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Induction of NAD(P)H:quinone reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: A model system for the study of anticarcinogens

(Sudan dyes/butylated hydroxyanisole/polycyclic aromatic hydrocarbons/cancer protection)

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ABSTRACT **Exposure of murine hepatoma (Hepa 1c1c7) cells to a variety of chemical agents known to protect animals against the neoplastic, mutagenic, and other toxic effects of chemical carcinogens results in dose- and time-dependent inductions of NAD(P)H:quinone reductase (EC 1.6.99.2). This enzyme protects against quinone toxicity by promoting obligatory two-electron reductions that divert quinones from oxidative cycling or direct interactions with critical nucleophiles. Quinone reductase levels are stable in culture, are easily measured, and are useful markers for the inductive effects of chemoprotective agents. The Hepa 1c1c7 system responds to chemoprotective compounds such as phenolic antioxidants {e.g., BHA [3(2)-*tert*-butyl-4-hydroxyanisole], BHT (3,5-di-*tert*-butyl-4-hydroxytoluene), and *tert*-butylhydroquinone}, lipophilic azo dyes belonging to the 1,1'-azonaphthalene, Sudan I (1-phenylazo-2-naphthol), and Sudan III [1-(4-phenylazophenylazo)-2-naphthol] families, polycyclic aromatic hydrocarbons, coumarin and various other lactones, flavonoids, and certain sulfur compounds (e.g., benzylisothiocyanate, dithiolthiones, and dithiocarbamates), all of which are recognized enzyme inducers and chemoprotectors *in vivo*. Quinone reductase induction in Hepa 1c1c7 cells therefore provides a simple, versatile, and reliable system for the evaluation of the potency, kinetics, and mechanism of action of anticarcinogens.**

An astonishing variety of structurally unrelated compounds, including phenolic antioxidants, coumarins, azo dyes, flavonoids, polycyclic aromatics, and certain sulfur compounds, protect laboratory animals against the neoplastic, mutagenic, and other toxic effects of chemical carcinogens (1, 2). Clarification of the molecular mechanisms underlying these protective actions is of importance in devising strategies for protecting man against cancer. Much evidence indicates that protection by these agents depends on altering the metabolism of carcinogens (3, 4). Some protectors (e.g., polycyclic hydrocarbons, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and azo dyes) are potent inducers of both phase I and phase II drug-metabolizing enzymes†, whereas other protective agents (e.g., phenolic antioxidants) induce phase II enzymes only (for review, see ref. 3). Although the balance between these two types of enzymes required for protective action remains unclear, current evidence indicates that induction of such phase II enzymes as glutathione *S*-transferases, quinone reductase, and epoxide hydrolase is important for protection (1–4, 6–8).

We have developed a murine hepatic cell culture system (Hepa 1c1c7) that mimics many animal tissues in responding to a wide variety of chemoprotective agents by induction of cytosolic quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2; also known as menadione reductase, DT diaphorase, or vitamin K reductase]. These cells are a suitable model for studying chemoprotection because (*i*) they retain many characteristics of normal tissues, particularly the capacity for carcinogen activation and metabolism, and (*ii*) they are amenable to precise control of environmental, nutritional, and hormonal factors. Further, mutants defective in enzyme-induction pathways (9, 10) are available. Quinone reductase was selected as a marker for induction because (*a*) it protects against toxicity (3, 11–16), (*b*) its levels rise coordinately with other chemoprotective enzymes in many animal tissues in response to the administration of various anticarcinogens (4, 17, 18), and (*c*) it is induced by many chemically dissimilar substances that protect against carcinogens (3, 19). An abstract of the work presented here has been published (20).

MATERIALS AND METHODS

Hepa 1c1c7 cells (a gift of J. P. Whitlock, Jr., Stanford University) were grown in alpha minimal essential medium minus nucleosides (GIBCO), supplemented with 10% fetal calf serum, at 37°C in an atmosphere of 5% CO_2 in a humidified incubator. In our standard induction protocol, Hepa 1c1c7 cells were plated at 0.89×10^6 cells per 75-cm^2 plate, grown for 48 hr to late logarithmic phase, and then exposed (usually for 24 hr) to fresh medium containing the test compounds in dimethyl sulfoxide (0.1%, by volume). Cell layers were washed three times with 10 ml of cold 0.25 M sucrose, collected by scraping, frozen in liquid nitrogen, and stored at −80°C until enzyme activities were assayed.

