Disruption of the DT Diaphorase (NQO1) Gene in Mice Leads to Increased Menadione Toxicity*

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NAD(P)H:quinone oxidoreductase 1 (NQO1) is a flavoenzyme that catalyzes two-electron reduction of quinones and their derivatives leading to protection of cells against redox cycling and oxidative stress. To examine the in vivo role of NQO1, a NQO1-null mouse was produced using targeted gene disruption. Mice lacking NQO1 gene expression showed no detectable phenotype and were indistinguishable from wild-type mice. However, NQO1-null mice exhibited increased toxicity when administered menadione compared with wild-type mice. These results establish a role for NQO1 in protection against quinone toxicity. The NQO1-null mice are a model for NQO1 deficiency in humans and can be used to determine the role of this enzyme in sensitivity to toxicity and carcinogenesis.

NQO1, previously known as DT diaphorase, is a flavoprotein that catalyzes two-electron reduction of quinones and their derivatives including azo dyes and nitroaromatic compounds (1–3). NQO1 is a cytosolic protein that is found ubiquitously in euakarytes (1–4). In mammals, it is present in many organs but is most abundant in liver (1). However, in man, NQO1 activity is significantly higher in many extrahepatic tissues (5, 6). The expression of the NQO1 gene is induced in response to a variety of agents including xenobiotics, oxidants, antioxidants, UV light, and ionizing radiation (2, 7). The obligatory two-electron reduction of quinones catalyzed by NQO1 competes with the one-electron reduction of quinones by enzymes such as NADPH:cytochrome P450 oxidoreductase and protects cells against redox cycling and oxidative stress (8, 9). This protection is the result of conversion of quinones to hydroquinones compared with semiquinones and reactive oxygen species. The role of NQO1 in cellular protection is well documented and oxidative stress. To examine the role of NQO1 in protection against quinone toxicity. The NQO1-null mice are a model for NQO1 deficiency in humans and can be used to determine the role of this enzyme in sensitivity to toxicity and carcinogenesis.

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§ The abbreviations used are: NQO1, NAD(P)H:quinone oxidoreductase 1; bp, base pair(s); kb, kilobase pair(s); HSV, herpes simplex virus; TK, thymidine kinase; ES, embryonic stem.

NQO1 is expressed at higher levels in certain tumor types compared with normal tissues has been used to develop bioreductive chemotherapeutic agents (12).

Recent studies have characterized a C → T (nucleotide position 609, NQO1P203S) mutation in the NQO1 gene which results in loss of NQO1 activity (13). This mutation in the NQO1 gene has been found in a human bladder carcinoma cell line (14), colon cancer (15), and fibroblasts taken from a cancer-prone family (16). In addition, Rosvold et al. (17) demonstrated that the same C → T mutation was overrepresented significantly in lung cancer. More recently, Rothman et al. (18) reported an increased frequency of C → T mutations in the NQO1 gene associated with benzene poisoning. The presence of a mutation, together with the fact that the physiological function of NQO1 is to detoxify potentially mutagenic compounds, poses the question of whether or not a deficiency in NQO1 activity predisposes individuals to certain types of cancer.

In the present report, we used homologous recombination in embryonic cells to disrupt the NQO1 gene and generated knockout (NQO1−/−) mice that lack expression of the NQO1 gene. NQO1−/− mice showed no detectable phenotype and were indistinguishable from wild-type mice or heterozygous (NQO1+/−) and normal littermates. However, NQO1−/− mice exhibited increased toxicity to menadione compared with wild-type mice.

EXPERIMENTAL PROCEDURES

Isolation and Characterization of Mouse NQO1 Gene—A full-length rat NQO1 cDNA was used to screen a 129SVJ mouse λ genomic library from Stratagene (La Jolla, CA) by procedures described previously (19).

