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Menadione-induced cell death in *Ginkgo biloba* cell cultures

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Introduction

Programmed cell death (PCD) is a physiological process that occurs during development and in defense responses in animals and plants. One of the most widely investigated forms of PCD is apoptosis. Apoptosis is a well-defined form of PCD in mammalian cells; it is triggered by a wide variety of stimuli, such as hormones, depletion of growth factors, pro-oxidants, viral infection, and anticancer agents (SUSIN *et al.* 1998). The morphological hallmarks of apoptosis include cell shrinkage, chromatin condensation, fragmentation of the nucleus into membrane-bound vesicles called apoptotic bodies, and nuclear DNA degradation into large fragments (50-300 kbp) as well as multimers of about 180 bp that arise from the cleavage of chromatin at internucleosomal sites (LEIST and NICOTERA 1997). Recent studies have revealed that the loss of mitochondrial membrane potential and the release of cytochrome (Cyt) *c* into the cytosol from mitochondria lead to the activation of apoptotic degradation processes, including the activation of specific cysteine proteases, called caspases, followed by the activation of specific DNases (JABS 1999).

In plants, PCD occurs during development, sexual reproduction, senescence, and in response to infection by pathogens (GREENBERG 1996). Many attempts have been made to identify plant genes that are homologous to those involved in mammalian apoptosis, but with little success (HEATH 1998). Despite the sequencing of the complete *Arabidopsis thaliana* genome, no genes homologous to the key proteins in mammalian apoptosis have yet been identified from plant systems. Although the activation of caspase-like protease activity in plant PCD has been reported (del POZO and LAM 1998, SUN *et al.* 1999a, b), to date no genes for plant caspase-like proteases have been isolated (HOEBERICHTS and WOLTERING 2003).

Menadione (2-methyl-1,4-naphthoquinone) is a quinone compound that is widely used as a model compound in studies of oxidative damage. Quinone is metabolized to a semiquinone radical through one-electron reduction by flavoenzymes such as NADPH Cyt P-450 reductase, NADH Cyt b_5 reductase, and NADH-ubiquinone reductase. The resulting semiquinone is converted to the parent quinone in the presence of oxygen, causing superoxide radical formation and subsequent oxidative stresses, which lead to cellular injury (THOR *et al.* 1982, LIND *et al.* 1982, MONKS *et al.* 1992).

Menadione has recently been reported to cause apoptotic cell death in plant cells (SUN *et al.* 1999a, b). Menadione-treated tobacco protoplasts exhibited the release of Cyt *c* from

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mitochondria, the activation of caspase-like proteases, and the degradation of lamin-like proteins (SUN *et al.* 1999a, b). In the present study, we investigated menadione-induced cell death in ginkgo suspension-cultured cells. Ginkgo is one of the longest-lived woody plants, and a large amount of cell death occurs during its lifespan. Cell death mechanisms in woody plants have not been extensively studied, and analysis of chemically induced cell-death mechanisms in woody plant cell cultures has not been reported. This study reports the first evaluation of the cytotoxic effects of menadione in ginkgo cultured cells. The involvement of reactive oxygen species and caspase-like proteases and the role of mitochondria in menadione-triggered cytotoxicity were investigated.

Materials and Methods

Chemicals

All reagents used were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

Cell cultures

Ginkgo (*Ginkgo biloba* L.) cell suspension cultures were initiated from calli derived from mature embryos. Cultures were maintained in a liquid medium containing Linsmaier & Skoog's mineral salts (LINSMAIER and SKOOG 1965), 1.0 mg $liter^{-1}$ thiamine chloride, 100 mg $liter^{-1}$ *myo*-inositol, 3% sucrose, 2.3 μM 2,4-D, and 1.9 μM kinetin; the pH of this medium was adjusted to 5.9 before autoclaving. Cultures were grown in 500-ml Erlenmeyer flasks shaken at 150 rpm in darkness at 26.5°C. Subculturing was performed every three weeks by transferring about 10 ml of the suspension culture to 90 ml of fresh medium, to obtain a cell density of 20 mg (fresh weight) of cells per 1 ml of culture. The fresh weight of the cells was rapidly determined for a sample of cells by collecting them with gentle vacuum filtration onto KIRIYAMA No. 5B filter paper in a KIRIYAMA ROHTO (Kiriyama Seisakusyo, Tokyo, Japan).

Menadione treatment

On day 18 of the growth cycle, three or four cultures were combined, the fresh weight of the cells was measured and the number of cells in representative portions was counted. Then 5 ml of the suspension cells were transferred to test tubes of 16 mm diameter and 180 mm length. Menadione was diluted in DMSO to 200X concentration stock solutions and added to cultures at final concentrations of 30, 100, 300, and 1000 μM. Control cells were exposed to equivalent concentrations of DMSO. Cells were shaken at 300 rpm at 26.5°C in darkness.

Detection of reactive oxygen species

The generation of the reactive oxygen species O_2^- and H_2O_2 after treatment with 300 μM menadione was monitored by the chemiluminescence of 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA) and luminol, according to the method described by

KAWANO *et al.* (2000) with slight modifications. Briefly, 18-day-old suspension cells were transferred to sample tubes 12 mm in diameter and 75 mm in length, and the pH of the cell suspension was adjusted to 5.8 or 7.5 with 20-mM K-phosphate buffers. O_2^- generation was monitored using CLA-dependent chemiluminescence, by adding 10 μM CLA to cell suspensions adjusted to pH 5.8. CLA was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Luminol-dependent chemiluminescence was used to monitor H_2O_2 generation, by adding 0.2 units ml^{-1} of horseradish peroxidase and 10 μM luminol to cell suspensions adjusted to pH 7.5. Luminescence measurements were begun 10 s or 30 min after addition of menadione. CLA or luminol was added to cultures just before the measurements. Luminescence was measured for 120 s using a Berthold Lumat LB 9507 luminometer (Berthold Technologies GmbH & Co. KG, Germany). In this method, the emitted light was measured by a photomultiplier and the photoelectrons released from the photocathode by the light quanta were multiplied *via* a dynode chain, triggering a rapid pulse at the anode. The number of relative light units (RLU) was calculated by dividing the pulses by ten, and the light yields were expressed as RLU per second (RLU/s). To test the scavenging effects of catalase and superoxide dismutase (SOD), these enzymes were added 30 min before the addition of menadione.

Treatment with apoptosis inhibitors

Cells exposed to 300 μM menadione were pre- and co-treated with known apoptosis inhibitors. Catalase and SOD were dissolved in distilled water and sterilized before use by passage through a 0.45-μm filter. Cells were pre-treated for 30 min and co-treated for 3 h with catalase (1,000, 5,000 or 25,000 units ml^{-1}) or with a mixture of 500 units ml^{-1} of SOD and 25,000 units ml^{-1} of catalase. The reversible caspase-specific inhibitor, acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspart-1-al (Ac-DEVD-CHO), was added to a final concentration of 100 μM along with menadione. Cyclosporin A (CsA) was dissolved in DMSO and added to final concentrations of 5 or 25 μM 30 min before menadione was added. Cell injury and DNA fragmentation were assayed 3 h and 48 h after menadione addition. Equivalent volumes of DMSO or water were added to the corresponding control cells.

