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QUINONEIMINES AS SUBSTRATES FOR QUINONE REDUCTASE (NAD(P)H:(QUINONE-ACCEPTOR)OXIDOREDUCTASE) AND THE EFFECT OF DICUMAROL ON THEIR CYTOTOXICITY

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Abstract—Several quinoneimines have been shown to be substrates for partly purified rat liver cytosolic quinone reductase with either NADH or NADPH as cofactor. K_m and V_{max} values with NADH as cofactor for *N*-acetyl-*p*-benzoquinoneimine were 54.9 μM and 278 μmol/min/mg; for 2-amino-1,4-naphthoquinoneimine, 2.8 μM and 38 μmol/min/mg; for *N,N*-dimethylindoaniline, 1.7 μM and 22 μmol/min/mg; and 2-acetamido-*N,N*-dimethylindoaniline, 0.4 μM and 9 μmol/min/mg. All the quinoneimines showed substrate inhibition at high concentrations. At 30 μM dicumarol, an inhibitor of quinone reductase, potentiated the acute toxicity of quinoneimines to cultured phenobarbital-induced rat hepatocytes by 0.7- to 2.9-fold. Dicumarol was toxic to cultured non-induced rat hepatocytes and produced little or no increase in quinoneimine toxicity. Dicumarol potentiated the toxicity of 2-methyl-1,4-naphthoquinone (menadione) to cultured non-induced, as well as phenobarbital-induced, hepatocytes. Levels of quinone reductase in both types of hepatocytes were similar. Quinoneimines exhibited strong growth inhibitory properties with Chinese hamster ovary (CHO) cells and A204 human rhabdomyosarcoma cells. Dicumarol, 0.1 mM, potentiated growth inhibition by *N,N*-dimethylindoaniline and 2-acetamido-*N,N*-dimethylindoaniline in A204 but not in CHO cells. Growth inhibition by 2-amino-1,4-naphthoquinoneimine was inhibited by dicumarol in both cell lines. Dicumarol potentiated growth inhibition by 2-methyl-1,4-naphthoquinone in A204 and CHO cells. Quinone reductase activity in A204 cells was 48% and in CHO cells 1% of the activity in cultured hepatocytes. The lack of a correlation between the effects of dicumarol on quinoneimine and quinone growth inhibition and levels of cellular quinone reductase suggests that dicumarol has effects in cells in addition to, or other than, inhibition of quinone reductase. It is concluded that quinone reductase may protect cells against quinoneimine toxicity under certain conditions, as with phenobarbital-induced hepatocytes, but does not appear to play a major role in modifying quinoneimine toxicity in non-induced hepatocytes, or growth inhibition in CHO cells or A204 cells.

Quinoneimines are nitrogen analogues of quinones where one quinone oxygen is replaced by an imino group. Quinoneimines have similar chemical properties to quinones including the ability to undergo one-electron reduction to a semiquinone-type free radical and two-electron reduction to an aminophenol [1]. Quinones are widely distributed in nature and occur as environmental pollutants [2, 3]. They have been studied extensively for their cytotoxic and antitumor properties [4–6] and are among the most useful drugs in the treatment of human cancer [7, 8]. The cytotoxic effects of quinones are believed to be due to their enzymatic one-electron reduction by flavoenzymes such as NADPH–cytochrome P-450 reductase (EC 1.6.2.4.) to form a semiquinone free radical. The semiquinone free radical can give a species that alkylates critical macromolecules in the cell [9, 10], or it can transfer an electron to dioxygen to form superoxide anion radical and in the process the semiquinone free radical is oxidized back to quinone, a process known as redox cycling [11, 12]. Reaction of superoxide anion radical with hydrogen peroxide, formed by the enzymatic or spontaneous dismutation of superoxide anion radical in the presence of trace amounts of iron salts, gives hydroxyl radical, a powerful oxidizing species that is toxic to cells [13, 14]. Quinone reductase (NAD(P)H:(quinone-acceptor)oxidoreductase, EC 1.6.99.2), formerly known as DT-diaphorase, is unusual among flavoenzymes in that it promotes obligatory two-electron reduction of quinones [15] leading to formation of relatively nontoxic hydroquinones that can be conjugated with glucuronic acid or sulfate and eliminated from the cell [16–19]. Quinone reductase has been suggested to protect cells against the toxicity of quinones [17–24], and dicumarol, a potent and specific inhibitor of quinone reductase [16], has been reported to increase the cytotoxicity of a number of quinones or quinone forming compounds [6, 17, 25–27].

