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Menadione Biphasically Controls JNK-Linked Cell Death in Leukemia Jurkat T Cells

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ABSTRACT

Signals for cell-death induction by menadione were studied in Jurkat T cells. Low concentrations of menadione (10–20 μ*M*) and H_2O_2 (10–50 μ*M*) induced cell death accompanying low (menadione: <5%) or moderate (H_2O_2: 10–15%) levels of DNA fragmentation in Jurkat cells. These concentrations of menadione (10 μ*M*) and H_2O_2 also caused membrane (necrotic) cell death at unproportionally high (80%) and proportional (10–30%) levels, respectively. Higher concentrations (100–5,000 μ*M*) of H_2O_2 exclusively induced membrane cell death. Unexpectedly, 30–300 μ*M* menadione induced ever-decreasing levels of necrotic cell death in a concentration-dependent manner. An *in vitro* kinase assay showed that 20–50 μ*M*, but not >100 μ*M*, menadione induced activation of c-Jun NH_2-terminal kinase (JNK), whereas a striking activation of JNK was induced by 500–5,000 μ*M* H_2O_2. Induction of cell death by a low concentration of menadione was partially inhibited in dominant negative JNK gene-transfected Jurkat/VPF cells. A high concentration (300 μ*M*) of menadione was found to inhibit cell-death induction by high concentrations (200–5,000 μ*M*) of H_2O_2. The JNK inhibitory activity of menadione was also demonstrated in a cell-free system. However, menadione did not activate JNK *in vitro*. These results suggest that JNK is required for induction of not only apoptotic cell death, but also necrotic cell death in Jurkat T cells and that menadione biphasically controls this JNK-linked signal for inducing cell death. *Antioxid. Redox Signal.* 4, 371–378.

INTRODUCTION

LOW CONCENTRATIONS OF REACTIVE OXYGEN SPECIES (ROS), such as superoxide and H_2O_2, induce apoptosis through caspase activation in various cells, whereas high concentrations of ROS induce necrosis accompanying caspase inactivation and ATP depletion (6, 10, 15, 36). ROS activate a number of signal-transducing molecules, including protein tyrosine kinase Lck and mitogen-activated protein kinase (MAPK) family kinases such as extracellular signal-regulated kinase (ERK), c-Jun NH_2-terminal kinase (JNK), and p38 (10, 18, 29). JNK, which is activated by oxidative stress or inflammatory cytokine, has been reported to be involved in the signal transduction for apoptosis induction, whereas ERK, which is activated by mitogen or growth factors, has been shown to be required for cell proliferation, differentiation, and survival (9, 12, 43, 46, 50). For induction of apoptosis, JNK has been shown to inactivate the antiapoptotic molecule Bcl-2 in mitochondria through its phosphorylation for releasing cytochrome *c* from mitochondria, which is required for caspase activation (43, 52). However, it has also been reported that JNK is required for cell survival (2, 4, 32, 34). The roles of JNK in promotion of cell death or cell survival are therefore still controversial.

JNK is activated by signals through upstream signal pathways such as the MEK kinase 1 (MEKK1)-MAPK kinase 4 (MKK4 or SEK1)/MKK7 pathway and the apoptosis signal-regulating kinase 1 (ASK1)-MKK4 pathway (9, 17, 18). Acti-

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vated MEKK1 selectively transduces signals for JNK activation through complex formations between MEKK1 and MKK4 and between MKK4 and JNK (49). JNK activation signals are also transduced through complex formation with scaffold proteins and upstream kinases such as JIP, LMK, and MKK7 (11, 48) or β-arrestin, ASK1, and MKK4 (23). On the other hand, ROS may activate ASK1 by releasing its inhibitory molecule thioredoxin (35). Adler *et al.* have recently shown that JNK activity is down-regulated by the associated molecule glutathione *S*-transferase (GST) and is activated by the release of GST oligomerized by UV or H_2O_2 (1). Multiple signal pathways may therefore be initiated by different physiological or oxidative stress-mediating agents for regulating JNK activities that may decide cell death or cell survival.

