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ELECTRON TRANSPORT AND PROTECTION OF LIVER SLICES IN THE LATE STAGE OF PARACETAMOL INJURY

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Abstract—Cell injury by chemicals takes place in two stages, initial chemical interaction between the material and cell components, and subsequent stages of adaptation or failure of response to chemical modification of some parts of the cell apparatus. Paracetamol toxicity was studied in an *in vitro* system using liver slices incubated in two stages. During the first 2 hr slices were exposed to 10 mM paracetamol and this was absent during the following 4 hr of incubation. Damage was quantified at the end by measuring leakage of lactate dehydrogenase (LDH), increase in water content and potassium loss. Treatment of slices with 10 μM DCPIP or 2 mM ethanol in the second period of incubation prevented paracetamol-induced damage. The protective effect was not dependent on the activity of P450 since the second stage of incubation was insensitive to SKF-525A. The protective effect of ethanol was blocked by the presence of 1 mM pyrazole suggesting that ethanol needs to be metabolized to exert its effect. The addition of 20 mM pyruvate reversed the protection provided by ethanol. Nicotinamide did not alter toxicity or protection by ethanol. These findings suggest an increase of NADH and NADH/NAD ratio as the most probable explanation for the protective effect observed. The role of reducing equivalents in detoxification of quinones such as NAPQI by formation of hydroquinones, is discussed in relation to the mechanism of cell injury by paracetamol.

Paracetamol is a widely used analgesic antipyretic drug available without prescription in many countries. Overdoses produce centrilobular hepatic necrosis that can lead to death both in animals and humans [1, 2]. The requirement for hepatic metabolism for development of liver injury after dosage with paracetamol and other hepatotoxic drugs such as CCl_4 is well established [3–5]. Although the exact sequence of events that lead to hepatic cell death has not been elucidated, extensive studies have shown that one metabolite formed by the action of cytochrome P450-oxidases, *N*-acetyl-*p*-benzoquinoneimine (NAPQI†) covalently binds to critical hepatic proteins, depletes glutathione and may stimulate oxidative events under aerobic conditions leading to the formation of oxygen radicals, as do other quinones [6, 7]. It is notable that paracetamol, even in overdose, is metabolized and excreted in the course of 24 hr, but signs of liver damage and liver failure take much longer to develop. There is a latent period between paracetamol absorbtion and metabolism and the subsequent development of cell injury and necrosis [8, 9]. To study the effectiveness of protective agents against paracetamol intoxication it is essential to separate the early stages in which paracetamol is metabolized from the late events of cell damage during which cells run down as a consequence of the primary attack.

There is general agreement that the useful procedures for treatment of the early stages of paracetamol overdose are those that increase the synthesis of glutathione, or possibly other sulphydryl compounds. The early treatment of patients who have taken large overdoses of paracetamol, by giving methionine or *N*-acetyl cysteine have been shown to be highly effective [10, 11]. It is now important to get compounds capable of stopping the chain of events leading to cell necrosis and liver failure, in the late stages after paracetamol has been metabolized, since patients often present many hours after taking an overdose, when *S*-amino acids are no longer effective.

It is also important to gain understanding of the events that lead from the initial insult of the cells to the much later cell death. Many events have been described, the question is which are incidental and which play an important part in the process [12].

In previous work we introduced an *in vitro* model system that allows the separation of the toxic effects of paracetamol into two stages, incubating liver slices or isolated hepatocytes for 2 hr with the toxin and then changing into a fresh solution not containing paracetamol [9, 13].

Using this procedure we previously reported that a number of compounds could ameliorate liver injury, those included Methylene blue, DPPD and promethazine, all compounds with antioxidant activity. Nevertheless, some other antioxidants like EDTA, BHT and BHA were not effective in slices [9]. In contrast, isolated liver cells in suspension could be

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† Abbreviations: NAPQI, *N*-acetyl-*p*-benzoquinoneimine; DCPIP, dichloro phenol indophenol; DPPD, diphenyl para phenylene diamine; BHT, butylated hydroxy toluene; BHA, butylated hydroxy anisole; LDH, lactate dehydrogenase; PMS, phenazine metho sulphate.

protected by CaEDTA which penetrates these cells, but not liver cells *in vivo*, slices or isolated hepatocytes attached to surfaces in tissue culture [14].

