

Full Paper

 α -Mangostin Induces Ca^{2+} -ATPase-Dependent Apoptosis via Mitochondrial Pathway in PC12 CellsAyumi Sato¹, Hironori Fujiwara¹, Hisae Oku², Kyoko Ishiguro², and Yasushi Ohizumi^{1,*}¹Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan²School of Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya 663-8179, Japan

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Abstract. We investigated the cell death effects of eight xanthenes on PC12 rat pheochromocytoma cells. Among these compounds, α -mangostin, from the fruit hull of *Garcinia mangostana* L., had the most potent effect with the EC_{50} value of 4 μM . α -Mangostin-treated PC12 cells demonstrated typical apoptotic DNA fragmentation and caspase-3 cleavage (equivalent to activation). The flow cytometric analysis indicated that this compound induced apoptosis in time- and concentration-dependent manners. α -Mangostin showed the features of the mitochondrial apoptotic pathway such as mitochondrial membrane depolarization and cytochrome c release. Furthermore, α -mangostin inhibited the sarco(endo)plasmic reticulum Ca^{2+} -ATPase markedly. There was a correlation between the Ca^{2+} -ATPase inhibitory effects and the apoptotic effects of the xanthone derivatives. On the other hand, c-Jun NH_2 -terminal kinase (JNK/SAPK), one of the signaling molecules of endoplasmic reticulum (ER) stress, was activated with α -mangostin treatment. These results suggest that α -mangostin inhibits Ca^{2+} -ATPase to cause apoptosis through the mitochondrial pathway.

Keywords: α -mangostin, xanthone, apoptosis, Ca^{2+} -ATPase, mitochondrial pathway

Introduction

Apoptosis is a programmed cell death with certain features such as chromatin condensation, membrane blebbing, and typical DNA fragmentation (1). There are two major signaling pathways of apoptosis. One is the mitochondrial pathway that involves the mitochondrial release of factors such as cytochrome c. These factors induce caspase-9 activation (2, 3). The other is the death receptor pathway that is triggered by death ligands like TRAIL (4) or Fas ligand (5). In this pathway, caspase-8 activation is detected typically (6).

Intracellular Ca^{2+} concentration is regulated very tightly as a signaling messenger. Disruption of Ca^{2+} homeostasis can induce apoptosis (7). Thapsigargin (TG) is known to disrupt Ca^{2+} homeostasis by inhibiting the endo(sarco)plasmic reticulum Ca^{2+} -ATPase, and it can induce apoptosis in several cell lines (8–12). TG is also known as a potent endoplasmic reticulum (ER)

stress inducer leading to the activation of c-Jun NH_2 -terminal kinase (JNK, also known as SAPK) (13, 14). Furthermore, it has been shown that TG-induced JNK activation and apoptosis are blocked by *N*^ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), a nitric oxide synthase inhibitor, in Jurkat T lymphocytes (12).

We have been investigating various activities of natural products for studies of signal transduction. In the course of our survey of pharmacological tools from natural resources, much attention has been given to compounds inducing apoptosis (15, 16). The fruit hull of mangosteen (*Garcinia mangostana* L.) has been used as a traditional medicine for the treatment of skin infections, wounds, and diarrhea in Southeast Asia (17). The fruit hull contains various xanthone derivatives including α -mangostin and γ -mangostin. In our previous studies, these compounds indicated several pharmacological activities such as anti-inflammatory activity (18) and the inhibitory effect of Ca^{2+} -ATPase (19). Recently, the inhibitory effect of acidic sphingomyelinase (20) and apoptotic effect (21) of α -mangostin

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were reported by Okudaira et al. and Matsumoto et al., respectively. In this study, we demonstrated the apoptotic effects of α -mangostin and its analogues and the correlation with the inhibitory effect on sarco (endo)plasmic reticulum Ca^{2+} -ATPase. This is the first paper on the apoptotic signaling pathway induced by α -mangostin.

Materials and Methods

Materials

α -Mangostin and γ -mangostin were kind gifts from Lotte Co., Ltd. (Tokyo). Demethylpaxantoin, patulone, garcinone B, paxanthone B, tripteroside, and 1,3,5,6-tetrahydroxyxanthone were isolated from *Hypericum patulum* as previous described (22). For all experiments, compounds were dissolved in dimethyl sulfoxide (DMSO).

Fetal calf serum (FCS) and horse serum (HS) were from Cell Culture Laboratory (Cleveland, OH, USA) and ICN Biochemicals (Costa Mesa, CA, USA), respectively. Dulbecco's modified Eagle's medium (DMEM) was obtained from Nissui Pharmaceuticals (Tokyo). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Dojindo (Kumamoto). Proteinase K, RNase A, and L-NAME were from Sigma (St. Louis, MO, USA). Z-IETD-AFC was obtained from Calbiochem (La Jolla, CA, USA). 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethylbenzimidazol-carbocyanine iodide (JC-1) was from Molecular Probes (Eugene, OR, USA).

