Antioxidant activity of mangostin in cell-free system and its effect on K562 leukemia cell line in photodynamic therapy

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Mangostin (MAG), a kind of xanthone widely used in diet and medicine, has antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. On account of its antioxidant activity, MAG might protect cancer cells from free radical damage in photodynamic therapy (PDT) during which reactive oxygen species production was stimulated leading to irreversible tumor cell injury. In this study, the antioxidant activity of MAG was investigated and the influence of MAG on K562 cells in 5-aminolevulinic acid (ALA)-based PDT is demonstrated. The results showed that MAG could scavenge hydroxyl radical, superoxide anion, and hydrogen peroxide and inhibit the formation of malondialdehyde (MDA), but increase the amounts of singlet oxygen in cell-free systems. MAG inhibits cell proliferation and enhances cell apoptosis, lipid peroxidation, and DNA damage in ALA-PDT on K562 cells. NaN3, a singlet oxygen quencher, suppresses the MAG-induced cell apoptosis, lipid peroxidation, and DNA damage. In conclusion, MAG enhances the PDT-induced cytotoxicity in K562 cells and singlet oxygen was involved in this process. These results implied that the effect of antioxidants on PDT might be determined by its sensitization ability to singlet oxygen.

Keywords mangostin; reactive oxygen species; photodynamic therapy; NaN3; leukemia K562

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Introduction

Photodynamic therapy (PDT) is a treatment for cancer and certain non-malignant pathologies that are generally characterized by overgrowth of unwanted or abnormal cells [1]. It includes loading of the target cells with a photosensitizer and subsequent illumination with visible light. When oxygen is present, the combination of light and photosensitizer causes the generation of reactive oxygen species (ROS), in particular singlet oxygen, superoxide anion, and hydroxyl radical [2], resulting in target cell death either through necrosis or through apoptosis [3]. The initial photochemical processes leading to cell death may follow two principal pathways: upon light absorption, the triplet state of the photosensitizer transfers the energy to triplet state O2 (3O2) to yield singlet oxygen (1O2), a potent oxidizer (type II mechanism), or alternatively photosensitized formations of superoxide anion and hydroxyl radical engaging in charge transfer reactions with biomolecules (type I mechanism). Both pathways can occur simultaneously and the ratio between them depends on the photosensitizer and the nature of the substrate. However, direct and indirect evidences support a prevalent role for 1O2 in the molecular processes initiated by PDT [4]. 5-Aminolevulinic acid (ALA)-mediated photosensitization is one of the fastest developing areas in the field of PDT. Following the exogenous administration of ALA, tumor cells selectively accumulate photosensitizer protoporphyrin IX (PPIX), which, under suitable irradiation, generates cytotoxic species leading to cellular damage. Therefore, ALA is widely used as a topical drug in PDT [5,6].

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. Mangosteen, Garcinia mangostana L. (Guttiferae), is a tree that is widespread in Thailand, India and Sri Lanka and is known for its medicine properties. Mangostin (MAG) is a kind of xanthone that exists in the pericarp of a mangosteen. Xanthone derivatives have been
reported to possess several pharmacological activities [7,8], such as antimalarial activity [9], antimicrobial activity against methicillin-resistant *Staphylococcus aureus* [10], and anti-inflammatory activity [11]. Two types of MAG, α-MAG and γ-MAG, have been used for the treatment of skin infection, wounds, and diarrhea for many years (Fig. 1) [9].

MAG has antioxidant properties and it was popular in diet and medicine. It should be noticed that any antioxidant found to reduce the toxicity of tumor therapy on healthy tissue has the potential to decrease effectiveness of cancer therapy on malignant cells. To assess whether MAG interferes with ALA-PDT treatment, we investigated the antioxidant activity of MAG and demonstrated the influence of MAG on PDT.