Preparation of cytosols and enzyme assays have been described (17, 18). The specific activities were obtained from spectrophotometric measurements of the reduction of 2,6-dichloroindophenol by NADH. Many azo compounds were generously provided by C. Huggins and J. Pataki of The University of Chicago (21) and purified by recrystallization when appropriate. 1-(4-Hydroxyphenylazo)-2-naphthol was synthesized by mixing 4-aminophenol (10 mmol) in 25 ml of H_2O with 10 mmol of $NaNO_2$. This solution was introduced dropwise into 25 ml of 0.4 M HCl, and the diazonium salt was added dropwise to 10 mmol of 2-naphthol in 25 ml of H_2O,

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Abbreviations: BHA, 3(2)-*tert*-butyl-4-hydroxyanisole; BHT, 3,5-di-*tert*-butyl-4-hydroxytoluene.

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†Enzymes involved in the metabolism of xenobiotics have been grouped into two broad classes (5): Phase I enzymes (which include the cytochromes P-450) functionalize compounds by oxidation, reduction, or hydrolysis, whereas phase II enzymes carry out the conjugations of functionalized compounds with endogenous ligands (e.g., glucuronic acid, glutathione, and sulfate). We consider quinone reductase as a phase II enzyme, since it does not introduce new functional groups and is often induced coordinately with conjugation enzymes.

resulting in the immediate formation of a red precipitate. The mixture was stirred for 30 min, filtered, and the product was recrystallized from hot ethanol (m.p. 192–193°C) (22).

RESULTS

Baseline Studies. Under standardized conditions, the mean basal specific activity of cytosolic quinone reductase of Hepa 1c1c7 cells was 324 nmol/min per mg of protein (range 238 ± 16 to 485 ± 31, for the mean values of four plates ± SEM) in 10 experiments. The quinone reductase level of Hepa cells is thus about twice those of female mouse hepatic cytosols: 132 ± 15 (CD-1 mouse), 160 ± 16 (DBA/2J), and 213 ± 7 (C57BL/6J) nmol/min per mg of protein (ref. 11; unpublished observations). The specific activity of quinone reductase of Hepa 1c1c7 cell cytosols is consistent from plate to plate within a single experimental group, remains relatively constant with time during growth to confluence and for at least 48 hr thereafter (Fig. 2), and is unaffected by 0.1% (by volume) dimethyl sulfoxide.

Dose Response and Kinetics of Induction of Quinone Reductase by Azo Dyes. Under standard conditions, Hepa 1c1c7 cells exposed to 10 nM to 10 μM concentrations of 1,1′-azonaphthalene, Sudan I (1-phenylazo-2-naphthol), or Sudan III [1-(4-phenylazophenylazo)-2-naphthol] showed concentration-dependent increases in cytosolic quinone reductase activity. Significant inductions were observed at 10 nM, and the half-maximally effective concentrations (potencies) of all three compounds were similar (1–2 μM), as were the concentrations required for maximal induction (Fig. 1). However, with near saturating doses the maximal-induction plateaus (efficacies) observed differed widely: 4, 6, and 10 times control values for Sudan III, Sudan I, and 1,1′-azonaphthalene, respectively. The mechanisms responsible for the markedly different efficacies of these inducers is unclear. Rates of transport, of conversion to active metabolites, and of excretion or further metabolism of inducers may all contribute to the regulation of the maximal enzyme levels. Azo dyes are extensively metabolized by animal cells, mainly by reduction, hydroxylation, and conjugation (22). We have suggested elsewhere that metabolism of azo dyes may be essential for enzyme induction (23).