Assay of cell injury

After incubation with menadione for varying amounts of time, 0.5-ml aliquots of suspension cells were transferred to microcentrifuge tubes, and 50 μl of a 5 mg ml^{-1} 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution in phosphate buffered saline (Nacalai Tesque Inc., Kyoto, Japan) was added. The cells were incubated for 1 h at 26.5°C with rotation at 15 rpm in darkness. Next, 500 μl of 0.04 M HCl in 2-propanol were added and mixed thoroughly to dissolve the resulting purple crystals. After centrifugation at 19,000 x *g* for 5 min at room temperature, the absorbance of the supernatant was measured at 570 and 660 nm. The relative cell viabilities were calculated by the formula A_{570}-A_{660}.

DNA fragmentation analysis

Cells were collected by filtration of 0.5-ml aliquots of suspension culture onto filter paper as described above. The cell mass was removed from the filter paper, placed into liquid N_2, and stored at –80°C, and the stored cells were ground to a fine powder using a CRYO-PRESS (Microtec Co., Chiba, Japan). The pulverized cells were collected in a microcentrifuge tube and lysed in 50 μl of a buffer of 200 mM Tris (pH 8.0), 40 mM EDTA, 1.5% sodium lauroyl sarcosinate, and 1.7 mg ml^{-1} proteinase K (Sigma Chemical Co., USA), followed by incubation at 50°C for 2 h. Then 3 μl of an RNase A solution (20 mg ml^{-1}) were added, and the solution was incubated at 50°C for 30 min. The lysate was centrifuged at 19,000 x *g* for 5 min at room temperature, and the supernatant was collected. A sample consisting of one-thirtieth of the volume of the supernatant was electrophoresed on a 2% agarose gel in 40 mM Tris-acetate, 1 mM EDTA (pH 8.5) at 10.7 V cm^{-1} for 1 h. The gel was then stained with 0.5 μg ml^{-1} ethidium bromide and visualized under UV light.

Cytological methods

Cells were fixed overnight at 4°C in a 3:1 mixture of ethanol-acetic acid, washed with phosphate buffered saline and stained with 0.5 μg ml^{-1} 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) for 1 h. The cells were then viewed under UV excitation with an Olympus epifluorescence microscope (Olympus Optical Co, Tokyo, Japan).

Results

Assessment of menadione-induced cell injury

The viability of cells in gingko cell cultures was measured using the MTT assay after treatment with 0, 30, 100, 300, or 1000 μM menadione for 24 or 48 h. The cell density was 6.1×10^5 cells ml^{-1} and 152 mg ml^{-1} when menadione was added. As shown in Fig. 1A, treatment with menadione at concentrations of up to 100 μM for 24 h was not detrimental to cells, but cell viability was substantially lower after a 48-h treatment with 100 μM menadione. Incubation with menadione at concentrations greater than 300 μM caused almost a total loss of cell viability within 48 h.

DNA from aliquots of the above cell samples was extracted and electrophoresed. In samples incubated for 48 h, DNA ladders were detected in cells treated with 300 and 1000 μM of menadione, whereas no fragmented DNA was detected after only 24 h of treatment, regardless of the menadione concentration. The degree of DNA fragmentation in samples treated with 300 and 1000 μM of menadione was similar (Fig. 1B).

Based on these results, a concentration of 300 μM menadione was used to induce apoptotic cell death in subsequent experiments.

Cell injury, DNA fragmentation, and cytological analysis

The loss of cell viability and the level of DNA fragmentation were compared after treatment

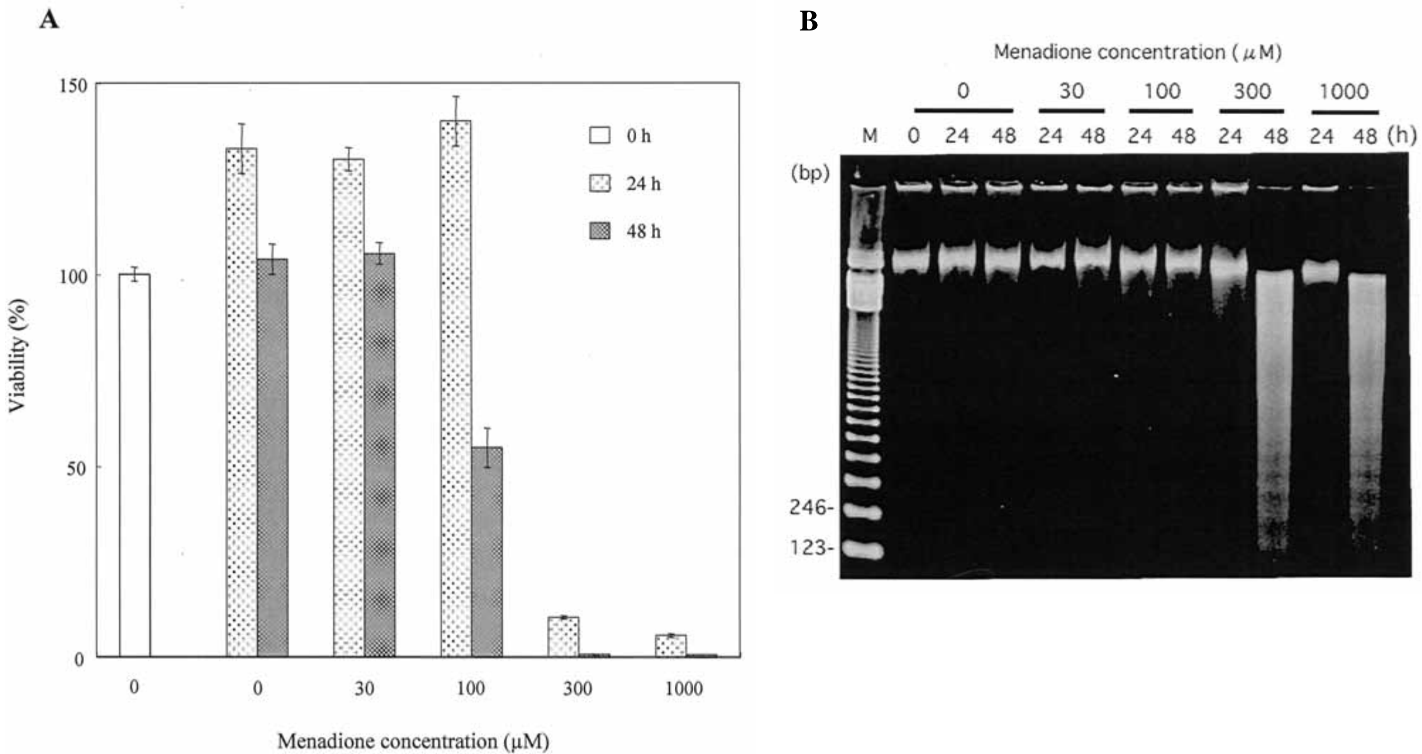

Fig. 1. Menadione-induced cell death and DNA fragmentation. Cells were treated with menadione at concentrations of 0 to 1000 μM for 24 and 48 h. (A) Cell viability was assayed using the MTT method. Values represent the mean of three experiments with the standard deviation. (B) Cellular DNA electrophoresed on a 2% agarose gel and stained with ethidium bromide. Three parallel cultures were combined and used for DNA analysis. M: molecular weight standards containing DNA multimers of 123 bp.