Liver slices, unlike isolated liver cells in suspension or in culture, have the problem of low oxygen tension in the centre of the slice, and injury to the cells at the cut surfaces. On the other hand, the cells have not had their surface stripped by exposure to proteases and have not been given an environment which forces a major change in morphology and activity. The slice cells are not permeable to molecules such as EDTA, which are excluded *in vivo*, but which enter freely into suspended hepatocytes, nor have inter cell adhesion and communication been broken.

In the present work we have studied the effects of some compounds that have the capacity to modify the intracellular redox state, and which we find are able to prevent the cell damage by paracetamol in the late stage of the process.

MATERIALS AND METHODS

Animals and slices. Male Wistar rats (OLAC, Bicester, U.K.) weighing 120–200 g were fed stock pellets (SDS, Witham, U.K.) and given phenobarbitone as a solution containing 1 mg of sodium phenobarbitone/mL as the sole source of drinking water. Phenobarbitone treatment was given for at least 5 days. Vitamin E (5 mg α-tocopherol acetate in two drops of olive oil) was given by mouth 12–24 hr before rats were killed. Rats were killed by exsanguination under anaesthesia using fentanyl citrate (0.01 mg/kg, i.m.) and diazepam (2.5 mg/kg, i.p.) (Janssen, Wantage, U.K.). The liver was rapidly removed and the liver slices of 0.3 mm thickness or less were cut by hand on a Stadie-Riggs stage with a long razor blade (A. H. Thomas Co. Philadelphia, PA, U.S.A.). Slices weighing about 100 mg were put into 25-mL Erlenmeyer flasks containing 5 mL of Ringer solution with the following composition NaCl 125 mM, KCl 6 mM, $MgSO_4$ 1.2 mM, NaH_2PO_4 1 mM, $CaCl_2$ 1 mM, glucose 10 mM, 15 mM Hepes buffer, pH 7.4 at 37° as previously described [9]. The slices were put into the Ringer solution at room temperature and the experiment started by placing the flasks into an incubator bath at 37° under oxygen with shaking (90 strokes/min). After 2 hr the slices were taken out of the first flask and reincubated in fresh Ringer solution for a further 4 hr.

Chemicals. Ethanol was bought from James Borroughs Ltd (Witham, U.K.). Paracetamol and all other reagents used were purchased from the Sigma Chemical Co. (Poole, U.K.) or BDH Ltd (Poole, U.K.) and were of analytical grade. SKF-525A was a gift from Smith Kline and French (Welwyn Garden City, U.K.).

Measurement of enzyme leakage, K^+ and water content. Injury was assessed by measuring leakage of lactate dehydrogenase from the slice into the incubation medium, and loss of potassium and increase of water content of the slices, as previously described [9, 13]. Lactate dehydrogenase activity (LDH) released into the Ringer solution was expressed as a percentage of the amount of enzyme originally present in the flask, based on the original slice weight LDH assays on homogenates of liver slices sampled before incubation [15].

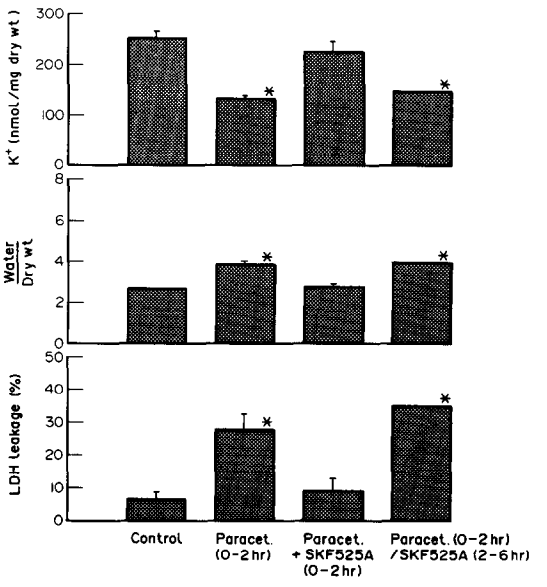

Fig. 1. Effect of 30 μM SKF-525A added together with, or after a 2 hr paracetamol exposure on cell injury in liver slices. Slice K^+, water content and leakage of LDH from slice into the medium were measured as described in Materials and Methods. LDH leakage is expressed as percentages of enzyme activity found in the medium in comparison with the amount originally present in the slice. K^+ shows the potassium content as nmol/mg of dry wt slice, while water is expressed as slice water content (mg water/mg dry wt of slice). Results represent mean values ± SD of at least three experiments performed in duplicate assays. Paracet. + SKF means both added during time 0–2 hr, Paracet./SKF means paracetamol incubated from 0–2 hr and then SKF from 2–6 hr. * $P < 0.05$ as compared to control group.

