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THE KILLING OF CULTURED HEPATOCYTES BY *N*-ACETYL-*p*-BENZOQUINONE IMINE (NAPQI) AS A MODEL OF THE CYTOTOXICITY OF ACETAMINOPHEN

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Abstract—The killing of isolated hepatocytes by *N*-acetyl-*p*-benzoquinone imine (NAPQI), the major metabolite of the oxidation of the hepatotoxin acetaminophen, has been studied previously as a model of liver cell injury by the parent compound. Such studies assume that the toxicity of acetaminophen is mediated by NAPQI and that treatment with exogenous NAPQI reproduces the action of the endogenously produced product. The present study tested these assumptions by comparing under identical conditions the toxicity of acetaminophen and NAPQI. The killing of hepatocytes by acetaminophen was mediated by oxidative injury. Thus, it depended on a cellular source of ferric iron; was potentiated by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), an inhibitor of glutathione reductase; and was sensitive to antioxidants. By contrast, the cytotoxicity of NAPQI was not prevented by chelation of ferric iron; was unaffected by BCNU; and was insensitive to antioxidants. Thus, the killing of cultured hepatocytes by NAPQI occurs by a mechanism different from that of acetaminophen. The killing by NAPQI was preceded by a collapse of the mitochondrial membrane potential and a depletion of ATP. Monensin potentiated the cell killing, and extracellular acidosis prevented it. These manipulations are characteristic of the toxicity of mitochondrial poisons, and are without effect on the depletion of ATP and the loss of mitochondrial energization. Thus, mitochondrial de-energization by a mechanism unrelated to oxidative stress is a likely basis of the cell killing by NAPQI. It is concluded that treatment of cultured hepatocytes with NAPQI does not model the cytotoxicity of acetaminophen in these cells.

The liver necrosis produced by acetaminophen depends on the metabolism of this hepatotoxin by mixed-function oxidation. Whereas there is general agreement that *N*-acetyl-*p*-benzoquinone imine (NAPQI‡) is the reactive metabolite of the biotransformation of acetaminophen [1, 2], the precise mechanism of its formation, as well as the manner by which the metabolism of acetaminophen leads to the necrosis of hepatocytes, is a matter of continuing study. For many years now, it has been widely held that the toxicity of acetaminophen is mediated by the reaction of NAPQI with cellular constituents. This hypothesis has prompted several recent studies of the killing of suspensions of isolated hepatocytes by NAPQI as a model of the mechanism of cell injury by the parent compound, acetaminophen [3–6].

NAPQI can react either as an electrophile or as an oxidant [2, 4]. As an electrophile, it can react with cysteine residues to deplete soluble as well as protein-bound thiols [4]. As an oxidant, NAPQI can react with soluble thiols (GSH) to form glutathione disulfide (GSSG) with an accompanying depletion of NADPH [4]. The latter effect presumably reflects the increased utilization of NADPH by glutathione reductase for reduction of GSSG, rather than a direct oxidation of NADPH by NAPQI [4]. An oxidation of protein-bound thiols by NAPQI was implied by the ability of dithiothreitol to prevent their loss [4]. Finally, whereas quinones are also capable of redox cycling, such a chemistry does not appear to play a significant role in the cytotoxicity of NAPQI [7, 8].

The study of the killing of isolated hepatocytes by NAPQI [3–6] in the attempt to understand the mechanism of action of acetaminophen makes at least two assumptions. It assumes that the toxicity of acetaminophen is, in fact, mediated by the reactivity of this metabolite, and that the exposure of hepatocytes to exogenous NAPQI reproduces the action of the endogenously produced metabolite. However, these assumptions have not been established and, thus, remain an issue in assessing the previous investigations of the toxicity of NAPQI. Both assumptions can be addressed by considering the mechanisms of the toxicity of acetaminophen and NAPQI under identical conditions. If exogenously added NAPQI kills hepatocytes by mechanisms that are clearly unrelated to those killing the same cells with acetaminophen, then one must question the validity of both assumptions.

