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MECHANISMS OF TOXIC INJURY TO ISOLATED HEPATOCYTES BY 1-NAPHTHOL

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Abstract—The mechanism(s) of toxicity of 1-naphthol and two of its possible metabolites, 1,2- and 1,4-naphthoquinone, to freshly isolated rat hepatocytes has been studied. 1-Naphthol and both naphthoquinones exhibited a dose-dependent toxicity to hepatocytes. [1-^{14}C]-1-Naphthol was metabolised by hepatocytes predominantly to its glucuronic acid and sulphate ester conjugates, but small amounts of covalently bound products were also formed. Blebbing on the surface of the hepatocytes was observed following exposure to 1-naphthol and the naphthoquinones, together with a dose-dependent decrease in intracellular glutathione (GSH), which preceded the onset of cytotoxicity. The toxicity of 1-naphthol and the naphthoquinones was potentiated by dicoumarol, an inhibitor of DT-diaphorase (NAD(P)H: quinone oxidoreductase). This enhanced toxicity was accompanied by a greater amount of surface blebbing, an increased depletion of intracellular GSH, particularly in the case of 1-naphthol and 1,4-naphthoquinone, and a decreased metabolism of 1-naphthol to its conjugates with variable effects on the amount of covalently bound products formed. These results support the suggestion that the toxicity of 1-naphthol may be mediated by the formation of 1,2-naphthoquinone and/or 1,4-naphthoquinone, which may then be metabolised by one electron reduction to naphthosemiquinone radicals. These, in turn, may covalently bind to important cellular macromolecules or enter a redox cycle with molecular oxygen thereby generating active oxygen species. Both of these processes appear to play a role in producing the cytotoxic effects of 1-naphthol.

Recently we reported that 1-naphthol is selectively toxic to short-term organ cultures of human colonic tumour tissue compared to normal colonic tissue and may be of value as a potential anti-cancer agent [1]. In order to learn more about the possible mechanisms of toxicity of 1-naphthol to both normal and tumour cells we decided to study the toxic effects of this compound on freshly isolated rat hepatocytes, a well-characterised cell model for toxicological studies [2, 3]. 1-Naphthol is also a major metabolite of naphthalene [4], which is used in many homes to control odours and insects, and may be responsible, at least in part, for its toxicological properties.

A previous study [5] has shown that incubation of naphthalene or 1-naphthol with rat liver microsomes in the presence of an NADPH generating system leads to the formation of reactive metabolites which irreversibly bind to protein. The nature of the covalently binding reactive intermediates was not established in this study, but it was suggested that either quinone or semiquinone metabolites were involved [5]. Naphthalene is also metabolised by rat liver microsomes and hepatocytes, via 1,2-naphthalene oxide to 1,2-dihydro-1,2-dihydroxynaphthalene [4, 6]. In isolated hepatocytes this dihydrodiol is further metabolised to a glucuronic acid conjugate [6], whereas in the eye it is metabolised by an active soluble dehydrogenase to 1,2-dihydroxynaphthalene which may spontaneously oxidise to 1,2-naphthoquinone [7]. Whilst 1-naphthol is conjugated to either its glucuronic acid or sulphate ester conjugate [8, 9], much less is known of its further metabolism by oxidative Phase I metabolism. One early study suggested indirectly that 1-naphthol may be metabolised by the microsomal mono-oxygenases to 1,2-dihydroxynaphthalene [10]. In addition, another likely metabolite is 1,4-dihydroxynaphthalene. Both these dihydroxynaphthalenes may be readily oxidised to their respective quinones, i.e. 1,2- and 1,4-naphthoquinone. Naphthoquinone metabolites could therefore mediate both 1-naphthol and naphthalene toxicity either by covalently binding (directly or as semiquinone radicals) to important cellular macromolecules or by redox-cycling to produce superoxide anion ($O_2^{\cdot-}$) and oxidative stress [11]. The results presented here suggest that naphthoquinone metabolites may be involved in 1-naphthol cytotoxicity. Moreover, both covalent binding and the formation of active oxygen species appear to play a role in producing its cytotoxic effects.