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Quinoneimines occur rarely in nature [28] but are represented among synthetic antitumor agents [29–33]. *N*-Acetyl-*p*-benzoquinoneimine is the putative hepatotoxic metabolite of acetaminophen [34]. We have shown recently that cytotoxic quinoneimines and quinonediimines are substrates for microsomal flavoenzymes catalyzing one-electron reduction and that some quinoneimines undergo redox cycling to form oxygen radicals [35].

We now report the ability of a number of cytotoxic quinoneimines to act as substrates for partly purified rat liver quinone reductase and the effects of dicumarol on the acute toxicity of quinoneimines to primary cultures of rat hepatocytes and on the growth inhibitory properties with cell lines in culture.

MATERIALS AND METHODS

Drugs and chemicals. 2-Amino-1,4-naphthoquinoneimine, *N,N*-dimethylindoaniline and 2-acetamido-*N,N*-dimethylindoaniline were synthesized as previously described [29]. *N*-Acetyl-*p*-benzoquinoneimine was obtained from Dr. S. D. Nelson, Department of Medicinal Chemistry, University of Washington, Seattle, WA. 2,6-Dichloroindophenol and menadione (2-methyl-1,4-naphthoquinone) were purchased from the Aldrich Chemical Co., Milwaukee, WI. NADPH, NADH and dicumarol were purchased from the Sigma Chemical Co., St. Louis, MO. Quinone reductase was prepared from liver cytosol from 200–250 g male Sprague–Dawley rats (Sprague–Dawley, Madison, WI) by a modification of the affinity chromatography method of Wallin and Little [36] with Cibacron Blue as the affinity ligand. Approximately 60 g of rat liver was homogenized in 4 vol. of 0.25 M sucrose and used to prepare a 110,000 *g* supernatant fraction. The supernatant fraction was mixed with 9 vol. of acetone at −20°, and the precipitate was collected, lyophilized for 1 hr to remove residual acetone, and then dissolved in 120 ml of 10 mM sodium phosphate buffer, pH 6.4. Approximately 65% of cytosolic quinone reductase activity was recovered at this stage. A 50–70% saturated ammonium sulfate fraction was prepared which contained 43% of the original quinone reductase activity. The precipitate, dissolved in a minimum amount of water, was dialyzed overnight against 8 liters of 10 mM sodium phosphate buffer, pH 7.4, before being applied to a 1 × 15 cm column of Blue Sepharose C1-6B (Pharmacia Inc., Piscataway, NJ). The ligand of Blue Sepharose is Cibacron Blue F3G-A which binds pyridine nucleotide requiring enzymes and, probably because Cibacron Blue F3G-A is a quinone, has a particularly high affinity for quinone reductase. The column was washed extensively with 10 mM sodium phosphate buffer, pH 7.4, followed by 100 ml of 2 M NaCl, 10 mM sodium phosphate buffer, pH 7.4, and quinone reductase eluted with 100 ml of 20 mM NADH in 2 M NaCl, 10 mM sodium phosphate buffer, pH 7.4. Recovery of quinone reductase was 61% of the activity applied to the column. The eluate was dialyzed against three changes of 16 liters of distilled water for 36 hr to remove NaCl and NADH. The purified enzyme was unstable, and dialysis resulted in loss of approximately 50% of the activity. The specific activity of the final preparation of quinone reductase with 0.1 mM 2,6-dichloroindophenol as substrate and NADH as cofactor at 21° was 161.2 μmol NADH oxidized/min/mg. This represented a 730-fold purification of quinone reductase from cytosol. Activity was inhibited completely by 30 μM dicumarol. Protein was measured by the dye-binding method of Bradford [37], using a commercial test kit (Bio-Rad Laboratories, Richmond, CA) and crystalline bovine serum albumin as a standard.