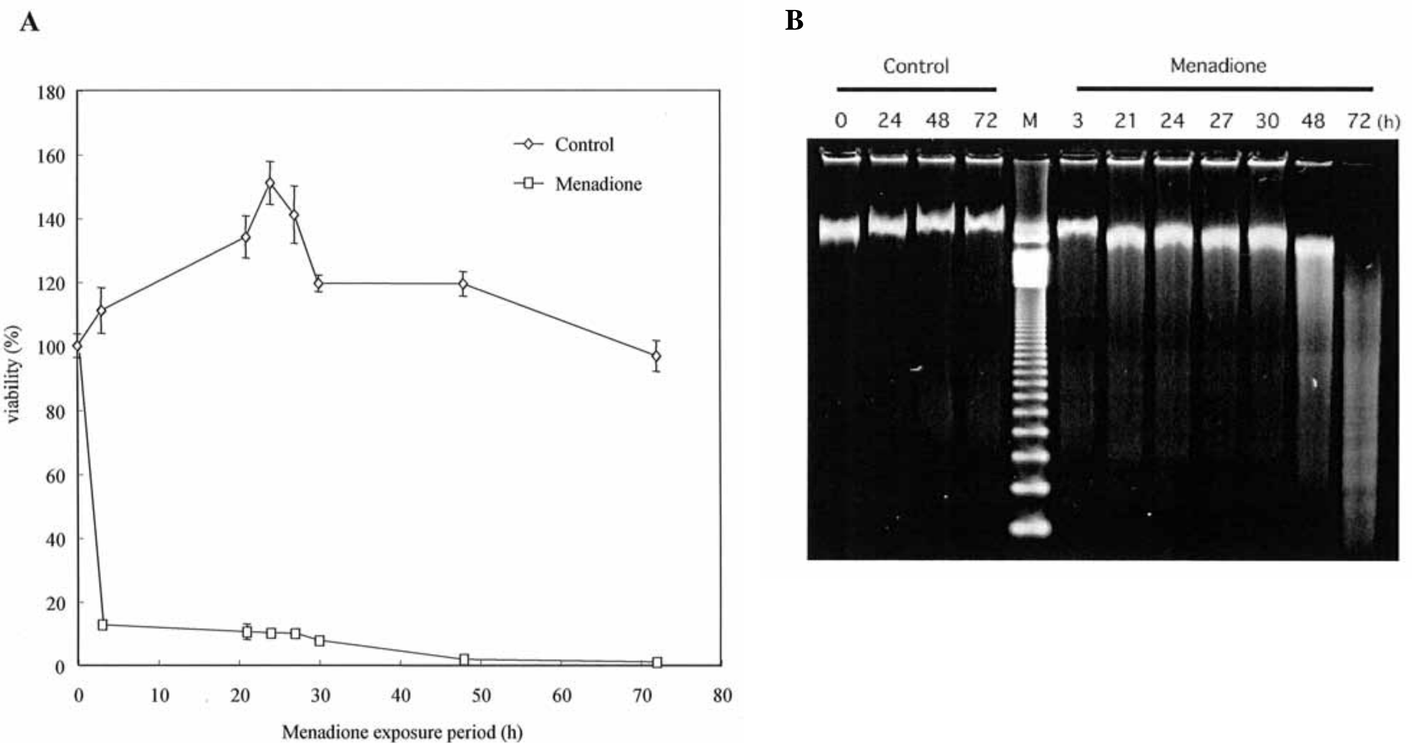

Fig. 2. Changes over time in cell viability, cellular DNA, and morphology in cultures containing 300 μM menadione. Cells were collected and assayed at various time points during a menadione treatment of 72 h. (A) Cell viability was assayed using the MTT method at 3, 21, 24, 27, 30, 48, and 72 h after menadione addition. Values represent the mean of three experiments with the standard deviation. (B) Electrophoretogram of cellular DNA on a 2% agarose gel. Three parallel cultures were combined and used for DNA analysis. (C) Changes in cell morphology. Cells were collected and fixed in a 3:1 mixture of ethanol-acetic acid, stained with 0.5 μg ml^{-1} DAPI, and viewed under UV excitation (right panels) or white light (left panels). Bar=250 μm.

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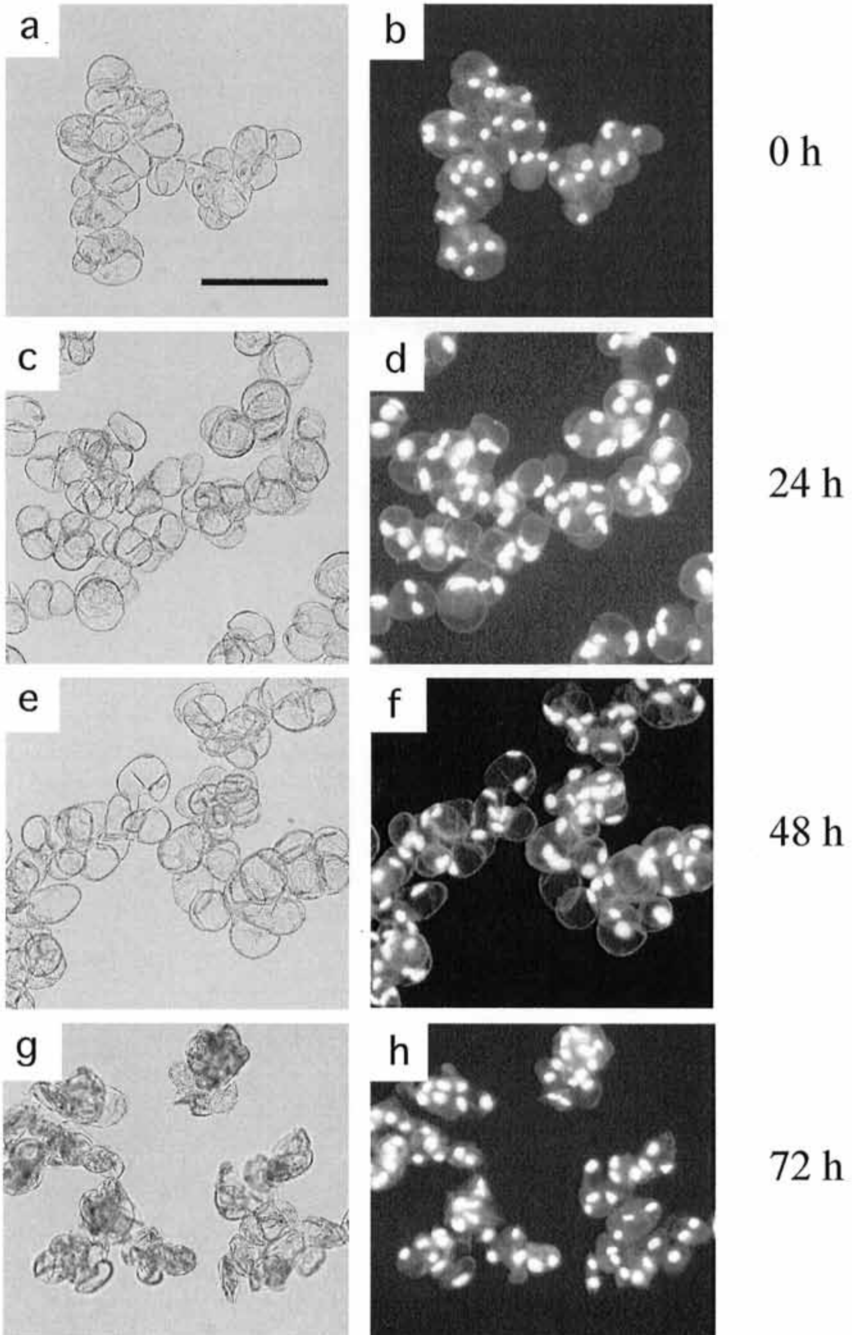


