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Role of NAD(P)H:Quinone Oxidoreductase 1 (DT Diaphorase) in Protection against Quinone Toxicity

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ABSTRACT. *NQO1*−/− mice, along with Chinese hamster ovary (CHO) cells, were used to determine the *in vivo* role of NAD(P)H:quinone oxidoreductase 1 (NQO1) in cellular protection against quinone cytotoxicity, membrane damage, DNA damage, and carcinogenicity. CHO cells permanently expressing various levels of cDNA-derived P450 reductase and NQO1 were produced. Treatment of CHO cells overexpressing P450 reductase with menadione, benzo[*a*]pyrene-3,6-quinone (BPQ), and benzoquinone led to increased cytotoxicity as compared with CHO cells expressing endogenous P450 reductase. In a similar experiment, overexpression of NQO1 significantly protected CHO cells against the cytotoxicity of these quinones. Knockout (*NQO1*−/−) mice deficient in NQO1 protein and activity had been generated previously in our laboratory and were used in the present studies. Wild-type (*NQO1*+/+) and knockout (*NQO1*−/−) mice were given i.p. injections of menadione and BPQ, followed by analysis of membrane damage and DNA damage. Both menadione and BPQ induced lipid peroxidation in hepatic and non-hepatic tissues, indicating increased membrane damage. Exposure to BPQ also resulted in increased hepatic DNA adducts in *NQO1*−/− mice as compared with *NQO1*+/+ mice. The skin application of BPQ alone and BPQ + 12-*O*-tetradecanoylphorbol-13-acetate (TPA) failed to induce papillomas, or other lesions, for up to 50 weeks in either *NQO1*+/+ or *NQO1*−/− mice. The various results from CHO cells and *NQO1*−/− mice indicated that NQO1 protects against quinone-induced cytotoxicity, as well as DNA and membrane damage. The absence of BPQ-induced skin carcinogenicity in *NQO1*−/− mice may be related to the strain (C57BL/6) of mice used in the present study and/or due to poor BPQ absorption into the skin and/or due to detoxification of BPQ by cytosolic NRH:quinone oxidoreductase 2 (NQO2). BIOCHEM PHARMACOL **60**;2:207–214, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. NAD(P)H:quinone oxidoreductase 1; menadione; benzo[*a*]pyrene-3; 6-quinone; benzoquinone; CHO cells; *NQO1*−/− mice; toxicity; carcinogenicity

Benzo[*a*]pyrene and benzene are environmental carcinogens [1, 2]. Cytochrome P450 and P450 reductase catalyze metabolic reductive activation of these chemicals into products that include quinones [1, 2]. Benzo[*a*]pyrene quinones (metabolic products of benzo[*a*]pyrene), benzoquinones (metabolic products of benzene), and other environmental and synthetic quinones are highly reactive molecules that readily undergo either one- or two-electron reduction [3, 4]. One-electron reduction of quinones and their derivatives by enzymes such as cytochromes P450, cytochrome P450 reductase, ubiquinone oxidoreductase, xanthine oxidoreductase, and cytochrome b_5 reductase generates unstable semiquinones, which undergo redox cycling in the presence of molecular oxygen. This leads to the formation of highly reactive oxygen species [2, 3]. The ROS§ cause oxidative stress, DNA damage, lipid peroxidation, membrane damage, cytotoxicity, mutagenicity, and carcinogenicity [3–5].

Quinone oxidoreductases (NQO1 and NQO2) are flavoproteins that catalyze two-electron reduction of quinones to hydroquinones [4, 6, 7]. The two-electron reduction of quinones does not result in the formation of free radicals (semiquinones) and the ROS that arise from them. Therefore, NQO1 and NQO2 are expected to protect cells against the adverse effects of quinones and their derivatives. Among the various cytosolic NQOs, NQO1 is the best studied enzyme [4, 6, 7]. Cytosolic NQO1 activities, purified from rat liver and human adipose tissue, have been cloned and characterized [8–12]. Several studies have suggested that NQO1 protects against the toxicity and mutagenicity of quinones and their precursors (reviewed in

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§ *Abbreviations*: ROS, reactive oxygen species; NQO1, NAD(P)H:quinone oxidoreductase 1; NQO2, NRH:quinone oxidoreductase 2; BPQ, benzo[*a*]pyrene-3,6-quinone; DCPIP, 2,6-dichlorophenolindophenol; and TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

Refs. 4, 6, and 7). More recently, an NQO1-null mouse was produced in our laboratory using targeted gene disruption [13]. Mice lacking *NQO1* gene expression (*NQO1*−/−) showed no detectable phenotype and were indistinguishable from the wild-type (*NQO1*+/+) mice. However, when administered menadione, *NQO1*−/− mice exhibited increased toxicity, as compared with their wild-type (*NQO1*+/+) littermates.