We have been studying the killing of cultured hepatocytes by acetaminophen [9–13]. Our results implicate a metabolism-dependent generation of partially reduced oxygen species in the toxicity of this compound. Using this system, the present report

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‡ Abbreviations: NAPQI, *N*-acetyl-*p*-benzoquinone imine; GSH; reduced glutathione; DPPD, *N*,*N*′-diphenylphenylenediamine; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; LDH, lactate dehydrogenase; and $TPMP^+$, triphenylmethylphosphonium cation.

directly compares the toxicity of acetaminophen with that of NAPQI. The data document that exogenously added NAPQI kills hepatocytes by a mechanism readily distinguishable from the oxidative stress that lethally injures the same cells in the presence of acetaminophen.

MATERIALS AND METHODS

NAPQI was synthesized as described previously [14] except that butylated hydroxytoluene was omitted from the procedure. The chemical structure of the product was verified by NMR spectroscopy with a Bruker AM 8-45T spectrometer operating at 360 MHz; ^{1}H-NMR (C_6D_6) $\delta = 6.4307$ (d, 2H, $J = 9.9$ Hz), $\delta = 6.2210$ (d, 2H, $J = 10.3$ Hz). The purity of the product was assessed by HPLC on a 25 cm × 0.39 cm μ-Porosil column (Waters) using a mobile phase of 1% methanol in ethyl acetate:petroleum ether (40:60) and a flow rate of 1 mL/min. The retention time of *p*-benzoquinone, a potential contaminant [14], NAPQI, and acetaminophen were 3.1, 4.1 and 17 min respectively. Preparations of NAPQI with purity greater than 98% were routinely obtained. NAPQI was dissolved in anhydrous dimethyl sulfoxide (DMSO) and used immediately.

Male Sprague–Dawley rats (150–200 g) were obtained from the Charles River Breeding Laboratories (Wilmington, MA) and fed *ad lib*. On the day prior to use, rats were treated with 25 mg/kg of 3-methylcholanthrene (Sigma) as a 10 mg/mL solution in corn oil by intraperitoneal injection and then fasted overnight. Hepatocytes were isolated by collagenase perfusion according to Seglen [15]. Yields of 2–4 × 10^8 cells/liver with 90–95% viability by trypan blue exclusion were routinely obtained. The hepatocytes were plated in 25 cm^2 flasks (Corning Glass Works, Corning, NY) at a density of 1.33 × 10^6 cells/flask in 3 mL of Williams' E medium (GIBCO Laboratories, Chagrin Falls, OH) containing 10 I.U./mL penicillin, 10 μg/mL streptomycin, 0.05 mg/mL gentamicin, 0.02 units/mL insulin and 10% heat-inactivated fetal bovine serum (Hazelton Research Products, Lenexa, KA).

After incubation for 2 hr in an atmosphere of 5% CO_2–95% air, the cultures were rinsed twice with a prewarmed HEPES buffer (0.14 M NaCl, 6.7 mM KCl, 1.2 mM $CaCl_2$ and 2.4 mM HEPES, pH 7.4) to remove unattached dead cells. In some cases, deferoxamine (20 mM) was added to the culture medium during the last hour of this incubation period. Williams' E medium minus bovine serum (5 mL) was then replaced, and the cultures were treated as indicated in the text.

Acetaminophen, β-napthoflavone, monensin (all from Sigma), and *N*,*N*′-diphenylphenylenediamine (DPPD) (Eastman) were dissolved in DMSO and added to final concentrations of 1 mM, 10 μM, 20 μM and 2 μM respectively. 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) (Bristol Laboratories) was dissolved in dehydrated ethanol and added to the culture at a final concentration of 50 μM. In all cases, control cultures were treated with an equal amount of ethanol and/or DMSO. In experiments utilizing extracellular acidosis, the medium was adjusted to pH 6.0 with HCl prior to addition to the cultures.

Cell viability was determined by the release of lactate dehydrogenase (LDH) into the culture medium, and has been shown to correlate with trypan blue exclusion [16]. The extent of cell killing was quantitated as the percentage of the total LDH present in the hepatocytes released into the medium. This total is determined by the LDH released from the cells after treatment with Triton X-100. Acetaminophen and NAPQI did not directly affect LDH activity. The GSH content was determined as described previously [17]. The ATP content was determined by scraping the cells into 3 mL of ice-cold 3% trichloroacetic acid (TCA). After centrifugation, 2 mL of protein-free supernatant was neutralized with KOH and assayed for ATP as described previously [18].

All experiments were repeated at least three times on triplicate cultures, and unless indicated one representative experiment is illustrated in the figures and tables. The statistical significance of the data was determined by the paired Student's *t*-test.