with 300 μM menadione for 0 to 72 h. The cell density was 7.4×10^5 cells ml^{-1} and 142 mg ml^{-1} when the menadione was added. After 3 h of menadione treatment, the cell viability was about 13% that of 0-h control cells, and after 48 h and 72 h of treatment the cell viability had decreased to about 1.9% and 1%, respectively (Fig. 2A). DNA extracted from cultures exposed to menadione for 48 h displayed DNA laddering, although little or no fragmented DNA was detected in cells incubated with menadione for up to 30 h. After 72 h of exposure, no high molecular weight DNA could be detected and DNA ladders were prominent. Control samples that were treated with DMSO alone did not exhibit fragmented DNA (Fig. 2B). Cytological analysis was performed for samples treated with menadione for 0, 24, 48, and 72 h. As shown in Fig. 2C, no obvious alterations in cell morphology appeared in samples exposed to menadione for up to 48 h. After 72 h of exposure, the protoplasts appeared shrunken (Fig. 2C-g), but no nuclear fragmentation or condensation was apparent (Fig. 2C-h).

Detection of production of reactive oxygen species and scavenging effects of catalase and SOD

Increases in the amounts of H_2O_2 and O_2^- were observed within a few seconds of menadione exposure, and these reactive oxygen species were still abundant 30 min after menadione addition. The amount of H_2O_2 in the culture after 30 min of menadione treatment was much larger than immediately after menadione exposure, whereas the amounts of O_2^- detected a few seconds and 30 min after menadione administration were similar (Fig. 3A, B). Treatment with 5,000 or 25,000 units ml^{-1} of catalase reduced the H_2O_2 level in the culture to that of the control, even after 30 min of menadione treatment. Treatment with 500 units ml^{-1} SOD had no effect on the O_2^- level, but addition of a mixture of SOD (500 units ml^{-1}) and catalase (25,000 units ml^{-1}) did reduce the O_2^- concentration (Fig. 3).

Effects of apoptosis inhibitors on menadione-induced cell injury and DNA fragmentation

Treatment with catalase at concentrations of up to 25,000 units ml^{-1} did not inhibit the loss of cell viability in cultures that were exposed to menadione for 3 h (Fig. 4A). However, treatment with a mixture of SOD (500 units ml^{-1}) and catalase (25,000 units ml^{-1}) slightly improved the viability of the cells (Fig. 4B).

Treatment with 100 μM of the caspase-specific inhibitor Ac-DEVD-CHO did not reduce menadione-induced cell injury or DNA fragmentation (Fig. 5).

Co-treatment with 5 or 25 μM of CsA had little effect on the loss of viability caused by 3 and 48 h of menadione exposure (Fig. 6A). Treatment with CsA alone (5 or 25 μM) was not detrimental to the cells (Fig. 6A). CsA dose-dependently reduced DNA fragmentation and laddering after 48 h of menadione exposure (Fig. 6B).

Discussion

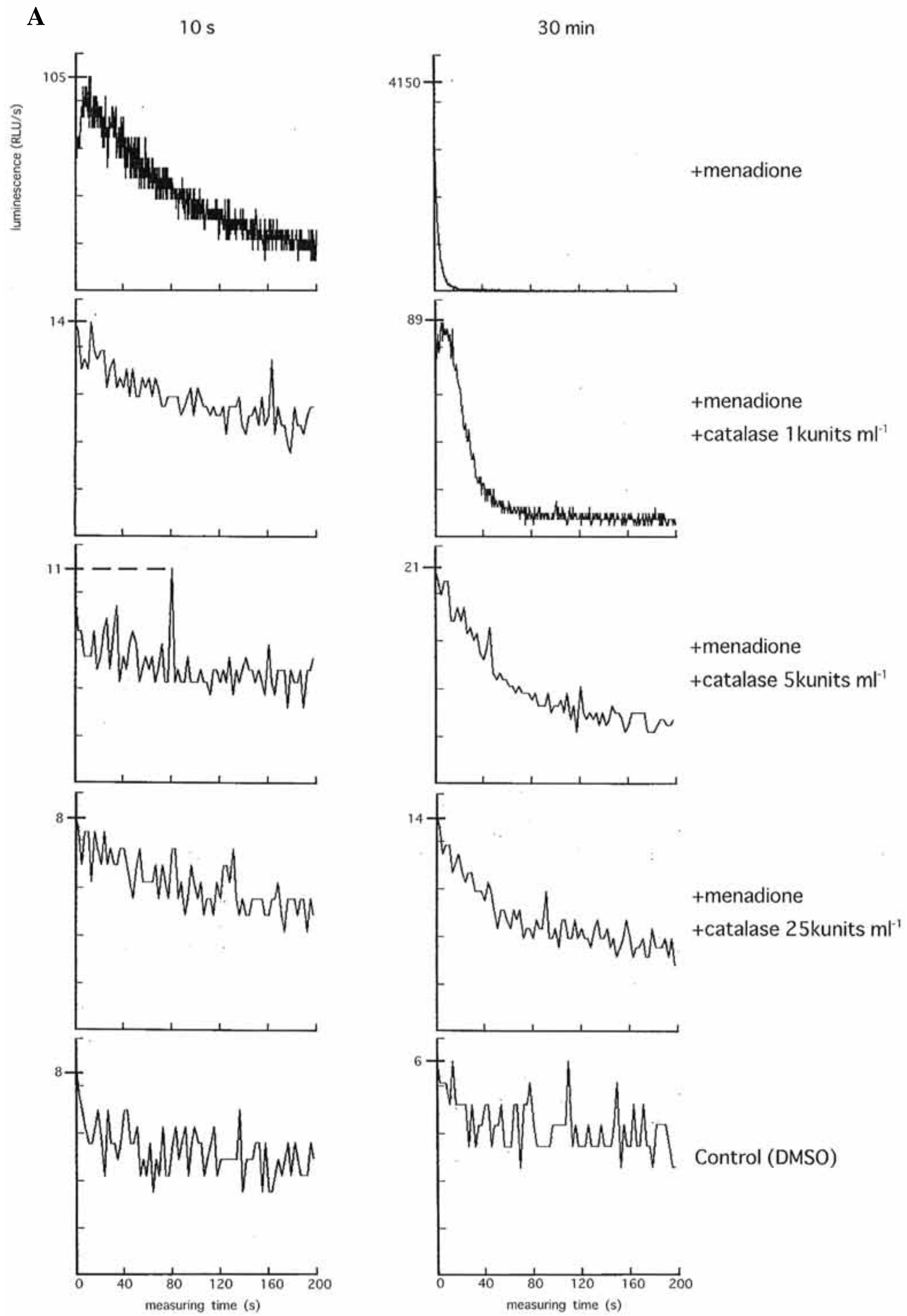
Menadione is a quinone compound that induces apoptotic cell death in animal and plant cells.