The human *NQO1* gene has been localized precisely to chromosome 16q22 [14]. Recent studies have characterized a C→T mutation in the *NQO1* gene, which results in the loss of NQO1 activity (reviewed in Refs. 15–17). This mutation has been found in human colon carcinoma (BE) cells, in human lung cancer cells (H596), and in fibroblasts taken from a cancer-prone family. It is also noteworthy that 2–4% of the human population do not express NQO1 [15–17]. However, some hydroquinones produced by NQO1 can autoxidize to generate ROS or alkylate DNA directly (reviewed in Refs. 15–17). In these instances, NQO1 catalyzes activation of such compounds to their ultimate toxic forms. This property of NQO1, along with the observation that NQO1 is overexpressed in certain tumor types, has been used to develop bioreductive chemotherapeutic agents (reviewed in Refs. 15–17). However, the role of NQO1 in metabolic activation of drugs is controversial [17].

In the present study, we have investigated the *in vivo* role of NQO1 in protection against cytotoxicity, membrane damage, DNA damage, and carcinogenicity caused by exposure to menadione (a vitamin K analogue), BPQ (a metabolite of benzo[*a*]pyrene), and benzoquinone (a metabolic product of benzene). CHO cells overexpressing P450 reductase showed higher cytotoxicity in response to these quinones than did CHO cells expressing normal levels of P450 reductase. Overexpression of NQO1 protected CHO cells against cytotoxicity from the various quinones. *NQO1*−/− mice demonstrated increased lipid peroxidation and DNA adducts when exposed to menadione and BPQ, as compared with wild-type (*NQO1*+/+) mice. However, BPQ and BPQ + TPA failed to induce skin tumors in wild-type and *NQO1*−/− mice. The various results clearly indicate that NQO1 protects against quinone toxicity. The absence of quinone-induced skin carcinogenicity in *NQO1*−/− mice may be related to the strain of mice (C57BL/6) used in the present study and/or due to poor BPQ absorption through the skin and/or due to detoxification of BPQ by cytosolic NQO2.

MATERIALS AND METHODS

Materials

The CHO cells were obtained from the American Type Culture Collection. The cell culture reagents were obtained from Gibco-BRL. BPQ was purchased from the NCI Chemical Carcinogen Repository. Menadione and benzoquinone were purchased from the Sigma Chemical Co. All other reagents used in the experiments were of the highest purity commercially available. Polyclonal antibodies against purified full-length rat NQO1 protein were raised in rabbits in our laboratory. The NQO1 antibodies cross-react with cytosolic NQO1 and NQO2 proteins from rodents and humans [18, 19].

Development of CHO Cells Stably Expressing High Levels of Microsomal P450 Reductase and Cytosolic NQO1 Activity

The cDNA encoding human microsomal P450 reductase (obtained as a gift from Dr. Frank Gonzalez) and human cytosolic NQO1 (cloned in our laboratory [12]) was subcloned separately in the pED4 vector [20] to generate pED4-P450 reductase and pED4-NQO1 recombinant plasmids. pED4 is a bicistronic vector carrying a dihydrofolate reductase gene downstream from the *NQO1* gene. The CHO cells were grown in monolayer culture in Iscove's DMEM containing 10% fetal bovine serum, 0.1 mM hypoxanthine, 0.01 mM thymidine, 50 μg/mL of streptomycin, and 2 mM glutathione. The cells were grown in 5% CO_2 at 37°. pED4-P450 reductase and pED4-NQO1 plasmid DNA were linearized by digestion with *Nde*I, cleaned with phenol:chloroform, and precipitated with ethanol. The CHO cells were transfected with 50 μg of linearized plasmid per dish by procedures described previously [19]. Sixty-two hours after transfection, the cells were washed with 1x PBS without calcium and magnesium, trypsinized, and plated in medium deficient in thymidine and hypoxanthine. The cells were plated at different dilutions of 1:5, 1:10, 1:20, and 1:40. A portion of the cells was analyzed for NQO1 activity by a previously described method using DCPIP as substrate [19]. The cells were allowed to grow in medium deficient in thymidine and hypoxanthine for 14 days. During this period, the medium was changed every third day. After 14 days, the colonies were visible. They were picked and expanded in 24-well plates. Selection of CHO cells continued in medium containing methotrexate at concentrations of 0.02, 0.1, and 0.5 μM. All of the clones selected at the various stages of methotrexate treatment were analyzed for P450 reductase and NQO1 activities. Two of these clones, CHO (RED-22) and CHO (RED-26), expressing 22- and 26-fold higher levels of P450 reductase, were selected and grown for further analysis. Similarly, three of the clones, CHO (NQO1–13), CHO (NQO1–38), and CHO (NQO1–3682), expressing 13-, 38-, and 3682-fold higher levels of NQO1, were selected for further studies. The selected CHO cell clones were grown in methotrexate-deficient medium for 4 weeks without a loss of NQO1 activity.

