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Is Activation of Lysosomal Enzymes Responsible for Paracetamol-induced Hepatotoxicity and Nephrotoxicity?

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Abstract

Paracetamol overdose (300 mg kg^{-1}) in mice resulted in a time-dependent increase in the liver weight; no change was seen for the kidney. The total acid phosphatase activities in the two tissues increased significantly 0·5 h after paracetamol overdose and remained elevated up to 3 h. Free as well as total cathepsin D activities increased significantly in both the tissues within 2–2·5 h of paracetamol treatment. Simultaneously tyrosine positive materials in the two tissues increased. RNAse II and DNAse II activities were low in liver and kidneys of the controls. Paracetamol treatment elevated both free and total RNAse II activity in the two tissues by 0·5 h. Maximum activity of DNAse II (free and total) was seen at 2·5 h after paracetamol administration.

The results suggest that concerted action of cathepsin D, RNAse II and DNAse II may be responsible for paracetamol-induced hepatotoxicity and nephrotoxicity.

Paracetamol (*N*-acetyl-*p*-aminophenol), also known as acetaminophen is an analgesic and antipyretic agent that is usually safe in therapeutic doses. However, upon overdose, the drug is hepatotoxic and nephrotoxic in man and in experimental animals (Kleinman et al 1980; Meyers et al 1988; Vermeulen et al 1992). Hepatic damage is generally thought to be initiated by the reactive metabolite *N*-acetyl-*p*-benzoquinonimine (NAPQI), which is believed to bind covalently to the cellular proteins (Vermeulen et al 1992). However, whether this is the major mechanism responsible for tissue damage has been debated and it is suggested that covalent binding to mitochondrial glutathione (GSH) and proteins might be a particularly important event in the induction of cytotoxicity by paracetamol (Vermeulen et al 1992). Mitochondrial dysfunction in paracetamol-induced hepatotoxicity has been well documented (Katyare & Satav 1989; Burcham & Harman 1990; Parmar et al 1995). The cytotoxic effects of paracetamol have also been attributed to alterations in intracellular Ca^{2+} homeostasis and activation of Ca^{2+} endonuclease in the nucleus (Shen et al 1992). The cytotoxic effects of paracetamol have been examined at the level of cellular membrane systems such as mitochondria, nuclei, plasma membranes and microsomes (Ginsberg & Cohen 1985; Tirmenstein & Nelson 1989; Vermeulen et al 1992). However, no work has been carried out to investigate the effects of paracetamol on the lysosomal membrane system or enzymes. The lysosomal enzymes increase under pathological conditions (Szego & Piefras 1984); hence it is possible that cytotoxic effects of paracetamol leading to necrosis and cell death could also be mediated by the action of lysosomal hydrolytic enzymes.

We have therefore investigated the effects of paracetamol overdose on lysosomal enzyme activities in mouse liver and kidneys, the two major tissues susceptible to paracetamol toxicity.

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Materials and Methods

Chemicals

Paracetamol was purchased from Aldrich. Yeast RNA, calf thymus DNA and bovine serum albumin fraction V were from Sigma Chemical Co. Sodium β-glycerophosphate was from Mallinkrodt, haemoglobin from British Drug Houses, Poole, Dorset, UK, and *p*-nitrophenyl-phosphate (PNPP) from SRL, India. All other chemicals were of analytical-reagent grade purchased locally.

Animals and treatment with paracetamol

Male albino mice (Swiss strain), 30–35 g, were fasted overnight and injected intraperitoneally with a saturated solution of paracetamol (35 mg mL^{-1}) in warm (45–50°C) saline at a dose of 300 mg kg^{-1} (Meyers et al 1988). The animals were killed at the end of 0·5, 1, 2, 2·5 h or 3 h after paracetamol injection. The controls received only warm saline and represent the 0 h group.

Enzyme assays

The animals were killed by decapitation and their livers and kidneys were quickly removed and placed in beakers containing chilled (0–4°C) 0·25 M sucrose. Ten percent (w/v) tissue homogenates were prepared after removal of gall bladder and medulla respectively from liver and kidneys.

β-Glycerophosphatase, *p*-nitrophenylphosphatase (PNPPase), cathepsin D, ribonuclease II (RNAse II) and deoxyribonuclease II (DNAse II) activities were measured in the whole homogenate within 15 min to obtain the free activities. The homogenates were diluted 1 : 5 times and subjected to three cycles of freezing and thawing in the presence of 0·1% Triton X-100 and used for measurement of total enzyme activities (Barret & Heath 1977).