In some experiments isocitrate dehydrogenase leakage was also measured and gave essentially the same results. For measurement of water and potassium content slices were removed from the incubation medium, blotted and weighed. They were then dried at 90° overnight, and weighed again. Water content was expressed as milligram water per milligram dry weight of liver slice. Potassium content was measured in an HCl extract of the dried piece of liver, by atomic absorption, as previously described, and expressed as nmoles K^+ per milligram dry weight of liver slice [9].

Statistical analysis. Significance of the differences were determined using analysis of variance with a $P < 0.05$ being taken as indicating significant differences.

RESULTS

As before, we found that slices incubated with 10 mM paracetamol showed minimal sign of injury after 2 hr, but developed an extensive leak of soluble enzyme content when incubated for a further 4 hr in a paracetamol free, second incubation medium [9]. The leak of LDH was accompanied by a major and significant loss of potassium, and increase of water content of the slices, (Fig. 1). In contrast, slices

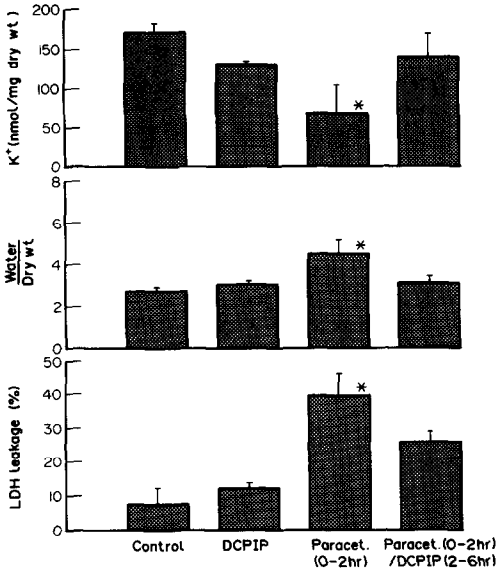

Fig. 2. Effect of 10 μM DCPIP added in the second period of incubation (2–6 hr) in slices exposed for 2 hr to 10 mM paracetamol. LDH activity was measured in the medium while K^+ and water contents were determined in slices. Each bar represents the mean ± SD of at least three experiments performed in duplicate. * $P < 0.05$ as compared to control groups.

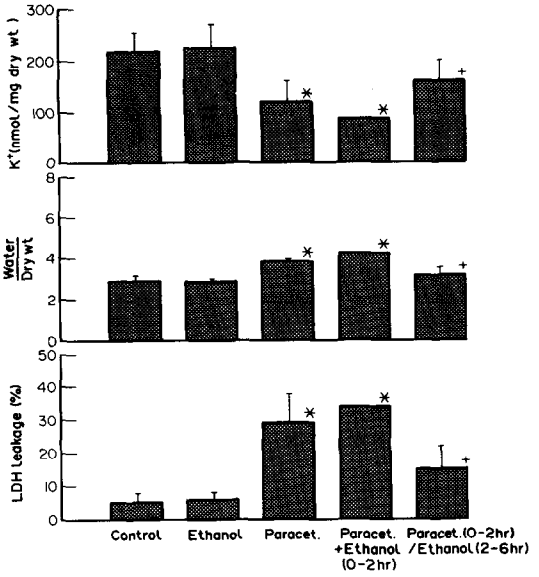

Fig. 3. Difference in effect of 2 mM ethanol in the first (0–2 hr) and second (2–6 hr) incgation periods on slices incubated with 10 mM paracetamol (0–2 hr). Slice K^+, water content and leakage of LDH from slice into the medium were measured as described in Materials and Methods. Results are expressed as the mean ± SD with data from at least three experiments in duplicate. * $P < 0.05$ as compared to control groups. ‡ $P < 0.05$ as compared to paracetamol treated groups.

incubated without paracetamol exposure showed a minimal leakage of enzyme and maintained a high potassium and low water content. Leakage of isocitrate dehydrogenase followed a similar pattern to that of LDH (data not shown).

Figure 1 clearly shows that only the first stage of incubation is dependent on P450-dependent oxidases. Thirty micromolar SKF-525A completely abolished the damage when added simultaneously with paracetamol. On the other hand, it did not have any effect on injury if this P450 inhibitor was added in the second incubation stage.