Cytotoxicity Assays

The wild-type control CHO cells and the five CHO cell clones expressing various levels of cDNA-derived P450 reductase or NQO1 were grown in dishes without methotrexate. The various cells were treated with different

concentrations of menadione, BPQ, and benzoquinone. The growth and survival of the cells, following 3 days of exposure to the drugs, were measured by procedures described previously [21, 22].

Generation of NQO1−/− *Mice*

We used targeted gene disruption to generate *NQO1*−/− mice in a hybrid 129SV/C57BL/6 background, which do not express NQO1 [13]. The hybrid *NQO1*−/− mice were backcrossed with C57BL/6 mice to generate *NQO1*−/− mice in a C57BL/6 background. The *NQO1*−/− mice were found to be normal in appearance and showed no discernible difference in their weight, development, or behavior, when compared with their wild-type (*NQO1*+/+) littermates. This was true for both male and female mice. In addition, the *NQO1*−/− animals appeared to have normal reproductive capacity when compared with the wild-type mice. The *NQO1*+/+ and *NQO1*−/− mice were bred separately in our animal colony at Baylor College of Medicine to generate sufficient mice for our studies. Some of these animals were selected at random and analyzed for the absence of NQO1 protein and activity by procedures described previously [19].

Measurement of NQO1 Activity and Western Blotting

The various tissues (liver and kidney) from wild-type and *NQO1*−/− mice were homogenized in 50 mM Tris, pH 7.4, containing 0.25 M sucrose, and centrifuged at 105,000 *g* for 1 hr to obtain cytosolic fractions. Dicoumarol-sensitive NQO1 activity was measured in all cytosolic fractions by a method reported earlier [19]. The final reaction mixture contained 25 mM Tris–HCl, pH 7.4, 0.18 mg/mL of BSA, 5 μM FAD, 0.01% Tween 20, 200 μM NADH or NADPH, 50 μM DCPIP, and 0.2 to 2.0 μg homogenate protein. The reaction rate was monitored by measuring the decrease in absorbance at 600 nm due to self-reduction of DCPIP. The various cytosolic fractions were also analyzed for the presence or absence of NQO1 protein by western blot analysis as previously described, using antibodies against purified rat liver NQO1 protein [19]. The rat NQO1 antibody is known to cross-react with mouse and human proteins [19]. Western blots were developed with ECL (Amersham) reagents by the procedure suggested by the manufacturer.

Lipid Peroxidation and DNA-Adduct Analysis

Six- to eight-week-old wild-type (*NQO1*+/+) and *NQO1*−/− mice were used. Menadione (vitamin K_3) and BPQ were dissolved in DMSO and administered i.p. at doses of 0 and 10 mg/kg body weight. Animals were given a single dose every day for 3 consecutive days. Animals were observed daily for symptoms of toxicity and mortality. Twenty-four hours following the last dose of the chemical, the animals were killed, and their livers and kidneys were analyzed for lipid peroxidation by a thiobarbituric acid/colorimetric procedure described previously [23]. Liver homogenates from *NQO1*+/+ and *NQO1*−/− mice, exposed to 10 mg/kg body weight of BPQ, were also analyzed for DNA adducts by a ^{32}P-postlabeling procedure as described previously [24].

Skin Carcinogenesis

Seven- to nine-week-old knockout (*NQO1*−/−) and wild-type (*NQO1*+/+) mice were used for the skin carcinogenesis experiments. The backs of the mice were shaved with surgical clippers 2 days before treatment. Ten animals were used in each group.

BPQ as Complete Carcinogen

The wild-type (*NQO1*+/+) and *NQO1*−/− mice were treated with 50, 100, 250, 500, 1000, and 3000 nmol of BPQ dissolved in DMSO, once a week, for 30 weeks. The control mice received DMSO alone. All the mice were fed exactly the same food *ad lib*. The animals were observed twice a week for 50 weeks from the date of the start of the experiment for the development of skin tumors. At the end of the study, histological analysis was done on two mice from each group to look for the presence of lesions at the place of BPQ application.

BPQ with TPA

The wild-type and *NQO1*−/− mice were treated once with 1000 or 3000 nmol of BPQ. This was followed by application of 10 μg of TPA twice a week for 30 weeks. The animals were observed twice a week for 50 weeks. The treated areas of skin, from two mice in each group, were analyzed histologically for the presence of lesions at the end of the study.