Several λ clones were characterized, and one, designated AEMLB3mNQO1g-10, was used for further studies and analysis. To determine the nucleotide sequences encompassing the exon-intron junctions of mouse NQO1 gene, oligonucleotides 15–17 bp in length were selected at random from the corresponding mouse NQO1 cDNA (20) and used for sequencing NQO1 gene fragments subcloned into pUC18 by procedures described previously (21). The process of sequencing continued until the coding region from mouse NQO1 gene was completely covered.

Construction of the Targeting Vector—The targeting vector was constructed using the pPNT vector having positive selection neomycin (G418) resistance and negative selection thymidine kinase markers (22). To disrupt the NQO1 gene, a targeting plasmid containing a deletion of exon 6 of the NQO1 gene was replaced by the 2.0-kb BamHI fragment from the pPNT vector containing the bacterial phosphoribosyltransferase II gene conferring G418 resistance (Fig. 1). The targeting vector contained 3.4 kb of homologous 5′-sequence and 1.4 kb of homologous 3′-sequence of the neo-cassette. A herpes simplex virus thymidine kinase (HSV-TK) gene inserted at the 3′-end of the construct allowed the use of a positive-negative selection scheme. To construct the targeting vector, a 7.0-kb XbaI-EcoRI fragment was isolated and subcloned at the XbaI-EcoRI site of plpUC19 to convert an XbaI-SalI site. In the plasmid pUC19, the polylinker region contains an SalI site 5′ to the XbaI site. Therefore, the SalI site was simply added at the XbaI site during subcloning of the 7.0-kb XbaI-EcoRI fragment in plpUC19. The

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resultant plasmid pUC19-mNQO1 gene (SalI-EcoRI) was opened at the EcoRI site, made blunt ended, and SalI adapters were added. These manipulations resulted in conversion of the XbaI and EcoRI ends to SalI ends and loss of the BamHI site in the polylinker region of pUC19. The loss of the BamHI site from pUC19 was achieved during digestion of pUC19 with XbaI-EcoRI to generate a vector plasmid to subclone the 7.0-kb mNQO1 gene. The BamHI restriction site is located between the XbaI and EcoRI sites of the polylinker region in pUC19. The digestion of pUC19 with XbaI and EcoRI removed the portion of the polylinker region between XbaI and EcoRI containing the BamHI site, resulting in loss of the BamHI site. The plasmid pUC19-mNQO1 gene (SalI-SalI) was digested with BamHI to remove 2.0 kb of the mouse NQO1 gene containing a portion of intron 5, exon 6, and a small portion of the 3’-flanking region. The 2.0 kb of the mNQO1 gene in the plasmid pUC19-mNQO1 gene was replaced with 2.0 kb of neo-cassette (derived from pPNT vector by digestion with XhoI, addition of BamHI linkers, and redigestion with BamHI to obtain a 2.0-kb neo-cassette). The 7.0 kb of the SalI-SalI fragment from the NQO1 gene containing 2.0 kb of the neo-cassette instead of exon 6 of the mNQO1 gene was isolated and subcloned at the SalI site of the pMC1tk-pA plasmid to generate the targeting vector pPNT-mouse NQO1 gene (Fig. 1). Four plasmids were produced with various orientations of neo- and HSV-TK cassettes. The HSV-TK cassette was under the control of the HSV-TK promoter/enhancer.

Electroporation, Selection of ES Cells, and Generation of Chimeric Mice—Genome Systems (St. Louis, MO) mouse embryonic stem (ES) cells were used for homologous recombination and deletion of exon 6 from the NQO1 gene. The ES cells were thawed and diluted with ES cell medium (HEPES-buffered Dulbecco’s modified Eagle’s medium, 15% fetal bovine serum (Hyclone), 55 μM β-mercaptoethanol, 0.1 mM nonessential amino acids, penicillin-streptomycin, 1,000 units of leukemia inhibitory factor/ml), pelleted by centrifugation, and resuspended in ES cell medium. The ES cells were plated onto a 60-mm Petri dish previously seeded with mitotically inactive mouse embryonic fibroblasts. After 2 days, fresh medium was added to the ES cells, incubated for 4 h, treated with 0.25% trypsin and EDTA buffered with HEPES, resuspended in 2.5 × 10^5/60-mm-diameter plates with electroporation buffer (Hanks’ balanced salt solution, 20 mM HEPES buffer, 0.11 mM β-mercaptoethanol, pH 7.2) and used for electroporation.

