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Hydroquinone-induced Apoptosis in HL-60 Cells

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Abstract. *To clarify the mechanisms by which hydroquinone (HQ; 1,4-benzenediol) produces apoptosis, HQ-induced cytotoxicity, internucleosomal DNA fragmentation, activation of superoxide dismutase (SOD), expression of Mn and Cu/ZnSOD mRNA and activation of caspase-3, -8 and -9 were investigated in the human promyelocytic leukemic cell line HL-60. Electrophoresis and activity staining of the SOD-enriched fraction showed that HQ reduced MnSOD activation more than Cu/ZnSOD activation, suggesting that it induces mitochondrial dysfunction at an early stage of apoptosis. Furthermore, the expression of MnSOD mRNA was suppressed to a greater extent than that of Cu/ZnSOD mRNA, implying that HQ causes apoptosis by inhibiting MnSOD induction. Release of cytochrome c and activation of procaspase-3 and -9, but not of procaspase-8, occurred more rapidly (as early as 6 h) in HQ-treated cells, suggesting that HQ activates the intrinsic pathway of apoptosis. Addition of the antioxidant N-acetyl-L-cysteine (NAC) significantly reduced the cytotoxicity of HQ. At a concentration that was cytotoxic to 50% of the cells (approximately 0.05 mM), HQ activated caspase-3; this effect was reduced in the presence of NAC. Interestingly, higher concentrations of HQ (0.1-0.2 mM) caused direct cell death; however, when combined with 5 mM NAC, the activation of caspase-3 was strongly enhanced, suggesting the promotion of apoptosis. The activation of caspase-3 by HQ/NAC combinations suggests that NAC, a precursor of intracellular glutathione synthesis, acts as a co-catalyst during HQ-induced apoptosis.*

Hydroquinone (1,4-benzenediol; HQ), a major marrow metabolite of the leukemogen benzene, is a widely used chemical that can also be found in the environment. In the chemical industry, it is used as a reducing agent and antioxidant, a chemical intermediate and a polymerization inhibitor in plastic dental materials (1). Less frequently, it is employed as a bleaching agent in certain dermatological conditions (2). Thus, HQ is frequently placed in direct contact with living tissues, albeit in very small quantities. However, the United States Environmental Protection Agency (U.S. EPA) has not classified HQ (*p*-hydroquinone) with respect to its potential carcinogenicity (3), while the International Agency for Research on Cancer (IARC) has placed it in Group 3: not classifiable (4). We have previously reported that HQ is an efficient scavenger of radicals such as cyanoisopropyl and benzoate radicals (5), but that, conversely, HQ itself readily produces semiquinone radicals, even under physiological conditions (6). These radicals are, however, efficiently inhibited by antioxidants such as *N*-acetyl-L-cysteine (NAC), catalase and sodium ascorbate (5). Based on a comparative study of HQ in myeloperoxidase (MPO)-containing HL-60 cells and MPO-deficient Jurkat T-lymphoblastic leukemia cells (7, 8), MPO-catalyzed bioactivation of HQ appears to be a prerequisite for HQ toxicity. HQ can act not only as an antioxidant, but also as a prooxidant, and this latter activity may be involved in its cytotoxicity and the induction of apoptosis. Although many investigators have studied the apoptosis-inducing effects of HQ and related compounds in HL-60 cells (7-15), the mechanism underlying these effects remains unclear. Therefore, as an extension of our previous studies (6, 7), we investigated HQ-induced cytotoxicity and apoptosis in HL-60 cells, and the effect of NAC on these actions.

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Materials and Methods

Materials. The following chemicals and reagents were obtained from the companies indicated: HQ and 1,4-benzoquinone (BQ; Tokyo Kasei Kogyo Ltd, Tokyo, Japan); Dulbecco's modified Eagle medium (DMEM) and RPMI1640 medium (GIBCO BRL, New York, USA); fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS, USA), NAC, bovine serum albumin (BSA), phenylmethyl-

sulfonylfluoride (PMSF), 3-[4,5-dimethylthiazo-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), riboflavin and nitro blue tetrazolium (NBT; Sigma Chemical Co., St. Louis, MO, USA); and dimethyl sulfoxide (DMSO), RNase A and proteinase K (Boehringer, Mannheim, Germany).

Cell culture. The HL-60 cells were cultured in RPMI1640 medium supplemented with 10% FBS.

Assay for cytotoxic activity. Near-confluent HL-60 cells were incubated for 24 h without (control) or with various concentrations of HQ in the presence of 0, 2.5, 5 or 10 mM NAC. Numbers of viable HL-60 cells were determined by trypan blue exclusion (15).

Assay for DNA fragmentation. DNA fragmentation was assayed by agarose gel electrophoresis. The cells were washed once with phosphate-buffered saline (PBS), then lysed in 20 μL of lysis buffer [50 mM Tris-HCl, pH 7.8, 10 mM EDTA and 0.5% (w/v) sodium *N*-lauroyl sarcosinate]. This solution was incubated sequentially with 0.5 mg/mL RNase A at 50˚C for 60 min and 0.5 mg/mL proteinase K at 50˚C for 60 min. DNA was extracted with chloroform/isoamyl alcohol, precipitated and subjected to 2% agarose gel electrophoresis. DNA from apoptotic HL-60 cells (apoptosis induced by UV irradiation) was run in parallel. The DNA fragmentation pattern was examined in photographs taken under UV illumination.

Assay of superoxide dismutase (SOD) activity. MnSOD and Cu/ZnSOD activity were detected by acrylamide gel electrophoresis using a modification of the method of Beauchamp and Fridovich (16). The cells were dissolved in sample buffer (1% Triton X-100, 0.25 M sucrose and 10 mM Tris-HCl, pH 7.4) and used as an enzyme source. An aliquot equivalent to $1x10^6$ cells was subjected to 9% polyacrylamide gel electrophoresis (PAGE) for 60 min. The gel was stained first with 4 mg/mL NBT, then with riboflavin (10 μg). It was left in the dark for 15 min after the addition of each staining agent, then illuminated overnight. During illumination, the gel became uniformly blue except for the areas containing SOD. The gel was destained by washing in water and subjected to image processing using a scanner (Canon N656U, Canon Inc., Tokyo, Japan).

Determination of MnSOD and Cu/ZnSOD mRNA by the reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was isolated using a Pure Script RNA Isolation kit (Gentra Systems, Minneapolis, MN, USA). HL-60 cells ($1x10^6$) were lysed in 300 μL cell lysis solution, then 100 μL protein-DNA precipitation solution was added. The lysis fluid was centrifuged at 15,000 x g for 3 min, then the pellet was washed in 300 μL 75% isopropanol. After further centrifugation at 15,000 x g for 1 min, the pellet was air-dried for 15 min, then dissolved in diethyl pyrocarbonate (DEPC)-treated H_2O_2.