Tyrosine-positive materials in the tissue were determined after deproteinization with cold (0–4°) trichloroacetic acid, as described earlier (Satav & Katyare 1981).

Results

Paracetamol treatment caused significant increase in liver weight which was evident as early as 0·5 h (+30%) following drug administration; at the end of 3 h, the liver weight had increased by 70%. The kidney weights were not significantly changed (Table 1).

The patterns for acid phosphatase activities measured with either β-glycerophosphate or *p*-nitrophenylphosphate as substrate were comparable. Thus in the liver, the free activity was generally unaltered but the total activity increased substantially, (3–4-fold) 1 h post paracetamol injection and decreased thereafter with time. The same trend was also observed for the kidneys (Fig. 1).

In the controls, the free cathepsin D activity in the liver was negligible and the total activity was marginal. The free as well as total activities increased maximally at 2 h and declined thereafter; increase in total activity was discernible as early as 0·5 h. Compared with liver, the kidneys were characterized by higher basal level of cathepsin D activity. Nevertheless, this activity increased significantly at 1–2 h following paracetamol treatment and then declined (Fig. 2). Interestingly, the tyrosine-positive materials in the two tissues increased (Table 2).

The RNAse II activity was low in both the tissues from controls but a significant rise in both free as well as total activity was noted at 0·5 h following paracetamol intoxication. The free and total activities declined with time but were always higher than in the controls (Fig. 3). The DNAse II activities (free and total) were undetectable up to 1 h after paracetamol treatment; both the activities increased substantially 2·5 h after drug treatment (Fig. 3).

Discussion

The results of the present studies have shown that overdose of paracetamol in mice caused increased activities of all the lysosomal enzymes studied, although the time at which peak activity was seen was not the same for all the enzymes. This may suggest that paracetamol toxicity specifically induced the synthesis of the lysosomal enzymes. Interesting to note is the increase in the free cathepsin D activity in both tissues; especially for the liver the free and total activities were

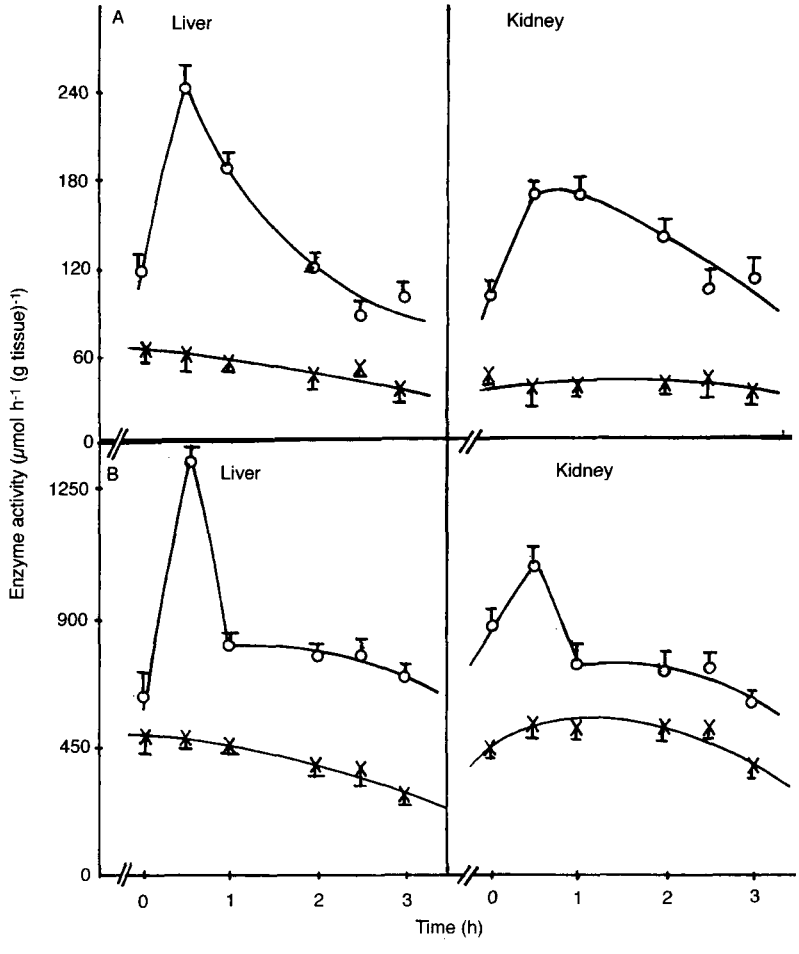

FIG. 1. Paracetamol-induced alteration in β-glycerophosphatase (A) and *p*-nitrophenylphosphatase (B) activities in mouse liver and kidney. Each point represents mean ± s.e.m. of 6 independent observations. ×, Free activity; ○, total activity.