Anti-caspase-3 and anti-14-3-3 β antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho-SAPK/JNK and secondary anti-rabbit IgG antibodies were from Cell Signaling (Beverly, MA, USA). Anti-SAPK/JNK and secondary anti-mouse IgG antibodies were obtained from New England Bio Labs (Beverly, MA, USA). Anti-cytochrome c antibody was from BD PharMingen (San Diego, CA, USA).

Cell culture

PC12 cells were cultured in DMEM supplemented with 10% FCS and 5% HS, 4 mM L-glutamine, 50 units/mL penicillin, and 50 $\mu\text{g}/\text{mL}$ streptomycin at 37°C in 5% CO_2 .

Cell viability assay

Cell viability was assessed by the MTT method as previously reported (15). PC12 cells were seeded in 96-well plates at a density of 1×10^4 cells per well. After 24 h, cells were treated with each drug. MTT (0.5 mg/mL) was added to each well and cells were

incubated at 37°C for 4 h. The medium was replaced with DMSO, and plates were read on the microplate reader (Model 450; Bio-Rad, Richmond, CA, USA) at a test wavelength of 595 nm.

Detection of DNA fragmentation

PC12 cells were seeded in 6-well plates at the density of 1×10^6 cells per well and were treated with or without 30 μM α -mangostin for 24 h. Cells (three wells per a sample) were washed with phosphate-buffered saline (PBS) and lysed in 150 μL of cell lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM EDTA, and 1% Triton X-100) at 4°C for 30 min. The lysates were centrifuged at $15,000 \times g$ for 5 min, and the supernatant was incubated with 20 $\mu\text{g}/\text{mL}$ RNase A at 37°C for 1 h and then with 20 $\mu\text{g}/\text{mL}$ proteinase K at 50°C for 30 min. DNA was precipitated by adding 30 μL of 5 M NaCl and 180 μL of isopropanol at -20°C . The DNA was dissolved in TE buffer (10 mM Tris-HCl, pH 7.4, and 1 mM EDTA) and electrophoresed on a 2% agarose gel. The gel was stained with 0.1 $\mu\text{g}/\text{mL}$ ethidium bromide for 1 h and visualized under UV light.

Immunoblot analysis

PC12 cells were seeded in 6-well plates at the density of 1×10^6 cells per well and were treated with or without 30 μM α -mangostin. Cells were washed with PBS and lysed in 100 μL of cell lysis buffer (20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 1% sodium dodecyl sulfate (SDS), 10% glycerol, 5 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonylfluoride (PMSF), 10 $\mu\text{g}/\text{mL}$ leupeptin, 10 $\mu\text{g}/\text{mL}$ aprotinin, and 1 mM Na_3VO_4) on ice for 30 min. Protein concentrations were estimated by BCA assay. The lysates were heated in SDS sample buffer (50% glycerol, 4% SDS, 25 mM Tris-HCl, pH 6.8, 5% 2-mercaptoethanol, and 0.01% bromophenol blue), electrophoresed on a polyacrylamide gel, and transferred onto a polyvinylidenedifluoride membrane. After incubation with Tris-buffered saline (10 mM Tris-HCl, pH 7.4, and 100 mM NaCl) containing 0.05% Tween 20 and 1% bovine serum albumin for 2 h, the membrane was treated with primary antibodies against caspase-3 (diluted 1:1000), phospho-SAPK/JNK (1:500), and SAPK/JNK (1:1000) at 4°C overnight, followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:2000) for 2 h. The bands were detected with the enhanced chemiluminescence system.

Flow cytometric analysis

Apoptotic cells were detected according to the method of Darzynkiewicz et al. (23) with slight modifications. PC12 cells were seeded in 12-well plates at the density

of 2×10^5 cells per well and were treated with or without α -mangostin for the indicated periods. Cells were washed with PBS and fixed with 70% ethanol at -80°C for 3 h. The cells were rinsed with PBS, incubated with $50 \mu\text{g/mL}$ RNase A at 37°C for 20 min, and stained with propidium iodide (PI). Apoptotic analysis was performed using a flow cytometer (FACScan; Becton Dickinson Immunocytometry Systems, Franklin Lakes, NJ, USA).