RESULTS

Toxicity of NAPQI. Sensitivity of cultured hepatocytes to acetaminophen is induced by pretreatment of the animals with either phenobarbital or 3-methylcholanthrene [11, 12]. We have shown previously that the conditions of the killing of hepatocytes are similar when either 3-methylcholanthrene or phenobarbital is used to induce sensitivity to acetaminophen [14]. Rats in the present study were induced with 3-methylcholanthrene, since it enhances more substantially than does phenobarbital the hepatotoxicity of acetaminophen [14]. Hepatocytes isolated from male rats pretreated with 3-methylcholanthrene were cultured for 2 hr, washed, and then exposed to increasing concentrations of NAPQI. Figure 1 (left panel) details the extent of cell killing after 4 hr. The number of dead cells increased with concentrations of NAPQI greater than 250 μM. The data in Fig. 1 (left panel) are similar to the previously reported sensitivity to NAPQI of suspensions of freshly isolated hepatocytes [3, 6]. Figure 1 (right panel) illustrates the time course of the cell killing by 1000 μM NAPQI. No significant cell killing was evident within 30 min. Between 1 and 4 hr the number of dead cells increased from 30% to over 70% of the total.

By contrast, the hepatocytes were less sensitive to the cytotoxicity of acetaminophen. No loss of viability was observed with up to 20 mM acetaminophen over the same 4 hr time course in which NAPQI killed 70% of the cells (data not shown).

At physiologic pH in aqueous media, NAPQI has been reported to decompose to *p*-benzoquinone [14]. The latter killed 30–40% of the hepatocytes within 30 min at concentrations as low as 100 μM (data not shown). The toxicity of NAPQI (Fig. 1) could not be attributed to its decomposition to *p*-benzoquinone. Liquid chromatographic analysis of the culture medium upon addition of 1 mM NAPQI did not reveal significant concentrations of *p*-benzoquinone after 60 min.

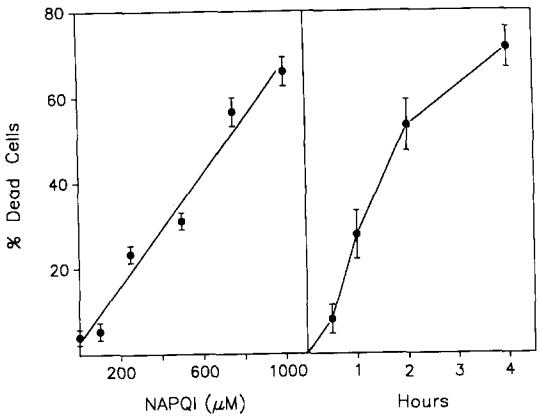

Fig. 1. Concentration and time dependency of the killing of hepatocytes by NAPQI. NAPQI was added to cell cultures in 25 μL DMSO to give final concentrations of 100, 250, 500, 750 and 1000 μM. The concentration of NAPQI used for the time course was 1000 μM. Control cultures received 25 μL DMSO only. The extent of cell killing was assessed after 4 hr by the release of LDH into the culture medium. Values are the means ± SD of six separate experiments.

Effect of BCNU on the toxicity of NAPQI. Killing of 3-methylcholanthrene-induced hepatocytes by acetaminophen within 4 hr depends upon the inhibition of glutathione reductase [12]. Table 1 documents the effect of BCNU, an inhibitor of glutathione reductase, on the toxicity of acetaminophen. Whereas over 80% of the hepatocytes were killed within 4 hr in the presence of BCNU, there was no cell killing in its absence. By contrast, there was no potentiation of the toxicity of NAPQI by BCNU (Table 1). With either 250, 500 or 1000 μM NAPQI, the extent of cell killing at 4 hr was the same in the presence or absence of BCNU. Furthermore, BCNU did not potentiate the rate of cell killing by NAPQI. The time course of the toxicity of either 250, 500 or 1000 μM was the same in the presence or absence of BCNU (data not shown). Since BCNU was without effect on the toxicity of NAPQI, all subsequent experiments with NAPQI were carried out in the absence of BCNU.