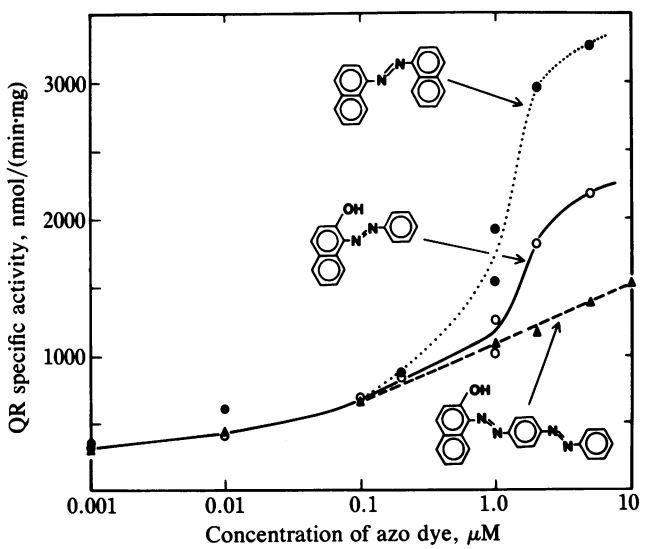


FIG. 1. Concentration-dependence of induction of quinone reductase (QR) in Hepa 1c1c7 cells by the azo dyes 1,1′-azonaphthalene (●), Sudan I (○), and Sudan III (▲). The cells were grown for 48 hr to late log phase and then exposed for 24 hr to fresh culture medium containing the indicated concentrations of the azo dyes, and the specific activities of quinone reductase in cytosols were measured. The graphs were constructed from four separate experiments, for which the mean control value was 363 ± 20 nmol/min per mg of protein. Each experimental point represents the mean of determinations on cells obtained from four plates.

The time courses of induction of quinone reductase by 1,1′-azonaphthalene, Sudan I, and Sudan III are shown in Fig. 2. For all three compounds, increases in enzyme activity were observed after 4 hr and continued linearly with time for at least the first 24 hr, after which the enzyme levels declined with 1,1′-azonaphthalene and Sudan I but continued to increase with Sudan III for the duration of the experiment (48 hr). Sudan III appears to be metabolized more slowly than the other azo dyes, since its red color did not disappear from the cell mass, whereas the other dyes were reduced to colorless products.

Relation of Structure of Azo Dyes to Induction of Quinone Reductase. Representative members of the three aforementioned classes of azo dyes were studied at 2 μM concentrations according to our standard protocol. Because the time-course of enzyme induction differs for various azo dyes (Fig. 2), the following observations apply only to the specified protocol. The most effective inducers of quinone reductase were 1,1′-azonaphthalenes (compounds **1** and **2**, Table 1 and Fig. 3), which elevated enzyme levels 10- to 11-fold. The 2-hydroxyl group has little effect on the induction in this class of agents. A two-electron reduction of the azo linkage to the hydrazine (**4**) reduced the inductive activity to less than one-half. A similar decrease in activity was observed when the azo linkage was replaced by a *trans*-olefin, irrespective of whether the linkage between the two naphthalene rings was 1 to 1′ (**5**) or 1 to 2′ (**3**). The geometry of the olefinic linkage appears to be important because the 1,1′*cis*-olefin analogue (**6**) was considerably less active than the 1,1′*trans*-olefin (**5**), possibly because the *cis*-olefin molecules are nonplanar. If the linkage between both naphthalene rings is 2,2′, considerable loss in inductive activity is observed (compare **7** with **5**).

Under similar conditions, Sudan I (**9**, Table 1 and Fig. 3) produced only about one-half the elevation of quinone reductase activity observed with 1,1′-azonaphthalene (**1**). Replacement of the phenyl by a 2-pyridyl ring (**8**) did not affect inductive activity. Unlike the 1,1′-azonaphthalenes, the Sudan I analogues require the 2-hydroxyl group for activity, since its elimination (**17**) or movement to the 4 position of the naphthalene ring (**19**) markedly reduced the

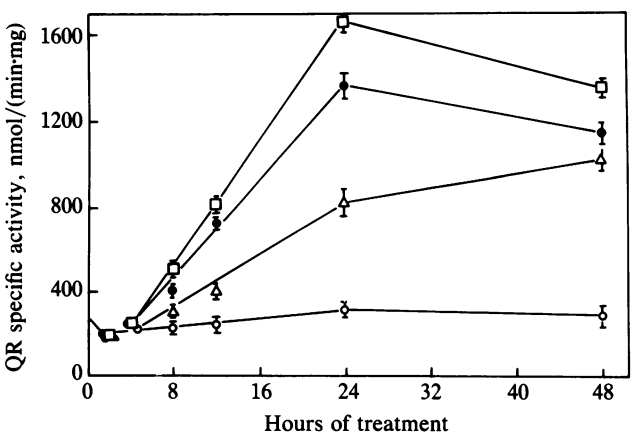


FIG. 2. Time-dependence of the induction of quinone reductase (QR) in Hepa 1c1c7 cells by 1,1′-azonaphthalene (1 μM, ●), Sudan I (2 μM, □), and Sudan III (2 μM, △). The cells were grown for 48 hr and then exposed to the azo dyes for 1.5–48 hr, and the specific activities of quinone reductase in cytosols were measured. Control specific activities (○) were relatively constant (range 202 ± 10 to 302 ± 18 nmol/min per mg of protein) during the experimental period. Mean values for four plates (± SEM) are shown.