MATERIALS AND METHODS

Materials

Collagenase was obtained from Boehringer-Mannheim GmbH, Mannheim, F.R.G. Bovine serum albumin (Fraction V), reduced glutathione (GSH), dicoumarol (bis-hydroxycoumarin), 1-naphthol, 1-naphthyl sulphate and HEPES (bis-hydroxyethyl-1-piperazine ethane sulphonic acid) were purchased from Sigma Chemical Company, London, U.K. 1,2- and 1,4-naphthoquinone were

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obtained from Fluka, Switzerland and 1-naphthyl-β-D-glucuronide from Koch-Light Laboratories Ltd., Colnbrook, U.K. [1-^{14}C]-1-Naphthol (Radiochemical Centre, Amersham, U.K.) was supplied at a specific activity of 19.2 Ci/mole and diluted 10–20-fold with cold 1-naphthol prior to use.

Animals and pretreatments

Male Sprague–Dawley rats (200–250 g) were used in all experiments and allowed food and water *ad libitum*. All rats were given phenobarbitone (1 g/l) in their drinking water for 5–10 days prior to use.

Hepatocyte isolation and incubation

Hepatocytes were isolated by collagenase perfusion as previously described [2]. The yield of each preparation was 2–4 × 10^8 cells/liver, and immediately after isolation 90–95% of the washed hepatocytes excluded trypan blue. Krebs–Henseleit buffer, pH 7.4, containing 25 mM HEPES was used for all incubations. The hepatocytes were incubated at 37° at 1–2 × 10^6 cells/ml in rotating round-bottom flasks [12]. 1-Naphthol and the naphthoquinones were added in 10–25 μl dimethyl sulphoxide.

GSH determination

Intracellular GSH was assayed after acid extraction by the *o*-phthaldialdehyde method [13].

1-Naphthol metabolism in isolated hepatocytes

The metabolism of [1-^{14}C]-1-naphthol to conjugates was studied by thin layer chromatographic analysis of the extracellular medium as previously described [14].

1-Naphthol covalent binding

The total amount of irreversible covalent binding of products associated with radioactivity to cellular protein, following incubation of hepatocytes for 4 hr, at 37° or 4°, with [1-^{14}C]-1-naphthol was determined essentially as described in [15], with the following modifications. The cells were lysed by repeated freeze thawing and precipitated by the addition of 2 volumes of methanol followed by serial 2 ml washes with 70% methanol, acetone : chloroform (4 : 1, v/v), 70% methanol and 100% methanol. The cell pellets were washed until no radioactivity above background levels could be detected in the washes and then dissolved in 1 N NaOH. Aliquots were taken for protein determination by the method of Lowry *et al.* [16], and liquid scintillation counting, in Aquasol, following neutralisation.

Phase-contrast light microscopy

Bleb formation on the surface of the hepatocytes was observed directly using Hoffman phase-contrast microscopy in the presence of 0.2% (w/v) trypan blue.

RESULTS

Effect of dicoumarol on the toxicity of 1-naphthol, 1,2- and 1,4-naphthoquinone in isolated hepatocytes

Initial experiments showed that 1-naphthol exhibited a dose-dependent toxicity, as assessed by trypan blue exclusion, to suspensions of freshly isolated hepatocytes from phenobarbitone-treated rats (Fig. 1(a)). If 1-naphthol toxicity is mediated by the formation of naphthoquinone metabolites, one might expect that the cytotoxicity of 1-naphthol in hepatocytes will be potentiated by the presence of dicoumarol, a potent inhibitor of DT-diaphorase (NAD(P)H : quinone oxidoreductase) [17]. Dicoumarol has recently been shown, for example, to potentiate the toxicity of menadione (2-methyl-1,4-naphthoquinone) in isolated hepatocytes [18]. The onset and time course of toxicity of two concentrations of 1-naphthol, i.e., 100 and 250 μM, which were slightly and moderately toxic respectively at 4 hr of incubation (Fig. 1(a)), were studied in the presence and absence of dicoumarol. A time-dependent increase in 1-naphthol-induced toxicity was