**Materials and Methods**

**Chemical reagents**

5-ALA, L-glutamine, and Trypan blue were purchased from Sigma Co., Ltd (St. Louis, USA). RPMI 1640 medium was from Gibco Co., Ltd (Carlsbad, USA). Dimethyl sulfoxide (DMSO), penicillin, and streptomycin were obtained from Solarbio (Shanghai, China). Newborn calf serum (NCS) was from Sijiqing (Hangzhou, China). Annexin V-FITC-PI apoptosis detection kit was obtained from Centre-Bio Co., Ltd (Beijing, China). Luminol was obtained from Aldrich and used as received (97%) (Sigma-Aldrich Fine Chemicals). Tetraethylammonium perchlorate was from Bioanalytical System Co., Ltd (California, USA). Other chemicals used in the present study were of analytical grade.

**Preparation of MAG**

MAG was prepared by the literature method with some minor modifications [12]. The crude material of α-MAG was extracted in toluene and glycol for 1 h at 80°C and successively the solution was filtered. Then the solution was extracted by glycol and the extract was dried in vacuum desiccator for complete removal of solvents. The solvent-free extracts were used for the present study. γ-MAG used in this study was purified as described in our previous study [13].

**Antioxidant activity in cell-free systems**

*Hydroxyl radical assay.* Hydroxyl radical assay was performed as described previously [14] according to the Fenton-type reaction. The reaction mixture contained 1 ml of 0.1 mM methyl violet, 0.5 ml of 5 mM FeSO₄, 0.5 ml of 1% H₂O₂, and 2 ml Tris buffer (pH 4.0), and the reaction volume being 10 ml. 0.5 ml MAG was added, and the absorbance of the reaction mixture was measured at 565 nm by a spectrophotometer. The absorbance with MAG was set as 

\[ A_s \]

the absorbance without MAG was set as 

\[ A_0 \]

and the absorbance without FeSO₄ and H₂O₂ was set as 

\[ A_0 \]

Scavenging activity (D) of MAG on hydroxyl radical was calculated according to the following formula:

\[
D = \frac{A_s - A_0}{A_0} \times 100\%
\]

DC₅₀ was defined as the concentration of MAG required to scavenge 50% of ROS.

*Superoxide anion assay.* Superoxide anion assay was performed as described previously [15]. In brief, 100 μl of 3 mM pyrogallol and 3 ml Tris buffer (pH 8.2) were mixed with 0.5 ml MAG. And the auto-oxidation rate of pyrogallol was measured by determining the changes in the absorbance at 325 nm in 4 min by UV–Vis spectrophotometer. The absorbance with MAG was set as 

\[ A_2 \]

and that without MAG was set as 

\[ A_1 \]

The scavenging activity (D) of the MAG on superoxide anion was calculated by comparing 

\[ \frac{DA_1/\text{min (without MAG)}}{DA_2/\text{min (with MAG)}} \]

of the pyrogallol system, according to the following formula:

\[
D = \frac{\Delta A_1 - \Delta A_2}{\Delta A_1} \times 100\%
\]

*Hydrogen peroxide assay.* Hydrogen peroxide assay was performed as described previously [15]. After 1.5 ml of 10 mM sodium hydroxide and 200 μl of 0.05 mM luminol were fully mixed in a chemiluminescence detector, 100 μl MAG (or methanol only as control) was added, then 100 μl of 0.2 M H₂O₂ was injected, and the...
chemiluminescence intensity was recorded. The intensity of the reaction with MAG was set as \( I_0 \) and that without MAG as \( I \). The scavenging activity (\( D \)) was calculated by the following formula:

\[
D = \left( \frac{I_0 - I}{I_0} \right) \times 100\%
\]

Singlet oxygen assay. Singlet oxygen assay was performed as described previously [16]. First, 0.5 ml of 0.2 M sodium molybdate was dissolved in 1.5 ml PBS (pH 8.4), 0.5 ml MAG was added, and 0.4 M \( \text{H}_2\text{O}_2 \) in 0.2 M sodium molybdate was dissolved in 1.5 ml PBS formed as described previously [16]. First, 0.5 ml of \( \text{TBA} \) and incubated for 1 h at 60°C. Then, 3.0 ml of 1.2 M HCl, 3.0 ml thio-barbituric acid (TBA), and 0.2 ml oleic acid were mixed and stirred thoroughly. The oleic acid phase was removed. The aqueous phase obtained was further extracted by 1-butanol, and the fluorescence intensity of the upper layer was determined after 5 min, \( \text{Ex/Em} = 541\text{nm}/563\text{nm} \), the bandwidth of excitation and emission filters being 10 nm. The intensity of the reaction with MAG was set as \( I_0 \) and that without MAG as \( I \). The scavenging activity (\( D \)) was calculated by the following formula:

\[
D = \left( \frac{I_0 - I}{I_0} \right) \times 100\%
\]