Table 1. Induction of cytosolic quinone reductase in Hepa 1c1c7 hepatoma cells by 1,1′-azonaphthalene, Sudan I, and Sudan III analogues

	Inducing agent*	Quinone reductase specific activity, nmol/(min·mg)	Ratio of specific activities (treated/control)
		1,1′-Azonaphthalenes	
1		2960 ± 219	10.6 ± 0.8
2		2780 ± 211	9.91 ± 0.75
3		1280 ± 14	4.58 ± 0.05
4		1180 ± 43	4.20 ± 0.15
5		1160 ± 50	4.15 ± 0.18
6		600 ± 24	2.14 ± 0.09
7		426 ± 7	1.52 ± 0.02
		Sudan I analogues	
8		1810 ± 54	6.47 ± 0.19
9	(Sudan I)	1720 ± 46	6.13 ± 0.16
10		1620 ± 61	5.80 ± 0.22
11		1550 ± 199	5.54 ± 0.71
12		1250 ± 81	4.46 ± 0.29
13		1230 ± 68	4.38 ± 0.24
14	(Sudan II)	1220 ± 52	4.34 ± 0.19
15		952 ± 31	3.40 ± 0.11
16		564 ± 25	2.64 ± 0.12
17		523 ± 30	2.46 ± 0.14
18		550 ± 21	1.96 ± 0.07
19		593 ± 86	1.83 ± 0.27
		Sudan III analogues	
20	(Sudan IV)	1150 ± 88	4.11 ± 0.31
21	(Sudan III)	1100 ± 39	3.92 ± 0.14
22		682 ± 52	2.43 ± 0.19
23		677 ± 43	2.42 ± 0.15
24		671 ± 10	2.40 ± 0.04
25		617 ± 8	2.20 ± 0.03
26		555 ± 35	1.98 ± 0.12
27		353 ± 14	1.26 ± 0.05

All enzyme specific activities are based on duplicate assays on cytosols of cells obtained from each of four plates and are expressed as means ± SEM. The treated/control ratios are based on a control specific activity of 280 ± 23 nmol/min per mg of protein for all determinations except for compounds **16** and **17** (specific activity 213 ± 21) and compound **19** (specific activity 324 ± 17). The SEM values of the treated/control ratios were obtained by dividing the SEM of each treated group by the control value.

*Inducing agents were present at 2 μM in culture medium. Structures are shown in Fig. 3.

inductive activity. Replacement of this hydroxyl group by an amino group (**13**) was far less damaging. Substituting the phenyl ring of Sudan I with an amino group at the 4′ position (**11**) reduced inductive activity slightly, whereas the simultaneous presence of 2′- and 4′-methyl substituents (**14**) resulted in a marked reduction of activity. Among the unsubstituted phenylazonaphthalenes, a 2,1′ linkage (**12**) was a more effective inducer than a 1,1′ linkage (**17**). Replacement of the azo linkage by a *trans*-olefin in the phenylazonaphthalenes showed a dichotomous effect; inductive activity was reduced if the ring linkage was from 2 to 1′ (compare **15** and **12**), whereas it was enhanced if the linkage was from 1 to 1′ (compare **17** and **10**). A *cis*-olefin linkage markedly reduced the inductive capacity in comparison to the *trans*-olefin (compare **10** and **18**).

Sudan III (**21**) and its analogues are of particular interest because they were most potent in protecting rats against mammary cancer, leukemia, and adrenal hemorrhage produced by polycyclic aromatic compounds (19, 22, 24). In the Hepa 1c1c7 system, Sudan III and its analogues were less than one-half as effective as 1,1′-azonaphthalene (**1**) and