Effect of deferoxamine and DPPD on the toxicity of NAPQI. The killing of cultured hepatocytes by acetaminophen depends on a cellular source of ferric iron [10, 12]. Pretreatment of the hepatocytes with the ferric iron chelator deferoxamine prevented the toxicity of 1000 μM acetaminophen in the presence of BCNU (Table 2, lines 1 and 2). By contrast, pretreatment with deferoxamine had only a slight effect on the toxicity of NAPQI (Table 2, lines 1 and 2).

The killing of cultured hepatocytes by 1000 μM acetaminophen plus BCNU is accompanied by the peroxidation of membrane lipids. The antioxidant DPPD prevents both the lipid peroxidation and the cell killing [10]. DPPD had only a slight effect on the toxicity of NAPQI (Table 2, lines 1 and 3), whereas it completely prevented that of acetaminophen plus BCNU (Table 2, lines 1 and 3). It is important to emphasize that the protective effect of both deferoxamine and DPPD was evident despite a greater number of dead cells with acetaminophen than with NAPQI (Table 2, line 1).

Mixed-function oxidation and the toxicity of NAPQI. The killing of cultured hepatocytes by acetaminophen depends on the metabolism of this toxin by mixed-function oxidation [10, 12]. Thus, addition to the culture medium of 10 μM β-napthoflavone, an inhibition of cytochrome P450-dependent metabolism, reduced substantially the cell killing by acetaminophen (Table 2, lines 1 and 4). By contrast, β-napthoflavone had only a slight effect on the cell killing by NAPQI (Table 2, lines 1 and 4).

The data with β-napthoflavone were confirmed by a study of the toxicity of NAPQI with hepatocytes incubated overnight prior to their use. Prolonged culture of rat hepatocytes decreases the cytochrome P450 activity [19]. Whereas an 18 hr incubation of the cultured hepatocytes resulted in a complete loss of their sensitivity to acetaminophen (Table 2, lines 1 and 5), the extent of the killing by NAPQI was similar to that of freshly cultured cells (Table 2, lines 1 and 5).

Effect of NAPQI on glutathione metabolism. Figure 2 illustrates the effects of NAPQI and acetaminophen on the metabolism of GSH. In the presence of 1000 μM acetaminophen, the content of GSH was reduced compared to control levels throughout the time course of the experiment (Fig. 2). This degree of depletion of GSH agrees closely

Table 1. Effect of BCNU on the toxicity of NAPQI

	% Dead cells			
	Acetaminophen		NAPQI	
Treatment	(1000 μM)	(250 μM)	(500 μM)	(1000 μM)
Minus BCNU	4 ± 1	6 ± 2	26 ± 3*	73 ± 3
Plus BCNU	83 ± 5	8 ± 2	31 ± 8*	74 ± 1

Hepatocytes were exposed to either NAPQI (250, 500, or 1000 μM) or acetaminophen (1000 μM) in either the presence or absence of 50 μM BCNU. The extent of cell killing was assessed after 4 hr by the release of LDH into the culture medium. The results are the means ± SD of the determinations on three separate cultures.

* Significantly different from 250 μM NAPQI ($P < 0.001$).

Table 2. Effect of deferoxamine, DPPD and β-napthoflavone on the cell killing produced by NAPQI and acetaminophen

	% Dead cells	
	NAPQI	Acetaminophen
1. No treatment	66 ± 6	81 ± 4
2. Deferoxamine (20 mM)	51 ± 4*	5 ± 1
3. DPPD (2 μM)	50 ± 4*	4 ± 1
4. β-Napthoflavone (10 μM)	53 ± 6*	14 ± 1
5. 18 hr Cultures	60 ± 3	2 ± 1

Hepatocytes were exposed to either NAPQI (1000 μM), or acetaminophen (1000 μM) plus BCNU (50 μM), in the presence or absence of either deferoxamine, DPPD, or β-napthoflavone. The extent of cell killing was assessed after 4 hr by the release of LDH into the culture medium. The results are the means ± SD of the determinations on three separate cultures. The cell killing by NAPQI and acetaminophen was also assessed in these hepatocytes after they had been cultured for an additional 18 hr.

* Significantly different from no treatment ($P < 0.05$).