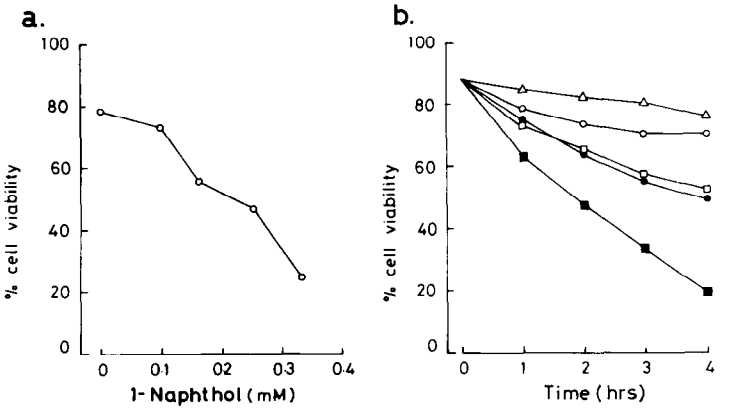

Fig. 1. Dose-dependent toxicity of 1-naphthol to freshly isolated rat hepatocytes (a) and its potentiation by dicoumarol (b). Hepatocytes were incubated with 1-naphthol for up to 4 hr and the percentage of viable cells was determined by trypan blue exclusion. After 4 hr, 1-naphthol exhibited a dose-dependent toxicity (a). The results are the means of 2–10 determinations. (b) Shows the time course of toxicity of 1-naphthol (100 and 250 μM), in the presence and absence of dicoumarol (27 μM). The results are from one experiment typical of three. Key: (△) control; (○) 100 μM 1-naphthol; (●) 100 μM 1-naphthol + dicoumarol; (□) 250 μM 1-naphthol; (■) 250 μM 1-naphthol + dicoumarol.

observed in all cases (Fig. 1(b)). Dicoumarol clearly potentiated the toxicity of 1-naphthol to hepatocytes at both concentrations (Fig. 1(b)). Dicoumarol (27 μM) alone had no effect on hepatocyte viability.

In addition to potentiating the toxicity of 1-naphthol to hepatocytes, dicoumarol also markedly potentiated the early effects of 1-naphthol on the surface morphology of the hepatocytes. After 1 hr of incubation, a large number of blebs was observed on the surface of hepatocytes exposed to 1-naphthol (100 μM) in the presence of dicoumarol. At this time, 1-naphthol (100 μM) alone had caused only relatively minor surface perturbing effects compared to the control hepatocytes. However, at 4 hr of incubation, 1-naphthol (100 μM) caused more extensive surface blebbing (results not shown). These surface blebs were similar in appearance to those observed previously following exposure of hepatocytes to a combination of menadione and dicoumarol and as reported previously dicoumarol (27 μM) alone had no effect on hepatocyte surface morphology [18].

Both 1,2- and 1,4-naphthoquinone exhibited a dose-dependent toxicity to isolated hepatocytes (results not shown). Dicoumarol (27 μM) potentiated the toxicity of both 1,2- and 1,4-naphthoquinone to isolated hepatocytes (Fig. 2). 1,4-Naphthoquinone was more toxic than 1,2-naphthoquinone and the presence of dicoumarol potentiated its toxicity to a greater extent (Fig. 2). In addition to 1-naphthol, both 1,2- and 1,4-naphthoquinone also caused surface blebbing but at lower concentrations (results not shown). These results support the suggestion that 1-naphthol may be toxic to isolated hepatocytes via its conversion to naphthoquinone metabolites and that the potentiating effect of dicoumarol on 1-naphthol toxicity in hepatocytes may be explained by its inhibition of DT-diaphorase. Other effects of dicoumarol not related to DT-diaphorase inhibition could, however, be involved. For example, dicoumarol may either interfere with the conjugation of 1-naphthol in hepatocytes since it is itself conjugated or it may uncouple the mitochondria [19].

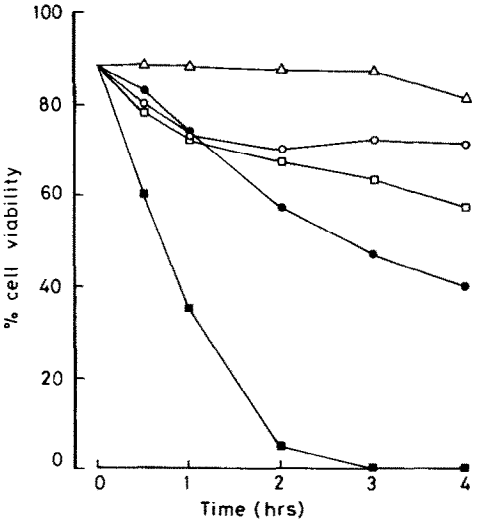

Fig. 2. Potentiation of the toxicity of 1,2- and 1,4-naphthoquinone to freshly isolated hepatocytes by dicoumarol. Hepatocytes were incubated with naphthoquinones (50 μM) in the presence or absence of dicoumarol (27 μM). Samples were withdrawn at various times and the % cell viability assessed by trypan blue exclusion. Dicoumarol (27 μM) alone was not toxic. Key: control (△); 1,2-naphthoquinone alone (○) or with dicoumarol (●); 1,4-naphthoquinone alone (□) or with dicoumarol (■). The results of one experiment typical of three are shown.