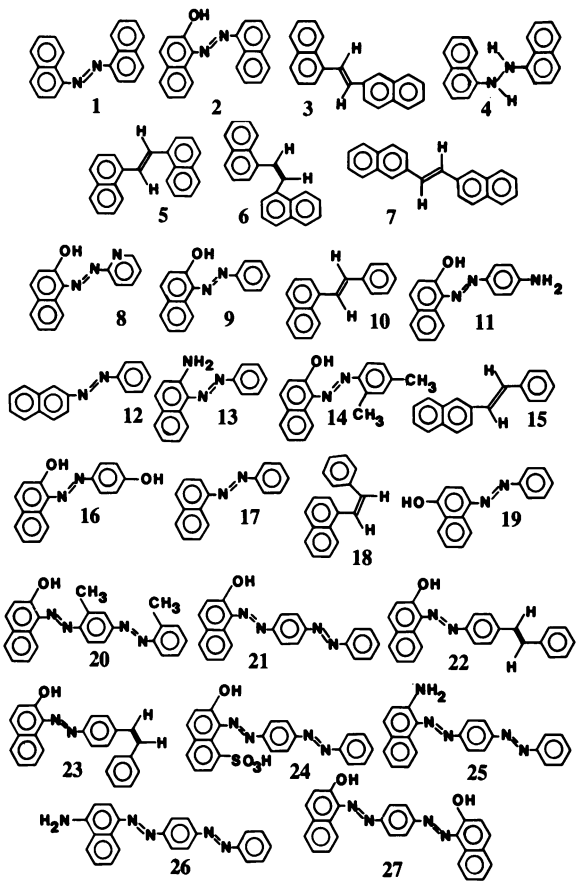


FIG. 3. Structures of compounds **1–27**.

about two-thirds as effective as Sudan I (**9**) in inducing quinone reductase. In this class of compounds, as in the Sudan I analogues, the presence of a hydroxyl group *ortho* to the azo linkage is important for induction. Thus, replacement of the 2-hydroxyl group by a 2-amino group (**25**) or by a 4-amino group (**26**) reduced the inductive activity 50%. If the additional phenylazo group is replaced by a *cis*- (**23**) or a *trans*-styrene (**22**) moiety, the inductive activity is diminished even further than by the presence of the phenylazo linkage alone. The presence of methyl groups on both phenyl rings of Sudan III (**20**) barely affected the inductive activity, whereas it was almost completely abolished by replacing the terminal phenylazo group by azonaphthol (**27**).

Induction of Quinone Reductase by Phenolic Antioxidants. Phenolic antioxidants, and in particular BHA [3(2)-*tert*-butyl-4-hydroxyanisole] and BHT (3,5-di-*tert*-butyl-4-hydroxytoluene), which have been studied extensively for their protective effects (1, 2, 25), induce quinone reductase in Hepa 1c1c7 cells (Table 2). However, substituted phenols (commercial BHA and its component isomers, BHT, and 4-hydroxyanisole) are much less efficient inducers than those carrying unsubstituted phenolic groups, such as *tert*-butylhydroquinone and 3,5-di-*tert*-butylcatechol, both of which are chemoprotectors and inducers of protective enzymes *in vivo* (4, 7, 8, 26, 27). These findings suggest that the ultimate inducers are probably hydroquinones or catechols (17, 18, 26). *tert*-Butylhydroquinone elevates the quinone reductase specific activity in Hepa 1c1c7 cells in a dose-dependent manner: 20 μM concentrations doubled the enzyme levels, and 50–60 μM more than tripled them.

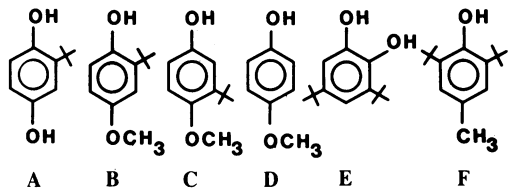
Induction of Quinone Reductase by Coumarins, Flavonoids, and Other Lactones. Coumarin, α-angelicalactone, quercitin, and especially 5,6-benzoflavone all induce quinone reductase in the Hepa 1c1c7 system (Table 3). These compounds are all

Table 2. Induction of cytosolic quinone reductase in Hepa 1c1c7 hepatocytes by phenolic antioxidants