Figure 2 shows the effect of the electron acceptor DCPIP added to the incubation system after the period of paracetamol metabolism was ended. Ten micromolar DCPIP was able to protect the slices against the development of damage caused by previous exposure to paracetamol. Leakage of LDH, K^+ loss and increase in water content were all significantly prevented, when compared with the slices incubated with paracetamol alone, ($P < 0.05$). Table 1 shows that another electron acceptor, PMS, did not protect, but made the damage worse.

The effect of ethanol is depicted in Fig. 3. As

Table 1. Effect of dicoumarol on LDH leakage, K^+ content and water content in liver slices incubated with paracetamol

Incubation time 0–2 hr	Incubation time 2–6 hr	LDH leakage (%)	K^+ content (nmol/mg dry wt)	mg water/mg dry wt
Ringer	Ringer	6.7 ± 3.8	260 ± 20	2.7 ± 0.2
Ringer	Dicoumarol	7.0 ± 2.0	239 ± 17	2.8 ± 0.2
Paracet.	Ringer	35.2 ± 7.3*	137 ± 10*	4.0 ± 0.1*
Paracet.	Dicoumarol	83.3 ± 4.2*†	85 ± 3*†	5.2 ± 0.1*†
Paracet.	PMS	44.3 ± 5.6*†	32 ± 4*†	4.4 ± 0.2*†

Each value represents the mean ± SD of at least three separate experiments each performed in duplicate assays.

The final concentrations of dicoumarol, PMS and paracetamol were 20 μM, 20 μM and 10 mM, respectively.

Cell injury was assessed as LDH leakage from slices at 6 hr, expressed as percentage of original content of LDH. K^+ content and water content of slices were measured as described in Materials and Methods.

* Statistically different from control values (Ringer), $P < 0.05$.

† Statistically different from paracet. treated group, $P < 0.05$.

Table 2. Effect of pyrazole and pyruvate on the protection offered by ethanol against paracetamol toxicity in liver slices

Incubation time 0–2 hr	Incubation time 2–6 hr	LDH leakage (%)	K^+ content (nmol/mg dry wt)	mg water/mg dry wet
Ringer	Ringer	6.7 ± 3.7	214 ± 51	2.9 ± 0.2
Paracet.	Ringer	30.9 ± 9.1*	119 ± 40*	3.8 ± 0.5*
Paracet.	Ethanol	16.6 ± 5.1†	161 ± 36†	3.1 ± 0.4†
Paracet.	Ethanol + pyrazole	23.7 ± 8.3*‡	126 ± 19*‡	3.6 ± 0.3*
Paracet.	Ethanol + pyruvate	24.5 ± 8.3*‡	125 ± 11*‡	3.6 ± 0.5*

Each value represents the mean ± SD of at least three separate experiments each performed in duplicate assays. The final concentrations used were: pyrazole 1 mM, pyruvate 20 mM, ethanol 2 mM and paracetamol 10 mM.

LDH leakage is expressed as the percentage of enzyme activity found in the medium at 6 hr in comparison with the amount originally present. K^+ content and water content of slices were measured as described in Materials and Methods and expressed as nmoles K^+/mg dry weight and mg water/mg dry weight of slice, respectively.

* Statistically different from control values (Ringer), $P < 0.05$.

† Statistically different from paracet. group, $P < 0.05$.

‡ Statistically different from paracet. + ethanol treated group, $P < 0.05$.

shown, the addition of 2 mM ethanol in the first incubation stage did not modify paracetamol toxicity, however, when added in the second stage it prevented tissue injury to a significant extent. LDH leakage was reduced, K^+ losses were prevented and the water content remained within control limits.

Table 2 shows that when 1 mM pyrazole was added to the incubation medium as an inhibitor of alcohol dehydrogenase, the protective effect of ethanol against paracetamol injury was greatly reduced, when compared to groups treated with paracetamol alone or paracetamol with subsequent ethanol. Similar results were obtained when 20 mM pyruvate was included in the incubation medium along with ethanol. Rapid consumption of the NADH formed by alcohol dehydrogenase, for the reduction of pyruvate, is a likely reason for the blocking of the protective effect of ethanol by pyruvate.