RT-PCR was performed on 1.0 μg of total RNA using the Rever Tra Ace system (Toyobo Co. Ltd, Osaka, Japan). Single-strand cDNA obtained from the RT reaction with an oligo $(dT)_{20}$ primer was amplified using the KOD Plus system (Toyobo), employing MnSOD-specific primers (5'-TCCCCGACCTGCCCTACGAC-3' and 5'-CATTCTCCCAGTTGATTACAT-3'), Cu/ZnSOD-specific primers (5'-ATGGCCACGAAGGCCGTGTG-3' and 5'-GGAA TGTTTATTGGGCGATCCA-3') and G3PDH-specific primers (5'-TCCACCACCCTGTTGCTGTA-3' and 5'-ACCACAGTCC ATGCCATCAC-3'), according to the protocol. The RT-PCR products were applied to 2% agarose gel, stained with ethidium bromide and photographed under UV light.

Assay for the release of cytochrome c. Cell pellets were suspended in 100 μL homogenizing buffer (100 mM Tris-HCl, pH 7. 4, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF and 0.5% Tween 20), and disrupted by passing through a 21.5-gauge syringe ten times. The cell homogenates were centrifuged at 10,000 rpm for 5 min at 4˚C to pellet down the mitochondria and nuclear fraction, and the supernatant was collected. The cytosolic fraction was obtained as a supernatant after centrifuging the post-mitochondrial supernatant at 105,000 x g for 15 min. The protein concentration was measured using a Protein Assay Kit (Bio Rad, Hercules, CA, USA). An aliquot equivalent to 20 μg of the protein was mixed with 2x sodium dodecyl sulfate (SDS) buffer (0.1M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 0.01% bromophenol blue and 1.2% 2-mercaptoethanol), boiled for 10 min, incubated with 15% skim milk in Tris-HCl-buffered saline plus 0.05% Tween 20 for 1.5 h, and incubated with antibodies against cytochrome c (BD Biosciences, Pharmingen, San Diego, CA, USA) and cytochrome oxidase subunit II (Molecular Probes Inc., Eugene, OR, USA). The immunoblots were developed using Lumi-Light and Western blotting substrate (Roche Molecular Biochemicals, Germany), according to the manufacturer's instructions.

Assay for caspase activation. Cells were washed with PBS and lysed in a lysis solution (Medical & Biological Laboratory (MBL) Co. Ltd, Nagoya, Japan). After standing on ice for 10 min and centrifugation at 10,000 x g for 5 min, the supernatant was collected. The lysate (50 μL, equivalent to 200 μg protein) was mixed with 50 μL MBL reaction buffer containing substrates for caspase-3 (DEVD)- *p*-nitroanilide (pNA), caspase-8 (IETD)-*p*NA or caspase-9 (LEHD)-*p*NA. After incubation for 3 h at 37˚C, the absorbance of the liberated chromophore-*p*NA at 405 nm was measured using a plate reader according to the manufacturer's instructions.

Detection of caspases (Western blot assay). The cells were suspended in PBS for the required number of hours. The required concentration of HQ was added to the cells, after which they were incubated for 0, 2, 3, 4 or 5 h at 37˚C. The cells were then suspended in lysis buffer [100 mM Tris-HCl, pH 7.4, 1% Triton X-100, 150 mM EDTA and 2 mM PMSF] and cooled with ice for 10 min. After centrifugation at 16,000 x g for 20 min at 4˚C, the supernatant was collected. The protein in the cell lysate was determined using a Protein Assay Kit (Bio Rad), then an aliquot equivalent to 20 μg protein was subjected to 15% SDS–PAGE and transferred to a PVDF membrane (Immobilon-P, Millipore Co., Bedford, MA, USA). The membrane was blocked with 5% skim milk in Tris-HCl-buffered saline plus 0.05% Tween 20 overnight at 4˚C, incubated with antibodies against caspase-3, -8 and -9 (1:1000; BD Biosciences) for 90 min at room temperature, then incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:4000) for 60 min at room temperature. The immunoblots were developed using Western Lightning™ Chemiluminescence Reagent Plus (Perkin Elmer Life Science, Boston, MA, USA) and analyzed on a Macintosh computer (Power Macintosh 7600/120) using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available from the Internet at zippy.nimh.nih.gov or on floppy disk from the National Technical

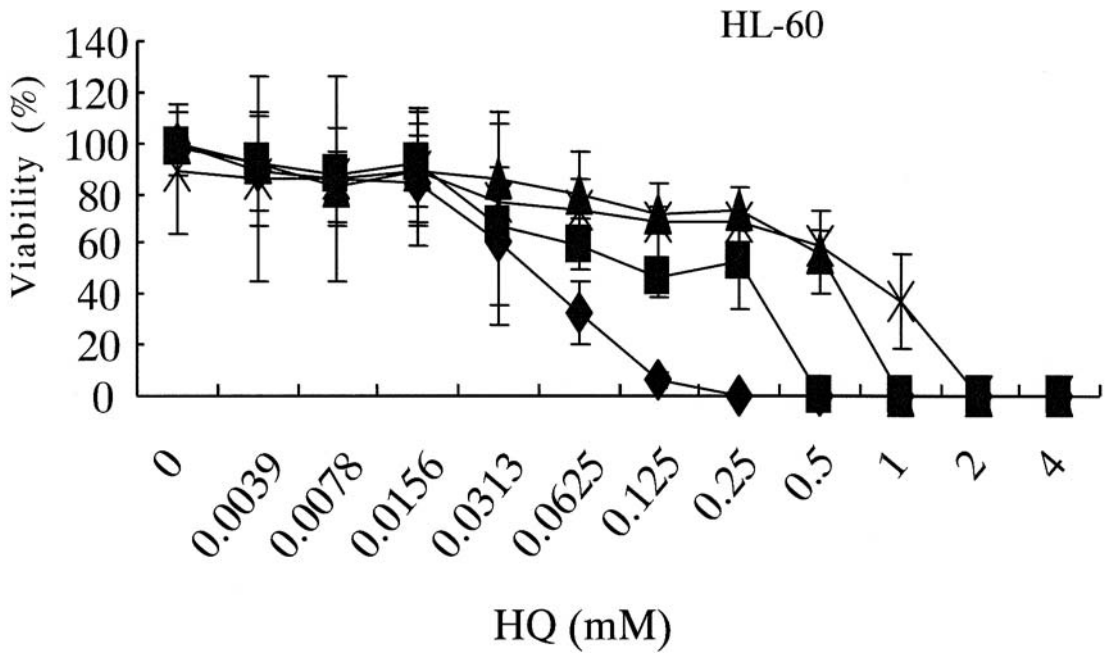

Figure 1. *Effect of N-acetyl-L-cysteine (NAC) on the cytotoxic activity of hydroquinone (HQ). Near-confluent HL-60 cells were incubated for 24 h without (control) or with the indicated concentrations of HQ in the presence of 0 (◆), 2.5 (■), 5 (▲) or 10 (*) mM NAC. The respective viable cell numbers were then determined and expressed as a percentage of the control value. Each value represents the mean ± S.D. of 4 determinations.*

Information Service, Springfield, Virginia, USA, part number PB95-500195GEI).