Inducing agent		Quinone reductase specific activity,	Ratio of specific activities
Compound*	Conc., μM	nmol/(min·mg)	(treated/control)
A	10	583 ± 30–650 ± 17 (n = 3)	1.37 ± 0.04–2.12 ± 0.13
	30	689 ± 35–1259 ± 71 (n = 6)	2.60 ± 0.14–3.62 ± 0.22
	50	1106 ± 35[a]	4.64 ± 0.15
B	30	388 ± 16[b]	1.42 ± 0.06
	30	638 ± 72[c]	1.32 ± 0.15
	60	784 ± 47[c]	1.62 ± 0.10
C	15	570 ± 34[c]	1.18 ± 0.07
	30	673 ± 74[c]	1.39 ± 0.15
	60	789 ± 37[c]	1.63 ± 0.08
D	30	555 ± 27[c]	1.14 ± 0.06
	60	589 ± 88[c]	1.21 ± 0.18
E	2	345 ± 17[d]	1.14 ± 0.06
	10	734 ± 41[e]	2.18 ± 0.12
	10	755 ± 4[f]	2.46 ± 0.01
	10	862 ± 38[d]	2.85 ± 0.13
F	10	268 ± 11[g]	0.97 ± 0.04
	30	281 ± 12[g]	1.01 ± 0.04
	50	516 ± 49[a]	2.17 ± 0.21

All enzyme specific activities are based on duplicate assays on cytosols of cells obtained from each of four plates and are expressed as means ± SEM. The treated/control ratios are based on the individual control values shown below. The SEM values of these ratios were obtained by dividing the SEM of each treated group by the control value. Control values: [a]238 ± 16; [b]274 ± 22; [c]485 ± 31; [d]302 ± 10; [e]337 ± 11; [f]307 ± 4; [g]227 ± 8.

*A, *tert*-butylhydroquinone; B, 3-*tert*-butyl-4-hydroxyanisole (major isomer of BHA); C, 2-*tert*-butyl-4-hydroxyanisole (minor isomer of BHA); D, 4-hydroxyanisole; E, 3,5-di-*tert*-butylcatechol; F, 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT). Structures are shown below.



chemoprotective (1, 2), although to varying degrees, and are also glutathione *S*-transferase inducers *in vivo* (7, 8).

Induction of Quinone Reductase by Polycyclic Aromatic Hydrocarbons. Low concentrations (2 μM) of benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, and 3-methylcholanthrene induced quinone reductase in Hepa 1c1c7 cells to 3.6–5.8 times control values, although 30 μM phenobarbital was completely inactive (Table 4). Although the polycyclic hydrocarbons are themselves carcinogens, it is well recognized that under suitable conditions these compounds protect against their own carcinogenicity and toxicity or those of other compounds (19, 28, 29).

Induction of Quinone Reductase by Sulfur Compounds. A number of sulfur-containing compounds (e.g., disulfiram, benzylisothiocyanate, dithiolthiones) are protectors against chemical carcinogenesis, as well as inducers of chemoprotective enzymes such as glutathione *S*-transferases and quinone reductase *in vivo* (1, 2, 30–32). Under conditions of our standard protocol, quinone reductase activity was elevated in Hepa 1c1c7 cells by benzylisothiocyanate (2.45-fold at 10 μM) and by oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] and anethole dithiolthione [5-(4-methoxyphenyl)-1,2-dithiol-3-thione] (1.6- to 1.9-fold at 10 μM and 2.1- to 2.4-fold at 30 μM). In preliminary experiments, diethyldithiocarbamate and bis(ethylxanthogen) [*O, O*-diethyl dithiobis(thioformate)], both chemoprotectors and enzyme inducers *in vivo* (2, 31), were also found to be inducers of quinone reductase in Hepa 1c1c7 cells.

Table 3. Induction of cytosolic quinone reductase in Hepa 1c1c7 hepatocytes by coumarin, flavones, and other lactones

Inducing agent	Quinone reductase specific activity, nmol/(min·mg)	Ratio of specific activities (treated/control)
Coumarin		
10 μM	235 ± 12[a]	1.10 ± 0.06
50 μM	383 ± 14[b]	1.61 ± 0.06
Quercitin		
10 μM	451 ± 34[c]	1.63 ± 0.12
30 μM	601 ± 14[c]	2.17 ± 0.05
50 μM	607 ± 14[b]	2.55 ± 0.06
α-Angelicalactone		
10 μM	303 ± 29[c]	1.09 ± 0.10
50 μM	454 ± 10[b]	1.91 ± 0.04
5,6-Benzoflavone		
2 μM	2270 ± 212[d]	5.42 ± 0.51
10 μM	3070 ± 244[e]	9.50 ± 0.76

All enzyme specific activities are based on duplicate assays on cytosols of cells obtained from each of four plates and are expressed as means ± SEM. The treated/control ratios are based on the individual control values shown below. The SEM values of these ratios were obtained by dividing the SEM of each treated group by the control value. Control values: [a]214 ± 21; [b]238 ± 16; [c]277 ± 8; [d]419 ± 10; [e]323 ± 17.