RESULTS

The stable transfection of CHO cells with pED4-P450 reductase and pED4-NQO1 resulted in the generation of CHO cell clones overexpressing different levels of cDNA-derived P450 reductase or NQO1. Five of these clones (two for P450 reductase and three for NQO1) were grown and analyzed for the levels of P450 reductase and NQO1 (Fig. 1). Untransfected CHO cells contained very low levels of NQO1 and moderate levels of P450 reductase. The CHO cells selected for overexpression of P450 reductase [CHO (RED-22) and CHO (RED-26)] demonstrated 22- and 26-fold higher levels of P450 reductase, as compared with untransfected CHO cells (Fig. 1A). Similarly, CHO cells selected for overexpression of NQO1 [CHO (NQO1–13), CHO (NQO1–38), and CHO (NQO1–3682)] expressed 13-, 38-, and 3682-fold higher levels of NQO1, as compared with untransfected CHO cells (Fig. 1B). The NQO1 activity in cells overexpressing P450 reductase and the P450 reductase activity in cells overexpressing NQO1

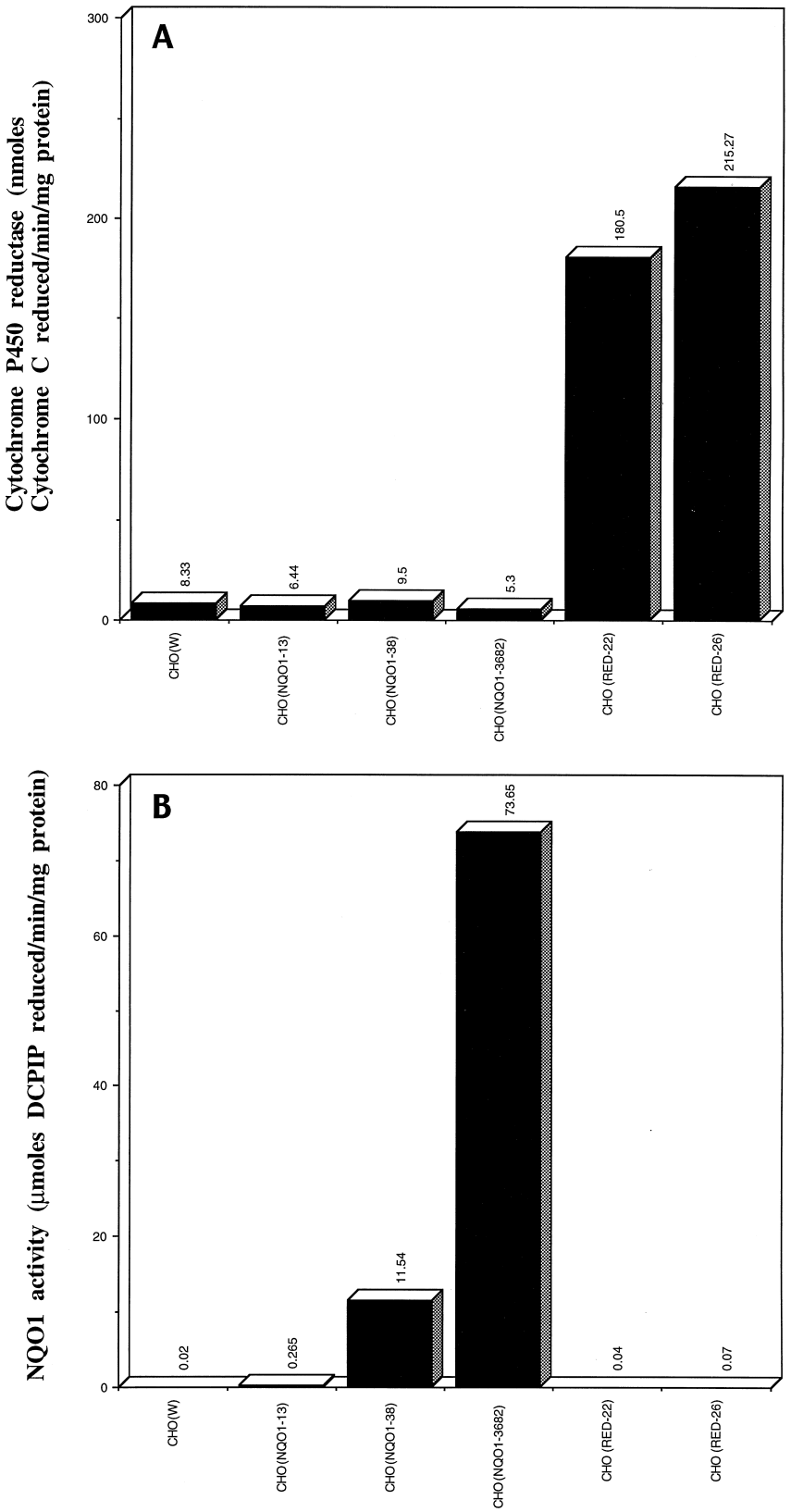


FIG. 1. Microsomal P450 reductase (A) and cytosolic NQO1 activity (B) levels in CHO cells and CHO cells stably expressing cDNAs derived from P450 reductase and NQO1. One unit of P450 reductase is equivalent to 1 nmol cytochrome c reduced/min/mg microsomal protein. The NQO1 activity is equivalent to 1 μmol DCPIP reduced/min/mg protein. The results shown are representative of three independent experiments.