SDS-PAGE was performed on the various samples, using a 7.5% separating gel overlaid with a 15% stacking gel. Western blotting experiments were carried out according to standard protocols. Briefly, the protein was transferred to a PVDF membrane at 200 mA over 1 h at room temperature. The blots were then equilibrated in 10 mM Tris-HCl plus 150 mM NaCl, pH 8.0 (TBST), for 15 min and blocked in 10 mL of 5% skim milk/TBST for 1 h at room temperature whilst being gently shaken. After being blocked, the blots were washed three times with TBST and incubated with appropriate first antibodies.

Statistical analysis. Unless stated otherwise, all results are expressed as the means of three separate experiments. Statistically significant differences between means were investigated using the Student's *t*-test.

Results

Cytotoxicity. The results of the cytotoxicity experiments are shown in Figure 1. The concentration at which HQ was cytotoxic to 50% of the HL-60 cells (CC_{50}) was 0.042 mM. The cytotoxicity of HQ was reduced by the addition of NAC in a dose-dependent manner.

Induction of internucleosomal DNA fragmentation. Next, we examined the induction of DNA fragmentation in HQ- and BQ-treated HL-60 cells (Figure 2). BQ was used as a reference for the activation of HQ because it is a metabolite of HQ. Neither HQ nor BQ induced DNA fragmentation during the first 3 h; however, at 6 h, DNA fragmentation was observed with both HQ and BQ at a concentration of 0.05 mM. Thus, HQ and BQ promoted the induction of apoptosis at a concentration close to the CC_{50}.

Determination of SOD activity by agarose gel electrophoresis. To assess biological functions during HQ-induced apoptosis, we examined the activity of SOD. Reduced activity of MnSOD was apparent as early as 1 h after treatment with 0.5 mM HQ, whereas the activity of Cu/ZnSOD was not reduced even at the higher concentration of 4 mM (Figure 3). The findings after 2 and 3 h were similar. The activity of MnSOD in BQ-treated HL-60 cells was similar to that in HQ-treated ones, except that a BQ concentration of 0.125 mM was sufficient to reduce activity at 1 h (Figure 4). Thus, the bioactivity of BQ was 4-fold greater than that of HQ, suggesting that it produces higher levels of mitochondrial dysfunction.

Expression of MnSOD and Cu/ZnSOD mRNA. Next, we examined changes in the expression of MnSOD and Cu/ZnSOD mRNA, a crucial factor in clarifying the mechanisms responsible for apoptosis. The results are shown in Figure 5. The expression of MnSOD mRNA was strongest

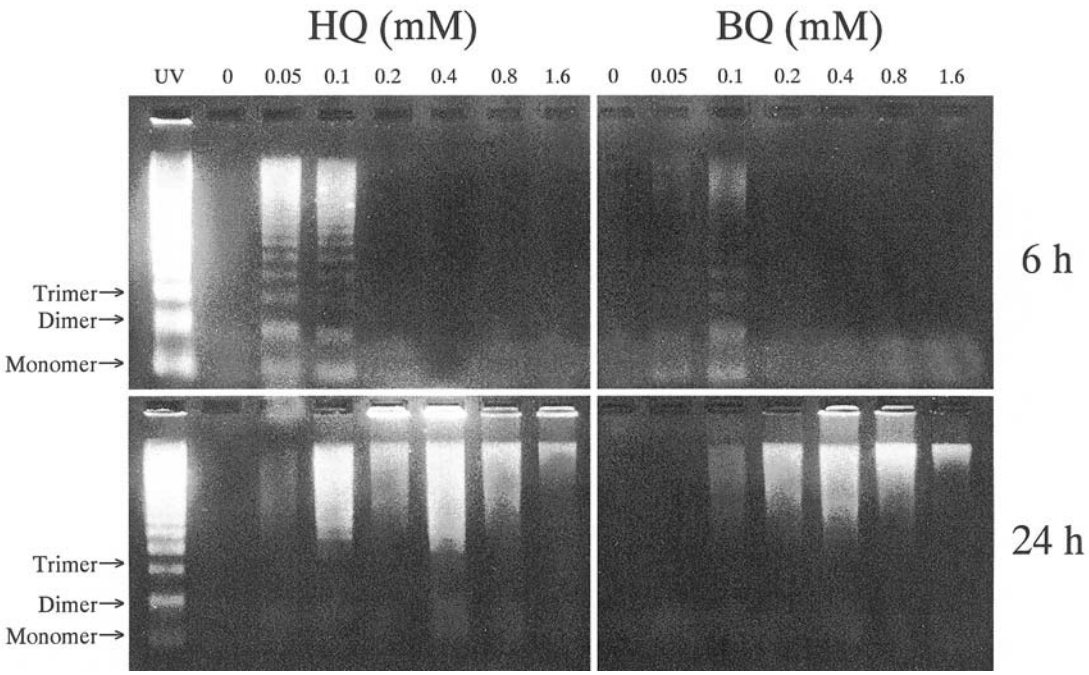

Figure 2. *Induction of internucleosomal DNA fragmentation by HQ and benzoquinone (BQ) in HL-60 cells. The upper panel shows the results after 6 h, while the lower panel shows the results after 24 h. HL-60 cells were incubated for 1, 3 or 6 h with the indicated concentrations of HQ. Their DNA was then extracted and analyzed by agarose gel electrophoresis. Marker: DNA size marker. UV: DNA from HL-60 cells in which apoptosis was induced by UV irradiation (6 J/m^2).*