DISCUSSION

Murine hepatic cells were chosen for these studies because inductions *in vivo* of chemoprotective enzymes by antioxidant anticarcinogens were most prominent in mouse liver (32, 33). Hepa cells were established in culture from a transplantable hepatoma (BW 7756) of the C57L/J mouse (34, 35), and the 1c1c7 clone was selected for a high inducibility of aryl hydrocarbon hydroxylase (36). This line retains many of the specific morphological and biochemical characteristics of liver (34, 35). The Hepa 1c1c7 cells also contain inducible ethoxyresorufin *O*-deethylase and cytochrome P-450 reductase, as well as ornithine decarboxylase and epoxide hydrolase (37–39). These cells can therefore presumably dealkylate substituted phenols and reduce azo linkages. Such

Table 4. Induction of cytosolic quinone reductase in Hepa 1c1c7 hepatocytes by polycyclic aromatic hydrocarbons

Inducing agent*	Quinone reductase specific activity, nmol/(min·mg)	Ratio of specific activities (treated/control)
DMBA[†]	1360 ± 120[a]	4.21 ± 0.37
Benzo[*a*]pyrene	1520 ± 76[b]	3.63 ± 0.18
	1800 ± 41[a]	5.57 ± 0.13
3-Methylcholanthrene	1670 ± 119[b]	3.99 ± 0.28
	1890 ± 220[a]	5.85 ± 0.68
Phenobarbital	425 ± 79[b]	1.01 ± 0.19

All enzyme specific activities are based on duplicate assays on cytosols of cells obtained from each of four plates and are expressed as means ± SEM. The treated/control ratios are based on the individual control values shown below. The SEM values of these ratios were obtained by dividing the SEM of each treated group by the control value. Control values: [a]323 ± 17; [b]419 ± 10.

*Concentrations were 2 μM, except for phenobarbital (30 μM).

[†]7,12-Dimethylbenz[*a*]anthracene.

metabolic functions are important for generating the signals for induction which depend on the formation of oxidation–reduction labile 1,2- or 1,4-diphenols or -diamines (23). The Hepa 1c1c7 cells have permitted analysis of the molecular control of the induction of aryl hydrocarbon hydroxylase. A number of mutants defective in this pathway (10, 40) will be useful in uncovering the relationship between the induction of phase I and phase II enzymes and their relative importance in chemoprotection.

Selection of quinone reductase as a marker of induction of chemoprotective enzymes was stimulated by the discovery of Huggins (19) that certain azo dyes (especially Sudan III), which protected rats against mammary cancer, leukemia, and adrenal hemorrhage produced by methylated benz[a]anthracene derivatives, also produced marked inductions of quinone reductase, and that both effects required synthesis of protein (41). These observations implied a functional relationship between induction of this enzyme and protection against carcinogenesis and toxicity (19). Furthermore, Fujita *et al.* (42) have demonstrated that, in rat liver, many azo dyes induce quinone reductase coordinately with cytochrome P-450 and associated monooxygenase activities, glutathione *S*-transferases, aldehyde dehydrogenase, and UDP-glucuronyltransferase. Thus, the azo dyes resemble polycyclic aromatics; both types of compounds bind to the receptor encoded by the *Ah* (aryl hydrocarbon) locus (43).

The toxic effects of quinones (which are ubiquitous constituents of human diet) have been ascribed to two mechanisms: first, direct interaction of electrophilic quinones with critical cellular nucleophiles; and second, facile one-electron reductions (promoted by many flavoproteins) to semiquinone free radicals which can participate in cyclic oxidation–reductions to generate reactive oxygen species (44). Quinone reductase is unusual among flavoproteins in that it promotes obligatory two-electron reductions of quinones (45), thus diverting quinones from oxidative cycling and preparing them for conjugation with glucuronic acid (13, 46, 47). Many different types of experiments attest to the importance of quinone reductase as a cellular protector against the toxicities of quinones. Further, this enzyme can be induced by a wide range of compounds, including BHA, polycyclic aromatics, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which, like azo dyes, can protect against chemical carcinogenesis (see ref. 3).

For these reasons, measurement of the cytosolic levels of quinone reductase of Hepa 1c1c7 hepatoma cells in culture provides a versatile, simple, and reliable method for identifying chemoprotective agents and for studying the mechanism of induction of chemoprotective enzymes.

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