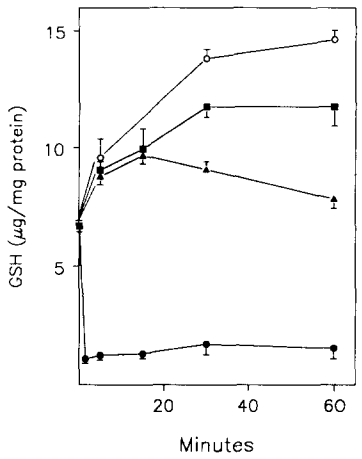

Fig. 2. Time course of GSH alterations following exposure to NAPQI or acetaminophen. Hepatocytes in culture for 2 hr were treated with either 1000 μM NAPQI (●), 1000 μM acetaminophen (■), or 1000 μM acetaminophen plus 50 μM BCNU (▲). Control cultures received DMSO only (○). GSH content was determined at the times indicated. The results are the means ± SD of the determinations from three separate cultures.

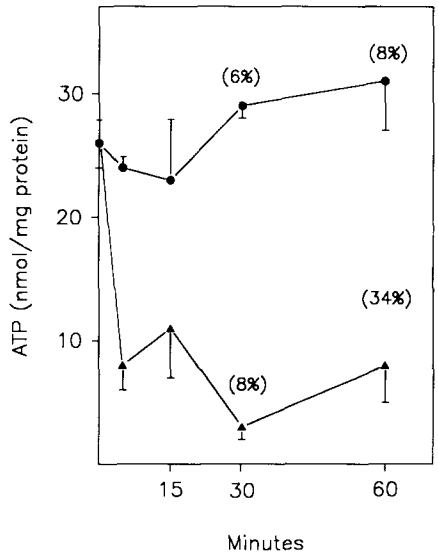

Fig. 3. Depletion of hepatocellular ATP by NAPQI. Hepatocytes in culture for 2 hr were exposed to either 100 μM NAPQI (▲) or DMSO only (●). ATP levels were determined at the times indicated. The numbers in parentheses represent percent dead cells. Results are the means ± SD of the determinations from three separate cultures. The data in the presence of NAPQI are significantly different ($P < 0.01$) from the corresponding DMSO control.

with the extent of formation of the GSH conjugate of acetaminophen reported previously [12] and reflects the generation of NAPQI *in situ*. On the other hand, direct exposure of the hepatocytes to a similar concentration of NAPQI resulted in a rapid fall in the intracellular GSH concentration. Within 1 min the GSH content fell to 15% of the intial value and remained at this level throughout the 60 min of the experiment.

The addition of BCNU to cultures receiving acetaminophen resulted in a further depletion of GSH compared to untreated controls or cells treated with acetaminophen alone (Fig. 2). This additional loss of GSH in the presence of BCNU reflects its oxidation to GSSG [13]. By contrast, BCNU was without effect on the loss of GSH with NAPQI treatment (data not shown).

Mitochondrial function and the toxicity of NAPQI in intact hepatocytes. NAPQI inhibits the function of isolated rat liver mitochondria [20]. Similarly, an inhibition of the function of mitochondria in intact hepatocytes was demonstrated in the present study. Figure 3 shows that 1000 μM NAPQI caused a loss of intracellular ATP content within 10 min. A comparison of these data with those in Fig. 1

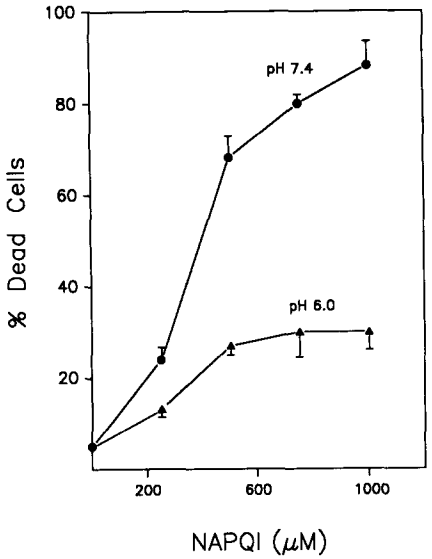

Fig. 4. Effect of extracellular acidosis on the toxicity of NAPQI. Hepatocytes were incubated in Williams' E medium at either pH 7.4 or 6.0 for 4 hr in the presence of NAPQI (250, 500, 750 and 1000 μM). The extent of cell killing was assessed by the leakage of LDH into the culture medium. The viability of control cells was unaffected by incubation in Williams' E at pH 6.0 for 4 hr. The results are the means ± SD of the determinations from three separate cultures. All data at pH 6.0 were significantly different ($P < 0.001$) from the corresponding results at pH 7.4.