Lipid peroxidation in cell-free system. Lipid peroxidation was evaluated by determining the malondialdehyde (MDA) produced in the oxidation of oleic acid with fluorophotometry. Briefly, 3.0 ml of 1.2 M HCl, 3.0 ml thio-barbituric acid (TBA), and 0.2 ml oleic acid were mixed and incubated for 1 h at 60°C. Then 0.5 ml MAG was added and stirred thoroughly. The oleic acid phase was removed. The aqueous phase obtained was further extracted by 1-butanol, and the fluorescence intensity of the upper layer was determined after 5 min, \( \text{Ex/Em} = 541\text{nm}/563\text{nm} \), the bandwidth of excitation and emission filters being 10 nm. The intensity of the reaction with MAG was set as \( F_i \) and that without MAG as \( F \), and a blank control without oleic acid and MAG was set as \( F_0 \). The inhibitory rate (\( I \)) was calculated according to the formula:

\[
I = \left( \frac{F_i - F_0}{F - F_0} \right) \times 100\%
\]

The concentration of MAG required for obtaining 50% inhibitory rate of MDA produced was defined as \( IC_{50} \).

Study on K652 cells

Cell culture. K652 cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) NCS, 1% (v/v) L-glutamine, and 100 U/ml antibiotics (penicillin and streptomycin) at 37°C in 5% CO₂ humidified incubator.

MAG treatment. MAG was dissolved in DMSO and stored at −20°C at a concentration of 1 mM. For the cell growth inhibition assay, cells were seeded into six-well plates at a density of \( 1 \times 10^5 \) cells/ml and were treated with or without MAG in triplicates. The final concentration of DMSO was kept at less than 0.05%. The cell cultures were stained by 0.2% Trypan blue solution and monitored on a hemacytometer by a light microscopy.

For the dose-dependent experiment, K562 cell cultures were treated with MAG or with DMSO only for 24 h, respectively. For the time-dependent inhibition of MAG, K562 cells were treated with MAG or with DMSO only and cells were detected at 12, 24, 36, and 48 h. All experiments were performed in triplicates.

Photodynamic treatment. K562 cells in the exponential phase of growth were harvested and suspended in the RPMI 1640 medium at a density of \( 1 \times 10^5 \) cells/ml. Cells were incubated for 4 h with 1 mM ALA at 37°C. Then, cells were seeded into six-well plates and illuminated with a light intensity of 350 mW/cm² and a light dose of 105 J/cm². The radiation source is a xenon lamp emitting over the wavelength range 400–800 nm, which encompassed the activation wavelength of PPIX (420–800 nm).

Lipid peroxidation on K562 cells. Large amounts of reactive oxygen intermediates result in lipid peroxidation.

The analysis of MDA as a marker of lipid peroxidation end products was carried out according to the previous report of Jentzsch et al. [17]. To assay the lipid peroxidation on K562 cells, 2 ml of cell suspensions (\( 3 \times 10^5 \) cells/ml) were centrifuged at 1000 rpm for 3 min, resuspended in 2 ml Hank’s, and mixed with 240 μl 10% SDS. 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. 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 10⁶ cells.
Cell apoptosis analysis. Cell apoptosis was assayed by using Annexin V-FITC-PI apoptosis detection kit. Cells were washed twice with cold PBS and resuspended in binding buffer at $1 \times 10^6$ cells/ml. First, 400 $\mu$l cell solution was incubated with 10 $\mu$l of 20 $\mu$g/ml Annexin V-FITC for 15 min at 4–8 °C in the dark, then incubated with 10 $\mu$l of 50 $\mu$g/ml PI for another 5 min at 4–8 °C in the dark, and finally analyzed by flow cytometry.