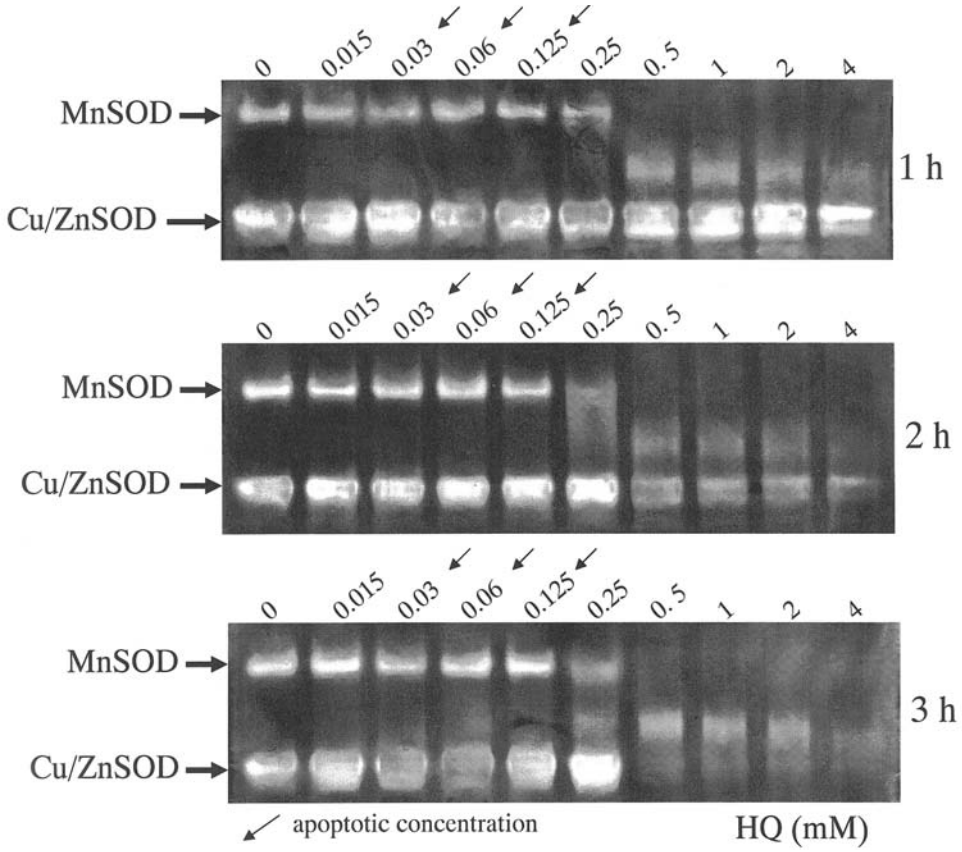

Figure 3. *Effect of HQ on Mn superoxide dismutase (SOD) mobility/activity in HL-60 cells. HL-60 cells were incubated for 1, 2 or 3 h with the indicated concentrations of HQ, then MnSOD and Cu/ZnSOD activity was measured by electrophoresis and activity staining.*

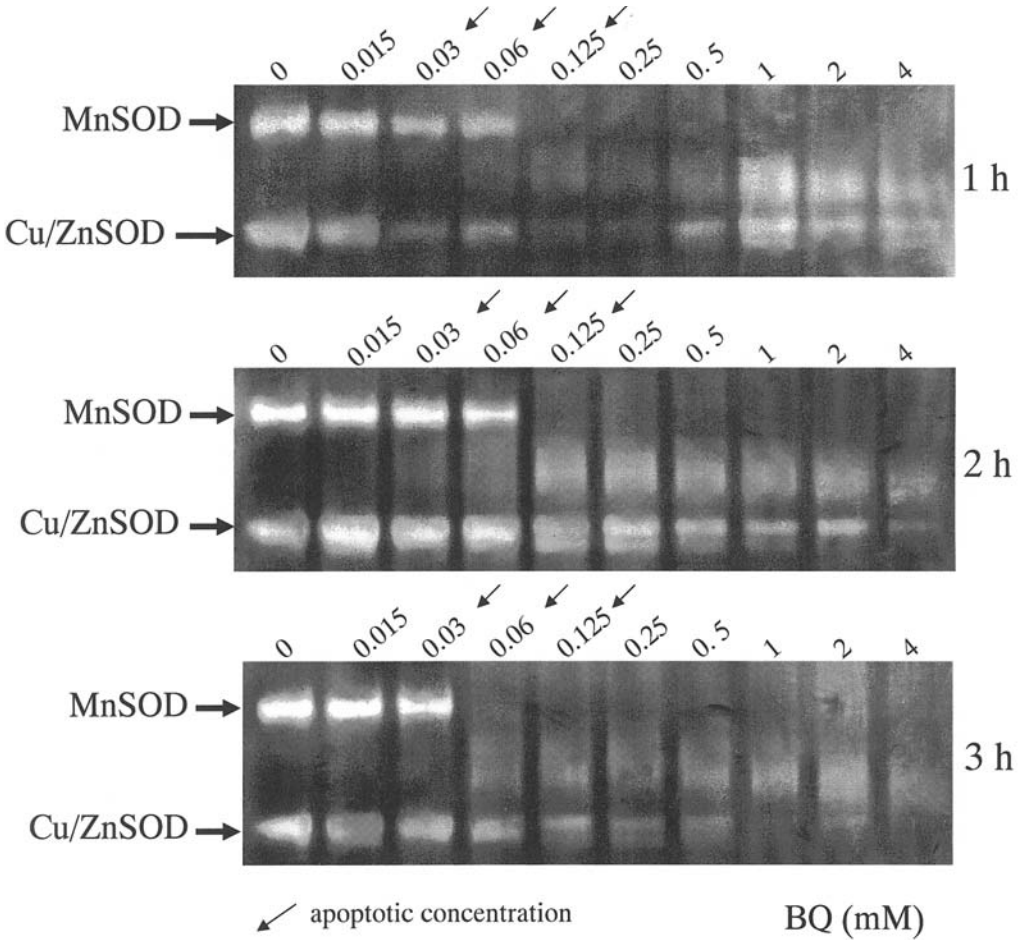

Figure 4. *Effect of BQ on MnSOD mobility/activity in HL-60 cells. HL-60 cells were incubated for 1, 2 or 3 h with the indicated concentrations of BQ, then MnSOD and Cu/ZnSOD activity was measured by electrophoresis and activity staining.*

at a HQ concentration of 0.03 mM, whereas that of Cu/ZnSODmRNA was strongest at the higher concentration of 0.1 mM and showed a reduction of about 40% at 0.3 mM. The expression of both SOD mRNAs, but particularly that of MnSOD mRNA, was reduced in a dose-dependent manner by higher HQ concentrations. These findings suggest that HQ may promote apoptosis in HL-60 cells by inhibiting MnSOD induction.

Activation of caspases. To clarify the effect of NAC on the apoptotic mechanism initiated by HQ, we examined the activities of various caspases (Figure 6). The activation of caspase-3, -8 and -9, but particularly that of caspase-3, was strongly induced by 0.025 and 0.05 mM HQ alone. However, this induction was not observed at the higher concentrations of 0.1 and 0.2 mM, which cause cell death. The strong activation of caspase-3 by 0.025 and 0.05 mM HQ was significantly reduced by adding 5 mM NAC. Furthermore, much more caspase-3 activation was apparent at the higher concentrations of 0.1 or 0.2 mM HQ in the presence of 5 mM NAC. The activation of caspase-9 also appeared to be enhanced by NAC as the concentration of HQ increased. The addition of NAC to HQ thus reduced the direct cytotoxicity of HQ and induced apoptosis instead.

Detection of cytochrome c. To clarify the sequence of the apoptotic cascade in HQ-treated HL-60 cells, we examined the release of cytochrome *c*. Cytochrome *c* was released into the cytoplasm of HQ-treated HL-60 cells after incubation for 6 h but not for 3 h (Figure 7).