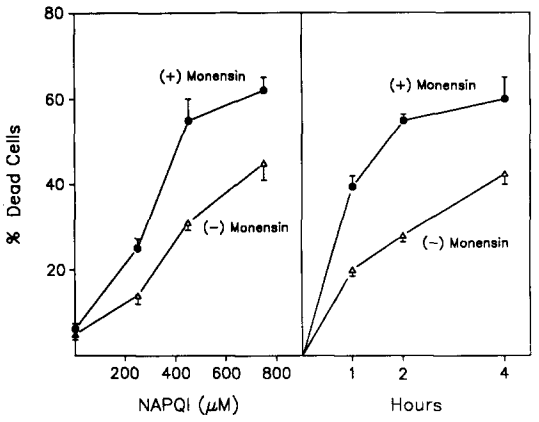

Fig. 5. Effect of monensin on the toxicity of NAPQI. In the left panel, hepatocytes were exposed to various concentrations of NAPQI in the presence or absence of 20 μM monensin for 4 hr. The right panel illustrates the time course of the cell killing by 750 μM NAPQI in the presence and absence of 20 μM monensin. The extent of cell killing was assessed by the leakage of LDH into the culture medium. The results are the means ± SD of the determinations from three separate cultures. Results in the presence of monensin were significantly different ($P < 0.01$) from the corresponding results in the absence of monensin.

indicates that this depletion of ATP preceded the loss of viability that is first evident between 30 and 60 min.

The loss of ATP was associated with a collapse of the mitochondrial membrane potential. The effect of NAPQI on the mitochondrial membrane potential was monitored by measurement of the release of [^{3}H]triphenylmethylphosphonium cation ([^{3}H]TPMP$^+$) from intact hepatocytes. A loss of mitochondrial energization is associated with release of TPMP$^+$ from the cells [21]. Treatment of cultured hepatocytes with 1000 μM NAPQI resulted in a release into the culture medium of 58% of the intracellular [^{3}H]TPMP$^+$ within 10 min as compared to a loss of only 3% from the control cells.

The toxicity of known mitochondrial poisons is modified by manipulation of the pH of the intracellular and extracellular environments [22, 23]. Extracellular acidosis protects cultured rat hepatocytes from the toxicity of cyanide [22, 23]. Figure 4 indicates that lowering of the pH of the culture medium from pH 7.4 to 6.0 substantially reduced the cell killing by 1000 μM NAPQI. This manipulation had no effect on the rate or extent of ATP depletion by NAPQI (data not shown). Thus, the protective effect of extracellular acidosis cannot be attributed to a change in the uptake and/or intracellular distribution of NAPQI.

By contrast to acidification, alkalinization of the cytosol by monensin, a H^+/Na^+ ionophore, potentiates the toxicity of cyanide [23]. Similarly, Fig. 5 illustrates that monensin potentiated the toxicity of NAPQI. Again, the potentiation by monensin was not accompanied by a parallel effect on the depletion of ATP by NAPQI (data not shown).

DISCUSSION

We have been using cultured rat hepatocytes to study the mechanism of lethal cell injury by acetaminophen [9–13]. Such an approach assumes that this culture system is an appropriate model with which to pursue the mechanism of action of acetaminophen. We have shown that the essential conditions of the killing of cultured hepatocytes [9–13] are reproduced in the hepatotoxicity of acetaminophen in the intact rat [11, 24, 25]. In turn, the present study has considered whether the toxicity of NAPQI can be used to model the action of acetaminophen. The data presented above document that acetaminophen kills cultured hepatocytes by a mechanism different from that which mediates the cytotoxicity of exogenously added NAPQI. Thus, one must interpret with caution the attempt to use NAPQI [3–6] as a model of the mechanism of the cytotoxicity of acetaminophen.

The metabolism of acetaminophen imposes an acute oxidative stress on the cultured hepatocytes [9–13]. When the antioxidant defenses of the cells are perturbed by inhibition of glutathione reductase, an iron-dependent mechanism initiates the peroxidative decomposition of cellular membranes. Cell death ensues. By contrast, there is little evidence that the cytotoxicity of exogenous NAPQI relates to an oxidative stress. By a BCNU-insensitive, iron-independent mechanism, NAPQI interferes with mitochondrial function such that the inner membrane

potential collapses, ATP stores are depleted, and the cells lose viability.