In an animal cell line, treatment with low concentrations of menadione caused apoptosis, and treatment with higher concentrations caused necrosis, which is a non-apoptotic form of cell death (SATA *et al.* 1997). Therefore, we first determined an appropriate menadione concentration to induce apoptosis-like cell death in ginkgo cultured cells. Menadione cytotoxicity was evaluated using the MTT assay, which is based on the reduction of the water-soluble yellow MTT tetrazolium salt to an insoluble purple-colored MTT formazan by the redox activity of living cells. A decrease in cellular MTT reduction is an indicator of cell injury (ABE and MATSUKI 2000). Based on MTT assays, menadione treatment at concentrations higher than 300 μM were found to be very toxic to ginkgo cultured cells. At these levels, almost none of the cells were viable, and DNA laddering was observed in cells treated with 300 or 1000 μM menadione for 48 h (Fig. 1). We therefore used treatments of 300 μM menadione to induce apoptotic cell death in cultured ginkgo cells.

We next investigated the effects over time of 300 μM menadione on the levels of DNA fragmentation and cell viability (Fig. 2). DNA degradation was already apparent after 21 h of exposure, but oligonucleosomal fragments were not visible until 48 h, suggesting that high molecular weight DNA begins to degrade as early as 21 h after menadione addition, and continues to degrade into oligonucleosomal-sized fragments. Nuclear DNA degradation into large fragments of about 50 to 500 kbp, and the subsequent appearance of oligonucleosomal fragments, have been reported to be associated with apoptosis in animals and in some plant programmed cell death processes (LEIST and NICOTERA 1997, KOUKALOVÁ *et al.* 1997, MITTLER *et al.* 1997). Menadione-induced cell degradation processes in ginkgo cells may occur *via* similar mechanisms. To prove this hypothesis, separation of high-molecular-weight DNA fragments using pulsed-field gel electrophoresis will be essential, and the best source of DNA for such experiments will be protoplasts. The isolation of protoplasts from menadione-treated dying cells or the addition of menadione to isolated protoplast cultures should provide a source for such protoplasts.

Our cytological assays revealed that changes in cell morphology occurred only during the final stage of the menadione-induced cell degradation process. Apoptotic bodies form in menadione-treated tobacco protoplasts and mycotoxin-treated tomato cells (SUN *et al.* 1999b, WANG *et al.* 1996), and cultured tobacco cells exposed to low temperatures exhibit protoplast shrinkage and chromatin condensation (KOUKALOVÁ *et al.* 1997). In the present study, protoplast shrinkage was observed after 72 h of menadione treatment (Fig. 2C-g), but no morphological changes were apparent in the nuclei of these cells (Fig. 2C-h). Degradation of the DNA into small fragments was apparent after 48 h of menadione treatment, and after 72 h of exposure the high-molecular-weight DNA had completely disappeared and DNA ladders were strongly detected (Fig. 2B). Nevertheless, no changes in nuclear morphology were apparent at these points. These results indicate that DNA fragmentation and laddering in apoptotic cell death are not exclusively associated with the condensation or fragmentation of nuclei.

Menadione cytotoxicity is thought to be caused mainly by the oxidative stresses that result from the metabolism of menadione in the cell. Depletion or arylation of cellular thiols are also thought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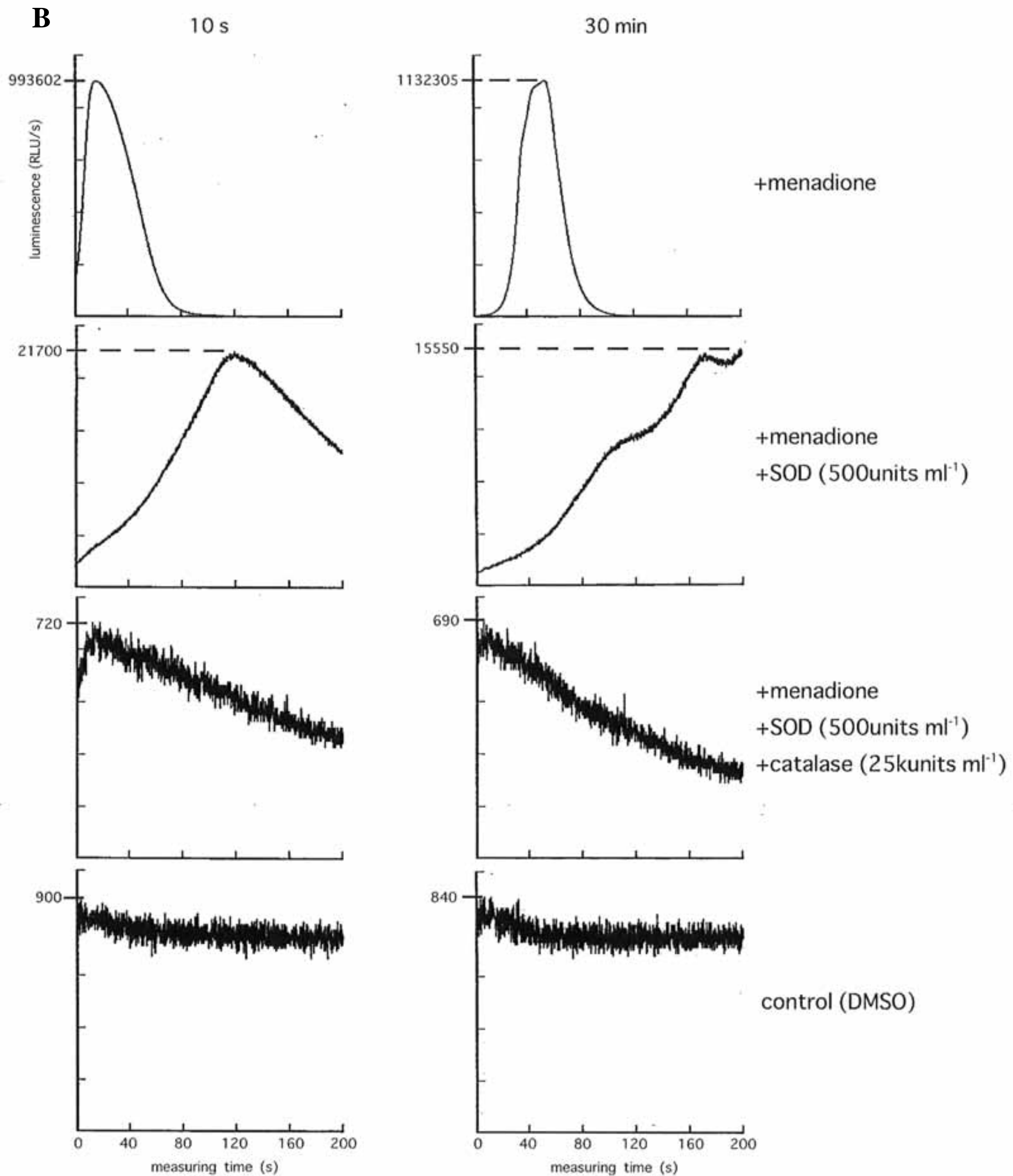