Menadione, which is also known as vitamin K_3, has a 2-methyl-1,4-naphthoquinone structure and is known to display a cytotoxic action for inducing cell death when added to cultures of various cell types (25, 26, 37, 42). However, the mechanism and potential signal pathway for cell-death induction are not clear. Menadione generates superoxide through autooxidation of one electron-reduced menadione, which is induced by enzymes such as NADPH-cytochrome P450 reductase, or through inhibition of the mitochondrial respiratory chain, and subsequently generates H_2O_2 as a metabolic product (6, 26, 33, 42, 47). Menadione also induces superoxide generation through nonenzymatic interaction with thiols such as reduced glutathione (GSH) or protein-SH (33, 41, 47). In addition, menadione inhibits glutathione reductase and induces severe depletion of GSH (3, 45). Those properties of menadione to induce ROS/H_2O_2 suggest that menadione shares a common signal pathway with H_2O_2 for cell-death induction. However, menadione has also been shown to display some unique properties that are not shared with H_2O_2. Menadione induces cell death through modification of protein-SH and GSH by oxidation (42) or arylation (26) or through alteration of cellular Ca^{2+} homeostasis (25). Membrane damage is characteristic of menadione-induced cytotoxicity (25, 26, 42), and a high concentration of menadione inactivates Fas-induced caspase activation and induces necrosis (36). However, recent studies have shown that a low concentration of menadione induces apoptosis in various cells such as pancreatic acinar cells (37), T cells (5), cardiac muscle cells (44), and hepatocytes (19). Menadione induced apoptosis of cardiac muscle cells through activation of JNK and caspases (44). Menadione-induced apoptosis of a Jurkat T-cell leukemia subclone was mediated by the Fas/Fas ligand system (5). Menadione-induced apoptosis was partially prevented by Bcl-2 (16) and by cytokines such as interleukin-3 (31). Menadione also inhibited cell growth by reducing activities of both $p34^{cdc2}$ kinase and phosphatase (20). On the other hand, a synthetic compound of vitamin K, 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone (Cdp 5), induced necrosis through activation of ERK (21). Jones *et al.* showed that, whereas both menadione and H_2O_2 induced caspase activation and apoptosis, caspase inhibition blocked H_2O_2- but not menadione-induced apoptosis (19). However, there have been few studies in which the signals for menadione-induced cell death in T cells have been characterized.

In the present study, we characterized the menadione-mediated signal for cell-death induction in human leukemia Jurkat T-cells in comparison with the H_2O_2-mediated one. The results show that menadione-induced cell death is at least in part JNK-dependent and that JNK is biphasically regulated by menadione in a concentration-dependent manner.

MATERIALS AND METHODS

Cells and reagents

Human leukemia Jurkat T cells and dominant negative JNK gene-transfected Jurkat cells (Jurkat/VPF cells) were used. Jurkat/VPF cells were established as described previously (12). The medium used for the cell culture (culture medium) was RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Menadione was purchased from Sigma (St. Louis, MO, U.S.A.).

Assay of viability

Cells (10^5 cells/well) were cultured with or without oxidants in 0.2 ml of culture medium in 96-well plates for 16 h. Viable cells and dead cells were counted by the trypan blue exclusion test, and viability was presented as the ratio of viable cells to total cells.

Analysis of DNA fragmentation by flow cytometry

Cells were lysed in a hypotonic solution (0.1% sodium citrate, 0.1% Triton X-100) containing 50 μg/ml propidium iodide (PI), and both intact and fragmented nuclei were measured for propidium iodide by fluorescence intensity. The percentages of fragmented nuclei were cultured as described previously (30).