Superciled targeting plasmid DNA was prepared by banding twice with cesium chloride and linearized with HindIII. The DNA was cleaned, precipitated, and resuspended at a concentration of 2 μg/μl sterile distilled water. 50 μg of linearized plasmid was ethanol precipitated and resuspended in 50 μl of electroporation buffer and electroporated in ES cells with a Bio-Rad gene pulser. The electroporation was carried out at 250 V, 250 microfarad capacitance. The electroporated ES cells were plated onto 60-mm Petri dishes containing mitotically inactive mouse embryonic fibroblasts with ES cell medium. One day after electroporation, cells were doubly selected with the drugs G418 (300 μg/ml) and gancyclovir (2 μg/ml). After 1 week of double selection, the ES cell clones were picked up under a dissecting microscope, transferred to individual wells of a 96-well plate containing 25 μl of 0.25% diluted HEPES-buffered trypsin-EDTA, and dissociated by pipetting up and down six times. Dissociated clones were transferred to wells of a 24-well plate containing mitotically inactive mouse embryonic fibroblasts with continued double-selection pressure. The clones were expanded and treated with trypsin. One-fourth of each clone was used to make DNA for analysis, and three-fourths were frozen in 10% dimethyl sulfoxide and 20% fetal bovine serum in ES medium in liquid nitrogen.

The ES cells with homologous recombination were detected by genomic Southern analysis using an EcoRI-NcoI fragment (280 bp) of the mouse gene from the 3’-flanking region as probe (the position of the probe is shown in Fig. 1). Positive ES cell clones with homologous recombination and deletion of exon 6 were detected in the ratio of 1:140 to ES cell clones. The ES cells from the positive clone mNQO1g-46 were injected into blastocysts, and chimeric animals were then bred to produce F1 mice. Southern blots were used to identify germ line transmission, and the heterozygous animals were interbred to produce homozygous knockout mice that do not express the NQO1 gene.

Southern and Northern Blot Analysis—DNA was isolated from ES cells and mouse tails by the procedure described by Laird et al., (23). DNAs were digested overnight with NcoI, electrophoresed on 1.0% agarose gel, blotted, and hybridized with a 280-bp EcoRI-NcoI fragment from the 3’-flanking region of the mouse NQO1 gene by standard procedures (24). Southern blots were washed, exposed to x-ray films, and subjected to autoradiography. In a related experiment, the DNAs from wild-type (NQO1+/+), heterozygous (NQO1+/−), and NQO1−/− mice were digested with BamHI, run on agarose gel, blotted, and hybridized with 1.1 kb of human NQO1 cDNA (complete coding region) and the 2.0-kb neo-cassette probes. The NQO1 cDNAs are highly conserved among humans, rats, and mice and are known to hybridize each other in Southern analysis (25, 26). The blots were washed and autoradiographed.

Total RNA was isolated from various tissues including liver, lung, kidney, colon, and skeletal muscle by the procedures described (26, 25). 30 μg of the RNA was subjected to electrophoresis on a 1.0% agarose gel containing formaldehyde, blotted, and hybridized with 310 bp of exon 6 probe from human NQO1 cDNA (27).