Detection of procaspase-3, -8 and -9 and corresponding caspases (Western blot assay). Next, we examined the activation of procaspase-3, -8 and -9 and the presence of their active forms in HQ-treated HL-60 cells. The results are shown in Figure 8. Expression of the active forms of procaspase-3 (A),

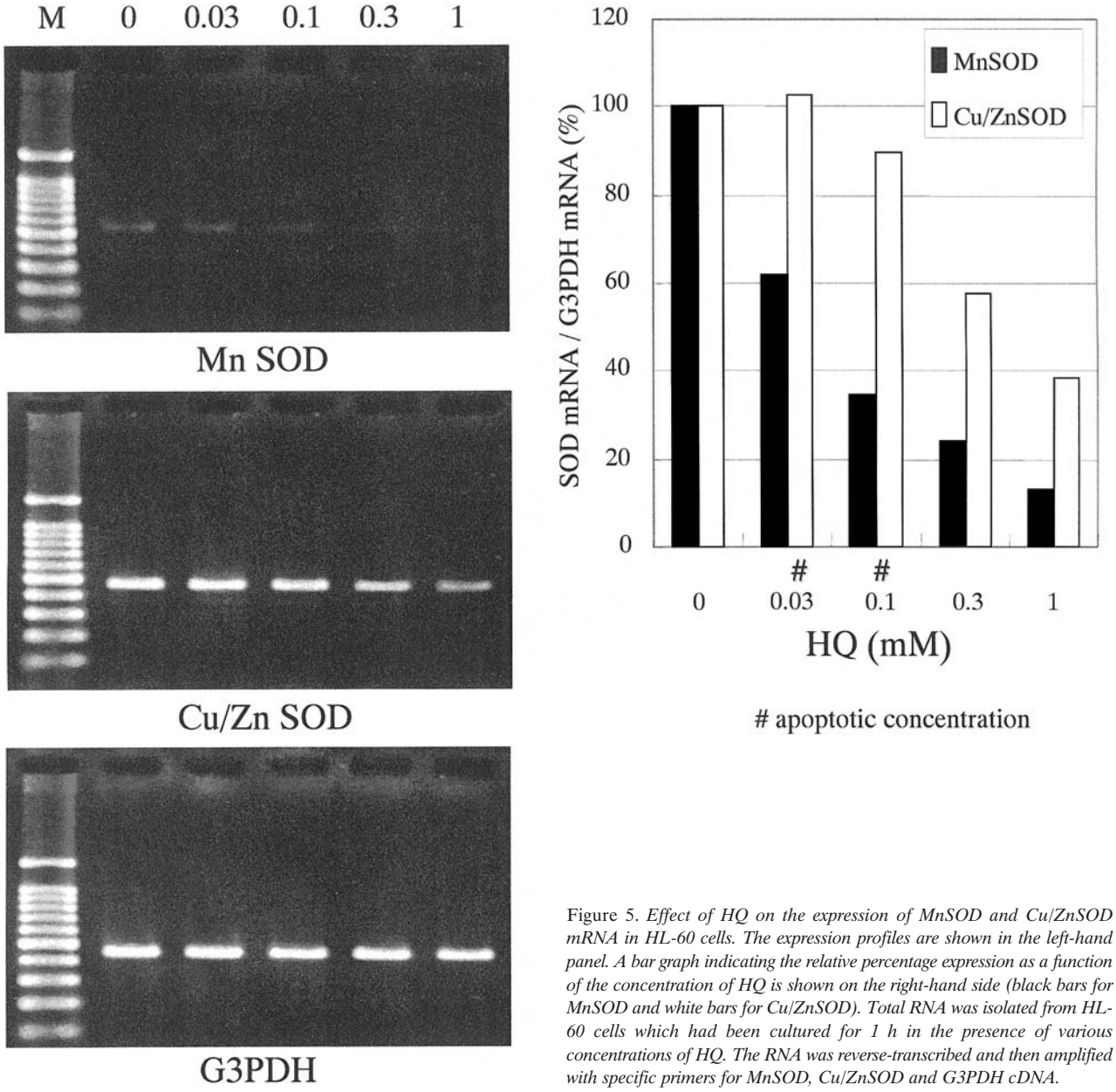

Figure 5. *Effect of HQ on the expression of MnSOD and Cu/ZnSOD mRNA in HL-60 cells. The expression profiles are shown in the left-hand panel. A bar graph indicating the relative percentage expression as a function of the concentration of HQ is shown on the right-hand side (black bars for MnSOD and white bars for Cu/ZnSOD). Total RNA was isolated from HL-60 cells which had been cultured for 1 h in the presence of various concentrations of HQ. The RNA was reverse-transcribed and then amplified with specific primers for MnSOD, Cu/ZnSOD and G3PDH cDNA.*

procaspase-8 (B) and procaspase-9 (C) was observed after treatment with 0.05 mM HQ. Similarly, expression of the active forms of procaspase-3 and -9, but not of procaspase-8, was found in cells treated with 0.05 mM HQ. As shown in the bar graph on the right of Figure 8, the active form of procaspase-9 was produced in a time-dependent manner, with peak expression at 5 h. Similarily, the active form of procaspase-3 was produced after 4 h, with peak expression at 5 h. In contrast, the active form of procaspase-8 was not produced, even after 5 h. Taken together, these findings indicate that HQ rapidly activates common apoptotic cascades in HL-60 cells.

Discussion

We have previously reported that HQ induces inter-nucleosomal DNA fragmentation in HL-60 cells, but not in other human cancer cell lines such as human oral squamous cell carcinoma (HSC-2) and human submandibular gland carcinoma (HSG) cells (7). This suggests that leukemic cells are particularly sensitive to HQ, a benzene metabolite. However, the addition of Cu (II) was shown to markedly enhance the cytotoxicity of HQ in both HSC-2 and HSG cells, whereas the addition of NAC markedly reduced