Several essential features of the lethal injury of cultured hepatocytes by acetaminophen have emerged from our previous studies. First, the metabolism-dependent cell killing by acetaminophen requires a cellular source of ferric iron and superoxide anions [9, 10, 12, 13]. Chelation of hepatocyte ferric iron by deferoxamine, or addition to the culture medium of superoxide dismutase, prevents the loss of viability caused by acetaminophen. Inhibition of glutathione reductase by BCNU sensitizes the cells to acetaminophen [9–13]. Peroxidation of membrane lipids occurs in cultures intoxicated with acetaminophen, and inhibition of such lipid peroxidation with antioxidants prevents the associated cell killing [12].

The data in the present study (Tables 1 and 2) confirm the previously documented conclusion [9–13] that acetaminophen injures cultured hepatocytes by an oxidative stress. However, the same criteria that implicate an oxidative stress in the cytotoxicity of acetaminophen imply that NAPQI, the major oxidative metabolite of acetaminophen, did not lethally injure cultured hepatocytes by an oxidative stress. To a major extent, the toxicity of exogenously added NAPQI was not dependent on a cellular source of ferric iron, was not potentiated by BCNU, and was not prevented by DPPD.

The inability of BCNU in the present study to potentiate the toxicity of NAPQI would seem to disagree with previous reports [4, 6]. In one study [4], BCNU produced only a small increase in the release of LDH after 60 min from hepatocytes intoxicated with 250 μM NAPQI. Neither was the significance of such a difference indicated nor the extent of cell killing after longer exposure times reported [4]. In the other study [6], BCNU increased by about 20% the cell killing by 300 μM NAPQI. However, the number of dead cells in the case of BCNU seemed to be greater by more than 10% at the start of the experiment, and no statistical analysis of the small increases in cell killing were presented.

The inability of BCNU to potentiate the toxicity of NAPQI observed here cannot be attributed to an exhaustion of GSH stores. Previous studies have documented BCNU potentiation in the killing of cultured hepatocytes by diethylmaleate, dinitrofluorobenzene and allyl alcohol [26, 27]. These compounds deplete GSH to the same rate and extent as did NAPQI in the present study.

Although NAPQI does not appear to redox cycle [7], it has been postulated that a component of NAPQI toxicity may result from an uncoupling of cytochrome P450 by NAPQI with the subsequent generation of reactive oxygen species [28]. Whereas this may occur in reconstituted microsomal systems, the major portion of the killing of cultured hepatocytes by NAPQI would seem unrelated to such a mechanism. Inhibition of cytochrome P450 with β-napthoflavone, or the use of hepatocytes with low cytochrome P450 activity, had only a small effect on the toxicity of NAPQI.

While not accompanied by an oxidative stress, the toxicity of NAPQI was associated with evidence of a loss of mitochondrial function. A loss of ATP could be attributed to a de-energization of the inner mitochondrial membrane. Such changes are characteristic of the toxicity of well-established mitochondrial poisons, such as cyanide [23]. Furthermore, conditions shown recently to modify the toxicity of cyanide similarly affected the cell killing by NAPQI. Extracellular acidosis prevents the killing of cultured hepatocytes by both cyanide [23] and NAPQI (Fig. 4). Conversely, alkalinization of the cytosol by monensin potentiates the toxicity of cyanide [23]. Monensin also increased the cell killing by NAPQI (Fig. 5). Neither the effect of extracellular acidosis nor that of monensin could be attributed to a parallel effect on the loss of ATP, a result again consistent with the previous experience with cyanide [23]. Interestingly, NAPQI has been shown recently to inhibit irreversibly the respiration of isolated rat liver mitochondria [20].

In summary, the data presented in this report document that acetaminophen and NAPQI kill cultured hepatocytes by different mechanisms. The cytotoxicity of acetaminophen is mediated by partially reduced oxygen species. By contrast, little evidence was found for the participation of such species in the cell injury by NAPQI. Our data do not negate the importance of the oxidative metabolism of acetaminophen to the genesis of lethal cell injury. Indeed, the oxidation of acetaminophen to NAPQI is required for its toxicity. However, our data do imply that it is questionable to pursue the mechanism of action of, at least exogenous NAPQI, as a model of the action of acetaminophen.

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