DNA damage determination by comet assay. DNA damage was quantified by the comet assay as described previously [18]. Cell solution at $3 \times 10^5$ cells/ml in cool PBS 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 ice-cold lysis buffer [200 mM NaOH, 2.5 M NaCl, 100 mM EDTA$\text{Na}_2\cdot2\text{H}_2\text{O}$, and 1% (w/v) N-lauroyl sarcosine (sodium salt), pH 12.5–13] for 1 h. Slides were then washed and incubated in ice-cold alkaline unwinding buffer (300 mM NaOH, 1 mM EDTA$\text{Na}_2\cdot2\text{H}_2\text{O}$, pH 12.5–13) 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. Digital images of 30 cells were randomly captured for analysis by Casp-2.2 analysis software.

Statistical analysis
Data were expressed as mean ± 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

Scavenging activity of MAG on hydroxyl radical
Methyl violet reacted as a trap with the hydroxyl radical produced in the Fenton system was decolorized. The absorbance was monitored by an UV–Vis spectrophotometer. The scavenging activity of MAG was measured by determining the change in the absorbance with the addition of MAG. As shown in Fig. 2, the scavenging activity of MAG on hydroxyl radical was concentration-dependent, and the DC$_{50}$ were 0.8 and 2.6 mM, respectively, for $\alpha$-MAG and $\gamma$-MAG, indicating that the antioxidant activity of $\alpha$-MAG was higher than that of $\gamma$-MAG.

Scavenging activity of MAG on superoxide anion
The pyrogallol auto-oxidation method is widely used for superoxide anion measurement. Superoxide anion was rapidly produced by the auto-oxidation of pyrogallol in alkaline solution. Its amount was measured by determining the rate of absorbance’s change in the solution at 325 nm, and $\Delta A$ increases with time in 4 min. The scavenging activity of the antioxidant was determined by the relative change in the auto-oxidation rate ($\Delta A$/min). Figure 3 shows that $\Delta A$/min decreased as the concentration of both MAGs increased. In other words, both of them showed evident inhibitory effects on superoxide anion; compared with $\gamma$-MAG (DC$_{50}$ was 5.7 mM), $\alpha$-MAG had a relatively strong scavenging activity, with a DC$_{50}$ of 3.9 mM.

Scavenging activity of MAG on hydrogen peroxide
The measurement of H$_2$O$_2$ was based on recording the chemiluminescence signal, rapidly produced by the mixture of hydrogen peroxide and luminol in alkaline solution, and the chemiluminescence intensity was reduced with the addition of MAG because of their
antioxidant activities. As can be seen in Fig. 4, both α-MAG and γ-MAG had a strong scavenging activity on H$_2$O$_2$, the activity of α-MAG was higher than that of γ-MAG obviously.

**Sensitization action of MAG on singlet oxygen**
The singlet oxygen was formed essentially as described by Véronique [16]. It is generated in the disproportionation reaction of hydrogen peroxide catalyzed by molybdate. The chemiluminescence signal corresponding to the singlet oxygen was monitored. The scavenging activity was measured by determining the change in chemiluminescence intensity with the addition of MAG. As shown in Fig. 5, the intensity increased with the addition of both MAGs, suggesting that MAG has a sensitization activity toward the formation of singlet oxygen. The sensitization action of MAG was enhanced with the increase in its concentration. The activity of α-MAG was higher than that of γ-MAG obviously.

**Inhibitory effect of MAG on the lipid peroxidation in cell-free systems**
Cell oxidation process was imitated by the oleic acid system instead of the real cell membrane. MDA produced in this process was measured to evaluate the injury degree by the fluorophotometry, based on the formation of a colored complex between MDA and TBA in the acid medium. MAG can inhibit the oxidized process of oleic acid and prevent the formation of MDA. The scavenging activity was determined by measuring the change of fluorescence intensity. Figure 6 indicates that the inhibitory rate changes with the concentration of MAGs. MAG has strong inhibition effects on lipid peroxidation. The inhibitory effect of α-MAG was greater than that of γ-MAG. The IC$_{50}$ was 8.9 mM for α-MAG and 16.6 mM for γ-MAG.