NQO1 Activity and Western Blot Analysis—The various tissues (liver, lung, kidney, colon, and skeletal muscle) from wild-type, NQO1+/+, 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 h to obtain cytosolic fractions. Dicoumarol-sensitive NQO1 activity was measured in all cytosolic fractions by a method reported earlier (28). The various...
Human NQO1 RNA contains a very long 3'-untranslated region compared with rat and mouse NQO1 RNA. Human RNA also contains four polyadenylation signals compared with one in rat and mouse (25). All of the exons are shown in capital letters. Lowercase letters represent the beginning and end of introns.

| Exon no. | Exon size | Sequence at the beginning of exon | Sequence at the end of exon |
|----------|-----------|----------------------------------|-----------------------------|
| 1        | Human     | 118 CCCACA                       | TGGTCCGgt                   |
|          | Mouse     | 115 CTGAGC                       | TGGCGGgt                   |
| 2        | Human     | 165 ggCCGAGAA                    | TCACAGtgt                   |
|          | Mouse     | 165 ggCCGAGAA                    | TCACAGgt                   |
| 3        | Human     | 131 agGTAAAC                     | TTCCAGgt                   |
|          | Mouse     | 131 agGTGAGC                     | TTCCAGgt                   |
| 4        | Human     | 114 agTTCCCC                     | TTCCGGgt                   |
|          | Mouse     | 114 agTTCCCC                     | TTCCAGgt                   |
| 5        | Human     | 102 agGTAAGA                     | ATTCAAggt                  |
|          | Mouse     | 102 agGTAAGA                     | ATTCAAggt                  |
| 6        | Human     | 1833 agAGTGGGC                   | CTCGCAAgtn                 |
|          | Mouse     | 901 agAGTGGGC                    | CACATCAAgtn                |

cytosolic fractions were also analyzed for the presence or absence of NQO1 protein by Western blot analysis as described previously using antibodies against purified rat liver NQO1 protein (28). The rat NQO1 antibody is known to cross-react with mouse and human proteins (28, 29). Western blots were developed with ECL (Amersham) reagents by

**Genomic Structure of Mouse NQO1 Gene**

The structure of the mouse NQO1 gene was found to be similar to that reported for the human NQO1 gene. Like the human NQO1 gene, the mouse NQO1 gene contained six exons interrupted by five introns. The splice junctions and nucleotide sequences in the various exons were highly conserved between the human and mouse genes (Table I).

**Production of NQO1−/− Mice**

The structure of the targeting vector pPNMT-mouse NQO1 gene is shown in Fig. 1. This construct was used successfully to generate NQO1−/− mice. The 5′- and 3′-homologous genomic sequences were 3.4 kb and 1.4 kb long, respectively. In the targeting vector, a 2.0-kb BamHI fragment containing exon 6 of the NQO1 gene was replaced with 2.0 kb of neo-cassette. This replacement was engineered to delete the carboxyl 101 amino acids of the NQO1 enzyme. This design would effectively disrupt NQO1 gene function. The decision to delete exon 6 was based on two important observations. First, deletion of 73 amino acids from the COOH terminus of the NQO1 cDNA resulted in a shorter protein and complete loss of NQO1 activity in transfected COS1 cells. Second, the C → T mutation resulting in the loss of NQO1 activity was reported to be in exon 6 of the NQO1 gene (13).