Analysis of mitochondrial membrane potential

The analysis was carried out using JC-1 by the method of Dörrie et al. (24) with slight modifications. PC12 cells were seeded in 6-well plates at the density of 4×10^5 cells per well and were treated with or without α -mangostin for the indicated periods. Cells were washed with PBS and stained with $2 \mu\text{g/mL}$ JC-1 for 20 min at 37°C . Cells were washed with PBS twice, resuspended with PBS, and analyzed by FACScan, referring to the method of Cossarizza et al. (25)

Detection of cytochrome c release

Isolation of cytosolic fractions was done by the method of Anantharam et al. (26) with slight modifications. PC12 cells were seeded in 6-well plates at the density of 1×10^6 cells per well and were treated with or without $30 \mu\text{M}$ α -mangostin. Cells were washed with PBS and resuspended in homogenization buffer (20 mM Tris-HCl, pH 8.0, 10 mM EGTA, 2 mM EDTA, 2 mM DTT, 1 mM PMSF, $10 \mu\text{g/mL}$ leupeptin and $25 \mu\text{g/mL}$ aprotinin). The suspensions were sonicated for 10 s and then centrifuged at $100,000 \times g$ for 1 h at 4°C . The supernatants were collected as cytosolic fractions. Protein concentrations were estimated by BCA assay, and the fractions ($3 \mu\text{g/lane}$) were electrophoresed on a 15% polyacrylamide gel. Immunoblotting was performed using mouse anti-cytochrome c antibody (diluted 1:1000) and then reprobed with rabbit anti-14-3-3 β antibody (1:1000).

Caspase-8 activity

Caspase-8 activity was measured according to the method of Viswanath et al. (27) with slight modifications. PC12 cells were seeded in 12-well plates at the density of 5×10^5 cells per well, and were treated with or without drug for the indicated periods. Cells were washed with PBS and lysed in $150 \mu\text{L}$ of cell lysis buffer (10 mM HEPES-KOH, pH 7.2, 2 mM EDTA, 5 mM DTT, 1 mM PMSF, 0.1% CHAPS, $10 \mu\text{g/mL}$ leupeptin, $10 \mu\text{g/mL}$ aprotinin, and $1 \mu\text{g/mL}$ pepstatin A) and freeze-thawed four to five times. Lysates were centrifuged at $13,000 \times g$ for 30 min at 4°C , and the supernatants were collected. Protein concentrations were

estimated by Bradford assay. Supernatant aliquots containing $100 \mu\text{g}$ protein were incubated with $50 \mu\text{M}$ caspase-8 fluorescent substrate (Z-IETD-AFC) at 37°C for 2 h. Free AFC accumulation was measured at 360 nm excitation and 515 nm emission wavelengths.

Purification of SR Ca^{2+} -ATPase

Vesicular sarcoplasmic reticulum (SR) and purified SR Ca^{2+} -ATPase were prepared from rabbit skeletal muscle by the methods of Morii et al. (28) and Meissner et al. (29) respectively.

Ca^{2+} -ATPase activity

The reaction buffer solution (50 mM MOPS-KOH, pH 7.0, 90 mM KCl, 5 mM MgCl_2 , 0.75 mM CaCl_2 , 1 mM EGTA, and $13 \mu\text{g/mL}$ rabbit SR Ca^{2+} -ATPase) was incubated for 5 min at 4°C , and then the drug was added. After 5 min, the reaction was started by adding 1 mM ATP and stopped by adding 10% trichloroacetic acid. Liberated inorganic phosphate was measured by the Martin-Doty method (30).

Statistical analyses of the data

The data are expressed as means \pm S.E.M. Statistical comparisons were made by using Student's *t*-test.

Results

The cell death induced by α -mangostin analogues

The effects of eight xanthone derivatives (Fig. 1) on the viability of PC12 rat pheochromocytoma cells were tested by MTT assay. α -Mangostin [1], γ -mangostin [2], demethylpaxanthoin [3], garcinone B [5], and paxanthone B [6] were significantly effective (Fig. 2). α -Mangostin had the highest potency in these compounds with the EC_{50} value of $4 \mu\text{M}$. We used α -mangostin for the subsequent experiments.

Detection of α -mangostin-induced apoptosis

We examined DNA fragmentation for the detection of apoptosis. α -Mangostin at a concentration of $30 \mu\text{M}$ induced typical apoptotic DNA fragmentation (DNA ladder) (Fig. 3A). We also observed apoptotic morphological changes as cell shrinkage, chromatin condensation, and apoptotic body (data not shown). Furthermore, a DNA ladder was detected in the $30 \mu\text{M}$ -treated L1210 murine leukemic cells (data not shown).