Considering that the amount of NADH formed is dependent on the availability of NAD inside the cell we performed experiments to study the effect of increasing the nicotinamide content of the liver cells. The results indicate that nicotinamide itself did not modify paracetamol toxicity either when added at the beginning of the incubation or during the second stage of incubation. When slices were exposed to nicotinamide plus ethanol the protection afforded by ethanol was still present (see Table 3).

Table 1 shows the effect of dicoumarol, an inhibitor of DT-diaphorase, on the toxicity of paracetamol. As shown, the addition of 20 μM dicoumarol in the second stage of incubation led to a significant increase in damage as measured by LDH leakage, and by increase in water content and potassium loss in the paracetamol pretreated slices.

DISCUSSION

The experiments we have described show that paracetamol injury can be prevented, even when addition of the protective agent is delayed until the stage after paracetamol metabolism has ended. Our previous studies *in vivo* [16] and using rat liver slices [9] demonstrated that the addition of exogenous sulphur-containing amino acids increased the cellular GSH concentration thus providing protection in the first stage of paracetamol metabolism. However, methionine was not effective in the second stage of cell rundown in the slice system, even though GSH levels could still be raised [9].

Prevention of damage by free radical trapping agents added in the second stage of injury, is found for some but not all compounds with this property [9]. In the present study we have demonstrated protection with an electron acceptor DCPIP, and also with ethanol, suggesting that an alternative line of therapy can be based on a change in intracellular redox balance. Tee *et al.* [17] have shown similar effects with dithiothreitol and attribute the protection to reduction of oxidized protein sites, especially in the Ca^{2+} transport ATPases. Sato *et al.* [18] and Wong *et al.* [19] have shown protection against paracetamol toxicity by ethanol *in vivo*. If paracetamol injury to cell function is through oxidative attack on proteins by a quinone metabolite, the targets could well include mitochondrial, as well as other sites. The ability of both reducing agents such as dithiothreitol and NADH generators such as ethanol, and also radical trapping agents to block the injury sequence in the second stage suggests that the oxidative injury is not terminated when the slices or cells are taken away from the paracetamol, so there must be a residual process going on in the cells leading to further oxidative attack. The mitochondrial location of at least some key site of injury is suggested by the ability of ATP generating systems to protect (Mourelle and McLean, unpublished data submitted to *Biochem Pharmacol*; [20]).

We have examined how ethanol may influence paracetamol toxicity. Sato *et al.* [18] and also Wong *et al.* [19] suggest that ethanol protects because of decreased metabolism of paracetamol by the cytochrome P450 system. Our findings demonstrate that ethanol does not modify paracetamol toxicity to slices when added simultaneously with paracetamol, but does protect when added after the stage of paracetamol metabolism. We suggest that both *in vivo*, and in the slices *in vitro*, ethanol is likely to protect by altering electron flow in the late stage of cell injury. The known ability of ethanol to act as a free

Table 3. Effect of nicotinamide on LDH leakage, K^+ content and water content in liver slices incubated with 10 mM paracetamol

Incubation time 0–2 hr	Incubation time 2–6 hr	LDH leakage (%)	K^+ content (nmol/mg dry wt)	mg water/mg dry wt
Ringer	Ringer	4.1 ± 1.3	215 ± 24	2.9 ± 0.3
Paracet.	Ringer	29.9 ± 7.0*	125 ± 26*	3.9 ± 0.3*
Paracet. + nicotinamide	Nicotinamide	31.5 ± 14.8*	113 ± 17*	3.5 ± 0.9*
Paracet.	Ethanol	16.6 ± 5.1†	161 ± 16†	3.0 ± 0.2‡
Paracet. + nicotinamide	Ethanol + nicotinamide	10.6 ± 5.9	166 ± 25†	3.2 ± 0.3‡

Each value represents the mean ± SD of at least three separate experiments each performed in duplicate assays.

The final concentration of nicotinamide and paracetamol was 10 mM.

LDH leakage is expressed as the percentage of enzyme activity found in the medium at 6 hr in comparison with the amount originally present in the slice. K^+ content and water content of slices were measured as described in Materials and Methods.

* Statistically different from control values (Ringer), $P < 0.05$.

† Statistically different from paracet. + nicotinamide treated group, $P < 0.05$.