**Influence of MAG on cell survival of K562 cells**
To analyze the effect of MAG on K562 cell growth/proliferation, we treated K562 cell cultures with different concentrations of MAGs (from 0 to 10 μM) for 48 h. As shown in Fig. 7, MAG inhibited cell proliferation in a time-dependent manner. After 48 h of incubation, the viable cells in the cultures treated with 10 μM γ-MAG were greatly reduced when compared with those in control [Fig. 7(B)]; no cell growth was observed when treated with 10 μM α-MAG [Fig. 7(A)]. These data confirmed that MAG was a potent inhibitor of cell growth in K562 cells in vitro.

Cell apoptosis analysis indicated that only 10 μM α-MAG could induce apoptosis in K562 cells 24 h after ALA-PDT irradiation and γ-MAG (0–10 μM) could not induce apoptosis in K562 cells (Fig. 8).
Influence of MAG on cell survival in ALA-PDT treatment

Cell survival was significantly decreased after ALA-PDT treatment (Fig. 9). Approximately 35% cells survived in the ALA-PDT. A dose-dependent decrease in cell survival was obtained by supplementing α-MAG (Fig. 9(A), \( P < 0.05 \)), and 10 \( \mu \text{M} \) γ-MAG also decreases the cell survival in PDT (Fig. 9(B), \( P < 0.05 \)). In comparison with untreated cells (control), the increase in apoptosis (∼20%) was observed after ALA-PDT treatment. α-MAG (0–10 \( \mu \text{M} \)) and 10 \( \mu \text{M} \) γ-MAG enhanced the K562 cell apoptosis induced by ALA-PDT. When MAG was supplemented together with 100 \( \mu \text{M} \) NaN₃ under the same condition, only 10 \( \mu \text{M} \) α-MAG enhanced the apoptosis in K562 cells induced by ALA-PDT (Fig. 10).

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

To investigate whether MAG interferes with ALA-PDT-generated cell damage, we measured its influence on cellular lipid peroxidation and DNA damage 24 h after ALA-PDT irradiation. MDA produced was measured to evaluate the lipid peroxidation. The amount survival was obtained by supplementing α-MAG (Fig. 9(A), \( P < 0.05 \)), and 10 \( \mu \text{M} \) γ-MAG also decreases the cell survival in PDT (Fig. 9(B), \( P < 0.05 \)). In comparison with untreated cells (control), the increase in apoptosis (∼20%) was observed after ALA-PDT treatment. α-MAG (0–10 \( \mu \text{M} \)) and 10 \( \mu \text{M} \) γ-MAG enhanced the K562 cell apoptosis induced by ALA-PDT. When MAG was supplemented together with 100 \( \mu \text{M} \) NaN₃ under the same condition, only 10 \( \mu \text{M} \) α-MAG enhanced the apoptosis in K562 cells induced by ALA-PDT (Fig. 10).
of MDA produced was determined by measuring the change in fluorescence intensity by the fluorophotometry. DNA damage was quantified with the comet assay. ‘%DNA in tail’ was calculated as the extent of DNA damage. γ-MAG supplementation alone did not affect the MDA concentration and the percentage of DNA in tail compared with the control cells as shown in Table 1 ($P > 0.05$). α-MAG supplementation alone enhanced the lipid peroxidation and DNA damage in K562 cells. Significant increase in the amounts of MDA and the 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 (Table 2, $P < 0.05$). α-MAG (0–10 μM) and 10 μM γ-MAG enhanced the lipid peroxidation and DNA damage in K562 cells induced by ALA-PDT ($P < 0.05$). With regard to the effect of γ-MAG supplemented together with sodium azide on PDT-induced DNA damage and lipid peroxidation in K562 cells, DNA Table 1 Influence of MAG on lipid peroxidation and DNA damage in K562 cells (24 h of incubation)