Fig. 3. Detection of H_2O_2 and O_2^- generation 10 s and 30 min after addition of 300 μM menadione. Typical luminol (A) and CLA (B) chemiluminescence signals are shown, indicating the presence of H_2O_2 and O_2^-, respectively. Catalase (0-25 kunits ml^{-1}) or SOD (0-500 units ml^{-1}) were added 30 min before the addition of menadione. The number in each vertical axis represents the maximum RLU/s value.

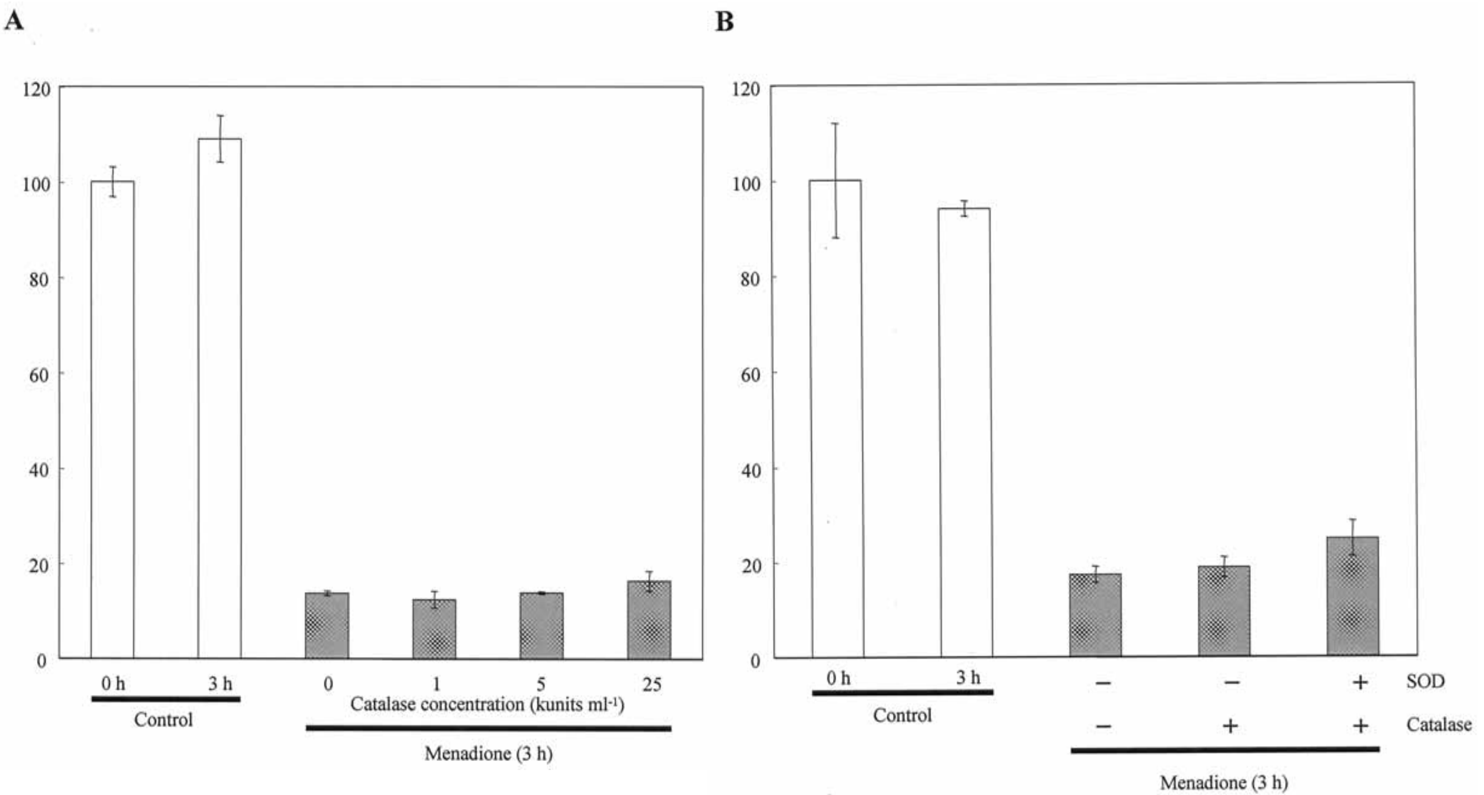

Fig. 4. Effects on menadione-induced cell injury of enzymes that scavenge reactive oxygen species. Catalase (0-25 kunits ml^{-1}) or a mixture of SOD (0 or 500 units ml^{-1}) and catalase (25 kunits ml^{-1}) were added 30 min before the addition of menadione (300 μM). Cell viability was assayed using the MTT method after 3 h of exposure to menadione plus either catalase (A) or a mixture of SOD and catalase (B). Values represent the mean of three experiments with the standard deviation.