Western blotting

Western blotting was carried out as described previously (28). In brief, cells (5×10^6/100 μl) were lysed with an equal volume of 2× sample buffer and boiled for 3 min. The cell lysates were passed through a syringe with a 26G needle before being applied on 10% sodium dodecyl sulfate (SDS)–polyacrylamide gels. The protein-transferred membrane was incubated with the first antibody, followed by goat anti-rabbit IgG or sheep anti-mouse IgG conjugated to horseradish peroxidase (Amersham Life Science, Boston, MA, U.S.A.), which was visualized using a western blot chemical reagent, Renaissance (NEN, Boston, MA, U.S.A.). The autograph of luminescence on x-ray film was analyzed by comparing with protein molecular weight standards (New England Biolabs, Beverly, MA, U.S.A.). Rabbit antibodies to phospho-ERK and phospho-MKK4 were purchased from Cell Signaling Technology (Beverly, MA), and anti-phospho-JNK rabbit antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

In vitro *kinase assay*

The *in vitro* kinase assay was done as described previously (14). Cells were lysed with 1.0 ml of ice-cold RIPA buffer (10 m*M* Tris-HCl, pH 8.0, 1% Triton X-100, 150 m*M* NaCl, 1 m*M* EDTA, 0.5 m*M* Na_3VO_4, and 1 m*M* phenylmethylsul-

fonyl fluoride). The lysate was cleaned by centrifugation at 15,000 rpm for 20 min at 4°C, and anti-JNK rabbit antibody (Santa Cruz Biotechnology) was added to the supernatant. The immunoprecipitates were collected by incubating with protein A–Sepharose beads (Pierce, Rockford, IL, U.S.A.). The reaction mixture for the kinase assay was prepared by adding 1.5 μg of GST-c-Jun (Cell Signaling Technology), an exogenous substrate, 370 kBq [$\gamma-^{32}$P]ATP (NEN, Wilmington, DE, U.S.A.), and kinase buffer (10 m*M* Tris-HCl, pH 7.4, 5 m*M* $MgCl_2$) with a final volume of 30 μl. The kinase reaction was carried out for 20 min at 30°C and was stopped by adding 30 μl of 2× SDS sample buffer with 2-mercaptoethanol. The immunoprecipitates were then heated in a boiling water bath for 3 min, and phosphoproteins were analyzed on 10% SDS-polyacrylamide gels. Gels were dried and exposed to x-ray film at −80°C for autoradiography.

RESULTS

Induction of apoptotic and necrotic cell death by menadione and H_2O_2 in Jurkat cells

Jurkat cells were stimulated with 5–100 μ*M* menadione or 20–200 μ*M* H_2O_2 for 16 h. Low concentrations of menadione (10 μ*M*) and H_2O_2 (20–50 μ*M*) induced DNA fragmentation in Jurkat cells as detected by flow cytometry (Fig. 1). The menadione-induced DNA fragmentation was, however, much weaker than the H_2O_2-induced DNA fragmentation. Correspondingly, 20 μ*M* menadione, which induced low levels (<5%) of DNA fragmentation (Fig. 1), caused a striking level (50–80% in repeated experiments) of necrotic cell death, as

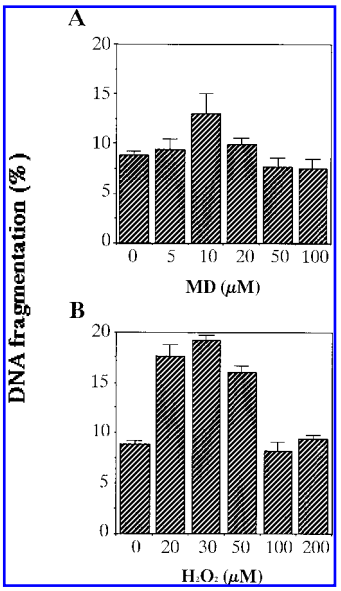

FIG. 1. Induction of DNA fragmentation by menadione and H_2O_2 in Jurkat cells. Jurkat cells (10^5 cells in 0.2 ml) were stimulated with or without 5–100 μ*M* menadione (MD; **A**) or 20–200 μ*M* H_2O_2 (**B**) for 16 h in triplicate cultures. Percentage of DNA fragmentation (mean ± SD) as assayed by flow cytometry is shown.