In many cases of apoptosis, caspase-3 activation is observed (31). We examined whether α -mangostin induced caspase-3 cleavage (equivalent to caspase activation). Decline in pro-caspase-3 and appearance of cleaved-caspase-3 were observed (Fig. 3B). Thus, α -mangostin-induced apoptosis is mediated by caspase-3

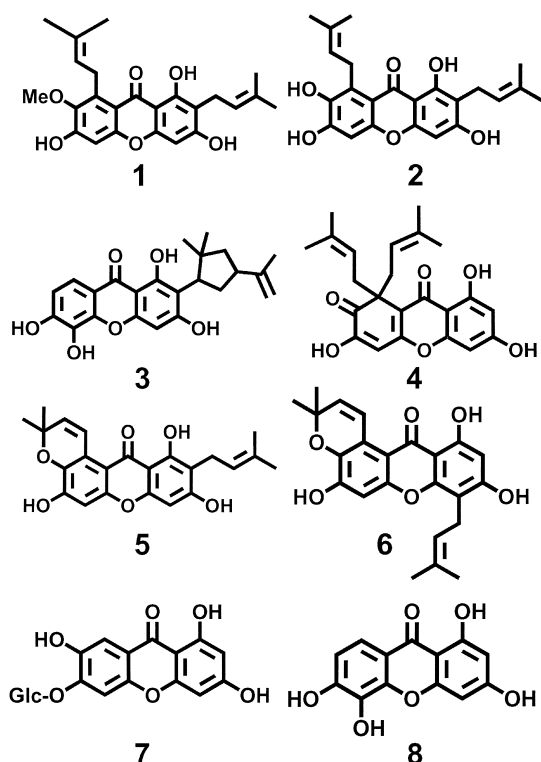


Fig. 1. Chemical structures of α -mangostin and xanthone derivatives. 1, α -mangostin; 2, γ -mangostin; 3, demethylpaxantoin; 4, patulone; 5, garcinone B; 6, paxanthone B; 7, tripteriside; 8, 1,3,5,6-tetrahydroxanthone.

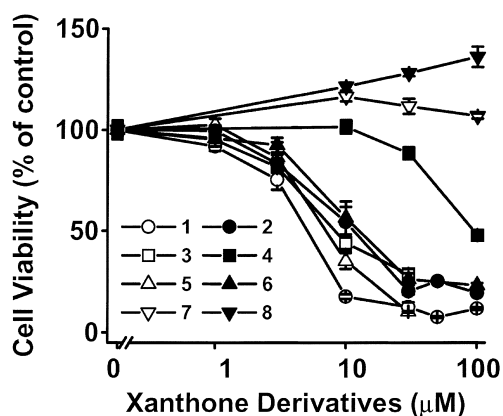


Fig. 2. Effects of the xanthone derivatives on the viability of PC12 cells. PC12 cells were treated with each compound for 24 h. Cell viability was measured by MTT assay as described in Materials and Methods. Each value is the mean \pm S.E.M. (%) of six determinations.

activation.

Flow cytometric analysis of apoptosis in α -mangostin-treated PC12 cells

Darzynkiewicz et al. showed that measurements of

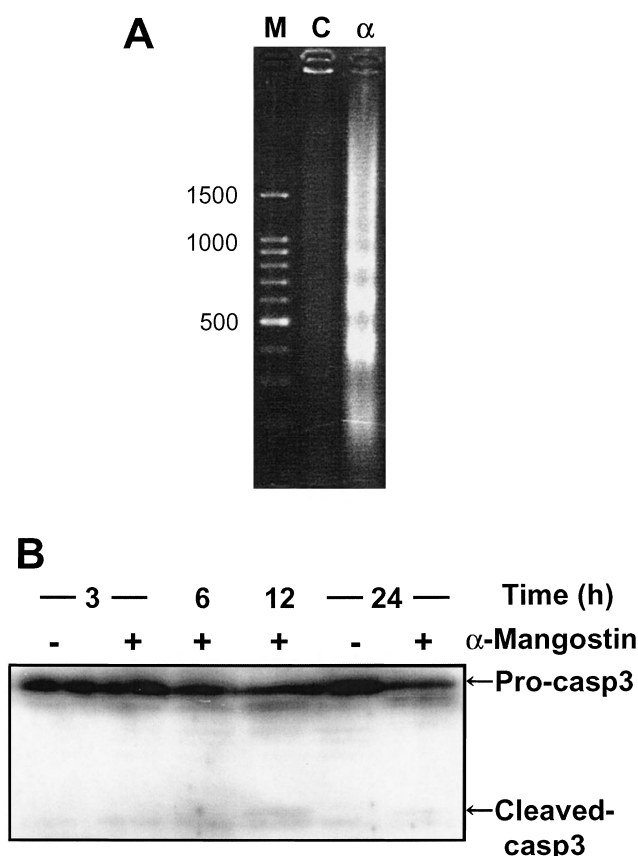


Fig. 3. Induction of DNA fragmentation (A) and the cleavage of caspase-3 (B) by α -mangostin. A: DNA fragmentation of PC12 cells. PC12 cells were treated with vehicle control (C) or 30 μ M α -mangostin (α) for 24 h. M: 100-bp marker. B: Immunoblot analysis of caspase-3. Each sample (20 μ g of protein/lane) was fractionated on a 15% gel, followed by immunoblotting with anti-caspase-3 antibody.