DNA from selected ES cells was analyzed by Southern blotting to screen for homologous recombinants (Fig. 2A). The digestion of DNA from ES cells (NQO1+/+) and wild-type mouse with NeoI and hybridization with 280 bp of EcoRI-NcoI fragment from the mouse NQO1 gene revealed the presence of a 9.4-kb band (Fig. 2A). Of the 140 ES clones analyzed, one NQO1−/− heterologous ES cell clone (mNQO1g-46) was identified. The presence of a 2.4-kb NcoI fragment in a genomic Southern (Fig. 2A) indicated that exon 6 is replaced with the neo-cassette as depicted in Fig. 1. The homologous recombination-positive ES cells were used to generate chimeric mice, and germ line transmission was detected. Heterozygous mice from the F1 generation were normal and were interbred to generate homozygous NQO1−/− mice. The heterozygous and homoyzous mice DNAs were analyzed for the presence of mutant allele(s) of NQO1 gene carrying deletion of exon 6 (Fig. 2A). The absence of a 9.4-kb NcoI band and the presence of a 2.4-kb NcoI band clearly indicated that homozygous NQO1−/− mice were born (Fig. 2A mice). In addition, the absence of exon 6 and the presence of the neo-cassette were confirmed by digestion of genomic DNA with BamHI followed by Southern analysis and hybridization with NQO1 cDNA and the neo-cassette probes (Fig. 2B). Southern analysis of DNA from the wild-type mice digested with BamHI and hybridization with NQO1 cDNA showed two bands of 8.0 and 2.0 kb (Fig. 2B). The 8.0-kb band contains exons 1–5 of the mouse NQO1 gene, and the 2.0-kb band contains exon 6 of the mouse NQO1 gene as shown in Fig. 1. The wild-type mice DNA did not hybridize to the neo-cassette probe (Fig. 2B). In a similar Southern analysis experiment, the NQO1−/− mice DNA showed only an 8.0-kb BamHI band hybridizing to the NQO1 cDNA (Fig. 2B). The 2.0-kb BamHI band containing exon 6 of the mouse NQO1 gene was absent in NQO1−/− mice DNA upon hybridization with NQO1 cDNA (Fig. 2B). However, the NQO1−/− mice DNA hybridized with the neo-cassette probe and, as expected, showed a band of 2.0 kb upon digestion with BamHI. This clearly indicated that the 2.0-kb neo-cassette has replaced the 2.0-kb BamHI fragment containing exon 6 from the mouse NQO1 gene in NQO1−/− mice. In the same experiment, as expected, NQO1+/+ DNA hybridized with both exon 6 and neo-cassette probes.

### Analysis of NQO1−/− Mice

**Viability and Fertility**—The NQO1−/− mice were found to be normal in appearance and showed no discernible difference in their weight, development, or in their behavior compared with their wild-type NQO1+/+ littermates. This was true for both male and female mice. At 6 weeks of age, wild-type NQO1+/+, heterozygous NQO1+/−, and homozygous NQO1−/− mice were killed for gross and histological examination. The organs and tissues examined histologically included liver, lung, kidney, colon, stomach, duodenum, spleen, thymus, lymph nodes, heart, brain, and skeletal muscle. No obvious anatomical differences in these organs were seen. In addition, the knockout animals appeared to have normal reproductive capacity compared with wild-type mice.

**Northern Analysis**—Analysis of RNA from five different tissues (liver, lung, kidney, colon, and skeletal muscle) by hybridization with the exon 6 probe indicated that NQO1 mRNA was present in wild-type and heterozygous NQO1+/− animals and absent in NQO1−/− mice (Fig. 3A). The NQO1 mRNA was lower in heterozygous mice compared with wild-type mice.

**Analysis of NQO1 Protein**—Analysis of cytosolic proteins from the various tissues (liver, lung, kidney, colon, and skeletal muscle) by SDS-polyacrylamide gel electrophoresis and Western blotting indicated the absence of the 32-kDa NQO1 protein.

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2 P. Joseph and A. K. Jaiswal, unpublished observations
in all of the tissues of NQO1−/− mice (Fig. 3B). In similar experiments, NQO1 protein was detected in all of the tissues of wild-type mice. The NQO1 protein was also detected in all of the tissues of heterozygous mice. However, the NQO1 wild-type mice demonstrated the presence of intermediate amounts of NQO1 protein between NQO1−/− and wild-type mice. Western analysis of the various tissues with NQO1 antibody also showed the presence of a 30-kDa cross-reacting band in all tissues of wild-type, heterozygous NQO1+/−, and NQO1−/− mice (Fig. 3B).