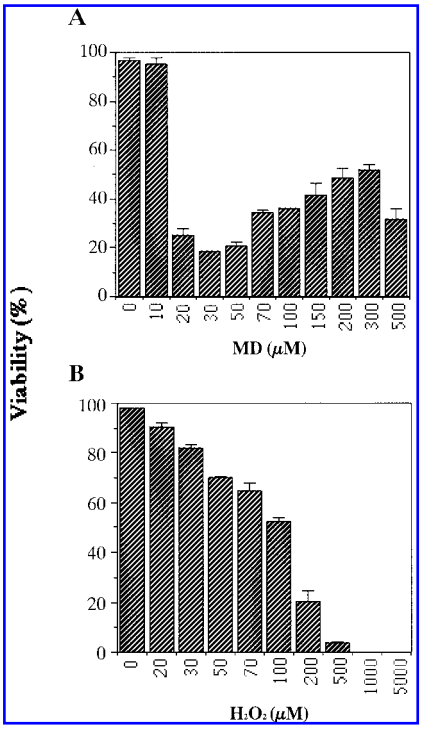

FIG. 2. Induction of cell death by menadione and H_2O_2. Jurkat cells (10^5 cells in 0.2 ml) were stimulated with or without 10–500 μ*M* menadione (MD; **A**) or 20–5,000 μ*M* H_2O_2 (**B**) for 16 h. Cell viabilities (mean ± SD) in triplicate cultures are shown.

determined by the trypan blue dye exclusion test (Fig. 2). This result contrasted with another observation that 20–30 μ*M* H_2O_2, which induced more extensive DNA fragmentation (10–15%) than did the same concentration of menadione, caused necrotic cell death at a level (20%) roughly corresponding to that of DNA fragmentation. Higher concentrations of menadione (50 μ*M*) and H_2O_2 (200 μ*M*), which barely induced DNA fragmentation (Fig. 1), caused high levels (~80%) of necrotic cell death (Fig. 2). Unexpectedly, however, the levels of necrotic cell death induced by 70–300 μ*M* menadione decreased in a concentration-dependent manner when compared with that induced by 30–50 μ*M* menadione (Fig. 2A). Such biphasic cytotoxity was not observed with higher concentrations of H_2O_2 (500–5,000 μ*M*), which caused complete necrotic cell death (almost 100%). These results demonstrated a clear difference between menadione and H_2O_2 in cell-death induction in Jurkat cells.

Induction of phosphorylation and activation of JNK by menadione and H_2O_2

In order to determine the correlation between menadione/H_2O_2-induced cell death and potential activation of JNK, Jurkat cells were stimulated with 10–100 μ*M* menadione or 50–5,000 μ*M* H_2O_2 for 30 min for western blotting analysis of phosphorylation levels of kinase proteins (Fig. 3A). Definite JNK phosphorylation was induced by 500–5,000 μ*M* H_2O_2, but not by 100 μ*M* menadione. JNK phosphorylation

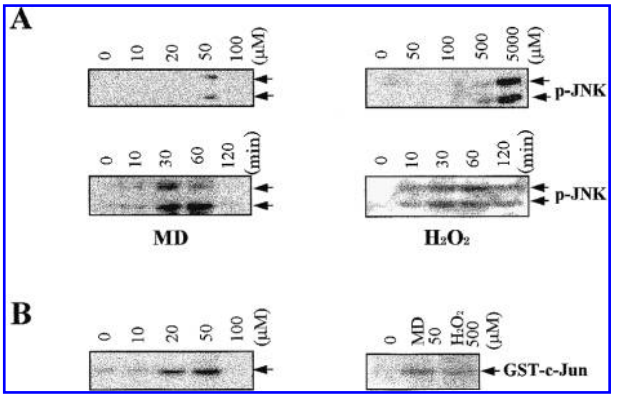

FIG. 3. Induction of phosphorylation and activation of JNK by menadione and H_2O_2. (**A**) Jurkat cells (5×10^6 cells) were stimulated with or without 10–100 μ*M* menadione (MD) or 50–5,000 μ*M* H_2O_2 for 30 min, or with 50 μ*M* menadione or 5 m*M* H_2O_2 for 0–120 min. Phosphorylation levels of JNK were assayed by western blotting. (**B**) Jurkat cells were stimulated with or without 10–100 μ*M* menadione (**left panel**) or with 50 μ*M* menadione or 500 μ*M* H_2O_2 (**right panel**) for 30 min, and kinase activity of JNK in cell lysates was assayed using GST-c-Jun as a substrate.