Quinone reductase activity with quinoneimines as substrates was measured using freshly prepared enzyme at 21° by oxidation of NADPH and NADH at 340 nm in an incubation mixture containing 150 μmol Tris–HCl buffer, pH 6.0, 2.1 mg bovine serum albumin, and 1.1 μg quinone reductase in a volume of 3 ml. Quinoneimine, dissolved in 10 μl dimethyl sulfoxide, was added immediately before 0.3 μmol NADH or NADPH dissolved in 10 μl water. Enzyme activity was corrected for slow non-enzymatic oxidation of NADH or NADPH by quinoneimine. All measurements were made in triplicate. Quinone reductase activity in cell sonicates and soluble quinone reductase activity were measured by oxidation of NADH with 0.1 mM 2,6-dichloroindophenol as a substrate in the presence and absence of 1 μM dicumarol. Quinone reductase activity was expressed as nmol/min/10^6 viable cells, viability being determined by trypan blue exclusion.

Hepatocytes were isolated from livers of male Sprague–Dawley rats by perfusion with low Ca^{2+} medium and collagenase as previously described [12]. Hepatocyte viability immediately after isolation was determined by trypan blue exclusion and was routinely greater than 93%. Some rats were pretreated with phenobarbital in the drinking water (0.1%, w/v) for 5 days. Hepatotoxicity of the quinoneimines was measured by leakage of cytosolic lactate dehydrogenase from primary cultures of hepatocytes [38]. Freshly prepared hepatocytes were plated under asceptic conditions in 60 mm plastic culture dishes (Corning Tissue Culture Products, Corning, NY) at a density of 5×10^6 viable cells in 5 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 0.1% bovine serum albumin, 0.37% sodium bicarbonate, 0.8 mM niacinamide, 100 μg gentamicin sulfate/ml, 10 μg insulin/ml, and 50 μg hydrocortisone/ml. The culture dishes were maintained in an incubator at 37°, with 5% CO_2:95% air at a relative humidity of 100% for 24 hr. Medium and unattached cells were removed and replaced with 5 ml of warmed sterile DMEM without supplementation containing quinoneimine at various concentrations, with and without dicumarol. The culture dishes were placed in incubators at 37° for 2 hr. The medium was then decanted and centrifuged at 800 *g* for 10 min at room temperature to remove any free cells. Lactate dehydrogenase was assayed using a commercially available test kit (Sigma Diagnostics, St. Louis, MO) and expressed as a percent of the total enzyme activity released from the hepatocytes by alternately freezing and thawing the cells three times. Cultures were performed in triplicate at each of six drug concentrations.

Chinese hamster (HA-1) ovary (CHO) cells were obtained from Dr. George Hahn, Stanford Medical Center, Stanford, CA. A204 human rhabdomyosarcoma cells were obtained from Dr. Michael Lieber, Mayo Clinic, Rochester, MN. CHO cells were maintained as bulk culture monolayers in multiple 75 cm^2 flasks containing Eagle's minimum essential medium (EMEM) with 10% fetal calf serum, 100 μg streptomycin/ml, and 2 mM glutamine. A204 cells were maintained in a similar manner except that DMEM replaced EMEM. The medium was changed three times per week, and cells in exponential growth were passaged each week for a maximum of 15 weeks using medium containing 0.05% trypsin and 0.01% EDTA. The cell lines were Mycoplasma-free by culture (Virology Laboratory, Mayo Clinic). Growth inhibitory activity was measured using colony formation of CHO or A204 cells. Cells in log-phase growth were plated in 60 mm plastic culture dishes at multiple densities such that final counts of between 100 and 200 colonies per culture dish were obtained following exposure to compounds. Culture dishes containing cells and 5 ml of growth medium were placed in an incubator at 37° with 5% CO_2:95% air at 100% relative humidity for 24 hr to allow attachment of cells. Medium was removed and replaced with 5 ml of warmed medium containing quinoneimine at various concentrations, with or without dicumarol. After a further 24 hr in the incubator, drug and medium were removed, and culture dishes were washed five times with warmed medium before allowing the cells to grow for 10 days. After this time medium was removed and dishes were washed with warmed 0.9% NaCl solution. Colonies were stained with 0.25% Coomassie Blue in methanol for 10 min, rinsed with tap water, and counted manually. Quadruplicate culture dishes were used for each compound concentration.