Table 1. Effect of paracetamol treatment on liver and kidney weight.

Time (h)	Body weight	Liver weight		Kidney weight	
	(g)	(g)	% body weight	(g)	% body weight
0	32·3 ± 1·7	1·38 ± 0·07	4·30 ± 0·17	0·32 ± 0·04	0·96 ± 0·08
0·5	31·6 ± 2·1	1·80 ± 0·14[a]	5·72 ± 0·40[c]	0·39 ± 0·03	1·25 ± 0·13
1	32·0 ± 2·0	1·72 ± 0·10[b]	5·38 ± 0·31[b]	0·32 ± 0·05	1·00 ± 0·04
2	31·7 ± 1·9	1·91 ± 0·09[d]	6·01 ± 0·32[d]	0·35 ± 0·03	1·10 ± 0·03
2·5	31·8 ± 2·8	2·06 ± 0·05[d]	6·44 ± 0·42[d]	0·36 ± 0·03	1·12 ± 0·09
3	30·8 ± 0·8	2·32 ± 0·02[d]	7·51 ± 0·07[d]	0·34 ± 0·04	1·10 ± 0·06

Values are expressed as the mean ± s.e.m. of 6 independent observations in each group. [a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$, [d] $P < 0.001$ compared with control.

Table 2. Effect of paracetamol treatment on the free amino acid pool in liver and kidney.

Time (h)	Tyrosine positive material (μmol g^{-1})	
	Liver	Kidney
0	21·9 ± 0·9	13·2 ± 0·2
0·5	35·6 ± 1·8[a]	39·2 ± 1·6[a]
1	33·1 ± 0·9[a]	47·4 ± 1·1[a]
2	28·2 ± 1·0[a]	53·8 ± 1·6[a]
2·5	22·3 ± 0·9	64·5 ± 0·6[a]
3	24·7 ± 1·4	44·1 ± 3·3[a]

Values are expressed as the mean ± s.e.m. of 6 independent observations in each group. [a] $P < 0.001$ compared with control.

almost comparable at 2 h. For RNAse II also the free activities increased substantially in both tissues and remained elevated. The DNAse II activities which were negligible in controls increased several fold in liver and kidney. Thus it would seem that the lysosomal membrane became selectively permeable to cathepsin D, RNAse II and DNAse II. Increased cathepsin D activity can indiscriminately bring about protein degradation. The eventual decrease in all the enzyme activities with time (Figs 1–3) may be attributed to this factor. Indeed, we found that the tyrosine-positive materials increased significantly in the paracetamol treated mice (Table 2).

The early increase in the free RNAse II activity at 0·5 h may be significant in pathophysiology of paracetamol action since it can interfere with the protein-synthesizing activity by indiscriminately degrading RNAs. The increase in DNAse II activity was a relatively late event. However it is noteworthy that the free enzyme activity increased very substantially especially in the liver. Increased DNAse II free activity can lead to indiscriminate DNA strand breaks and fragmentation which can ultimately lead to cell necrosis and cell death. Indeed, we have seen that the mortality rate became very high beyond 3 h after paracetamol intoxication. Taken together, the results would thus imply that indiscriminate protein, RNA and DNA breakdown by lysosomal enzymes may play a crucial role in pathogenesis of paracetamol action. In this connection it is important to note that hepatic failure rather than kidney failure is the major cause of death in paracetamol overdose (Vermeulen et al 1992).

The role of Ca^{2+} endonuclease in DNA fragmentation and cell death has been inferred from studies on mouse hepatocyte cultures (Shen et al 1992). It is possible that in their studies these authors were measuring the lysosomal DNAse II, which we report here.