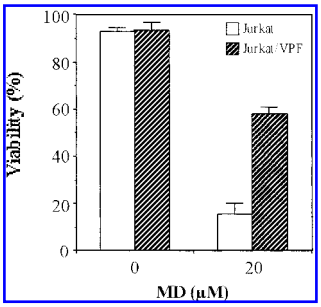

FIG. 4. Inhibition of induction of cell death by a low concentration of menadione in dominant negative JNK gene-transfected Jurkat/VPF cells. Jurkat cells (10^5 cells in 0.2 ml) and Jurkat/VPF cells were stimulated with 20 μ*M* menadione (MD) for 16 h. Cell viabilities (mean ± SD) in triplicate cultures are shown.

was, however, induced by 50 μ*M* menadione (Fig. 3A). Correspondingly, the results of the *in vitro* kinase assay showed that 20–50 μ*M*, but not 100 μ*M*, menadione increased the catalytic activity of JNK to phosphorylate c-Jun as the substrate (Fig. 3B). The *in vitro* kinase assay also showed that 500 μ*M* H_2O_2 induced a level of catalytic activity of JNK comparable to that induced by 50 μ*M* menadione (Fig. 3B). These results indicate that menadione biphasically regulates JNK activity by increasing its concentration, whereas H_2O_2 induced marked JNK phosphorylation/activation in a concentration-dependent manner.

Dependency of menadione-induced cell death on JNK

The requirement of JNK activity for induction of cell death by menadione was investigated. As shown in Fig. 4, Jurkat/VPF cells, which contain dominant negative JNK cDNA, were partially resistant to induction of cell death by 20 μ*M* menadione (Fig. 4). This result suggested that JNK activity was required for induction of cell death by a low concentration of menadione.

Inhibition of H_2O_2-induced cell death by a high concentration of menadione

We next investigated whether a high concentration of menadione inhibits induction of necrotic cell death by a high concentration of H_2O_2 (Fig. 5). Jurkat cells were stimulated with 200–5,000 μ*M* H_2O_2 with or without 300 μ*M* menadione for 16 h. H_2O_2 at a concentration of >300 μ*M* induced almost complete cell death, whereas 300 μ*M* menadione induced only ~50% cell death. However, menadione, which was added to the cell suspension together with H_2O_2, partially inhibited the induction of complete cell death by 200–5,000 μ*M* H_2O_2, and the extent of cell death induced by menadione plus 5,000 μ*M* H_2O_2 was only slightly more than that induced by menadione alone. These results demonstrated that a high concentration of menadione not only failed to induce a high level of cell death, but also actively suppressed the signal for induction of necrotic cell death.

Inhibition of H_2O_2-induced phosphorylation and activation of JNK by menadione

We next investigated whether a high concentration of menadione, which barely induced activation of JNK, inhibits induction of JNK phosphorylation by a high concentration of H_2O_2. As shown in Fig. 6, 500 μ*M* menadione, which was added together with 5 m*M* H_2O_2, inhibited H_2O_2-induced JNK phosphorylation, whereas it, rather, augmented H_2O_2-induced ERK phosphorylation as a specific control (Fig. 6A). Such an inhibitory effect was also observed with 50–100 μ*M*, but not with 10–20 μ*M*, menadione (Fig. 6B). H_2O_2 (5 m*M*) induced MKK4 phosphorylation. Menadione at 100 μ*M*, but not 50 μ*M*, inhibited induction of MKK4 phosphorylation by