‡ Statistically different from paracet. treated group, $P < 0.05$.

radical trapping agent is observed only at concentrations far higher than those used here. Our findings point towards the increase in concentration of NADH and NADH/NAD ratio in the hepatocytes as the most probable explanation for the protection provided [21]. Pyruvate was able to block the protective effect possibly because it is able to consume the NADH as it is formed. Moreover, the inhibition of alcohol dehydrogenase with pyrazole clearly demonstrated that ethanol needs to be metabolized to exert its protective effect against paracetamol injury.

The redox dye DCPIP (redox potential +0.217 V) is able to accept electrons from the flavoprotein step between NADH and ubiquinone. The dye can in turn reduce other substances including O_2, but possibly including electron carriers downstream from ubiquinone in the mitochondria [22, 23]. It may well be able to bypass a block in mitochondrial electron flow or alternatively be able to reduce an oxidized essential protein, or even NAPQI. Phenazine methosulfate, another redox dye with a very different redox potential (+0.08 V) which is also able to accept electrons from flavoproteins and able to reduce O_2 to superoxide, does not protect. Presumably its redox potential or intracellular pharmacokinetics are such that it could neither provide a useful bypass to block electron flow, allowing ATP generation, nor act as a reducing agent for oxidized macromolecules or quinones.

These results pose the further question "by what mechanism could changes of electron flow through DCPIP or ethanol administration alter paracetamol toxicity?"

It is generally accepted that toxicity is initiated by the intracellular damage caused by the short lived quinone metabolite, NAPQI. NAPQI is formed at physiological pH and temperature when hydroxy acetaminophen loses 1 mole of H_2O [6]. It is well known that NAPQI is detoxified by the reaction with GSH but when the system is overwhelmed, free NAPQI remains and binds to macromolecules, or causes oxidation of essential sites [17]. The cytoxicity of free, or even protein bound NAPQI could well be dependent upon its metabolism via one electron reduction which can occur in the presence of flavoproteins, followed by re-oxidation. This redox cycle reaction would then be responsible for the formation of a semiquinone radical. Several semiquinone radicals are toxic *per se* others also reduce O_2 to form superoxide anion radical ($\cdot O_2^-$) with a consequent formation of reactive reduced oxygen molecules (H_2O_2) and ($\cdot OH$). The oxidizing radicals formed outside the normal, well regulated, oxidative pathways of the mitochondria can then cause injury to the next tier of cell defences such as Ca^{2+} regulation, and mitochondrial ATP generation.

Only traces of NAPQI could remain in the cells after transfer of slices to fresh medium without any paracetamol. We have previously shown that the second stage is not accompanied by any change in the amount of paracetamol bound to macromolecules [13]. Any postulated mechanism of toxicity in the second stage of injury that is put in terms of redox cycling would have to involve NAPQI already bound to cell macromolecules or some redox cycling substance generated in the cells.

A detoxication pathway can be that NAPQI undergoes $2e^-$ transfer, catalysed by DT-diaphorase [24], with the formation of a hydroquinone. Such a stable hydroquinone could then remain harmless in the cell or be further conjugated. If DT-diaphorase is part of a detoxication mechanism then its inhibitor, dicoumarol should make the injury worse. As predicted, when dicoumarol was added in the second stage of incubation, toxicity was greatly increased. It seems possible that NAPQI remains in the cells, perhaps bound to proteins, and there catalyses a destructive redox cycle until detoxified by reduction to the hydroquinone.

Our results strongly suggest that by increasing the flow of reducing equivalents it is possible to prevent liver damage during the late stages of exposure to

paracetamol. This would fit in with the recent work of Tee *et al.* [17]. However, it seems unlikely that the radical trapping agents like DPPD act similarly as reducing agents, and makes us think that the oxidative damage continues after cessation of paracetamol metabolism.

While the early stage of generation of reactive metabolite presumably NAPQI is understood, the lesions caused by release of the quinone inside the cell are not. It may well be that several points of cell function are injured (e.g. mitochondrial electron flow, endoplasmic reticulum ATPase calcium pump). In order to prevent breakdown of the cell after attack by NAPQI we may have to protect the several sites of injury which have been indicated by the different *in vitro* models. It may be for this reason that no convincing *in vivo* protection has been found so far using single substances given after the stage of paracetamol metabolism is over but before the liver cells have died. Not only pharmacokinetic considerations but also the need for protection of several different "points" in the cell have to be considered.