DNA content using PI make it possible to identify apoptotic cells and to recognize the cell cycle phase (23). They also described that necrotic cells are not detected by this method. We tested flow cytometric analysis with PI-stained PC12 cells for quantitative determination of apoptosis. α -Mangostin induced apoptosis in concentration- and time-dependent manners (Fig. 4: A and B).

Involvement of mitochondrial pathway, but not death receptor pathway, in α -mangostin-induced apoptosis

The disruption of the mitochondrial transmembrane potential is an early event of programmed cell death (32). Furthermore, cytochrome c release from mitochondria plays an important role in the apoptotic mitochondrial pathway (3, 32). Thus, we examined the effects of α -mangostin on these factors. To determine whether α -mangostin altered mitochondrial membrane potential, we used JC-1, a specific indicator of mito-

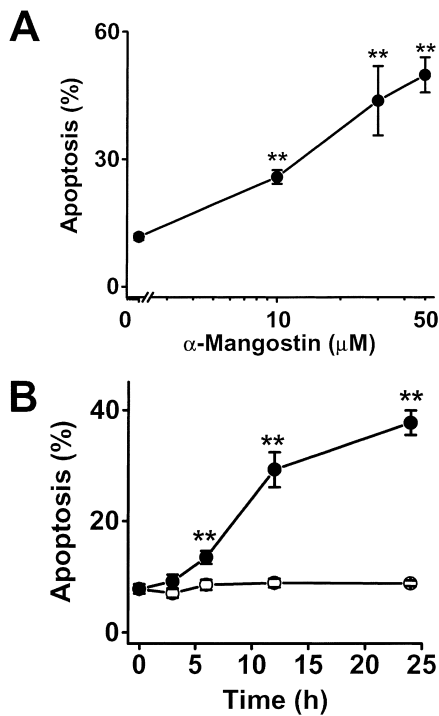


Fig. 4. Concentration (A)- and time (B)-dependent increases in apoptosis by α -mangostin. A: PC12 cells were treated with several concentrations of α -mangostin for 24 h. Apoptosis is expressed as the percentage of the number of apoptotic cells in 5,000 cells. Values represent means \pm S.E.M. (%) of three determinations. Significance: ** P <0.01 vs the level in the vehicle treatment. B: Cells were treated with vehicle control (open circle) or 30 μ M α -mangostin (closed circle). Apoptosis is expressed as described above. Values represent means \pm S.E.M. (%) of three determinations. Significance: ** P <0.01, compared with the control.

chondrial potential (25). α -Mangostin at 30 μ M rapidly depolarized mitochondria in PC12 cells (Fig. 5: A and B). Cytochrome c was also detected in the cytoplasmic fraction in a similar time course (Fig. 5C).

Since a cross-talk between the death receptor pathway and the mitochondrial pathway was reported (33), we detected the caspase-8 activity to determine whether the death receptor pathway existed in the upstream of the mitochondrial pathway on α -mangostin-induced apoptosis. No significant caspase-8 activity was observed in the cells treated with 30 μ M α -mangostin (Fig. 6).

Structure-activity correlation between the cell death effects and the Ca^{2+} -ATPase inhibitory effects in xanthenes

In the previous study, our laboratory demonstrated the concentration-dependent Ca^{2+} -ATPase inhibitory effect of α -mangostin (19). Since there were the reports that Ca^{2+} -ATPase inhibitors like TG induced

