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# RAPID COMMUNICATIONS

## PEROXISOME PROLIFERATORS PROTECT AGAINST PARACETAMOL HEPATOTOXICITY IN MICE

Felicity A. Nicholls-Grzemski, Ian C. Calder[1] and Brian G. Priestly

Department of Clinical and Experimental Pharmacology, University of Adelaide, Adelaide, 5001, South Australia, and [1]South Australian Health Commission, Adelaide, 5000, South Australia, Australia

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The hepatotoxicity of paracetamol is thought to be mediated by an electrophilic intermediate N-acetyl-p-benzoquinone imine (NAPQI). Conjugation of NAPQI with reduced glutathione (GSH) is a primary detoxication mechanism, but it is possible that other cellular antioxidants may play a role, by scavenging oxyradicals produced by a NAPQI-related redox cycling reaction. Peroxisome proliferation is a condition in which excess oxyradical formation occurs when the $H_2O_2$-producing enzymes involved in fatty acid ß-oxidation are markedly induced while the $H_2O_2$-degrading enzymes are either increased marginally (catalase) or reduced in activity (GSH peroxidase) [1].

The purpose of this study was to determine whether a change in oxidant status associated with hepatic peroxisome proliferation in mice could alter susceptibility to paracetamol-induced hepatotoxicity.

### MATERIALS AND METHODS

Mice (Swiss white male, 25-35g) were randomly assigned into groups receiving daily intraperitoneal injection of either 0.2ml of olive oil (vehicle), 500mg/kg clofibrate, 2g/kg diethylhexylphthalate (DEHP) or 100mg/kg 2-(2,4,5-trichlorophenoxy) propionic acid (Silvex). Treatment was maintained for 10 days. On day 11, mice were injected with paracetamol or saline and liver and plasma were collected 24 hours later. Alanine aminotransferase, sorbitol dehydrogenase and lactate dehydrogenase levels were determined by the commercially available Sigma kits. Reduced glutathione [2], palmitoyl Co-A oxidase [3] and catalase [4] were determined by literature methods.

### RESULTS AND DISCUSSION

Treatment of mice with clofibrate, DEHP and Silvex all produced hepatic peroxisome proliferation as shown by increases in liver weight and the activity of palmitoyl CoA oxidase and catalase, although there were no changes in hepatic GSH levels (Table1).

| | Liver Wt% Body Wt | P CoA *mmolNADH/min/mg* | Catalase *k/mg* | Glutathione *(umol/g liver)* |
|---|---|---|---|---|
| Control | 5.30 ±0.34 | 0.146.± 0.02 | 5.03 ± 1.04 | 6.59 ± 0.35 |
| Clofibrate | 6.92 ±0.46 | 0.527 ± 0.14 | 10.14 ± 1.92 | 7.50 ± 0.47 |
| DEHP | 5.80 ±0.30 | 0.178 ± 0.02 | 6.12 ± 0.90 | 7.27 ± 0.77 |
| Silvex | 6.60 ±0.23 | 0.390 ± 0.07 | 8.21 ± 3.05 | 6.73 ± 0.76 |

Table1: Effect of peroxisome proliferators on liver weight, hepatic palmitoyl CoA oxidase (PCoA), catalase and glutathione levels. These mice received saline vehicle, but not paracetamol (n = 3 or 4, mean ± sem).

Paracetamol produced hepatotoxicity, as demonstrated by increases in the activities of plasma SDH, LDH and ALAT (Figure 1) and the appearance of extensive necrosis. Protection against paracetamol hepatotoxicity was consistently observed with all three peroxisome proliferators and for clofibrate, was demonstrated across a range of doses (Table 2).

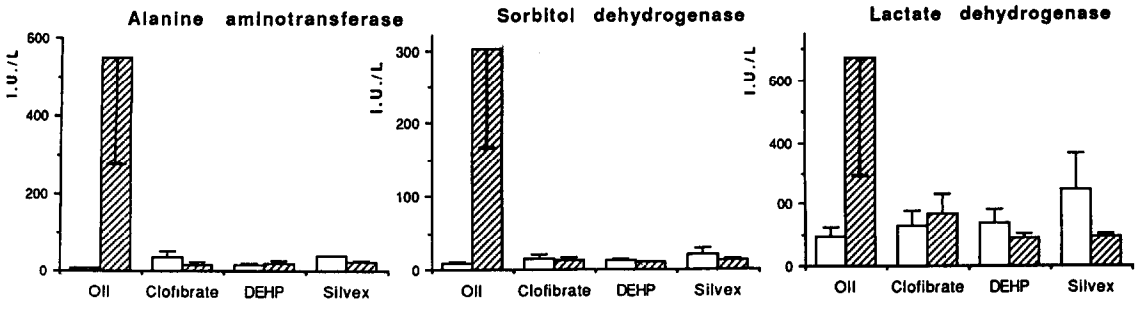


Figure 1: Effect of peroxisome proliferator pretreatment on plasma enzyme activities following 250mg/kg paracetamol dose 24 hours earlier (saline: open and paracetamol: cross hatched columns n = 3-5, mean ± sem).

| Paracetamol dose | 0 mg/kg | 200mg/kg | 300mg/kg | 400 mg/kg | 500 mg/kg |
|---|---|---|---|---|---|
| Oil | 33.4 ± 3.2 | 37.3 ± 8.5 | 324 ± 164 | 1338 ± 1120 | 1035 ± 542 |
| Clofibrate | 20.6 ± 1.6 | 22.4 ± 3.6 | 27.7 ± 7.5 | 31.8 ±12.8 | 18.5 ± 4.4 |

Table 2: Paracetamol dose related hepatotoxicity (plasma ALAT activity (I.U./L) n = 3-5, mean ± sem) and the effect of clofibrate.

The basal levels of hepatic GSH were unchanged by peroxisome proliferation in this study. However, paracetamol induced hepatic GSH depletion was less while paracetamol-thiol conjugate urinary output was unchanged (Nicholls-Grzemski et al - unpublished observations). Nafenopin has been reported to alter antioxidant status, increasing plasma oxidised glutathione, reducing hepatic Vitamin E, but leaving hepatic ascorbate unchanged [1]. These observations suggest that peroxisome proliferation has had some, as yet undetermined, effect on GSH metabolism.

Peroxisome proliferation is postulated to produce oxidative stress, and might thereby be expected to increase susceptibility to paracetamol hepatotoxicity. The unexpected protective effect adds another dimension to the already controversial nature of peroxisome proliferation. While the mechanism remains to be determined, there are some reports in the literature which are consistent with a cytoprotective effect associated with peroxisome proliferation. For example, nafenopin treatment has been reported to protect against oxidant stress-related iron induced uroporphyria in mice [5]. Furthermore, peroxisome proliferators bind to HSP-70 [6], a member of the heat shock protein family, which have been implicated as having a protective role in oxidative cell damage [7]. Further work in hand on the relationship between peroxisome proliferation, hepatic GSH, heat shock proteins and other cellular antioxidants may provide a better understanding of the protective effects and the general phenomena of peroxisome proliferation.

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