to contribute to menadione cytotoxicity (CHO *et al.* 1997). In this study, menadione-induced rapid generation of H_2O_2 and O_2^- in the culture was confirmed using luminol and CLA as indicators (Fig. 3). Luminol and CLA are not immediately taken up by cultured tobacco cells, and these agents can thus be used to detect extracellular reactive oxygen species (KAWANO *et al.* 2000). Neither H_2O_2 nor O_2^- was detected with the addition of menadione to culture medium from which the cells had been removed (data not shown), indicating that these reactive oxygen species are generated exclusively *via* cellular menadione metabolism. Addition of catalase effectively prevented increases in H_2O_2 levels in the culture, whereas addition of SOD alone did not completely prevent the increase in O_2^- levels (Fig. 3). Since SOD eliminates O_2^- and the resultant H_2O_2 may itself affect SOD activity, we also treated cells with a mixture of SOD and catalase, which eliminated the O_2^- in the cultures. Although this treatment fully reduced both the H_2O_2 and O_2^- levels, cell viability was not appreciably higher (Fig. 4), which contradicts previously published results (SUN *et al.* 1997), in which extracellularly added catalase greatly increased the viability of menadione-treated rat osteoblast cells. It is probable that in ginkgo cells treated with menadione, O_2^- and H_2O_2 are generated in an apoplastic region or at the outer surface of the plasma membrane. As catalase cannot effectively scavenge H_2O_2 in this region due to its high molecular size, it thus fails to inhibit the cell damage triggered by extracellularly generated H_2O_2. It is also probable that O_2^- and H_2O_2 are generated intracellularly, and that these and the resultant reactive oxygen species may damage the cell from the inside. Alternatively, the decrease in gingko cell viability may be the result of direct actions of menadione, such as thiol depletion or arylation, rather than the result of damage to the cell caused by reactive oxygen species.

Recent studies have revealed that mitochondria have important roles in animal-cell apoptotic processes (SUSIN *et al.* 1998, PASTORINO *et al.* 1998). The loss of mitochondrial transmembrane potential through a mitochondrial permeability transition (MPT) and subsequent Cyt *c* release from the mitochondrial intermembrane space into the cytosol are key events that occur upstream of the caspase cascade. CsA is known to inhibit apoptosis in animal cells (SUGANO *et al.* 1999, PRITCHARD *et al.* 2000). CsA prevents MPT by binding to a cyclophilin-family protein associated with the MPT pore, increasing the probability of closure of the pore (NICOLLI *et al.* 1996). We have shown that CsA reduces menadione-induced DNA fragmentation in a dose-dependent manner, but does not appreciably inhibit the loss of cell viability (Fig. 5). However, the caspase-specific inhibitor Ac-DEVD-CHO had no effect on cell viability or DNA fragmentation (Fig. 4). These results indicate that in ginkgo cells treated with menadione, MPT may trigger cell degradation processes, as it does in many animal systems, but that caspase-like proteases do not participate in this process.

In contrast to our results, elevation of caspase-like protease activity and inhibition of cell death or degradation by caspase-specific inhibitors have been reported in some studies of plant apoptotic cell death (del POZO and LAM 1998, SUN *et al.* 1999b, DE JONG *et al.* 2000). Nevertheless, no caspase-like protease has been isolated from plants, and there are no obvious homologs of caspase-like protease genes in the public plant genome databases (JONES 2000). It is possible that unrelated proteolytic enzymes with substrate specificities similar to those of

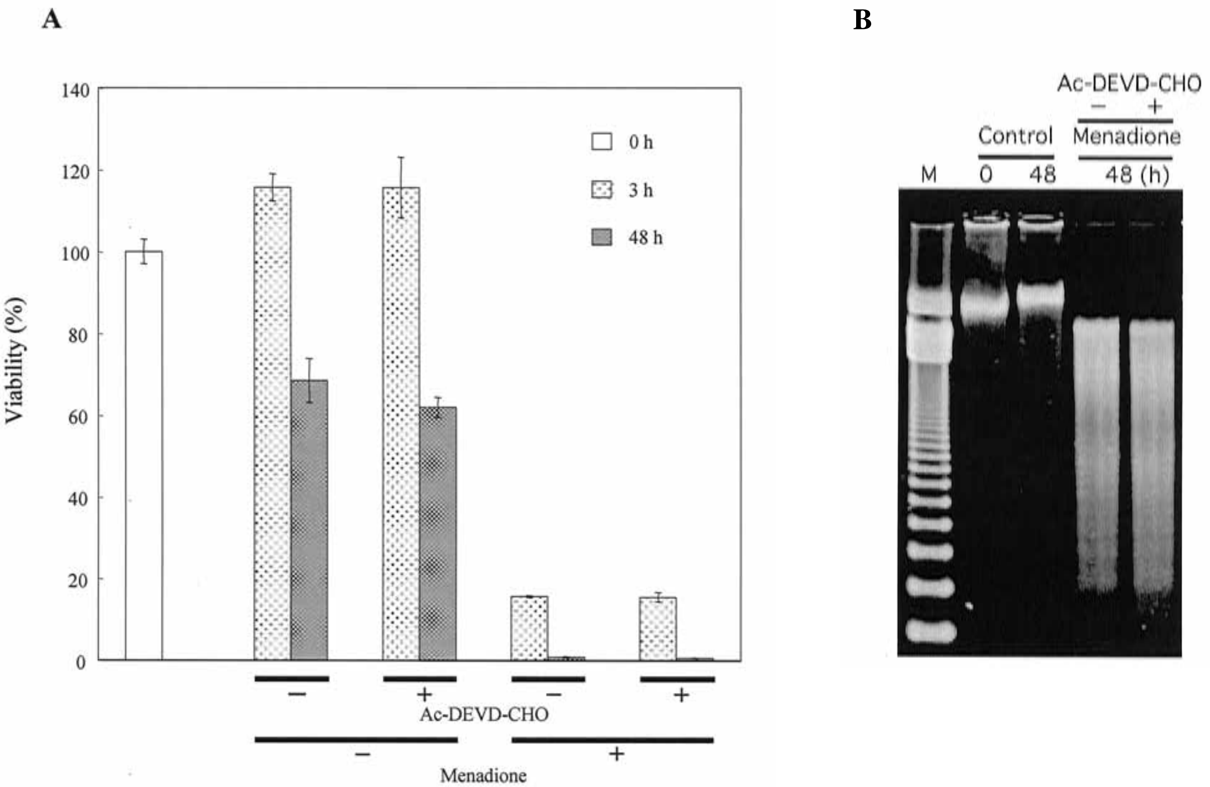

Fig. 5. Effect of the caspase-specific inhibitor Ac-DEVD-CHO on cell viability and cellular DNA fragmentation in menadione-treated cells. (A) Cell viability assayed using the MTT method. Values represent the mean of three experiments with the standard deviation. (B) Cellular DNA electrophoresed on a 2% agarose gel and stained with ethidium bromide. Three parallel cultures were combined and used for DNA analysis.