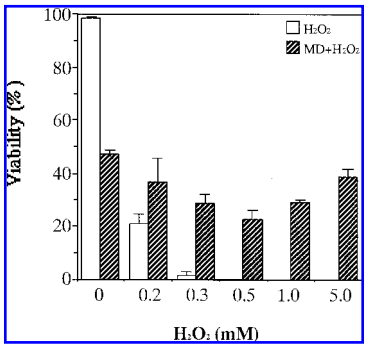

FIG. 5. Inhibition by a high concentration of menadione of induction of cell death by a high concentration of H_2O_2. Jurkat cells (10^5 cells in 0.2 ml) were stimulated with 0.2–5 m*M* H_2O_2 in the presence or absence of 300 μ*M* menadione (MD) for 16 h. Cell viabilities (mean ± SD) in triplicate cultures are shown.

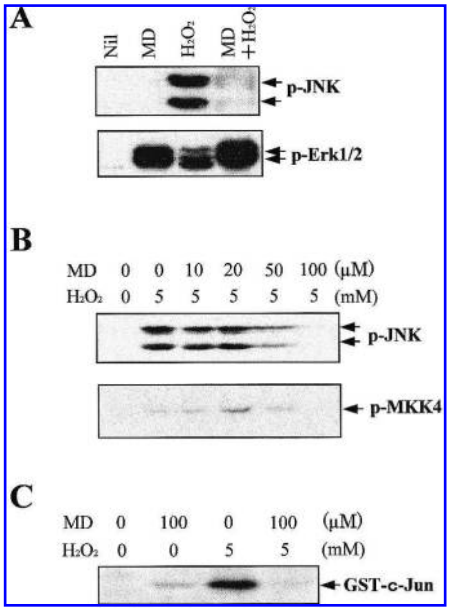

FIG. 6. Inhibition by menadione of induction of JNK phosphorylation and activation by H_2O_2. Jurkat cells (5×10^6 cells) were stimulated with or without 5 m*M* H_2O_2 for 30 min after pretreatment with or without 500 μ*M* (**A**) or 10–100 μ*M* (**B**) menadione (MD) for 10 min. Phosphorylation levels of JNK, ERK, and MKK4 were assayed by western blotting. (**C**) Jurkat cells were stimulated with or without 5 m*M* H_2O_2 for 30 min after pretreatment with or without 100 μ*M* menadione for 10 min. Kinase activity of JNK in cell lysates was assayed using GST-c-Jun as a substrate.

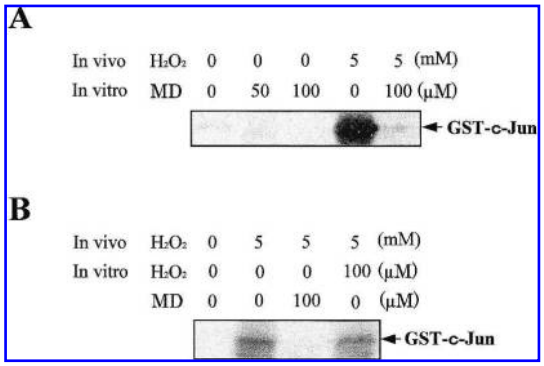

FIG. 7. Inhibition of JNK by menadione in a cell-free system. Jurkat cells (10^7 cells) were pretreated *in vivo* with or without 5 m*M* H_2O_2 for 30 min, JNK immunoprecipitated from these lysates was *in vitro* stimulated with 50–100 μ*M* menadione (MD; **A**) or with 100 μ*M* menadione or 100 μ*M* H_2O_2 (**B**) for 30 min, and kinase activities of these precipitates were assayed.

H_2O_2, indicating that the concentration of menadione required for inhibition of MKK4 phosphorylation was slightly higher than that for inhibition of JNK phosphorylation. These results suggested that inhibition of MKK4 is not necessarily required for inhibition of JNK phosphorylation by menadione. Inhibition of H_2O_2 activity by menadione was tested by the *in vitro* kinase assay (Fig. 6C). Treatment of Jurkat cells with 5 m*M* H_2O_2 greatly increased the catalytic activity of JNK, and this action of H_2O_2 was almost completely inhibited by 100 μ*M* menadione added together with H_2O_2 to activate JNK. These results demonstrated a marked difference between the abilities of menadione and H_2O_2 to activate JNK. The demonstrated action of menadione in suppressing H_2O_2-mediated JNK action might be correlated with its action in partially rescuing the Jurkat cells from a high level of cell death induced by H_2O_2.