<table>
<thead>
<tr>
<th>Group</th>
<th>DNA damage (% DNA in tail)</th>
<th>MDA (ng/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-MAG</td>
<td>γ-MAG</td>
</tr>
<tr>
<td>Control</td>
<td>5 ± 0.4</td>
<td>6 ± 0.3</td>
</tr>
<tr>
<td>1 μM MAG</td>
<td>10 ± 0.9 a</td>
<td>6 ± 0.5</td>
</tr>
<tr>
<td>5 μM MAG</td>
<td>12 ± 1.3 a</td>
<td>7 ± 0.6</td>
</tr>
<tr>
<td>10 μM MAG</td>
<td>23 ± 1.7 a</td>
<td>6 ± 0.3</td>
</tr>
</tbody>
</table>

Values are reported as mean ± SD ($n = 3$). a$P < 0.05$ vs control group.

Table 2 Influence of MAG on lipid peroxidation and DNA damage in K562 cells with PDT treatment (24 h of incubation)

<table>
<thead>
<tr>
<th>Group</th>
<th>DNA damage (% DNA in tail)</th>
<th>MDA (ng/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-MAG</td>
<td>γ-MAG</td>
</tr>
<tr>
<td>Control</td>
<td>7 ± 0.6</td>
<td>7 ± 0.5</td>
</tr>
<tr>
<td>Dark control</td>
<td>5 ± 0.5</td>
<td>6 ± 0.4</td>
</tr>
<tr>
<td>Light alone</td>
<td>7 ± 0.4</td>
<td>7 ± 0.5</td>
</tr>
<tr>
<td>PDT alone</td>
<td>62.9 ± 4.3</td>
<td>60.9 ± 5.4</td>
</tr>
<tr>
<td>PDT + 1 μM MAG</td>
<td>70.5 ± 6.3 a</td>
<td>62.3 ± 7.2</td>
</tr>
<tr>
<td>PDT + 5 μM MAG</td>
<td>71.5 ± 12.4 a</td>
<td>62.4 ± 10.2</td>
</tr>
<tr>
<td>PDT + 10 μM MAG</td>
<td>84.5 ± 9.8 a</td>
<td>72.5 ± 11.6 a</td>
</tr>
</tbody>
</table>

Values are reported as mean ± SD ($n = 3$). a$P < 0.05$ vs PDT alone group.

Figure 10 Effects of NaN₃ combined with MAG on apoptotic cells in ALA-PDT treatment K562 cells were incubated for 20 min prior ALA-PDT irradiation with different concentrations of MAG (0–10 μM) and NaN₃. Results represent the mean ± SD of three independent experiments. *$P < 0.05$ vs NaN₃ + PDT.
breakage and MDA productivity in control and supplemented cells were not significantly different (Table 3, P > 0.05). Although α-MAG supplemented together with sodium azide on PDT significantly increased DNA damage in control cells (P < 0.05), MDA amount in control and supplemented cells was not significantly different (P > 0.05).

**Discussion**

Natural products have been proved to be an infinite source of remedies for diseases. Among the different dietary substances, plant foods that contain MAG showing biological activities are used as a potential anticancer drug. As is well known, MAGs (α-MAG and γ-MAG) are regarded as two of the main xanthones and can produce significant in vitro antioxidant activities. In this study, it was found that MAG can scavenge hydroxyl radical, superoxide anion, and hydrogen peroxide and inhibit the formation of MDA in cell-free systems (Figs. 2–4 and 6). Such findings suggested that MAG may decrease the effectiveness of cancer therapy which produces ROS to kill malignant cells. The present study focuses on the effect of MAG on ROS-mediated PDT. 5-ALA-mediated PDT results in the formation of excessive amounts of ROS, which can significantly alter several crucial biomolecules, including DNA, proteins (enzyme inhibition), and lipids with undesirable consequences.