**NQO1 Activity**—The levels of dicoumarol-sensitive cytosolic NQO1 activity in the various tissues of wild-type, heterozygous NQO1+/−, and NQO1−/− mice are shown in Fig. 4. Among the five tissues tested, the highest levels of cytosolic NQO1 activity were observed in kidney followed by colon of wild-type mice. Livers from wild-type mice showed only one-fifth of the NQO1 activity observed in kidney. The level of NQO1 activity in lungs and skeletal muscles of wild-type mice was reduced further to 10% of NQO1 activity in kidney. The kidney and colon showed the complete absence of NQO1 activity in NQO1−/− mice. However, liver, lung, and skeletal muscle showed some NQO1 activity, which was no more than 15% of NQO1 activity in the respective tissues of wild-type mice. Heterozygous NQO1+/− mice tissues showed levels of NQO1 which were intermediate between NQO1−/− and wild-type animals.

**Menadione Toxicity**

**Survival of Animals**—Exposure of wild-type, heterozygous (NQO1+/−), and knockout (NQO1−/−) mice to different con-
A Northern

![Northern blot](image)

**Northern analysis.** Panel A, Northern analysis. 30 µg of RNA isolated from liver, lung, kidney, colon, and skeletal muscle of wild-type, heterozygous NQO1+/− and NQO1−/− mice were electrophoresed on formaldehyde gel, blotted to nylon membranes, hybridized with 310 bp of exon 6 probe from human NQO1 cDNA, and subjected to autoradiography. The blot after stripping off the NQO1 probe was rehybridized with a β-actin probe. Results are shown only for liver RNA. RNA from other tissues produced results similar to those of liver RNA. The exposure time for NQO1 and liver RNA. RNA from other tissues produced results similar to those of liver RNA. The exposure time for NQO1 and liver RNA. RNA from other tissues produced results similar to those of liver RNA.

B Western

![Western blot](image)

**Western analysis.** Cytosolic proteins from liver, lung, kidney, colon, and skeletal muscle were subjected to SDS-polyacrylamide gel electrophoresis and Western blotting. Western blots were probed with antibodies against rat NQO1 and developed with ECL (Amersham) reagents. +/+ wild-type; +/− heterozygous; −/− NQO1-null allele.

**FIG. 3.** Northern and Western analysis. Panel A, Northern analysis. 30 µg of RNA isolated from liver, lung, kidney, colon, and skeletal muscle of wild-type, heterozygous NQO1+/− and NQO1−/− mice were electrophoresed on formaldehyde gel, blotted to nylon membranes, hybridized with 310 bp of exon 6 probe from human NQO1 cDNA, and subjected to autoradiography. The blot after stripping off the NQO1 probe was rehybridized with a β-actin probe. Results are shown only for liver RNA. RNA from other tissues produced results similar to those of liver RNA. The exposure time for NQO1 and liver RNA. RNA from other tissues produced results similar to those of liver RNA. The exposure time for NQO1 and liver RNA. RNA from other tissues produced results similar to those of liver RNA.

Several lines of evidence indicate that modification of the NQO1 gene by replacing exon 6 with the neo cassette resulted in a null mutation. NQO1 mRNA and protein were not detected in NQO1−/− mice. The NQO1 activity dropped from very high levels in kidney and colon of wild-type mice to zero in NQO1−/− mice. The NQO1 activity also dropped significantly (>85%) in other tissues including liver, lung, and skeletal muscle. We believe that residual amounts of NQO1 activity observed in liver, lung, and kidney tissues of NQO1-null mice are caused by NQO1-related protein(s) rather than NQO1. This is clearly evident from the fact that the NQO1 protein was absent in these tissues of NQO1-null animals as determined by Western analysis. These NQO1-related protein(s) must be tissue-specific because NQO1 activity was not detectable in kidney and colon tissues of the NQO1-null animals. The small amount of NQO1 activity detected in liver, lung, and skeletal muscle of these mice is probably not caused by a <30-kDa band detected just below the 32-kDa NQO1 bands in Western blot analysis because this band was also present in kidney and colon tissues of NQO1-null mice when no measurable NQO1 activity was found. The <30-kDa band may be NQO1-related protein with no NQO1 activity or an artifact of cross-reaction of the NQO1 antibody with an unrelated protein. It remains a possibility that the <30-kDa band is cytosolic NQO2 (29). Hum- man NQO2 is a 27-kDa protein that cross-reacts with antibody against NQO1 (29). It is also known that NQO2 requires reduced dihydronicotinamide riboside instead of NAD(P)H as cofactor (30). Its activity with NAD(P)H as cofactor is also very low as measured in the present studies. The identification of the <30-kDa band as NQO2 awaits additional experimentation.