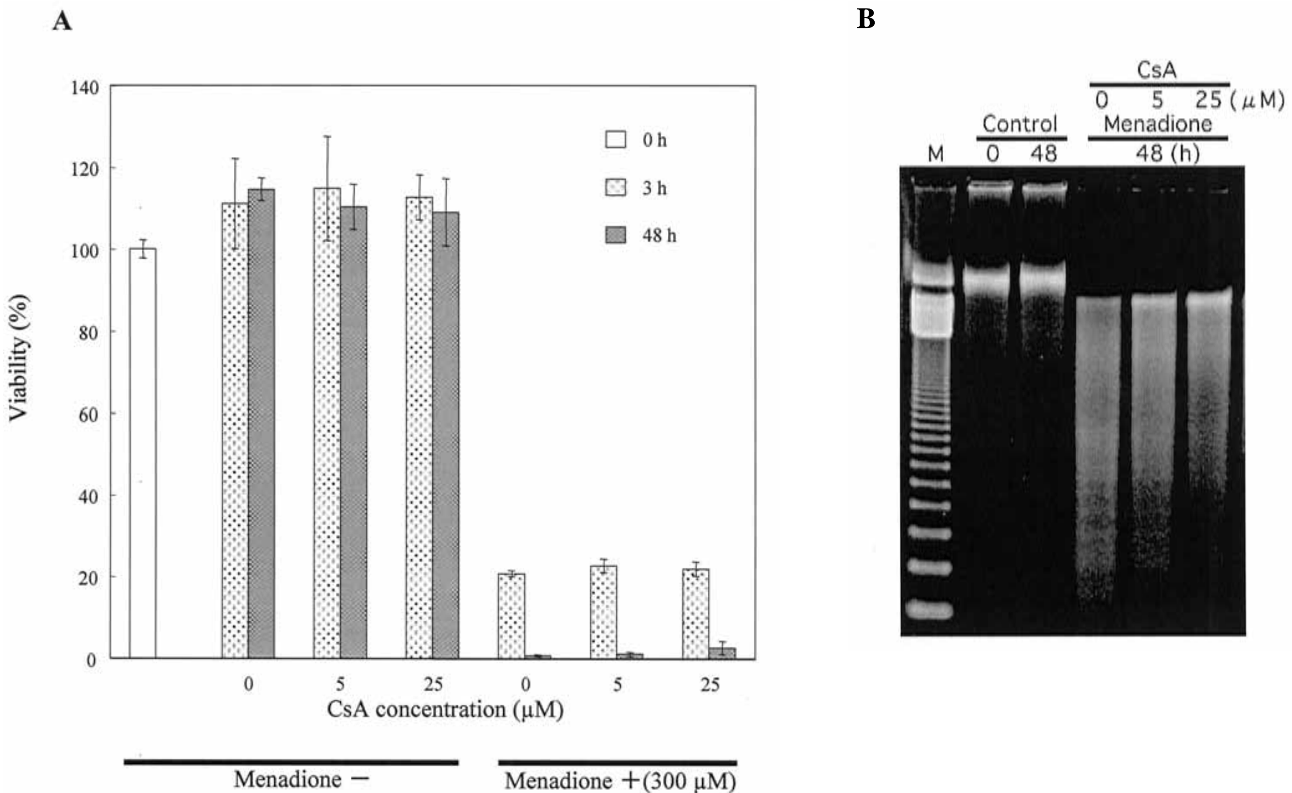

Fig. 6. Effect of CsA on menadione-induced cell injury and cellular DNA fragmentation. (A) Cell viability assayed using the MTT method. Values represent the mean of three experiments with the standard deviation. (B) Cellular DNA electrophoresed on a 2% agarose gel and stained with ethidium bromide. Three parallel cultures were combined and analyzed.

mammalian caspases operate in apoptotic cell death in some plant species. In ginkgo, enzymes with caspase-like activities may not be involved in this process, in contrast to results from other higher plants.

In summary, our results indicate that mitochondria may have a role in ginkgo cell death and degradation, as in mammals and other higher plant cells, but that the enzymes involved in these processes may differ from those of other higher plants. The discovery of the gingko enzymes involved in oxidant-induced cell death and degradation pathways could reveal factors that are specific to apoptosis in woody plants.

Acknowledgments

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Summary

Menadione induces apoptosis in the cells of many animal species and of some plant species. We report here on the detection of apoptotic features in menadione-treated ginkgo suspension-cultured cells. Treatment of gingko cells with menadione at concentrations of 300 and 1000 μM caused a rapid loss of cell viability, and agarose gel electrophoresis of DNA extracted from the dying cells revealed the ladder pattern that is one of the hallmarks of apoptosis. Increases in superoxide anion and hydrogen peroxide levels were observed in the culture within a few seconds after menadione addition. The effects of known apoptosis inhibitors on menadione-induced cell injury were also examined. Catalase, superoxide dismutase (SOD), and a caspase-specific inhibitor had little or no inhibitory effect on the loss of cell viability. Treatment with several concentrations of the immunosuppressive drug cyclosporin A (CsA), which prevents mitochondrial dysfunction, reduced DNA fragmentation in a dose-dependent manner, but did not inhibit the loss of cell viability. These results show that menadione induces apoptotic cell death in ginkgo cells and that mitochondria may play a role in the cell degradation process.

Key words: *Ginkgo biloba* cell cultures, menadione, programmed cell death

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イチョウ培養細胞におけるメナジオンによる細胞死の誘導

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要　　旨

メナジオンは，多くの動物細胞及びいくつかの植物細胞においてアポトーシスを誘導することが知られている。本稿では，イチョウ懸濁培養細胞をメナジオン処理した際に観察されるアポトーシス様の細胞死について報告する。

イチョウ懸濁培養細胞を300μM又は1000μMのメナジオンで処理することにより，細胞生存率の急速な低下が引き起こされるとともに，それらの細胞から抽出したDNAの電気泳動像が，アポトーシスの特徴のひとつであるラダー状のパターンを呈することが明らかになった。また，メナジオン処理の後数秒以内に細胞系中のスーパーオキサイドアニオン及び過酸化水素の濃度が上昇することが確認された。

メナジオンによる細胞傷害に対する，既知のアポトーシス阻害剤の影響についても検討した。カタラーゼ，スーパーオキサイドディスムターゼ，及びカスパーゼ特異的阻害剤は細胞生存率の低下をほとんど抑制しなかった。サイクロスポリンAは，用量依存的にDNAの分解を軽減したが，細胞生存率の低下は抑制しなかった。サイクロスポリンAは免疫抑制剤であり，ミトコンドリアの機能障害を抑制する作用を持つことが知られている。

これらの結果から，メナジオンはイチョウ培養細胞においてアポトーシス様の細胞死を誘導すること，及びメナジオン処理細胞での細胞の分解過程にミトコンドリアが関与している可能性があることが示された。

キーワード： イチョウ培養細胞・メナジオン・プログラム細胞死