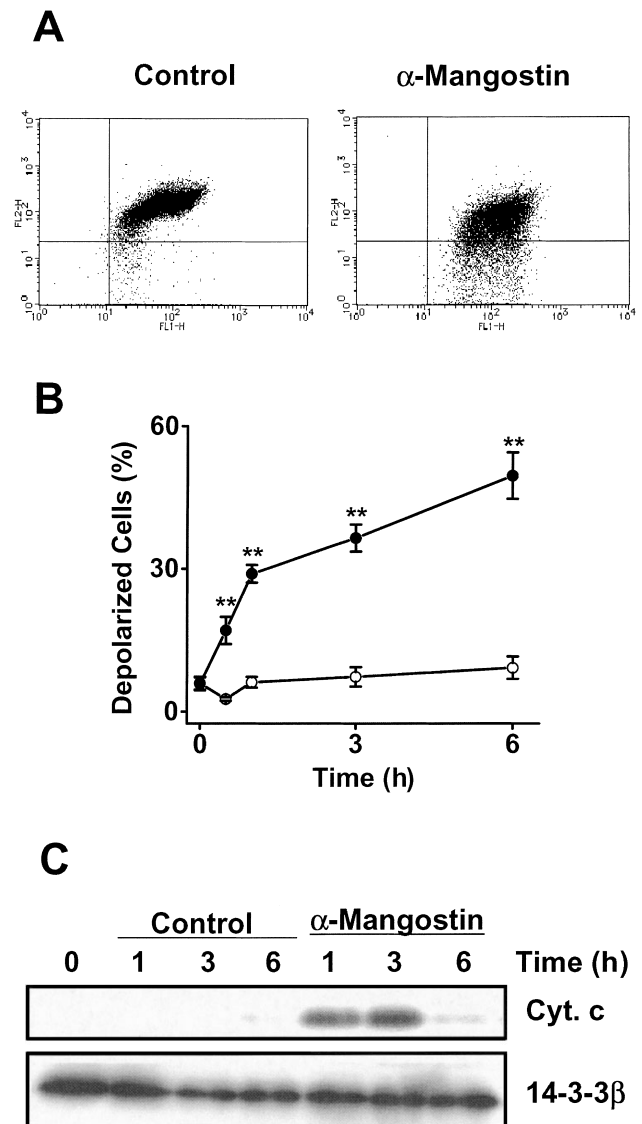


Fig. 5. Activation of the mitochondrial pathway by α -mangostin. A and B: Detection of mitochondrial membrane potential using JC-1. A: PC12 cells were treated with vehicle control or 30 μ M α -mangostin for 3 h. Cells with normal polarized mitochondrial membranes are in the upper right quadrant. Cells with depolarized mitochondrial membranes are in the bottom right quadrant. B: Cells were treated with vehicle control (open circle) or 30 μ M α -mangostin (closed circle). Depolarized cells are expressed as the percentages of cells in the bottom right quadrant as described in A. Values represent means \pm S.E.M. (%) of three determinations. Significance: ** P <0.01, compared with the control. C: Cytochrome c release from mitochondria. PC12 cells were treated with vehicle control or 30 μ M α -mangostin. The cytosolic fractions were subjected to immunoblot analysis with antibodies against cytochrome c, and 14-3-3 β was used as an internal control.

apoptosis in several cell lines (8–12), we examined the Ca^{2+} -ATPase activities with the eight xanthenes described (Fig. 7). Positive correlation between the Ca^{2+} -ATPase inhibitory effects and the cell death effects

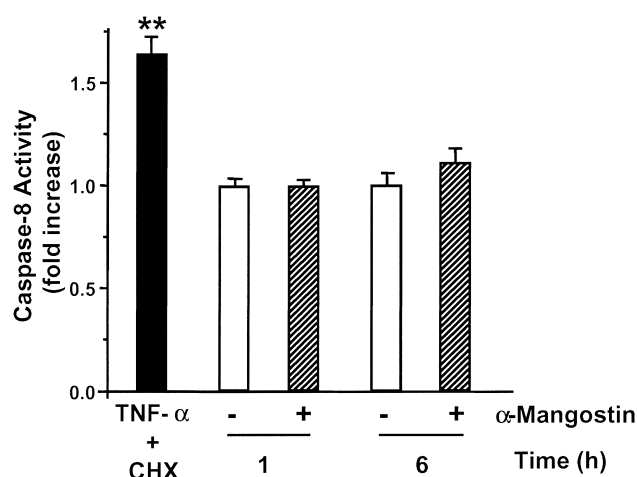


Fig. 6. Effect of α -mangostin on caspase-8 activity. PC12 cells were treated with 100 μ g/mL cycloheximide (CHX) and 10 ng/mL TNF- α for 24 h, vehicle control (DMSO), or 30 μ M α -mangostin for the indicated periods. Caspase-8 activity was measured by using the fluorogenic substrate Z-IETD-AFC as described in Materials and Methods. Each datum is expressed as a fold increase relative to vehicle control. Values represent means \pm S.E.M. (%) of six determinations. Significance: ** P <0.01, compared with the vehicle control.

was observed, except for the case of 1,3,5,6-tetrahydroxyxanthone [8] (Table 1). We also detected the parallel apoptotic effect by flow cytometric analysis (data not shown). These data suggest that Ca^{2+} -ATPase inhibition plays a pivotal role in the apoptosis induced by α -mangostin and its derivatives.