The loss of NQO1 in knockout mice did not affect the development and viability of mice. This was expected because a small percentage of the adult human population is known to be homozygous for mutant alleles of NQO1 (14–17). The C → T mutation in exon 6 of these individuals results in a proline → serine change and the loss of NQO1 activity. Humans with the NQO1-null genotype exhibited no developmental or physiological abnormalities. However, it is expected that NQO1−/− mice and humans with the NQO1−/−-null genotype would be more susceptible to free radical damage and development of toxicity from exposure to quinones, their derivatives, and redox cycling compounds.

Interestingly, the NQO1 was shown to play an important role in the maintenance of the reduced form of coenzyme Q in membranes which provides protection to the membranes against free radical damage (31, 32). Thus, NQO1−/− mice lacking the expression of NQO1 would be expected to have a significantly lower capacity to reduce coenzyme Q, leading to free radical-induced damage to membranes and DNA and premature aging of the animals. However, the extent of damage would also depend on the presence of other NQO1-related protein(s) that catalyze the reduction of coenzyme Q. This is clearly an area of great future interest.

NQO1 activity is also a part of defensive network within the cells which protects against redox cycling, oxidative stress, and other toxic effects caused by exposure to quinones and their derivatives (2, 3, 33, 34). NQO1 activity was shown to prevent the formation of highly reactive quinone metabolites (35), detoxify benzo(a)pyrene quinones (10, 36), and reduce Cr(VI) toxicity (37). Recently, benzene poisoning, a risk factor for hematological malignancy, has been shown to be associated with the NQO1 C → T mutation (18). It is a well known fact that induction of NQO1 and other detoxifying enzymes is one mechanism of critical importance in chemoprevention (2, 3, 38, 39). Therefore, many compounds that block toxic, mutagenic,
FIG. 4. NQO1 activity. The cytosolic fractions from the liver, lung, kidney, colon, and skeletal muscle of wild-type NQO1+/+, heterozygous NQO1+/−, and NQO1−/− mice were analyzed for dicoumarol-sensitive NQO1 activity by procedures as described (28). The NQO1 activity in various tissues is expressed as nmol of 2,6-dichlorophenolindophenol reduced/min/mg of cytosolic proteins.
and neoplastic effects of carcinogens share in common the ability to elevate levels of detoxifying enzymes including NQO1, glutathione S-transferase, and UDP-glucuronosyltransferases (2, 3, 40, 41). Studies with NQO1-null mice lacking the expression of NQO1 gene clearly support a protective role of NQO1 against menadione toxicity. NQO1-null mice were more sensitive to menadione than wild-type mice. 70% of the null mice died when exposed to 10 mg of menadione/kg of body weight. On the other hand, no deaths were observed with a similar dose of menadione administered to wild-type mice expressing the NQO1 gene. In addition, serum levels of the liver enzymes aspartate aminotransferase and alanine aminotransferase as markers for hepatotoxicity were found to be significantly different in NQO1-null animals compared with wild-type animals. Elevation of these liver enzymes, which are considered to be a measure of liver cell death, were detected at doses of 2.5 and 5 mg/kg of body weight in NQO1-null animals but were more or less unchanged at these doses in wild-type mice. These data indicated that liver damage is involved in mediating the toxicity of menadione in knockout mice and at higher doses in wild-type mice. The toxicity of menadione in other organs of the NQO1-null mice will be expected but remains to be determined.