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REFERENCES

1. Clark R, Thompson RPH, Goulding R and Williams R, Hepatic damage and death from overdose of paracetamol. *Lancet* i: 66–70, 1973.
2. Davis DC, Potter WZ, Jollow DJ and Mitchell JR, Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sci* **14**: 2099–2109, 1974.
3. Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillete JR and Brodie BB, Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J Pharmacol Exp Ther* **187**: 211–217, 1973.
4. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR and Brodie BB, Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *J Pharmacol Exp Ther* **187**: 211–217, 1973.
5. McLean AEM and McLean EK, The effect of diet and DDT on microsomal hydroxylation and on sensitivity of rats to CCl_4 poisoning. *Biochem J* **100**: 564–571, 1966.
6. Rosen GM, Singletary WV, Rauckman EJ and Killenberg PG, Acetaminophen hepatotoxicity, an alternative mechanism. *Biochem Pharmacol* **32**: 2053–2059, 1983.
7. Powis G and Appel PL, Relationship of the single electron reduction potential of quinones to their reduction by flavoproteins. *Biochem Pharmacol* **27**: 2567–2572, 1980.
8. Prescott LF, Wright N, Roscoe P and Brown SS, Plasma paracetamol half-life and hepatic necrosis in patients with paracetamol overdosage. *Lancet* i: 519–525, 1971.
9. McLean AEM and Nutall L, An *in vitro* model of injury using paracetamol treatment of liver slices and prevention of injury by some antioxidants. *Biochem Pharmacol* **27**: 425–430, 1978.
10. Vale JA, Meredith TJ and Goulding R, Treatment of acetaminophen poisoning, use of oral methionine. *Arch Int Medicine* **141**: 394–396, 1981.
11. Prescott LF, Clinical features and management of analgesic poisoning. *Human Toxicol* **3**: 75S–84S, 1984.
12. Beales D, Hue DP and McLean AEM, Lipid peroxidation, protein synthesis and protection by calcium EDTA in paracetamol injury to isolated hepatocytes. *Biochem Pharmacol* **34**: 19–23, 1985.
13. Devalia JD, Ogilvie RC and McLean AEM, Dissociation of cell death from covalent binding of paracetamol by flavones in a hepatocyte system. *Biochem Pharmacol* **31**: 3745–3749, 1982.
14. Hue DP, Beales D, Griffith K and McLean AEM, Permeability of isolated hepatocytes and impermeability of cultured hepatocytes and liver slices to EDTA. *Food Chem Toxicol* **24**: 801–802, 1986.
15. Amador E, Dorfman LE and Wacker WEC, Serum lactic dehydrogenase activity: an analytical assessment of current assays. *Clin Chem* **9**: 391–399, 1963.
16. McLean AEM and Day P, The effect of diet on the toxicity of paracetamol and the safety of paracetamol-methionine mixtures. *Biochem Pharmacol* **24**: 37–42, 1975.
17. Tee LBG, Boobis AR, Huggett AC and Davis DS, Reversal of acetaminophen toxicity in isolated hamster hepatocytes by dithiothreitol. *Toxicol Appl Pharmacol* **83**: 294–314, 1986.
18. Sato Ch, Nakano M and Lieber CS, Prevention of acetaminophen-induced hepatotoxicity by acute ethanol administration in the rat: comparison with carbon tetrachloride-induced hepatotoxicity. *J Pharmacol Exp Ther* **218**: 805–810, 1981.
19. Wong LT, Whitehouse LW, Solomonraj G and Paul CT, Effect of a concomitant single dose of ethanol on the hepatotoxicity and metabolism of acetaminophen in mice. *Toxicology* **17**: 297–309, 1980.
20. Nieminen A, Dawson TL, Gores GJ, Kawanishi T, Herman B and Lemasters J, Protection by acidotic pH and fructose against lethal injury to rat hepatocytes from mitochondrial inhibitors, ionophores and oxidant chemicals. *Biochem Biophys Res Commun* **167**: 600–606, 1990.
21. Lieber CS, Metabolism and metabolic effects of alcohol. *Med Clin North Am* **68**: 3–31, 1984.
22. Hochster RM, Morris K and Quastel JH (Eds.), *Metabolic Inhibitors*, Vol. IV, pp. 197. Academic Press, New York, 1973.
23. Lehninger AL, Oxidation reduction enzymes and electron transport. In: *Biochemistry* (Ed. Lehninger AL), pp. 477–508. Worth Publishers, New York, 1975.
24. Ernster L, Danielson L and Ljunggren M, DT-diaphorase. *Biochim Biophys Acta* **58**: 171–188, 1962.