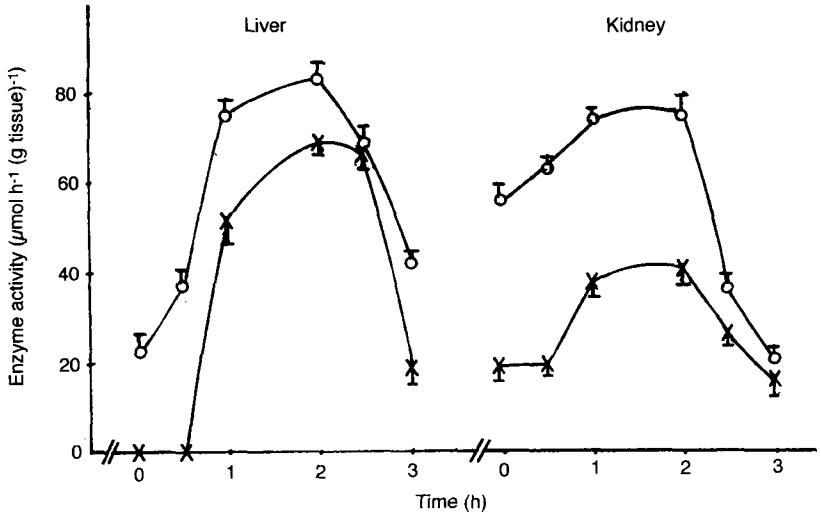

FIG. 2. Paracetamol-induced alteration in cathepsin D activity in mouse liver and kidney. Each point represents mean ± s.e.m. of 6 independent observations. ×, Free activity; ○, total activity.

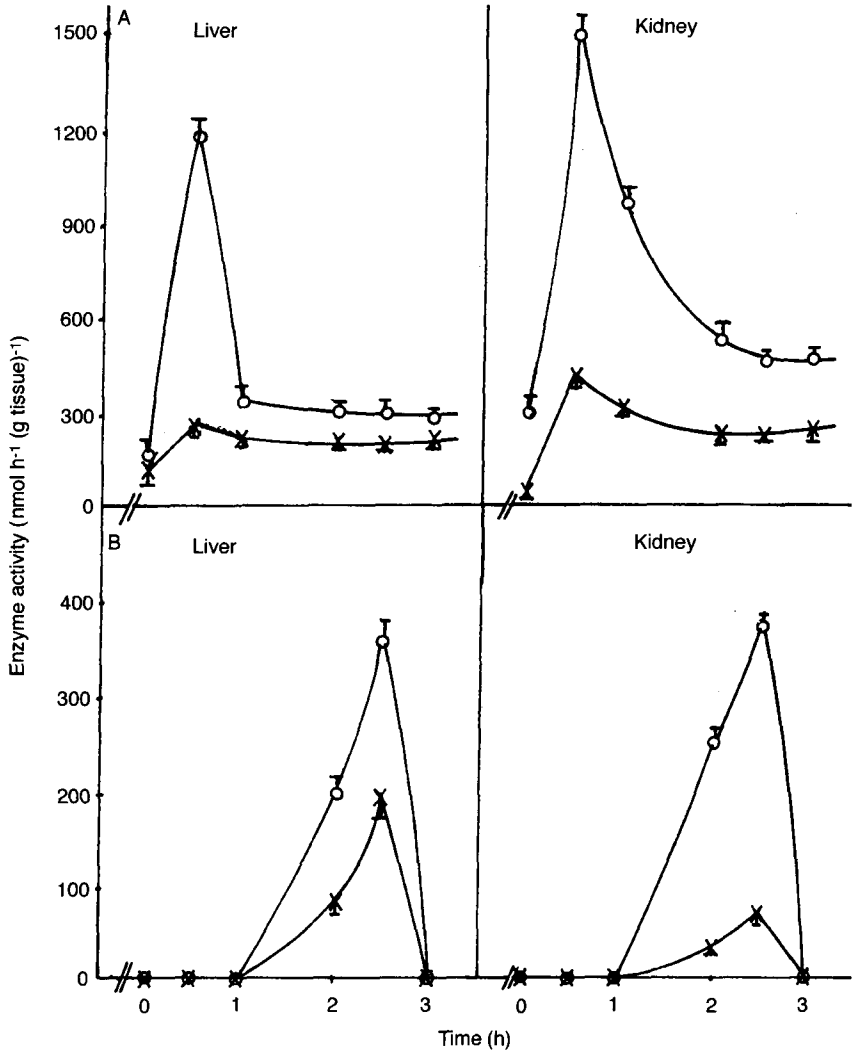

FIG. 3. Paracetamol-induced alteration in A. RNAse II and B. DNAse II activities in mouse liver and kidney. Each point represents mean ± s.e.m. of 6 independent observations. ×, Free activity, ○, total activity.

In conclusion we suggest that concerted action of lysosomal free cathepsin D, RNAse II and DNAse II may be responsible for cell necrosis and hepatic failure following paracetamol overdose.

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