In conclusion, a NQO1-null mutant mouse was produced which develops normally and is completely viable and fertile. However, NQO1<sup>−/−</sup> mice exhibit significantly increased sensitivities to menadione toxicity compared with wild-type mice. The generation and establishment of NQO1<sup>−/−</sup> mice provide very important tools in determining the in vivo role of NQO1 in protection against redox cycling-activated compounds and whether these mice are sensitive to cancer when exposed to environmental carcinogens including benzo(a)pyrene (benzo(a)pyrene quinones) and benzene (benzoquinone). The data obtained with NQO1-null mice may be used to design further studies in humans to determine if individuals carrying null alleles of the NQO1 gene are at risk for developing cancer related to exposure to chemicals via diet and occupation. In addition, the NQO1<sup>−/−</sup> mice will be an invaluable tool to study the role of NQO1 in activation of antitumor drugs such as mitomycin C and indoloquinone. This is especially important knowing that NQO1 gene is overexpressed in several kinds of tumors (13). Finally, it will be of interest to determine if mice lacking NQO1 have life spans that differ from wild-type mice since accumulation of oxidative damage has been considered as a factor in aging.

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was followed every day of the experiment. 24 h after the last dose, blood was drawn from the various surviving animals and analyzed for serum levels of aspartate aminotransferase and alanine aminotransferase activities. Panel A, all of the animals in the various groups receiving 2.5 mg and lower doses of menadione dissolved in dimethyl sulfoxide control; 2.5, 5.0, 10, 20, and 30 mg of menadione/kg of body weight (b.w.). 10 animals were injected in each group. The various doses were injected once a day for 3 consecutive days. Survival of the animals and neoplastic effects of carcinogens share in common the ability to elevate levels of detoxifying enzymes including NQO1, glutathione S-transferase, and UDP-glucuronosyltransferases (2, 3, 40, 41). Studies with NQO1-null mice lacking the expression of NQO1 gene clearly support a protective role of NQO1 against menadione toxicity. NQO1-null mice were more sensitive to menadione than wild-type mice. 70% of the null mice died when exposed to 10 mg of menadione/kg of body weight. On the other hand, no deaths were observed with a similar dose of menadione administered to wild-type mice expressing the NQO1 gene. In addition, serum levels of the liver enzymes aspartate aminotransferase and alanine aminotransferase as markers for hepatotoxicity were found to be significantly different in NQO1-null animals compared with wild-type animals. Elevation of these liver enzymes, which are considered to be a measure of liver cell death, were detected at doses of 2.5 and 5 mg/kg of body weight in NQO1-null animals but were more or less unchanged at these doses in wild-type mice. These data indicated that liver damage is involved in mediating the toxicity of menadione in knockout mice and at higher doses in wild-type mice. The toxicity of menadione in other organs of the NQO1-null mice will be expected but remains to be determined.

In conclusion, a NQO1-null mutant mouse was produced which develops normally and is completely viable and fertile. However, NQO1<sup>−/−</sup> mice exhibit significantly increased sensitivities to menadione toxicity compared with wild-type mice. The generation and establishment of NQO1<sup>−/−</sup> mice provide very important tools in determining the in vivo role of NQO1 in protection against redox cycling-activated compounds and whether these mice are sensitive to cancer when exposed to environmental carcinogens including benzo(a)pyrene (benzo(a)pyrene quinones) and benzene (benzoquinone). The data obtained with NQO1-null mice may be used to design further studies in humans to determine if individuals carrying null alleles of the NQO1 gene are at risk for developing cancer related to exposure to chemicals via diet and occupation. In addition, the NQO1<sup>−/−</sup> mice will be an invaluable tool to study the role of NQO1 in activation of antitumor drugs such as mitomycin C and indoloquinone. This is especially important knowing that NQO1 gene is overexpressed in several kinds of tumors (13). Finally, it will be of interest to determine if mice lacking NQO1 have life spans that differ from wild-type mice since accumulation of oxidative damage has been considered as a factor in aging.

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