Inhibition of JNK activity by menadione in a cell-free system

We next investigated whether menadione directly regulates JNK activation in a cell-free system. JNK was immunoprecipitated from cell lysates of Jurkat cells that had been pretreated with 5 m*M* H_2O_2 or untreated and then treated *in vitro* with 50–100 μ*M* menadione or 100μ*M* H_2O_2. As shown in Fig. 7, menadione (100 μ*M*), but not H_2O_2 (100 μ*M*), acted to down-regulate the catalytic activity of JNK that had been activated *in vivo* by H_2O_2. Menadione (50–100 μ*M*) did not induce JNK activation *in vitro*. These results indicate that menadione inhibits the catalytic activity of JNK, possibly through interaction with JNK or JNK-associated molecules, whereas JNK requires other cellular factors for its activation.

DISCUSSION

The present study showed for the first time that menadione biphasically induced cell death in Jurkat cells when its concentration was increased from 10 to 300 μ*M*. The menadione-induced cell death was basically necrotic, although a low concentration (10 μ*M*) of menadione also induced a low level of apoptotic cell death, partially correlating with the results of earlier studies on the cytotoxic action of menadione against different cell types (19, 36, 44). Unexpectedly, menadione at a concentration higher than 50 μ*M* (70–300 μ*M*) partially rescued the cells from necrotic death.

In the present study, we concluded that JNK activation is crucially involved in the signaling for induction of cell death by menadione because induction of cell death by menadione was inhibited in dominant negative JNK gene-transfected Jurkat/VPF cells. This observation partially agrees with the finding of Turner *et al.* that menadione induced apoptosis through JNK activation in cardiac muscle cells (44). Potential roles of JNK activation in induction of apoptotic cell death in other stimulator–responder combinations have also been reported (9, 12, 43, 52). These include demonstration of JNK-dependent disruption of mitochondrial membrane potential for cytochrome *c* release (43), possibly through inactivation of Bcl-2 (52). The present results suggest that activation of JNK plays a role in induction of not only apoptotic cell death, but also necrotic cell death, although the mechanism of the action of menadione for inducing necrotic cell death remains to be clarified. We also found that a high concentration of menadione, which is less cytotoxic than a low concentration of menadione, partially rescued Jurkat T cells from induction

of complete cell death by a high concentration of H_2O_2. Possibly correlating with this observation, we found that low (20–50 μM), but not high (100–500 μM), concentrations of menadione induce JNK activation. This contrasted with another finding that H_2O_2 induces JNK activation in a concentration-dependent manner. More interestingly, menadione at concentrations of 100–500 μM, when added to a cell suspension together with a high concentration (5,000 μM) of H_2O_2, suppressed the activation of JNK induced by a high concentration of H_2O_2. This demonstrated that menadione at a high concentration acts as an active inhibitor of JNK activation. Xu *et al.* showed that activation of AP-1 as well as JNK occurs during H_2O_2-induced necrosis and that inhibition of Jun by antisense *c-Jun* significantly increased survival (51). A close relationship is therefore thought to exist in the action of a high concentration of menadione between menadione-induced partial rescue of cells from complete death and active inhibition of JNK activation.