Hepatocyte toxicity and cell colony formation data were fitted to a monoexponential curve using the NONLIN nonlinear least-squares regression analysis program [39]. Variance of the effective drug concentration required to release 50% of hepatocyte intracellular lactate dehydrogenase over background release in the absence of compound (EC_{50}), or to produce 50% inhibition of cell growth (IC_{50}) was obtained from the variance of the intercept and slope of the dose–response relationship using a Taylor series expansion. Groups of data were compared using Student's *t*-test [40].

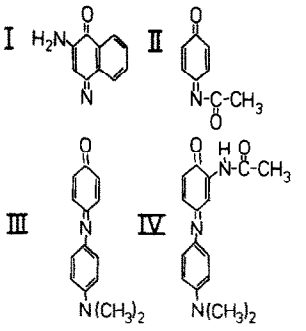

Fig. 1. Structures of the quinoneimines used: (I) 2-amino-1,4-naphthoquinoneimine; (II) *N*-acetyl-*p*-benzoquinoneimine; (III) *N,N*-dimethylindoaniline; and (IV) 2-acetamido-*N,N*-dimethylindoaniline.

RESULTS

Metabolism by quinone reductase. The structures of the quinoneimines used are shown in Fig. 1. The quinoneimines tested were all substrates for partly purified quinone reductase with either NADH or NADPH as cofactor (Table 1). *N*-Acetyl-*p*-benzoquinoneimine was the most rapidly metabolized quinoneimine but had a relatively high K_m, around 47 μM. The other quinoneimines were less rapidly metabolized but had a K_m in the range of 0.4 to 2.8 μM. All the quinoneimines showed substrate inhibition at high concentrations, above 30 μM for *N*-acetyl-*p*-benzoquinoneimine, 2-amino-1,4-naphthoquinoneimine and 2-acetamido-*N,N*-dimethylindoaniline and above 1 μM for *N,N*-dimethylindoaniline (Fig. 2). Substrate inhibition of quinone reductase by quinones and by 2,6-dichloroindophenol has been reported previously [16].

Hepatotoxicity. Quinone reductase activity in 24 hr cultured non-induced hepatocytes was 183.8 nmol/min/10^6 viable cells and in phenobarbital-induced hepatocytes 186.6 nmol/min/10^6 viable cells. These levels of quinone reductase were 68.7 and 76.3% of the activity in freshly prepared hepatocytes respectively. Approximately 80% of the quinone reductase activity was present in the soluble cell fraction of fresh and cultured hepatocytes. In non-induced hepatocytes, 30 μM dicumarol was itself toxic and with 2-hr exposure produced a 30% increase in the release of lactate dehydrogenase (Fig. 3). Dicumarol

Table 1. Metabolism of quinoneimines by quinone reductase

	NADH		NADPH	
Compound	K_m (μM)	V_{max} (μmol/min/mg)	K_m (μM)	V_{max} (μmol/min/mg)
N-Acetyl-*p*-benzoquinoneimine	54.9	277.8	40.0	238.1
2-Amino-1,4-naphthoquinoneimine	2.8	38.5	2.6	35.7
N,N-Dimethylindoaniline	1.7	22.2	0.9	14.3
2-Acetamido-*N,N*-dimethylindoaniline	0.4	9.1	0.5	12.5

Kinetic parameters for metabolism of quinoneimines by rat liver cytosol quinone reductase were determined as described in the text with either NADH or NADPH as cofactor. Metabolism was measured as the oxidation of cofactor.

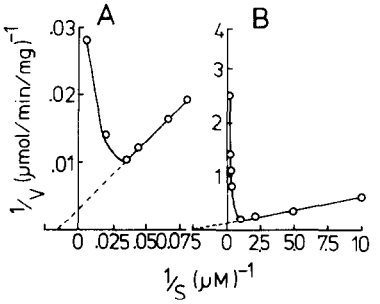

Fig. 2. Lineweaver–Burk plot of metabolism of (A) *N*-acetyl-*p*-quinoneimine and (B) 2-acetamido-*N,N*-dimethylindoaniline by partly purified rat liver quinone reductase with NADH as cofactor, showing substrate inhibition at high quinoneimine concentration.