Table 1. Metabolism of 1-naphthol by freshly isolated rat hepatocytes

Experiment	1-Naphthol (μM)	Dicoumarol (μM)	Cell death (%)	1-Naphthyl-β-D-glucuronide (nmoles/mg prot/4 hr)	1-Naphthyl sulphate (nmoles/mg prot/4 hr)	1-Naphthol remaining (nmoles/ml after 4 hr)	1-Naphthol unmetabolised (%)
A	100		4	20.5	3.7	1.3	1.3
	100	27	51	22.5	2.6	2.9	2.9
	250		24	39.1	15.2	19.3	7.7
	250	27	59	16.1	3.9	170.2	68.1
B	100		2	22.0	3.2	2.3	2.3
	100	27	68	15.3	3.6	28.5	28.5
	250		63	49.9	13.7	8.3	3.3
	250	27	70	8.9	3.3	200.1	80.0
C	100		8	13.6	3.1	1.8	1.8
	100	27	16	12.8	3.7	4.6	4.6
	250		22	34.7	6.9	4.1	1.7
	250	27	52	21.7	4.4	91.5	36.6
D	100		10	17.5	2.1	8.5	8.5
	100	27	80	14.0	2.1	27.0	27.0
	250		76	42.1	11.8	15.1	6.0
	250	27	74	9.9	2.6	186.5	74.6

Hepatocytes were incubated with [1-^{14}C]-1-naphthol in the presence or absence of dicoumarol for 4 hr. At this time cell viability was assessed by trypan blue exclusion and metabolites in the extracellular medium were analysed as described in Materials and Methods.

Effect of dicoumarol on the metabolism of 1-naphthol in isolated hepatocytes

1-Naphthol was metabolised by isolated hepatocytes to both its glucuronic acid and sulphate ester conjugates (Table 1) in agreement with other studies [6, 20]. At the higher concentrations of 1-naphthol (100 and 250 μM) used in this study, more 1-naphthyl-β-*D*-glucuronide than 1-naphthyl sulphate was formed (Table 1). Whilst conjugates of 1-naphthol were readily observed, we were unable, using high pressure liquid chromotography, to detect naphthoquinones possibly because of their high reactivity [7]. In the absence of dicoumarol, most of the 1-naphthol was conjugated, with little unmetabolised 1-naphthol (1.3–8.5% of the original) remaining in the incubation medium at the end of the 4 hr incubation (Table 1). In the presence of dicoumarol, the metabolism of 1-naphthol (250 μM) was markedly inhibited in all 4 experiments (last column, Table 1). Inhibition of the metabolism of lower concentrations of 1-naphthol (100 μM) was not as striking but was observed in two of the four experiments (B and D, Table 1). It was of interest that in experiments B and D the potentiation of toxicity by dicoumarol was greatest, lending some support to the suggestion that inhibition of 1-naphthol conjugation was due in part to increased toxicity in the presence of dicoumarol.

Effect of dicoumarol on the covalent binding of 1-naphthol in isolated hepatocytes