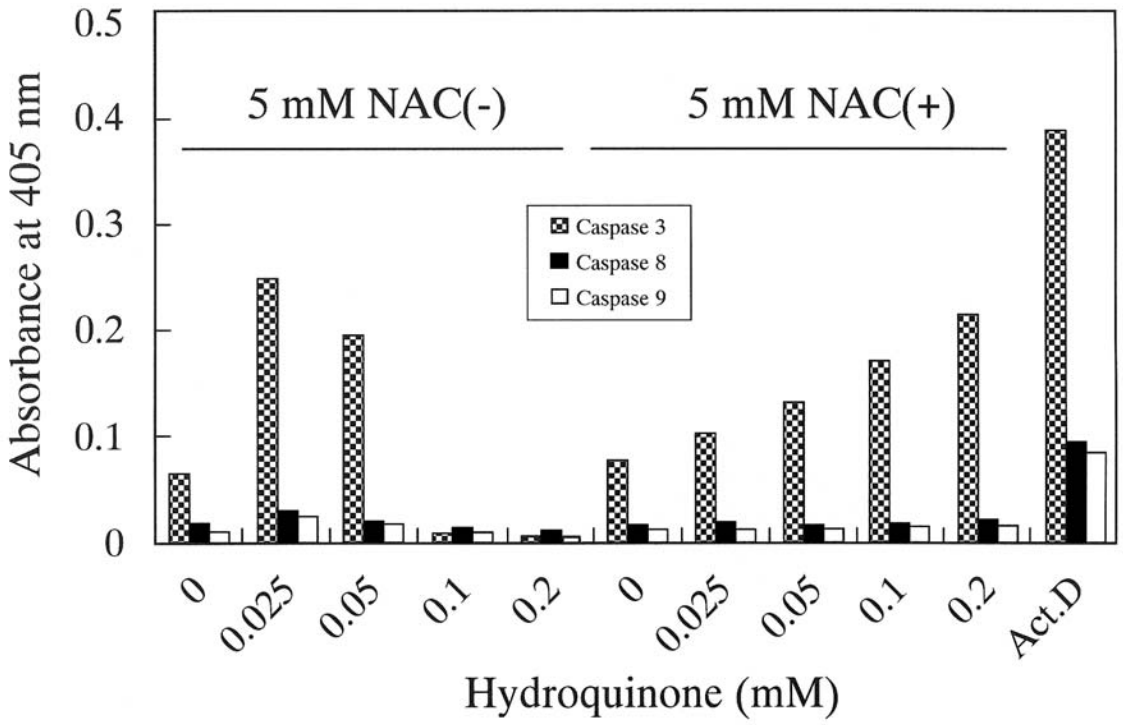

Figure 6. *Activation of caspases in HQ-treated HL-60 cells in the presence or absence of NAC. The experimental procedure is described in the text.*

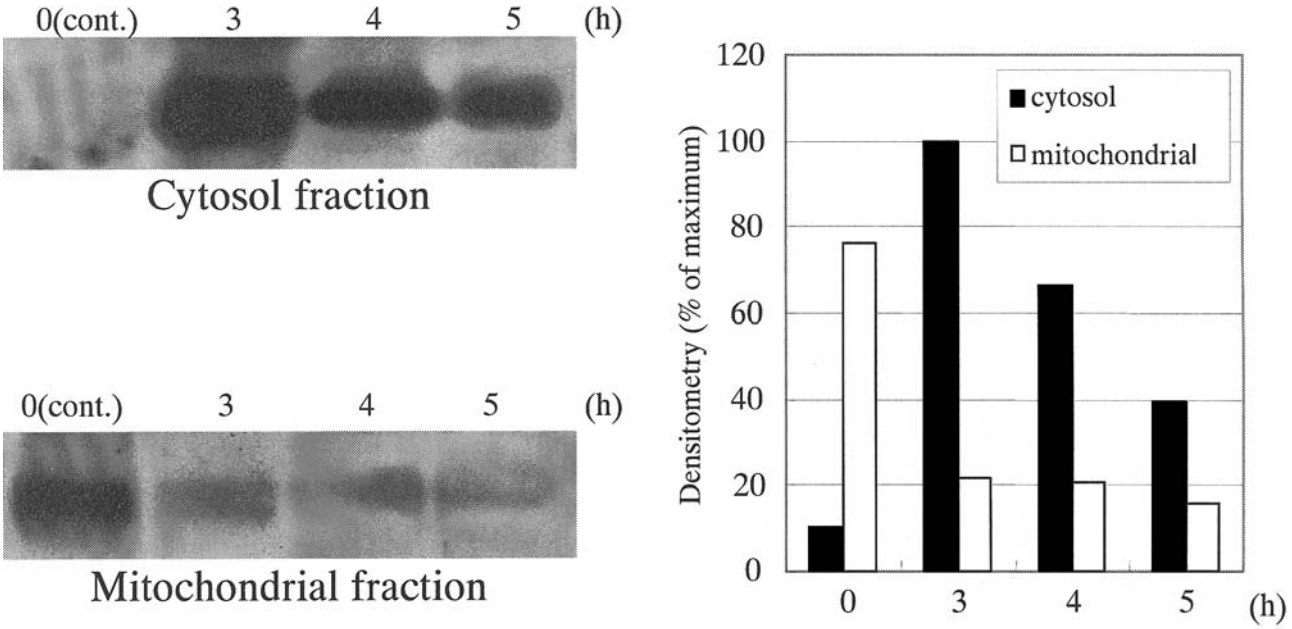

Figure 7. *Induction of cytochrome c release from mitochondria by HQ. The results for the cytosolic and mitochondrial fractions are shown in the upper and lower left-hand panels, respectively. A bar graph illustrating the relative activity as a function of the reaction time is shown on the right (black bars for the cytosolic fraction and white bars for the mitochondrial fraction). HL-60 cells were incubated with 0.05 mM HQ for the times indicated. Both the mitochondrial and cytosolic fractions were subjected to SDS-PAGE and analyzed by Western blotting, using specific antibodies against cytochrome c.*