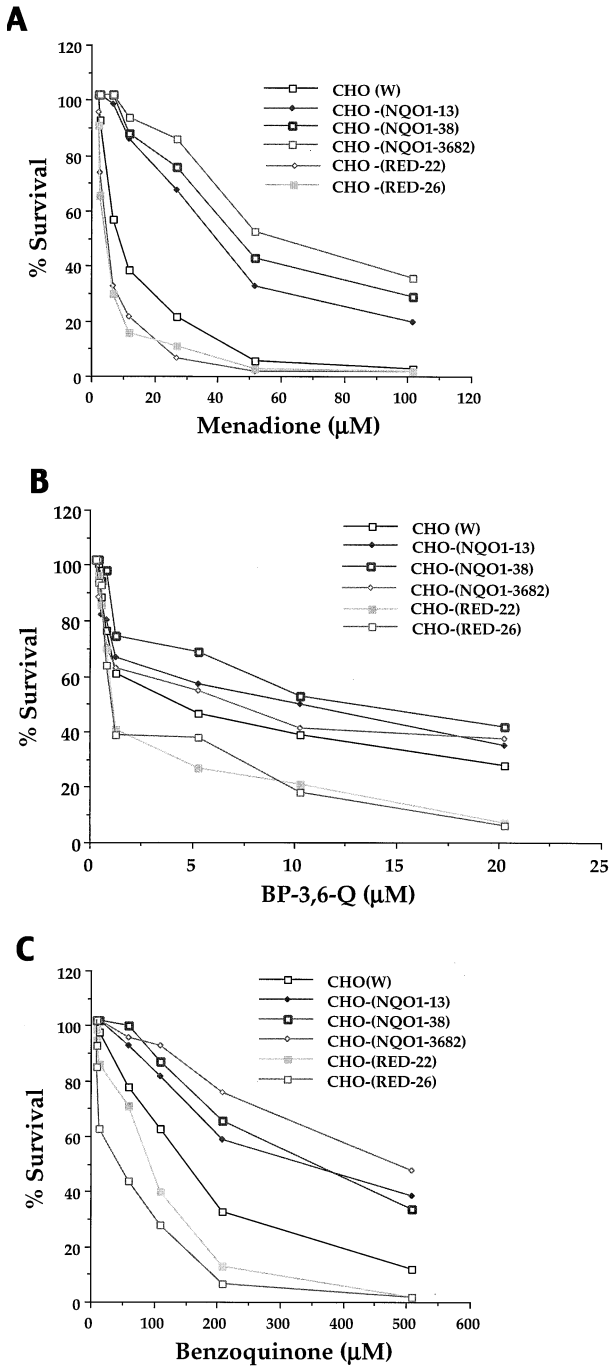
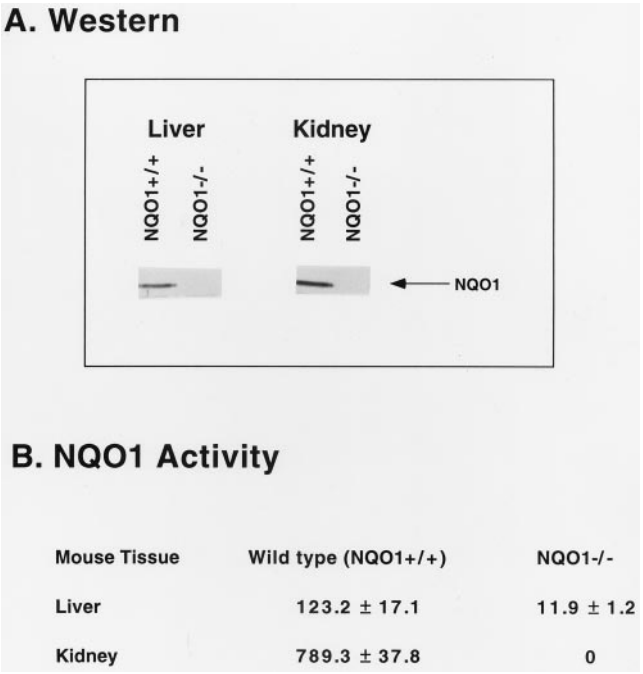