In an attempt to further elucidate the mechanism of the potentiation of 1-naphthol toxicity by dicoumarol, we determined the amount of radioactivity irreversibly bound to the hepatocytes after 4 hr incubation with [1-^{14}C]-1-naphthol (100 and 250 μM) in the presence and absence of dicoumarol (27 μM). Incubations were carried out at 37° and 4°, the latter was included in order to control for binding not due to metabolic activation of 1-naphthol by cellular enzymes. Incubation at 4° inhibited the metabolism of 1-naphthol greater than 98% (results not shown). At 37° when 100 μM [1-^{14}C]-1-naphthol was used, the amount of radioactivity covalently bound to hepatocyte protein was increased in the presence of dicoumarol (Table 2). However, when 250 μM [1-^{14}C]-1-naphthol was used the level of covalent binding was decreased in the presence of dicoumarol (Table 2). Dicoumarol did, however, potentiate 1-naphthol toxicity at both concentrations. The interpretation of this data is difficult, but if a linear regression analysis of toxicity (% cell death) versus the level of covalent binding was performed, an *r* value of 0.946 was obtained in the absence of dicoumarol. However, it was apparent that no such correlation existed in the presence of dicoumarol as the covalent binding decreased as the toxicity increased (cf. naphthol (250 μM) in the presence and absence of dicoumarol). This data may indicate that in the absence of dicoumarol a close correlation exists between the covalent binding of 1-naphthol metabolites and its toxic effects, but that in the presence of dicoumarol a second toxic mechanism also plays a major role. The latter may be the generation of active oxygen species by the redox-cycling of naphthoquinone metabolites. In order to further test this hypothesis, we studied the effects of 1-naphthol and the naphthoquinones, both in the presence and absence of dicoumarol, on intracellular glutathione (GSH) levels as a possible indicator of oxidative stress.

Effects of 1-naphthol, 1,2- and 1,4-naphthoquinone on intracellular GSH levels

1-Naphthol caused a rapid dose-dependent depletion of GSH in hepatocytes (Fig. 3(a)) which preceded the onset of cytotoxicity as assessed by trypan blue exclusion (Fig. 1(b)). In the presence of dicoumarol, the depletion of GSH was more extensive but not more rapid than in the absence of dicoumarol (Fig. 3(a)). Dicoumarol alone had no significant effect on GSH levels in hepatocytes.

A lower concentration (50 μM) of 1,2- and 1,4-naphthoquinone, the two most likely quinone metabolites of 1-naphthol, rapidly depleted hepatocyte GSH (Fig. 3(b)). Dicoumarol (27 μM) potentiated the toxicity of 1,4-naphthoquinone more readily than that of 1,2-naphthoquinone (Fig. 2), and this was reflected in a greater potentiation of GSH loss by dicoumarol in the case of 1,4-naphthoquinone as compared to 1,2-naphthoquinone. The potentiation of GSH depletion by 1,2-naphthoquinone in the presence of dicoumarol was more variable than 1-naphthol or 1,4-naphthoquinone. In some experiments little, if any, potentiation was observed (Fig. 3(b)). However, in experiments where a range of 1,2-naphthoquinone concentration (10–100 μM) was employed, a marked potentiation was observed at lower 1,2-naphthoquinone concentration (results not shown).

Table 2. Covalent binding of 1-naphthol in isolated hepatocytes

1-Naphthol (μM)	Dicoumarol (μM)	nmoles naphthol equivalents bound/mg prot/4 hr 37°	4°	37° − 4°	% Cell death
100		2.35 ± 0.33	1.85 ± 0.24	0.50	4.7 ± 1.8
100	27	3.39 ± 1.18	1.29 ± 0.33	2.10	45.0 ± 15.3
250		16.36 ± 4.30	2.66 ± 0.54	13.70	36.3 ± 13.4
250	27	9.48 ± 1.22	2.32 ± 0.65	7.16	60.3 ± 5.2

Hepatocytes were incubated with [1-^{14}C]-1-naphthol in the presence or absence of dicoumarol at either 4° or 37° for 4 hr. Cell viability was assessed by trypan blue exclusion and covalently bound products determined as described in Materials and Methods. (Values represent the means ± SE for 3 separate experiments.)