Involvement of stress-dependent signaling in α -mangostin-induced apoptosis

To explore whether ER stress-dependent signaling participated in α -mangostin-induced apoptosis, we first examined the effect of L-NAME on α -mangostin-induced cell death by MTT assay. L-NAME at 1 mM did not inhibit the cell death effect of α -mangostin (Fig. 8A). We next evaluated the effect of α -mangostin on the activation of JNK. α -Mangostin phosphorylated and activated JNK slightly with 1-h treatment and markedly with 3-h treatment (Fig. 8B).

Discussion

Natural products having apoptotic activity have been extensively studied by numerous investigators. Some of these compounds are clinically used as anti-cancer drugs (34, 35). There are some reports that caged (36) or prenylated (21, 37) xanthenes induce apoptosis. However, the detailed molecular mechanism of these compounds has not been elucidated yet.

Now in this report, we demonstrated that α -mangos-

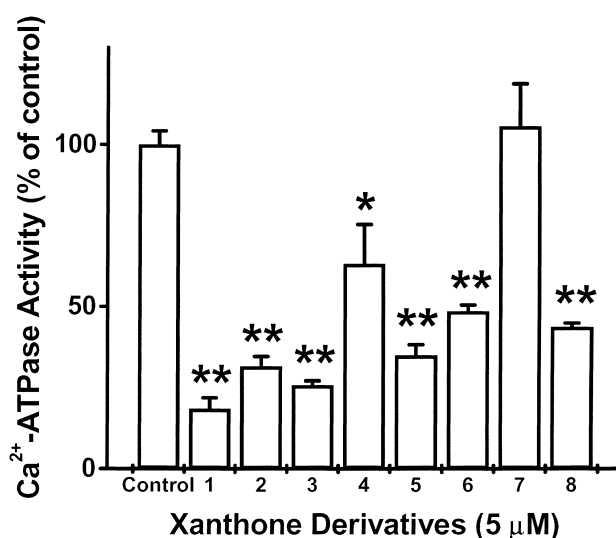


Fig. 7. Ca^{2+} -ATPase inhibitory effects of xanthone derivatives. 1, α -mangostin; 2, γ -mangostin; 3, demethylpaxantoin; 4, patulone; 5, garcinone B; 6, paxanthone B; 7, tripteroside; 8, 1,3,5,6-tetrahydroxyxanthone. Rabbit sarcoplasmic reticulum Ca^{2+} -ATPase activities were measured by the Martin-Doty method. Ca^{2+} -ATPase was incubated with 5 μ M of each drug for 5 min. Values represent means \pm S.E.M. (%) of three determinations. Significance: * P <0.05, ** P <0.01, compared with the control.

Table 1. Structure-activity correlation between cell viability and Ca^{2+} -ATPase inhibitory effects in xanthone derivatives

| Compound | Cell viability (%) | Ca^{2+} -ATPase activity (%) |
|----------|--------------------|---------------------------------------|
| 1 | 17.8 \pm 1.2 | 18.5 \pm 2.6 |
| 2 | 54.3 \pm 7.6 | 31.5 \pm 2.4 |
| 3 | 44.1 \pm 4.0 | 25.7 \pm 1.1 |
| 4 | 101.6 \pm 3.4 | 63.2 \pm 9.6 |
| 5 | 35.2 \pm 3.9 | 34.9 \pm 2.6 |
| 6 | 56.5 \pm 8.1 | 48.5 \pm 1.5 |
| 7 | 116.4 \pm 2.3 | 105.7 \pm 10.2 |
| 8 | 121.4 \pm 2.0 | 43.6 \pm 1.0 |

The percentages of viability in 10 μ M-xanthone-treated PC12 cells were determined by MTT assay. Xanthone (5 μ M)-treated Ca^{2+} -ATPase activities were determined by the Martin-Doty method. Each value is the mean \pm S.E.M. (%) of six determinations of viability and of three determinations of Ca^{2+} -ATPase activity.

tin, a prenylated xanthone from *Garcinia mangostana* L. induced cell death in PC12 cells. Furthermore, apoptotic DNA fragmentation and caspase-3 cleavage are observed. α -Mangostin induced apoptosis in concentration- and time-dependent manners.