FIG. 2. Sensitivity of CHO cells expressing different levels of cDNA derived from P450 reductase and NQO1 to menadione, BPQ, and benzoquinone. The wild-type CHO (W) cells and CHO cells permanently overexpressing the cDNA derived from P450 reductase or NQO1 were grown in dishes and treated with the various quinones. The growth and survival of cells following 3 days of exposure to drugs were measured by procedures described elsewhere [21–23]. (A) Treatment with menadione; (B) treatment with BPQ; and (C) treatment with benzoquinone. The results shown are the means of three independent experiments.



Mouse Tissue	Wild type (NQO1+/+)	NQO1-/-
Liver	123.2 ± 17.1	11.9 ± 1.2
Kidney	789.3 ± 37.8	0

FIG. 3. Analysis of NQO1 in wild-type and *NQO1*−/− mice. The wild-type (*NQO1*+/+) and *NQO1*−/− mouse liver and kidney cytosolic fractions were prepared by previously described procedures. (A) The cytosolic proteins were subjected to SDS–PAGE and western blot analysis with polyclonal antibodies against the full-length rat NQO1 protein. The 32-kDa NQO1 band is indicated. (B) NQO1 activity was measured in the various cytosolic fractions. One unit of NQO1 activity is equivalent to 1 μmol DCPIP reduced/min/mg protein. The results (means ± SEM) shown are representative of three independent experiments.

remained more or less the same when compared with untransfected CHO cells. Menadione, BPQ, and benzoquinone treatment of CHO cells overexpressing P450 reductase resulted in increased cytotoxicity (Fig. 2). This is because a lower number of cells survived after the treatment. The toxicity increased with increasing concentrations of quinones. The toxicity of the three chemicals on a molar basis was in the order of BPQ > menadione > benzoquinone. Interestingly, CHO cells overexpressing NQO1 were protected markedly against menadione, BPQ, and benzoquinone cytotoxicity, as evident by an increased number of cells surviving the treatment (Fig. 2).

Western and activity analyses of selected *NQO1*−/− mice are presented in Fig. 3. Western analysis showed the absence of the 32-kDa NQO1 protein in the livers and kidneys of *NQO1*−/− mice (Fig. 3A). NQO1 activity was absent in the kidneys and reduced to 10% in the livers of *NQO1*−/− mice, as compared with the wild-type (*NQO1*+/+) mice (Fig. 3B). Exposure of *NQO1*+/+ and *NQO1*−/− mice to menadione and BPQ led to a marked increase in membrane lipid peroxidation in the livers and kidneys of *NQO1*−/− mice, as compared with *NQO1*+/+ mice (Fig. 4). The lipid peroxidation was higher with menadione than with BPQ. In a similar experiment, the *NQO1*−/− mice treated with BPQ demonstrated increased DNA adducts as compared with *NQO1*+/+ mice, as determined by the ^{32}P-postlabeling method (Fig. 5). Interestingly, the application of BPQ (in doses of 50, 100, 250, 500, 1000, and 3000 μmol) to the skin of *NQO1*+/+ and

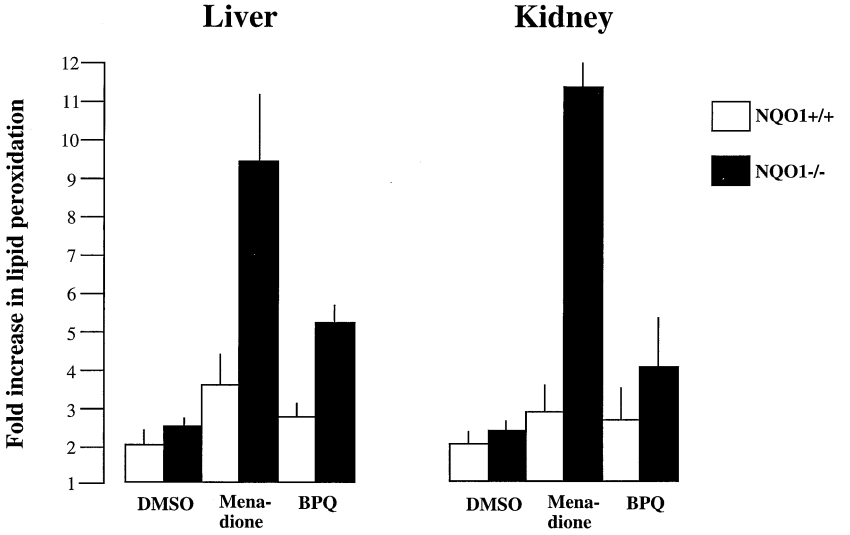


FIG. 4. Lipid peroxidation of membranes in wild type NQO1+/+ and NQO1−/− mice. The wild type (NQO1+/+) and NQO1−/− mice mice were administered intraperitoneally with either DMSO (control) or menadione and BPQ at doses of 0, 2.5, 5 and 10 mg per kg body weight. Animals were sacrified and the various tissues analyzed for lipid peroxidation by procedure as described (23). The values are mean ± SE of three independent experiments.