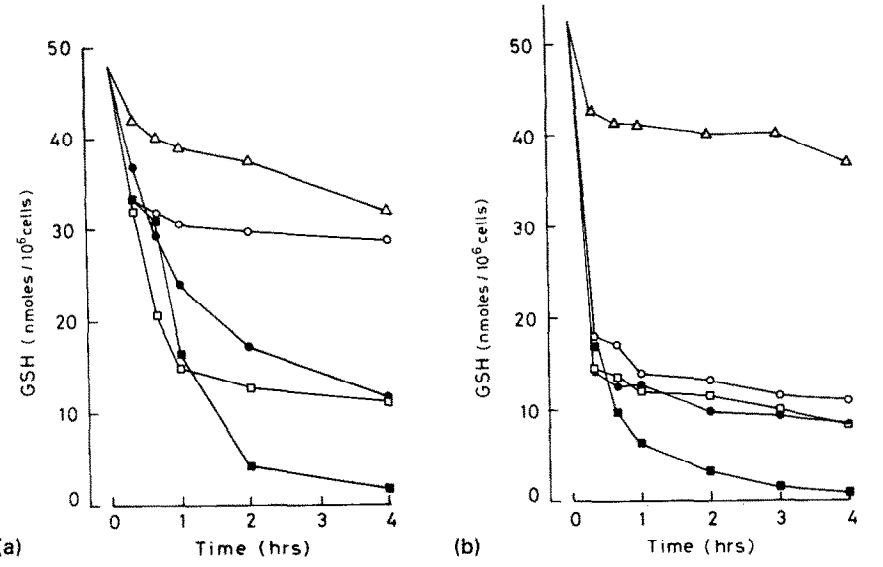

Fig. 3. Time course of GSH depletion in hepatocytes incubated with 1-naphthol (a), 1,2- or 1,4-naphthoquinone (b), in the presence and absence of dicoumarol (27 μM). At specified times, the GSH concentration was determined in control and treated suspensions. A small decrease in GSH concentration was observed in the control hepatocytes (△), but this was unaffected by dicoumarol. (a) Shows that 1-naphthol (100 μM) in the presence (●) and absence (○) of dicoumarol and 1-naphthol (250 μM) in the presence (■) and absence (□) of dicoumarol caused a time-dependent depletion of GSH. (b) Shows the effect on GSH of 1,2-naphthoquinone (50 μM) in the presence (●) and absence (○) of dicoumarol and 1,4-naphthoquinone (50 μM) in the presence (■) and absence (□) of dicoumarol. The results are from one experiment typical of three.

DISCUSSION

This study investigated the mechanism(s) of 1-naphthol toxicity to freshly isolated rat hepatocytes. 1-Naphthol exhibited a dose and time dependent toxicity in this system which was potentiated by dicoumarol (Fig. 1(b)), a potent inhibitor of DT-diaphorase [17]. This enzyme catalyses the reduction of quinones to diols without forming reactive semiquinone intermediates [18, 21, 22]. This two-electron reduction of quinones is therefore considered a non-toxic pathway under normal circumstances, whereas the one-electron reduction which leads to the formation of semiquinone intermediates and active oxygen species is considered a toxic route of metabolism [18, 23, 24]. The potentiation of 1-naphthol toxicity by dicoumarol, as assessed both by trypan blue exclusion (Fig. 1(b)) and increase in surface blebbing, suggested that this toxicity was mediated by the formation of naphthoquinone(s) which redox cycle within the cell to produce active oxygen species. In the presence of dicoumarol, more extensive redox cycling occurs due to the inhibition of the alternative two electron reduction of quinones to hydroquinones. This leads to the formation of increased amounts of active oxygen species, which require more GSH for their eventual detoxification mediated by glutathione peroxidase [11].

The cytotoxicity of menadione (2-methyl-1,4-naphthoquinone) to isolated hepatocytes has recently been shown to be potentiated by the presence of a low concentration of dicoumarol [18]. Results presented here show that dicoumarol also potentiated the toxicities of both 1,2- and 1,4-naphthoquinone, the most likely quinone metabolites of 1-naphthol, to isolated hepatocytes (Fig. 2). This was not surprising since these two quinones have similar affinities to menadione for DT-diaphorase [17]. Additionally, we observed that 1,2- and 1,4-naphthoquinone were more toxic, on an equimolar basis, than 1-naphthol to isolated hepatocytes. These results further supported the suggestions that: (a) 1-naphthol was toxic to isolated hepatocytes via the formation of naphthoquinone metabolites; and (b) DT-diaphorase may serve as a protective enzyme not only against quinone cytotoxicity, but also against the cytotoxicity of naphthols and possibly other phenols.

1-Naphthol, 1,2- and 1,4-naphthoquinone caused a rapid depletion in reduced cellular glutathione which preceded cytotoxicity (Fig. 3(a) and (b)). A more extensive GSH depletion was observed in each case in the presence of dicoumarol, although it was less striking in the case of 1,2-naphthoquinone (50 μM) as its toxicity was not as readily potentiated by dicoumarol at this concentration. From experiment to experiment, a variation in the degree of toxicity was observed with any particular concentration of the naphthoquinones, which may be related to the initial intracellular GSH levels. However, when compared at equimolar concentrations in any particular experiment, 1,4-naphthoquinone was always more toxic than 1,2-naphthoquinone.