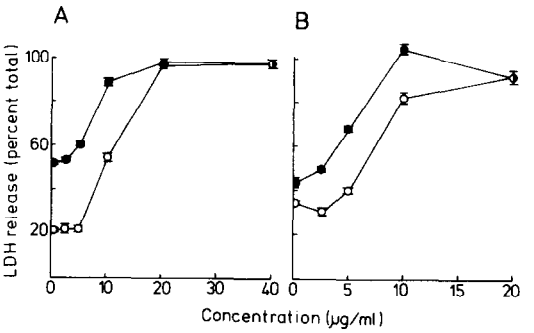

Fig. 3. Effect of dicumarol on the toxicity of 2-amino-1,4-naphthoquinoneimine in (A) control and (B) phenobarbital-induced cultured rat hepatocytes. Key: (○) without dicumarol and (●) with 30 μM dicumarol. Each point is the mean of three determinations; bars are SE of mean.

produced a further small increase in the toxicity of 2-amino-1,4-naphthoquinoneimine to non-induced hepatocytes but no increase in the toxicity of the other two quinoneimines (Table 2). Dicumarol, 30 μM, was non-toxic to phenobarbital-induced hepatocytes (Fig. 3) and produced a 0.7- to 2.9-fold increase in the toxicity of the quinoneimines (Table 2). The toxicity of menadione, which was included in the studies as a positive control, was increased by dicumarol in both non-induced and phenobarbital-induced hepatocytes.

Growth inhibition. Quinone reductase activity in sonicates of A204 cells was 88.7 nmol/min/10^6 cells and in CHO cells 2.0 nmol/min/10^6 cells. All the quinoneimines tested were potent inhibitors of cell growth and were more active than menadione (Table 3). A non-toxic concentration of 0.1 mM dicumarol was chosen to study the effect of quinone reductase inhibition upon quinoneimine and menadione dependent growth inhibition. The effect of dicumarol upon quinoneimine growth inhibition depended upon the cell line being studied. In A204 cells, dicumarol potentiated growth inhibition by *N,N*-dimethylindoaniline and 2-acetamido-*N,N*-dimethylindoaniline but had no significant effect upon growth inhibition in CHO cells by these agents. Growth inhibition by 2-amino-1,4-naphthoquinoneimine was inhibited significantly by dicumarol in A204 cells and CHO cells.

DISCUSSION

Quinone reductase is known to catalyze the reduction of a variety of compounds including quinones [16], azo compounds [41] and a transition metal [42]. There have also been reports of quinoneimines being substrates for quinone reductase. 2,6-Dichloroindophenol, a redox dye often used as a substrate for assay of quinone reductase [16], is a quinoneimine. Resorufin, a heterocyclic quinoneimine, is also metabolized by quinone reductase [43]. We are not aware of other quinoneimines having been reported as substrates for quinone reductase. The present study shows that the quinoneimines, *N*-acetyl-*p*-benzoquinoneimine, the putative hepatotoxic metabolite of acetaminophen [34], and 2-amino-1,4-naphthoquinoneimine, *N,N*-dimethylindoaniline and 2-acetamido-*N,N*-dimethylindoaniline, which have antitumor activity against sarcoma 180 in mice [29, 30], are substrates for partly purified quinone reductase.

Table 2. Effect of dicumarol on hepatocyte toxicity of quinoneimines and menadione

Compound	Non-induced hepatocytes		Phenobarbital-induced hepatocytes	
	EC_{50} (μM)		EC_{50} (μM)	
	− Dicumarol	+ Dicumarol	− Dicumarol	+ Dicumarol
2-Amino-1,4-naphthoquinoneimine	12.2 ± 0.4	8.3 ± 0.3*	11.9 ± 0.5	4.2 ± 0.2*
N,N-Dimethylindoaniline	15.5 ± 0.80	14.7 ± 0.64	20.4 ± 0.4	7.2 ± 0.4*
2-Acetamido-*N,N*-dimethylindoaniline	5.8 ± 0.3	9.1 ± 0.3*	23.7 ± 0.9	16.4 ± 0.8*
Menadione	50.1 ± 1.1	24.6 ± 0.6*	59.1 ± 2.5	19.9 ± 0.7*

The effect of 30 μM dicumarol on the toxicity of quinoneimines to non-induced and phenobarbital-induced hepatocytes cultured for 24 hr was measured by the release of lactate dehydrogenase. Menadione was included as a positive control. Results are expressed as the mean ± SE of the effective concentration of quinoneimine or menadione to produce a 50% increase in the release of total lactate dehydrogenase (EC_{50}).