NQO1−/− mice failed to induce any significant lesions. The mice treated with BPQ and TPA (0 μM BPQ + TPA, 1000 μM BPQ + TPA, and 3000 μM BPQ + TPA) and TPA-alone-treated wild-type and *NQO1*−/− mice also failed to show any lesions, with the exception of one wild-type mouse that developed a spontaneous skin tumor.

DISCUSSION

Quinones are diketones. They are highly reactive molecules and readily undergo either one- or two-electron reductions. Quinones are distributed widely in nature, and human exposure to them is extensive. Quinones of polycyclic aromatic hydrocarbons (e.g. BPQ and benzoquinone) are abundant in all burnt organic materials, including automobile exhaust, cigarette smoke, and urban air particulates. They are also found naturally in many foods we eat. Compounds containing the quinoid nucleus are also employed widely as antitumor agents [16].

NQO1, first discovered in 1958, is an enzyme that catalyzes the two-electron reduction of quinones to hydroquinones [25]. Expression of the *NQO1* gene is higher in some hepatic carcinomas and other types of tumors; it is induced in response to xenobiotics, antioxidants, and oxidants [4, 6, 7]. Many studies have suggested a role of NQO1 in protection against quinone-induced redox cycling, oxidative stress, and mutagenicity. As a protective agent, NQO1 activity has been shown to prevent the formation of highly reactive quinone metabolites [26, 27] and to detoxify quinones [28]. Recently, NQO1 was also shown to reduce benzo[*a*]pyrene- and BPQ-induced mutagenicity [29, 30]. A point mutation in the *NQO1* gene (C609T; P187S) has been reported that significantly reduces NQO1 activity due to instability of the protein [31]. Human individuals have been identified that carry one or both mutant alleles of the *NQO1* gene [15–17]. The presence of a mutation, together with the suggested role of NQO1 in cellular protection, raises an important question of whether individuals carrying the mutant *NQO1* alleles are at higher risk for diseases associated with free radicals, including cancer. Recent studies have revealed a positive correlation between the loss of *NQO1* due to a point mutation and benzene-induced leukemia [32, 33]. In addition, the *NQO1*−/− mice have shown increased sensitivity to menadione toxicity. The various results, as described above, suggest a role of NQO1 in chemoprevention and warrant further studies on the *in vivo* role of NQO1 in cellular protection.

In this study, we have analyzed the *in vivo* role of NQO1 in protection against menadione- (vitamin K_3), BPQ- (a metabolic product of benzo[*a*]pyrene), and benzoquinone- (a metabolic product of benzene) induced cytotoxicity, membrane damage, DNA damage, and carcinogenicity. Overexpression of P450 reductase in CHO cells increased the toxicity of the various quinones. This was expected because P450 reductase is a one-electron reducing enzyme that generates ROS. It is also known that NQO1 and P450 reductase compete with each other for the metabolism of quinones [24]. Overexpression of P450 reductase in CHO cells may have changed the balance in favor of P450 reductase, leading to the formation of semiquinones and ROS, increased cytotoxicity, and cell death. In contrast to cells overexpressing P450 reductase, CHO cells overexpressing NQO1 were protected from the toxicity of menadione, BPQ, and benzoquinone. This protection was presumably due to two-electron reduction and detoxification of the various quinones. The cellular studies on the role of NQO1 in protection against quinone toxicity also were supported by animal models. *NQO1*−/− mice exhibited increased lipid peroxidation and DNA adducts, as compared with the wild-type (*NQO1*+/+) mice. These results indicate that quinones, in the absence of NQO1, are diverted to the redox cycling pathway. This leads to their activation, which results in damage to macromolecules. Therefore, the various results from *in vivo* experiments clearly support the hypothesis that NQO1 protects against