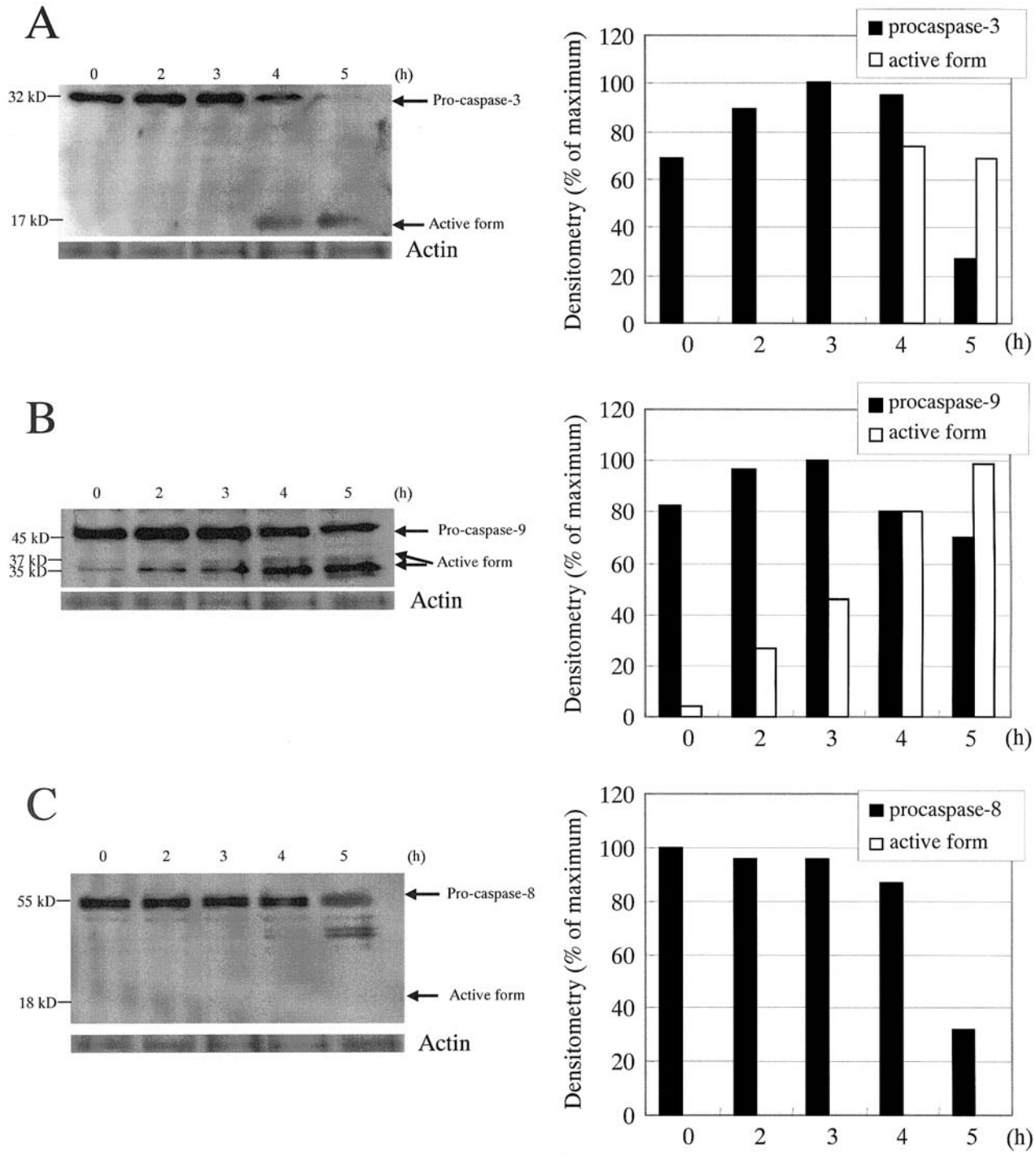

Figure 8. *Production of the active forms of procaspase-3 (A), procaspase-9 (B) and procaspase-8 (C) in HQ-treated HL-60 cells. Western blotting profiles for the procaspases, their active forms and actin in cells treated with 0.05 mM HQ are shown on the left-hand side. Bar graphs illustrating the relative expression of the active form as a function of time are shown on the right-hand side (black bars for procaspases and white bars for the active forms).*

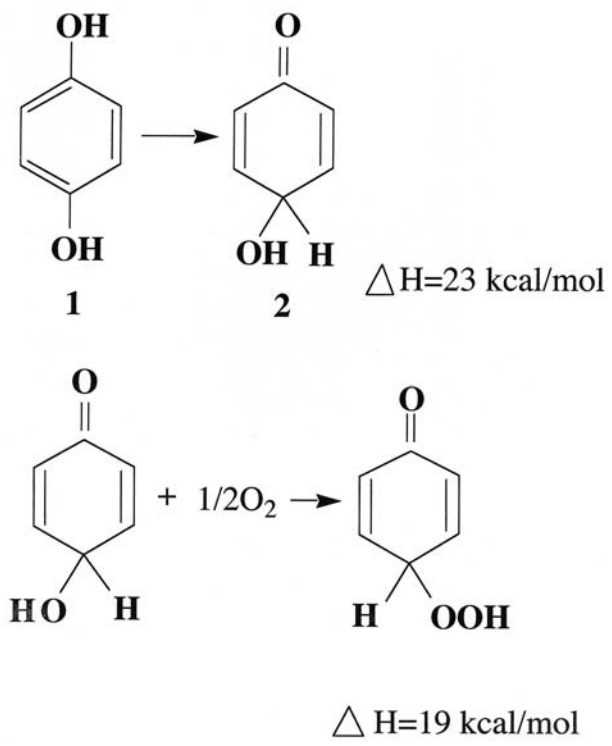

Figure 9. *The enthalpy (ΔH) was calculated from reactions in the gas phase and [$\Delta H_{f.\ ROO.}$ -$\Delta_{Hf.\ ROOH}$] and [$\Delta H_{f.phenoxy}$ - $\Delta H_{f.\ phenol}$] value of compound 1 (hydroquinone) and compound 2 (quinol) (21). The reaction occurs with a relatively small heat value. Also, compound 2 is easily oxidized.*

cytotoxicity. Similarly, HQ (or BQ)-induced DNA fragmentations in HL-60 cells have previously been reported to be accelerated in the presence of Cu (II), and the changes induced by BQ occurred at a much lower concentration than those produced by HQ (14). Li *et al.* also reported that Cu/ZnSOD is mediated by HQ-induced strand breaks in its plasmid DNA, possibly reflecting the fact that Cu (II) accelerates the oxidation of HQ. HQ is easily oxidized to its semiquinone radical, which is subsequently converted into BQ. This BQ can be back-converted into parent HQ in the presence of reducing agents such as NADH. However, the H_2O_2 generated by the Cu/ZnSOD-accelerated oxidation of HQ can be utilized by myeloperoxidase, and this promotes the conversion of HQ to BQ (13). Bioactivation of HQ is therefore implicated in the production of BQ. In the present study, the cytotoxicity of HQ in HL-60 cells, as well as in HSC-2 cells and HSG cells, was enhanced by the addition of Cu (II), but not of Co (II) or Fe (III) (data not shown). PAGE and activity staining for both MnSOD and Cu/ZnSOD in the SOD-enriched cell fraction after treatment with concentrations of HQ and BQ close to the CC_{50} indicated that mitochondrial dysfunction was induced during the early stages of apoptosis. In this respect, the effect of BQ was greater than that of HQ, despite the similar cytotoxicity of the two compounds. This finding is consistent with the results of a previous study (14).

The addition of NAC inhibited the cytotoxicity of HQ in HL-60 cells in a dose-dependent manner. Moreover, it reduced the activation of caspase-3 at concentrations of HQ close to the CC_{50}. This inhibitory effect of NAC on HQ-induced apoptosis in HL-60 cells has been reported previously (8, 9, 12, 13). NAC can prevent apoptosis and promote cell survival by activating the extracellular signal-regulated kinase pathway (18). Interestingly, in the present study, the activation of caspase-3 was greatly enhanced by combinations of high concentrations of HQ (0.01-0.02 mM) with 5 mM NAC. NAC is a precursor of glutathione in cells; however, the conjugation of glutathione with quinones does not necessarily result in detoxification, even when the resulting conjugates are more stable to oxidation (19). MPO-catalyzed bioactivation of HQ is not a prerequisite for toxicity, but the reaction of glutathione adducts with BQ derived from HQ may be a cause of toxicity (19). Reactive compounds of HQ and glutathione (*e.g.*, 2,3, 5-tris(glutathione-S-yl)hydroquinone [TGHQ], a putative metabolite of benzene) have been shown to induce apoptosis in HL-60 cells (20). Prior to the onset of apoptosis, TGHQ depletes intracellular glutathione in a reactive oxygen species-independent manner. Moreover, ceramide-mediated generation of H_2O_2 is necessary for the activation of caspase-3 and/or -7, both of which are effectors of apoptosis (20). The direct cytotoxicity of, and induction of apoptosis by, HQ may therefore be caused by the formation of adducts such as TGHQ and/or quinol-based toxins.