Mitochondria play an essential role in various forms of apoptosis. The release of cytochrome c from the inner mitochondrial membrane to the cytosol triggers the assembly of the apoptosome. The apoptosome consists of cytochrome c, Apaf-1, dATP (or ATP), and pro-

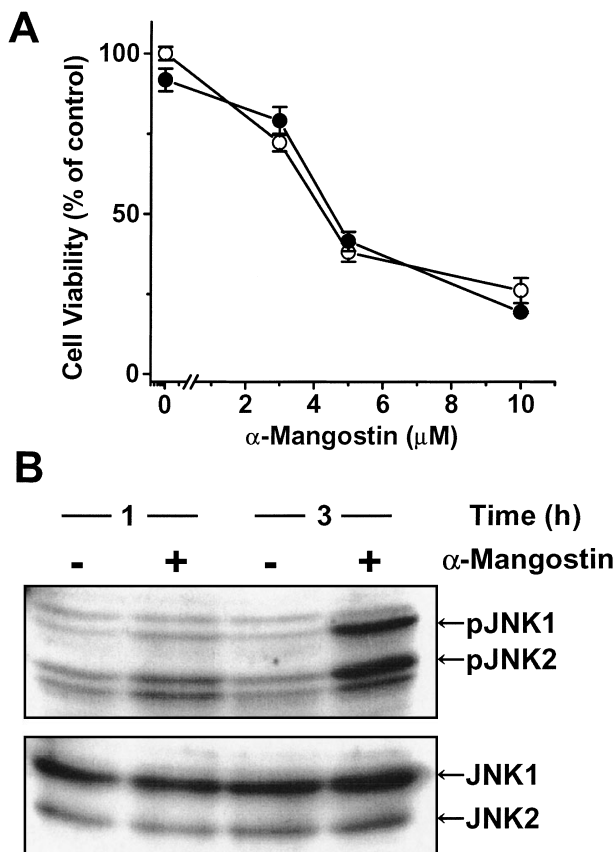


Fig. 8. Involvement of stress-mediated signaling on the effect of α -mangostin. **A:** The inhibitory effect of L-NAME on α -mangostin-induced cell death. PC12 cells were pretreated with saline (open circle) or 1 mM L-NAME (closed circle) for 1 h and then subjected to several concentrations of α -mangostin treatment for 12 h. Cell viability was measured by MTT assay. Each value is the mean \pm S.E.M. (%) of five determinations. **B:** JNK activation by α -mangostin. Cells were treated with or without 30 μ M α -mangostin. Each sample (20 μ g of protein/lane) was fractionated on an 11% gel, followed by immunoblotting with anti-phospho-JNK antibody (top panel) and then reprobred with anti-JNK antibody (bottom panel).

caspase-9. Pro-caspase-9 is activated by binding to Apaf-1. Then active caspase-9 can cleave and activate caspase-3 (3, 32). Meanwhile, mitochondrial membrane depolarization has been reported as an apoptotic signal (32). We further examined whether mitochondria were involved in α -mangostin-induced apoptosis. Treatment of PC12 cells with α -mangostin rapidly caused mitochondrial membrane depolarization and cytochrome c release. These results suggest that α -mangostin induces apoptosis via the mitochondrial pathway.

TG, a Ca^{2+} -ATPase inhibitor, is known to induce apoptosis in several cell lines (8–12). One of the plausible mechanisms of TG-induced apoptosis is the death receptor 5 (DR5) pathway. In this signaling pathway, TG upregulates DR5 and its ligand TRAIL

levels, activates caspase-8, and induces Bid cleavage (9). Bid cleavage bridges between the death receptor pathway and mitochondrial pathway (33). Another mechanism of TG-induced apoptosis is ER stress dependent. TG activates caspase-12 (11) or generates nitric oxide, leading to activation of the JNK pathway (12). We previously reported that α -mangostin also has a Ca^{2+} -ATPase inhibitory effect on SR (19). In order to elucidate the relationship between the cell death effect and the Ca^{2+} -ATPase activity, we investigated the structure-activity correlation using the eight xanthenes. All the compounds except 1,3,5,6-tetrahydroxanthone demonstrated the clear correlation in both activities. This correlation might be evidence that Ca^{2+} -ATPase inhibition plays a pivotal role in xanthone-induced apoptosis. Interestingly, α -mangostin did not display caspase-8 activation at least in the upstream of the mitochondrial pathway in spite of its Ca^{2+} -ATPase inhibitory effect. This result suggests that α -mangostin inhibits Ca^{2+} -ATPase to induce death-receptor-independent apoptosis in PC12 cells. On the other hand, L-NAME had no effect on the cell death induced by α -mangostin, whereas α -mangostin induced JNK activation. These results did not fit in the case of TG-induced apoptosis in Jurkat T cells (12). Nevertheless, α -mangostin seems to induce ER stress. Further experiments are needed to verify that α -mangostin-induced apoptosis is ER stress-dependent. Judging from the time course, we suppose that mitochondrial changes are prior to ER stress signaling.

In summary, α -mangostin inhibits Ca^{2+} -ATPase to induce cytochrome c release from mitochondria, resulting in apoptosis. α -Mangostin and its derivatives may become valuable pharmacological tools for clarifying the Ca^{2+} -ATPase-dependent apoptotic mechanism.

Acknowledgments

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