* $P < 0.01$ compared to absence of dicumarol.

Table 3. Effect of dicumarol on the growth inhibitory properties of quinoneimines and menadione

Compound	IC_{50} (μM)	
	Without dicumarol	With dicumarol
A204		
2-Amino-1,4-naphthoquinoneimine	0.36 ± 0.00	0.78 ± 0.05*
N,N-Dimethylindoaniline	4.83 ± 0.12	1.65 ± 0.04*
2-Acetamido-*N,N*-dimethylindoaniline	4.08 ± 0.20	0.97 ± 0.03*
Menadione	13.0 ± 0.46	7.55 ± 0.23*
CHO		
2-Amino-1,4-naphthoquinoneimine	1.15 ± 0.05	2.13 ± 0.05*
N,N-Dimethylindoaniline	0.66 ± 0.04	0.78 ± 0.04
2-Acetamido-*N,N*-dimethylindoaniline	1.07 ± 0.03	1.07 ± 0.03
Menadione	13.13 ± 0.46	5.69 ± 0.23*

The effect of 100 μM dicumarol on growth inhibition by quinoneimines was measured in cultured A204 human rhabdomyosarcoma and Chinese hamster ovary (CHO) cell lines. Menadione was included as a positive control. Results are expressed as the mean ± SE of the concentration of quinoneimine or menadione producing a 50% inhibition of cell growth (IC_{50}).
* $P < 0.01$ compared to absence of dicumarol.

Quinone reductase catalyzes two-electron reduction of quinones to dihydrodiols [15] and has been suggested to provide a cellular defense against quinone toxicity by removing quinones from one-electron redox cycling and electrophilic addition [17–20]. It is likely that quinoneimines undergo two-electron reduction to aminophenol derivatives, although this was not measured directly in this study. Aminophenols are less cytotoxic than quinoneimines [35] so that quinone reductase might also protect cells against quinoneimine cytotoxicity.

Dicumarol, an inhibitor of quinone reductase, has been reported to increase the toxicity of menadione to freshly isolated phenobarbital-induced rat hepatocytes [17] and to cultured human fibroblasts [6]. Dicumarol also increases the toxicity of mitomycin C to hypoxic EMT6 tumor cells [25]. The mechanism in all cases has been ascribed to inhibition of quinone reductase. Quinone reductase can also, under certain conditions, activate quinones and dicumarol has been reported to decrease the toxicity of halogenated dimethylnaphthoquinones to *Streptomyces typhimurium TA97* [44], to human tumor cells and to murine bone marrow cells [45]. Metabolism by quinone reductase has been suggested, although with little direct evidence, to be involved in the covalent binding of doxorubicin to DNA catalyzed by rat liver cytosol [9].

Dicumarol at concentrations previously reported to be non-toxic to freshly isolated phenobarbital-induced and cultured non-induced rat hepatocytes [17, 21] was toxic to cultured non-induced rat hepatocytes in our system. This may be related to the absence of serum albumin or other serum protein in our incubation medium. Serum albumin binds dicumarol 99% and will, thus, reduce the free concentration of dicumarol [46]. Menadione was also more toxic to cultured hepatocytes in our system than previously reported with either fresh or cultured rat hepatocytes [17, 21], again presumably due to lack of protein in our incubation medium.