HQ may be converted to a quinol-containing reactive oxygen species, but not BQ (21; Figure 9). During this process, the heat of reaction is exothermic. Cu/ZnSOD-mediated activation of HQ and the subsequent biological effects were previously proposed, suggesting that BQ forms DNA adducts in either isolated DNA or cells (13, 22). However, the formation of quinols from HQ may be more preferable from an energy viewpoint than that of BQ from HQ. The tertiary alcohol groups in the HQ-quinol may then attack nucleophiles in biological systems, thus enhancing cytotoxicity and the induction of apoptosis.

In summary, during the present investigations, DNA fragmentation, suppression of MnSOD mRNA expression and cytochrome *c* release, and activation of procaspase-3 and -9, but not procaspase-8, were observed in HQ-treated HL-60 cells. These findings suggest that HQ activates common apoptosis cascades, in agreement with previous reports (23).

Acknowledgements

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References

1 Koran A: Prosthetic applications of polymers. *In*: Restorative Dental Materials, 11th Edition (edited by Craig RG and Power JM). Mosby, London, pp 638, 2002.

2 Stulberg DL, Clark N and Tovey D: Common hyperpigmentation disorders in adults: Part II. Melanoma, seborrheic keratoses, acanthosis nigricans, melasma, diabetic dermopathy, tinea versicolor, and postinflammatory hyperpigmentation. Am Fam Physician *68*: 1963-1968, 2003.

3 US EPA: Hydroquinone, Toxic Air Contaminants Identification List Summaries ARB/SSD/SES. p 588, 1997.

4 IARC: Hydroquinone (group 3). Summaries & Evaluations *71*: 691, 1999.

5 Kadoma Y and Fujisawa S: Kinetic evaluation of reactivity of bisphenol A derivatives as radical scavengers for methacrylate polymerization. Biomaterials *21*: 2125-2130, 2000.

6 Satoh K, Ida Y, Tomioka M, Shiohara K, Sakagami H and Fujisawa S: Interaction between antioxidants and hydroquinone/bisphenol A. Anticancer Res *19*: 547-5462, 1999.

7 Terasaka H, Takayama F, Satoh K, Fujisawa S and Sakagami H: Effect of antioxidants on radical intensity and cytotoxicity of hydroquinone. Anticancer Res *20*: 3357-3362, 2000.

8 Inayat-Hussain SH, Winski SL and Ross D: Hydroquinone and catechol metabolites of remoxipride in HL-60 cells. Chem Biol *128*: 51-63, 2000.

9 Inayat-Hussain SH, Winski SL and Ross D: Differential involvement of caspases in hydroquinone-induced apoptosis in human leukemic HL-60 and Jurkat cells. Toxicol Appl Pharmacol *175*: 95-103, 2001.

10 Hazel BA, Baum C and Kalf GF: Hydroquinone, a bioreactive metabolite of benzene, inhibits apoptosis in myeloblasts. Stem Cells *14*: 730-742, 1996.

11 Saito M, Sakagami H and Fujisawa S: Cytotoxicity and apoptosis induction by butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Anticancer Res *23*: 4693-4702, 2003.

12 Kuo ML, Shiah SG, Wang CJ and Chuang SE: Suppression of apoptosis by Bcl-2 to enhance benzene metabolites-induced oxidative DNA damage and mutagenesis: a possible mechanism of carcinogenesis. Mol Pharmacol *55*: 894-901, 1999.

13 Li Y, Kuppusamy P, Zweier JL and Trush MA: Role of Cu/Zn-superoxide dismutase in xenobiotic activation. 1. Chemical reactions involved in the Cu/Zn-superoxide dismutase-accelerated oxidation of the benzene metabolite 1,4-hydroquinone. Mol Pharmacol *49*: 404-411, 1996.

14 Hiraku Y and Kawanishi S: Oxidative DNA damage and apoptosis induced by benzene metabolites. Cancer Res *56*: 5172-5178, 1996.

15 Sakagami H, Kuribayashi N, Ida M, Hagiwara T, Takahashi H, Yoshida H, Shiota F, Ohhara H, Momose K and Takeda M: The requirement for and mobilization of calcium during induction by sodium ascorbate and by hydrogen peroxide of cell death. Life Science *58*: 1131-1138, 1996.

16 Beauchamp C and Fridovich I: Superoxide dismutase: improved assays and an assay applicable to acryamide gels. Anal Biochem *44*: 276-287, 1971.

17 Hitosugi N, Hatsukari I, Ohno R, Hashimoto K, Mihara S, Mizukami S, Nakamura S, Sakagami H, Nagasaka H, Matsumoto I and Kawase M: Comparative analysis of apoptosis-inducing activity of codeine and codeinone. Anesthesiol *98*: 643-650, 2003.

18 Zafarullah M, Li WQ, Sylvester J and Ahmad M: Molecular mechanism of *N*-acetylcysteine actions. Cell Mol Life Sci *60*: 6-20, 2003.

19 Lau SS, Hill BA, Highet RJ and Monks TJ: Sequential oxidation and glutathione addition to 1,4-benzoquinone: correlation of toxicity with increased glutathione substitution. Mol Pharmacol *34*: 829-836, 1988.

20 Bratton SB, Lau SS and Monks TJ: The putative benzene metabolite 2,3,5-tris(glutathion-S-yl)hydroquinone depletes glutathione, stimulates sphingomyelin turnover, and induces apoptosis in HL-60 cells. Chem Res Toxicol *13*: 550-556, 2000.

21 Mahoney LR and Da Rooge MA: The kinetic behavior and thermochemical properties of phenoxyl radicals. J Am Chem Soc *97*: 4722-4731, 1975.

22 Ievay G, Pongracz K and Bodell WJ: Detection of DNA adducts in HL-60 cells treated with hydroquinone and p-benzoquinone by 32P-postlabelling. Carcinogenesis *12*: 1181-1186, 1991.

23 Okubo T, Yokoyama Y, Kano K and Kano I: Cell death induced by the phenolic antioxidant tert-butylhydroquinone and its metabolite tert-butylquinone in human monocytic leukemia U937 cells. Food Chem Toxicol *41*: 679-688, 2003.

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