The use of antioxidants during cancer therapy is currently a debated topic because of some contradicted findings [19–21]. Some data suggested that antioxidants can protect healthy cells and tissues from the damage of free radical without affecting treatment efficacy [22]. In contrast, other researchers suggested that antioxidant decreases the effectiveness of cancer therapy on malignant cells [23,24]. The role of antioxidants in PDT is only marginally examined. Only a few studies have been published to date concerning antioxidants in photodynamic treatment. Frank et al. [19] found that increasing intracellular concentrations of vitamin C contribute to the resistance of cultured cancer cells to pro-oxidant treatment modalities as 5-ALA-PDT. Shevchuk et al. [20] found that t-butyl-4-hydroxyanisole (BHA) can enhance the efficiency of PDT. Kelley et al. [21] demonstrated that loading the cells with antioxidants has no significant protection of Hypericin-photosensitized HL-60 cells.

MAG has been demonstrated to induce cell-cycle arrest and/or apoptosis in cultured cells, such as human leukemia HL60, K562, NB4, and U937 [9,25,26]. Matsumoto et al. [25] found that α-MAG showed the strong cytotoxic activity and induced apoptosis in K562 cell lines at 10 μM, the concentration at which no cytotoxic effect was observed in human peripheral blood lymphocytes. Just as shown in Fig. 1, there is an α-dihydroxy group in the structure of γ-MAG, but an α-methoxyl group in α-MAG instead. Some researchers found that the substitution of hydroxyl group for methoxyl group seemed to attenuate the antiproliferative potency of prenylated xanthones [27]. The exact explanation for this finding is not available, but the formation of an intramolecular hydrogen bond via a hydroxy group may be involved in the antiproliferative potency of the prenylated xanthones.

The structures of MAG resemble the mitochondrial-specific fluorescent dyes, dihydrorhodamine 123 and Mito Tracker Orange CM-H2TMRos, and it is reasonable to consider that the xanthones may interact with or act on mitochondria. Matsumoto et al. reported that the preferential target of xanthones is mitochondrion. MAG and other xanthones rapidly induce the loss of mitochondrial membrane potential rapidly, which resulted in cell apoptosis.
Nakagawa et al. [28] found that MAG-induced apoptosis was mediated by a caspase-independent pathway via mitochondria with the release of Endo-G. The signaling pathways including Erk1/2, Erk5, and Akt contributed to this α-MAG-induced apoptosis. Endo-G, a known 30 kDa nuclease residing in mitochondria, is able to induce nucleosomal DNA fragmentation. Erk5 is known to promote cell growth and proliferation. In α-MAG-induced apoptosis of DLD-1 cells, the levels of Erk5 and c-myc proteins gradually decreased during the period of α-MAG treatment.

Matsumoto et al. [27] also reported that MAGs’ antiproliferative effects were associated with cell cycle: G1 arrest was by α-MAG and S arrest by γ-MAG. As shown in Fig. 7, after 48 h of incubation, the viable cells in the cultures treated with 10 μM γ-MAG were greatly reduced when compared with those in the untreated cell cultures [Fig. 7(B)], but it was not reduced when compared with the amounts of inoculants (1.0 × 10^5 viable cells). No growth of cells was observed when treated with 10 μM α-MAG [Fig. 7(A)]. The present study suggests that MAG is a potent inhibitor of growth in the K562 cells in vitro and the growth inhibition by MAG may due to cell-cycle arrest. However, cell-cycle arrest was not the only reason for growth inhibition, because MAG also induce apoptosis in cells (Fig. 8).

MAG-induced cell-cycle arrest may induce K562 cells more sensitive to the toxicity of PDT. MAG may enhance the PDT toxicity by increasing the amounts of singlet oxygen because MAG can increase the amounts of singlet oxygen in cell-free system and the singlet oxygen quencher sodium azide suppresses cell death in K562 cells induced by MAG in PDT. The survival cells in MAG (from 0 to 10 μM) treatment groups were not reduced comparing with the amounts of inoculants (1.0 × 10^5 viable cells) after 48 h of incubation (Fig. 7). The decrease in cell survival was obtained by supplementing MAG comparison with PDT (Fig. 9). These results imply that the decrease in survival cells result from MAG potentiate ALA-PDT. α-MAG induced apoptosis at 10 μM but could not induce apoptosis at 5 and 1 μM (Fig. 8). γ-MAG (from 0 to 10 μM) could not induces apoptosis in K562 cells (Fig. 8). α-MAG (1–10 μM) and 10 μM γ-MAG enhanced the apoptosis in K562 cells induced by ALA-PDT (Fig. 9). These results imply that ALA-PDT-induced K562 cell was enhanced by MAG, indicating that MAG can potentiate the cell apoptosis effect of ALT-PDT.

