viable cells). The present study sustains that EA is a potent inhibitor of growth in the K562 cells *in vitro* and the growth inhibition by EA may due to cell cycle arrest. EA induce cell cycle arrest may induce K562 cells more sensitive to the toxicity of PDT.

In our study, we focused on the effect of EA on ROS-mediated cancer therapy. 5-Aminolevulinic acid-mediated photosensitization results in the formation of excessive amounts of ROS. ROS have been implicated in the induction of various types of oxidative damage to biomolecules that result several pathological events in living organisms (Ziech et al. 2010). It can induce changes in different biological tissues and cell biomolecules such as lipids, proteins, DNA or RNA (Cooke et al. 2003). Cell apoptosis was assayed by flow cytometry in present study. Oxidative damage induced by PDT was investigated by measurement of malondialdehyde (MDA), a marker of lipid peroxidation. Peroxidation of lipids is particularly destructive because the formation of lipoperoxidation products leads to a facile propagation of free radicals and membrane disintegration. Comet assay used to evaluate the potential genotoxic effect induced by PDT on the cells. It has been widely used in toxicology, radiation biology, and was introduced into the field of PDT. Comet assay is a useful technique for the detection of DNA single- and double-stranded breaks, and alkali-labile sites in individual cells after treatment with genotoxins. The major advantages of the comet assay over other methods of measuring DNA damage is that information is acquired about the distribution of DNA damage and repair in individual cells within the population, providing an intracellular distribution of damage.

The present study found that EA supplementation alone did not affect the cell apoptosis (Fig. 2), but it increased the apoptosis in K562 cells induced by ALA-PDT (Fig. 3). EA supplementation alone did not affect the MDA concentration and the level of DNA damage compared to control cells (Figs. 5A and 6A), but its supplementation increased the amounts of MDA and the level of DNA damage in ALA-PDT treatment compared to PDT treatment alone (Figs. 5B and 6B). These results imply that the direct effect of EA may not contribute to the enhancement of PDT-induced cytotoxicity in K562 cells and EA exhibits a synergistic effect on PDT. The exact reasons for the EA enhancement of radiation damage on cancer cells are unknown. We propose the following: (1) the treatment of tumor cells with antioxidant before irradiation can initiate changes in expressions of those genes which can cause differentiation, growth inhibition and/or apoptosis (Israel et al. 2000, Fig. 1), and this damage will continue to progress during the entire period of radiation therapy; and (2) antioxidant can inhibit the repair of radiation damage in cancer cells more than that in normal cells (Rutz and Little 1981).

Ellagic acid enhancements of the PDT-induced cytotoxicity in K562 cells are contradictory to the findings that it is an antioxidant that can scavenge hydroxyl radical, superoxide anion, and hydrogen peroxide. It is known that although both superoxide anion and hydroxyl radical are potentially cytotoxic, most of the oxidative damage in PDT is caused by the singlet oxygen (Agostinis et al. 2011). The different ROS have different signaling and damaging capabilities. Since singlet oxygen is highly reactive and cannot interconvert with endogenous ROS species, it is more likely to cause damage than to elicit signal transduction. Moreover, sodium azide suppresses apoptosis (Fig. 4), lipid peroxidation (Fig. 7) and DNA damage (Fig. 8) in K562 cells induced by EA in PDT support this hypothesis. These results imply that singlet oxygen could be involved in the enhance cytotoxicity effects of the EA in PDT. The finding of singlet oxygen involved in the enhance cytotoxicity effects of the EA in PDT implied that the contradicted findings on the use of antioxidants during cancer therapy may be resulted from the different sensitization activity of antioxidants on singlet oxygen. The antioxidants which can scavenge singlet oxygen may decrease the effectiveness of PDT (such as vitamin C (Frank et al. 2006)). The antioxidants which increase the amounts of singlet oxygen may exhibit a synergistic effect on PDT (such as genistein (Zhang et al. 2012), EA (in this research)).

Some research also found that EA showed significant free radical-scavenging activity which may not contribute to their antioxidant activity. Instead, EA may show antioxidant activity by inducing endogenous antioxidants such as GSH (Bhosle et al. 2005; Türk et al. 2010). This perspective may be another explanation why EA didn't have a protective effect on K562 cells. It is known that antioxidant levels are comparatively higher in normal cells than in tumor cells. Cell malignancy or transformation is often accompanied by a decrease in activity of antioxidant enzyme (SOD, catalase, GSH-Px), which increases the cell sensitivity to prooxidant compounds (Brevard et al. 2002). The susceptibility of tumor cells to radiation or drug is associated with decreased level of antioxidants (Zhang et al. 2002; Dal-Pizzol et al. 2003; Clichici et al. 2010). If EA had direct scavenging ability, a protective effect on cancer cells would be expected. In contrast, if EA revealed antioxidant activity by inducing endogenous antioxidants, a selective effect by different mechanisms could be expected in regards to inducing antioxidant enzymes between cancer cells and normal cells. Cells contain a large number of antioxidants to prevent, or repair the damage caused by ROS. Antioxidant enzyme levels are variable in most animal and human cancers, but often higher

in human tumors compared to normal tissue (Cairns et al. 2011). However, the exact mechanism of EA show antioxidant activity by inducing endogenous antioxidants in cells needed to be explored.

In conclusion, the results proved that EA enhanced the cell death in K562 cells induced by ALA-PDT. EA supplementation alone did not affect the lipid peroxidation, DNA damage and apoptosis in K562 cells. It increases the lipid peroxidation, DNA damage, apoptosis and decreases the survival rate in K562 cells induced by ALA-PDT. The singlet oxygen quencher sodium azide suppresses apoptosis, lipid peroxidation and DNA damage in K562 cells induced by EA in PDT. Consequently, these results implied that EA consumption during PDT did not decrease the effectiveness of cancer therapy on malignant cells and singlet oxygen could be involved in this process.

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