As pointed out by Thor *et al.* [17], whether quinone reductase will protect cells against quinone toxicity depends upon a higher affinity of the quinone for quinone reductase than its affinity for enzymes, such as NADPH–cytochrome P-450 reductase, that catalyze redox cycling of the quinone. The affinity of 2-amino-1,4-naphthoquinoneimine for quinone reductase, 2.7 μM, is similar to its affinity for microsomal NADPH–cytochrome P-450 reductase of 3.5 μM [35]. 2-Amino-1,4-naphthoquinoneimine undergoes redox cycling and stimulates microsomal and hepatocyte superoxide anion radical formation [35]. Quinone reductase might, therefore, be expected to protect hepatocytes against 2-amino-1,4-naphthoquinoneimine toxicity and, in the present study, dicumarol produced a 2.9-fold increase in the toxicity of 2-amino-1,4-naphthoquinoneimine to phenobarbital-induced cultured hepatocytes. This effect of dicumarol is circumstantial evidence for a role for quinone reductase in protecting phenobarbital-induced hepatocytes against quinoneimine toxicity. *N*-Acetyl-*p*-benzoquinoneimine, *N,N*-dimethylindoaniline and 2-acetamido-*N,N*-dimethylaniline are metabolized extensively by microsomal NADPH–cytochrome P-450 reductase but do not undergo redox cycling and do not form oxygen radicals or stimulate oxygen utilization [35, 47]. Unlike 2-amino-1,4-naphthoquinoneimine, these quinoneimines react directly with reduced glutathione [47, 48]. Metabolism to a semiquinoneimine free radical or direct reaction with cellular thiols might contribute to the toxic effect of these quinoneimines. We have found that dicumarol increases the toxicity of *N,N*-dimethylindoaniline and 2-acetamido-*N,N*-dimethylaniline to phenobarbital-induced cultured hepatocytes. The K_m of *N*-acetyl-*p*-benzoquinoneimine for NADPH–cytochrome P-450 reductase is around 1.8 to 4 μM [35], whereas the K_m for quinone reductase is 47 μM. Quinone reductase would not be expected to protect cells against metabolic activation of *N*-acetyl-*p*-benzoquinoneimine. Dicumarol has been reported not to protect hepatocytes against *N*-acetyl-*p*-benzoquinoneimine toxicity [48]. The

hepatotoxicity of *N*-acetyl-*p*-benzoquinoneimine may be due to a direct reaction with intracellular reduced glutathione and oxidation of protein thiol groups [48].

We found that dicumarol had different effects upon growth inhibition by quinoneimines, depending upon the cell line being studied. Two different cell lines were chosen, A204 human rhabdomyosarcoma which has high levels of quinone reductase and Chinese hamster ovary (CHO) which has very low levels of quinone reductase. Growth inhibition by *N,N*-dimethylindoaniline and 2-acetamido-*N,N*-dimethylindoaniline was potentiated by dicumarol in A204 but not in CHO cells. This might suggest that quinone reductase normally protects A204 cells against growth inhibition by these quinoneimines but does not protect CHO cells which have much less enzyme. However, dicumarol decreased growth inhibition by 2-amino-1,4-naphthoquinoneimine in both cell lines. It appears that dicumarol has effects, at least in the cell lines studied, in addition to inhibition of quinone reductase. Ackman *et al.* [27] have proposed a mechanism for potentiation of growth inhibition by menadione in L1210 murine leukemia cells by dicumarol, independent of inhibition of quinone reductase. In the present study, dicumarol potentiated growth inhibition by menadione in both A204 and CHO cells, despite their different levels of quinone reductase. This observation lends support, although is not conclusive evidence, for potentiation of menadione growth inhibition by dicumarol by a mechanism that does not involve, or in addition to, inhibition of quinone reductase. In any event the effect of dicumarol in altering quinoneimine-dependent growth inhibition was small, usually no more than a 4-fold change, so that quinone reductase does not appear to play a major role in modifying the response of A204 or CHO cells to quinoneimine growth inhibition.

In summary, cytotoxic quinoneimines have been shown to be substrates for partly purified quinone reductase. Dicumarol, an inhibitor of quinone reductase, potentiated the toxicity of quinoneimines to phenobarbital-induced rat hepatocytes but produced little potentiation of toxicity to non-induced rat hepatocytes. Dicumarol produced a small increase in growth inhibition by some quinoneimines in cell lines but decreased growth inhibition by another quinoneimine. It is concluded that the effect of quinone reductase in protecting cells against quinoneimine toxicity is small and that dicumarol has effects in cells that alters quinoneimine and quinone toxicity in addition to inhibition of quinone reductase.

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