The rapid GSH depletion caused by 1-naphthol,

1,2- and 1,4-naphthoquinone is consistent with the suggestion that 1-naphthol is metabolised to a naphthoquinone intermediate(s) which initiates redox cycling within the cell leading eventually to a depletion of cellular reduced glutathione. Additional support for this hypothesis comes from our recent studies using electron spin resonance trapping techniques which demonstrate the production of active oxygen species during the metabolism of 1-naphthol, 1,2- and 1,4-naphthoquinone by rat liver microsomes in the presence of a reduced pyridine nucleotide [25]. However, we have been unable to detect naphthoquinone formation from 1-naphthol in either hepatocytes or rat liver microsomes in the presence of NADPH, using high pressure liquid chromatography (M. d'Arcy Doherty and G. M. Cohen, unpublished observations). This may be due either to low conversion of 1-naphthol to naphthoquinones or their high reactivity if formed. 1,2-Naphthoquinone is known to bind covalently to proteins [7, 26].

Whilst we have been unable to detect naphthoquinones, the major metabolic fate of 1-naphthol (100 and 250 μM) in hepatocytes is conversion to its sulphate ester and glucuronic acid conjugates (Table 1), in agreement with previous studies [6, 20]. Very little unconjugated 1-naphthol remains at the end of the 4-hr incubation period (1.3–8.5%). Thus the majority of 1-naphthol in hepatocytes is detoxified as 1-naphthyl sulphate and 1-naphthyl-β-D-glucuronide which are not toxic to hepatocytes at concentrations in which they are formed (results not shown). The presence of dicoumarol inhibited the Phase II metabolism of 1-naphthol (250 μM) in all 4 experiments and had a variable effect at the lower concentration of 1-naphthol (100 μM) (Table 1). The degree of inhibition of metabolism was related to the severity of the observed toxicity. After 4 hr incubation with [1-^{14}C]-1-naphthol (250 μM), hepatocytes retained 17.7 ± 1.9% of the total radioactivity (mean ± SD ($n = 4$)), whereas in the presence of dicoumarol this increased significantly to 43.2 ± 3.6%. In preliminary studies, examination of the material associated with this radioactivity showed it to be predominantly unmetabolised 1-naphthol. This effect was most marked at the higher concentration of 1-naphthol (250 μM) but was also observed in some experiments at the lower concentration (100 μM). It is possible that this increased intracellular retention of 1-naphthol may contribute to the increased toxicity observed in the presence of dicoumarol or more likely that the increased toxicity may result in a decreased metabolism.

In a previous study, Hesse and Mezger [5] incubated [1-^{14}C]-1-naphthol with rat liver microsomes in the presence of an NADPH generating system and found that some product(s) associated with radioactivity was covalently bound to protein, which they postulated was either a naphthoquinone or semiquinone metabolite. In our studies we find a dose-dependent increase in covalently bound products in hepatocytes following incubation with [1-^{14}C]-1-naphthol for 4 hr. A good correlation ($r = 0.946$) between the level of covalent binding and the observed toxicity suggested a possible causal relationship. However, in the presence of dicoumarol (27 μM) (Table 2), this correlation was no longer apparent suggesting that some other mechanism of toxicity was also important. Whilst many studies have demonstrated a good correlation between covalent binding and toxicity [27, 28], several recent studies have shown a dissociation of these events [29, 30].

The rapid depletion of GSH in hepatocytes caused by 1-naphthol implicates the formation of active oxygen species during its metabolism. Both 1,2- and 1,4-naphthoquinone also depleted GSH in hepatocytes and this depletion was potentiated by dicoumarol, although to a lesser extent in the case of 1,2-naphthoquinone. These results further support the suggestion that 1-naphthol is metabolised to 1,2- and/or 1,4-naphthoquinone which then undergo redox cycling.

In conclusion then, the results of this study suggest that 1-naphthol is toxic to isolated hepatocytes via the formation of cytotoxic naphthoquinone metabolites and that both covalent binding and oxygen radical formation may be involved in the toxicity. The formation of quinonoid metabolites and oxygen radicals may also explain, in part, the selective toxicity of 1-naphthol to human tumor tissue compared to normal tissue *in vitro* [1].

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