MAG can increase the amounts of singlet oxygen in cell-free system (Fig. 5) and sodium azide suppresses apoptosis (Fig. 10), lipid peroxidation (Table 3), and DNA damage (Table 3) in K562 cells induced by MAG in PDT. These results imply that singlet oxygen could be involved in enhancing the cytotoxicity effects of the MAG in PDT. In the study, MAG enhances the PDT-induced cytotoxicity in K562 cells (Fig. 9) are contradictory to the findings that it is an antioxidant that can scavenger hydroxyl radical, superoxide anion, and hydrogen peroxide and inhibit the formation of MDA in cell-free systems (Figs. 2–4 and 6). The contradictory results may be caused by the fact that MAG increases the amounts of singlet oxygen in PDT. 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 [4]. The finding that singlet oxygen is involved in enhancing the cytotoxicity effects of the MAG in PDT implied that the contradictory findings on the use of antioxidants during cancer therapy may be resulted from the different sensitization activities of antioxidants on singlet oxygen. The antioxidants which can scavenge singlet oxygen may decrease the effectiveness of PDT (such as vitamin C [19]). The antioxidants which increase the amounts of singlet oxygen may exhibit a synergistic effect on PDT (such as MAG). The sensitization activity of α-MAG on singlet oxygen was greater than that of γ-MAG (Fig. 5). Similarly, after PDT treatment, cells survived in α-MAG groups are lower than those in γ-MAG groups (Fig. 9).

To investigate whether MAG interferes with ALA-PDT-generated cell damage, we also measured its influence on cellular lipid peroxidation and DNA damage. The results of MAG on MDA levels in cell-free system are contradictory to those in K562 cells. The contradictory results may be caused by different mechanisms of action result in production of MDA in K562 cells. For instance, MAG may increase the amounts of singlet oxygen in K562 cells. MAG also can rapidly induce the loss of mitochondrial membrane potential and regulate some signaling pathways in K562 cells. The signaling pathways include Erk1/2, Erk5, Endo-G, and Akt. As shown in Table 3, sodium azide suppresses DNA damage and lipid peroxidation in K562 cells induced by MAG in PDT. These results imply that singlet oxygen could be involved in enhancing DNA damage and lipid peroxidation by MAG in PDT.
Mitochondria play a pivotal role in apoptosis and are thought to be a novel target of anticancer chemotherapy. Actually, strategies for the development of anticancer agents acting on mitochondria are attracting much attention. Some studies [26] provided a useful clue to adopt α-MAG and other xanthones as a candidate for developing new anticancer drugs. α-MAG can lead to apoptotic cell death and exhibited a synergistic effect on fluorouracil-induced growth inhibition at the lower concentrations [27], fluorouracil is one of the most effective chemotherapeutic agents for colorectal adenocarcinoma. In a word, the results implied that MAG may increase the effectiveness of cancer therapy.

In conclusion, our results proved that MAG can increase the amounts of singlet oxygen in cell-free systems and enhance the cell death in K562 cells induced by ALA-PDT. Supplementation of MAG in K562 cells increases the lipid peroxidation, DNA damage, and decreases the survival rate in PDT treatment. The singlet oxygen quencher sodium azide suppresses apoptosis, lipid peroxidation, and DNA damage in K562 cells induced by MAG in PDT. These results implied that the effect of antioxidants on PDT may depend on its sensitization ability to singlet oxygen. MAG enhances the PDT-induced cytotoxicity in K562 cells and singlet oxygen could be involved in this process.

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