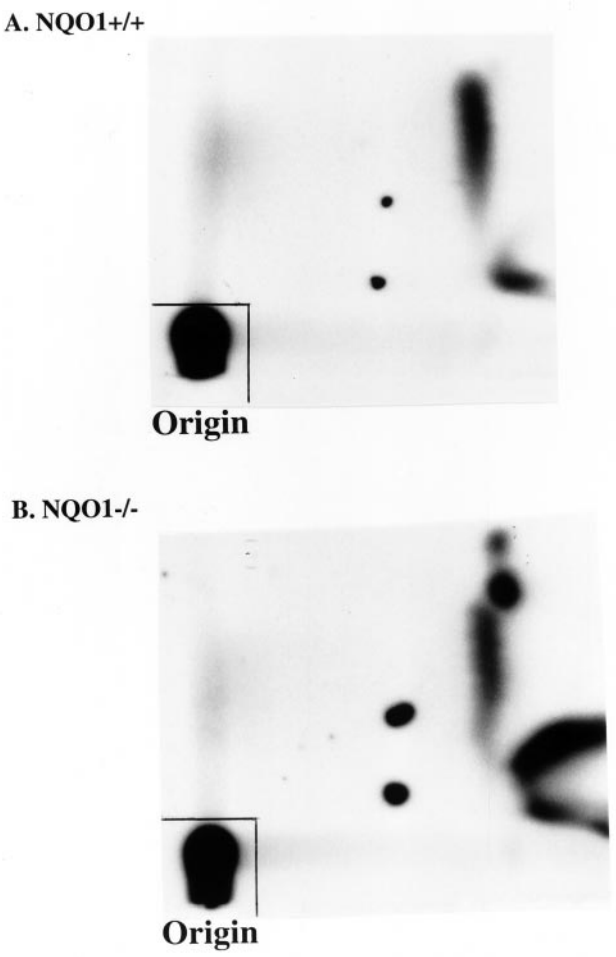


FIG. 5. BPQ–hepatic DNA adduct analysis in wild-type and *NQO1*−/− mice. Five wild-type (*NQO1*+/+) and five *NQO1*−/− mice were given i.p. injections of 10 mg of BPQ/kg body weight. The animals were given a single dose every day for 3 consecutive days. The animals were killed, and the livers were removed by surgery. Then the livers were homogenized, and DNA was isolated and analyzed for BPQ adducts by a ^{32}P-postlabeling method [24]. The results are shown for only one wild-type and one *NQO1*−/− mouse.

toxicity, membrane damage, and DNA damage caused by exposure to quinones. These results also reveal that a part of the toxicity of benzo[*a*]pyrene and benzene may be due to their quinone metabolites, especially in the absence of NQO1 activity.

BPQ has been reported to produce a low frequency of skin papillomas in CD-1 mice [34]. Surprisingly, exposure of the skin of *NQO1*−/− mice to BPQ and BPQ + TPA failed to induce any lesions. The absence of BPQ-induced skin carcinogenicity in *NQO1*−/− mice may be due to several reasons. The *NQO1*−/− mice were generated in a C57BL/6 background, and mice of this background are known to be more resistant to chemical carcinogenesis than CD-1 mice. It is also possible that BPQ is poorly absorbed in skin cells. Therefore, the BPQ concentration required to produce lesions may be sufficient in CD-1 mice to produce lesions even in the presence of NQO1. However, the BPQ concentration in C57BL/6 mice may not have reached a level sufficient to produce lesions in *NQO1*+/+ or *NQO1*−/− mice. It is also possible that BPQ was degraded at the time of application on the skin of the mice. Alternatively, this result may be due to the detoxification of BPQ by NQO2, the only other known isoenzyme of NQO1. We believe that the absence of BPQ-induced carcinogenicity in *NQO1*−/− mice may be more related to the strain (C57BL/6) of the mice used in the present study. However, the possibility of poor absorption or degradation of BPQ during application and/or of BPQ detoxification by NQO2 cannot be ruled out. We also believe that NQO2 may play only a limited role in the detoxification of BPQ due to several reasons. NQO2 differs from NQO1, not only in cofactor requirement, but also in substrate specificities [35, 36]. In addition, *in vitro* experiments have revealed that BPQ is a poor substrate for NQO2 (unpublished data). Furthermore, NQO2 expression remains unchanged in *NQO1*−/− mice as compared with *NQO1*+/+ mice, and *NQO1*−/− mice containing NQO2 activity demonstrated increased DNA and membrane damage due to exposure to BPQ. It will be interesting to further examine the sensitivity of the *NQO1*−/− mice to benzo[*a*]pyrene, the parent compound of BPQ, for skin carcinogenicity. Benzo[*a*]pyrene is a stable compound and is known to diffuse inside cells easily [37].

In conclusion, CHO cells overexpressing NQO1 and *NQO1*−/− mice were used to demonstrate that NQO1 protects against the toxic effects of menadione, BPQ, and benzoquinone. The exposure of *NQO1*−/− mouse skin to BPQ failed to produce significant lesions. It is presumed that the absence of lesions may be due to poor absorption or degradation of BPQ by unknown mechanisms.

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