Lastly, we demonstrated that menadione actively inhibits the activity of once-activated JNK through direct interaction with JNK or JNK-associated molecules in a cell-free system. Cross *et al.* previously showed that JNK activation is inhibited by an NAD(P)H quinone oxidoreductase (DT-diaphorase) inhibitor, dicoumarol, and by quinone-related compounds such as menadione, butylated hydroxyanisole, and hydroquinone, although the inhibition mechanism and effect on cell death of menadione were not determined (8). Dual-specific phosphatases of MAPK, such as M3/6 and MKP-1, were shown to strongly inhibit JNK and p38 activation (13, 27). However, inhibition of JNK through potential activation of phosphatases by menadione is not the case in the present study because inhibitors of phosphatases were added to a kinase buffer. Menadione also inhibited induction of MKK4 phosphorylation by H_2O_2 depending on its concentration (Fig. 6B). However, menadione suppressed the JNK action *in vitro* that had already been activated by H_2O_2 *in vivo,* suggesting MKK4-independent inhibition of JNK by menadione. These results suggest that menadione directly modifies the structure of JNK or JNK-associating molecules other than MKK4 for inactivation.

The factors known to be involved in the regulation of JNK activity are $p21^{WAF1/CIP1/Sdi1}$, a DNA-damage-inducible cell-cycle inhibitor (40), GST (1), a nuclear oncogene product, Evi-1 (22), and SHP72 (24). Adler *et al.* have recently shown that monomeric GST is associated with JNK for inhibition of its activation (1). GST (7), in addition to thioredoxin (35), is associated with ASK1, an upstream kinase of JNK activation pathways, for inhibition of its activation. Activation of JNK by oxidative stress such as H_2O_2 or UV was shown to require the release of GST from JNK through its dimerization, and *in vivo* induced JNK activity was inhibited *in vitro* by the addition of GST (1). GST inhibited phosphorylation of MKK4 and JNK, although the constitutively activated form of MEKK1 reversed GST inhibition of MKK4, but not JNK (1). GST inhibited H_2O_2 induction of cell death by increasing ERK, p38, and nuclear factor-κB activities together with suppression of JNK signaling (53). These results show that menadione and GST have a striking similarity in their inhibitory activities toward JNK and MKK4. This is intriguing because menadione and GST have completely different structures and functions: menadione is a prooxidant, and GST plays an important role as an antioxidant in detoxification of ROS and maintenance of the cellular redox state (38). Menadione modulates protein thiols through oxidation or arylation (26). It is therefore possible that menadione inhibits JNK activity through modulation of the thiols of JNK or interaction with JNK-associated molecules such as GST. Further study is needed to determine the precise mechanisms of menadione-induced modulation of JNK activity.

The mechanism of the action of JNK mediated by a low concentration of menadione remains to be clarified because menadione did not induce JNK activation in a cell-free system under the condition used in the present study. Meriin *et al.* showed that protein-damaging stresses such as heat shock, ethanol, and menadione activate JNK through repression of its dephosphrylation by putative JNK-phosphatases regulated by SHP72 in H9c2 myogenic cells (24). In their study, heat shock, but not UV, activated JNK without MKK4 phosphorylation. It was not clarified in the present study whether menadione activates JNK through inactivation of phosphatases or through activation of upstream MAPK kinases such as MKK7 in Jurkat cells. Taken together, the results suggest that menadione-induced activation and inhibition of JNK are regulated by different mechanisms.

Perspective

The findings obtained in the present study are unique and potentially useful for future study for the following reasons. Firstly, menadione-induced cell death is a good model for analysis of the shared role of JNK in induction of necrosis and apoptosis. The present study suggested that JNK activation is crucially involved in signaling for not only apoptotic, but also necrotic, cell-death induction. Secondly, menadione is useful for analysis of the molecular mechanism of JNK inhibition, because menadione has been shown to inhibit JNK activity in a cell-free system. Thirdly, some derivatives of menadione may be useful for a JNK-specific inhibitor. There is no inhibitor specific for JNK, whereas PD98059 and SB203580 are specific inhibitors for MEK and p38, respectively. It is our hope that menadione is reformed so as to be a much more useful derivative for application to clinical therapy.

ABBREVIATION

ASK1, apoptosis signal-regulating kinase 1; ERK, extracellular signal-regulated kinase; GSH, reduced glutathione; GST, glutathione *S*-transferase; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; MEKK1, MEK kinase 1; MKK4 and MKK7, MAPK kinase 4 and 7, respectively; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate.

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