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Protective effects of propylene glycol, a solvent used pharmaceutically, against paracetamol-induced liver injury in mice

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The most widely used antidote against paracetamol-induced liver injury is *N*-acetylcysteine [1], but many other compounds, such as alternative sulphydryl compounds and antioxidants, have been evaluated as potential protective agents [2, 3]. In this study we report the unexpected beneficial effects in mice of a solvent frequently used as a vehicle for water-insoluble compounds and pharmaceutical preparations.

Materials and Methods

Male C57 BL6 mice (19–23 g body wt), obtained from Charles River (Margate, Kent), were fed a standard diet (SDS R&M No. 1, Scientific Diet Service, Witham, Essex) up to 4 hr before the experiment. Access to drinking water only was then allowed until 1 hr after administration of paracetamol, when food was reinstated. Paracetamol (Sigma Chemical Co., Poole, Dorset) was dissolved in warm sterile water (25 mg/mL) by mixing on a heated stirrer and administered to the mice at a dose of 450 mg/kg by stomach tube. Propylene glycol BP (1,2-propanediol, Macarthy Medical, Romford, Essex) was administered at a dose of 4 mL/kg i.p.

Three groups of mice were studied: Group 1, mice were given paracetamol alone; Group 2, mice were given paracetamol and propylene glycol at the same time, i.e. 0 hr; Group 3, mice were given paracetamol followed by propylene glycol 3 hr later. Seven normal mice, without any treatment were used to obtain control samples.

Samples and assays. After 24 hr the mice were anaesthetized with ether and a capillary blood sample (10 μL) was collected from the tail vein. The Normotest blood clotting time (Nyegaard, Birmingham) was determined using a Fibrometer (Becton and Dickinson). The abdomen was opened and a blood sample was taken from the inferior vena cava with a heparinized syringe. The blood was kept on ice, centrifuged at 4° and the plasma

stored at −20°. After cervical dislocation the liver was removed, weighed and the macroscopic appearance examined to grade the liver on an arbitrary scale of 0 to 5 (0 = normal, 5 = massive haemorrhagic necrosis involving all liver lobes).

Plasma aspartate aminotransferase was determined using a Sigma UV assay kit in an LKB Ultrospec 4050 Spectrophotometer. Plasma glucose was determined using an IL 919 Analyser (Instrumentation Laboratories) in the microsample mode.

Administration of [^{14}C]*paracetamol.* Paracetamol labelled in the benzene ring with ^{14}C (>95% pure by thin layer chromatography, sp. act. 17.85 μCi/mmol) was added to the paracetamol dose solution. Mice were given paracetamol either alone or together with propylene glycol as described above. Capillary blood samples (10 μL) were obtained from the tail after 30 and 60 min. At 2 hr blood and the liver were removed as above. The blood samples were added directly to OptiPhase Safe scintillation fluid (Pharmacia LKB) in a minivial and counted in a Packard 460CD liquid scintillation counter. Duplicate samples of liver (100 mg) were solubilized in Soluene-100 (1 mL, Packard Instruments) 15 mL of scintillation fluid added and the samples counted.

Results are expressed as median with range, the Mann–Whitney test was used to detect significant differences and the Spearman Rank test for correlation.

Results

The dose of paracetamol used (450 mg/kg) caused severe liver injury as assessed by elevations in the Normotest blood clotting time and plasma aspartate aminotransferase, visual appearance of the liver and high mortality of the animals.

Propylene glycol administered at the same time as paracetamol and after 3 hr significantly reduced the increase in blood Normotest at 24 hr (median 29.6 sec, range 25.1 to 45.6, $N = 10$, $P < 0.01$) and (33.7 sec, range 29.6 to 45.6, $N = 10$, $P < 0.05$), respectively, as compared to paracetamol alone (46.1 sec, range 29.4 to 75.0, $N = 11$; normal range 22.6 to 27.6 sec; Fig. 1). No blood samples were available in mice which died within 24 hr. Significant protective effects were seen on the plasma aspartate aminotransferase activity at 24 hr when propylene glycol was given at the same time as paracetamol (median 58 I.U./L, range 13 to 86, $P < 0.01$) compared to paracetamol alone (3598 I.U./L, range 849 to 5719; normal range 0 to 38 I.U./L; Fig. 2). Significant but less marked protection was observed when propylene glycol administration was delayed by 3 hr (993 I.U./L, range 467 to 2301, $P < 0.01$). Propylene glycol prevented the reduction in blood glucose concentration at 24 hr due to paracetamol injury when given at 0 hr (median 9.9 mmol/L, range 7.1 to 12.1, $P < 0.01$) but not when delayed by 3 hr. The concentrations in this group (7.7 mmol/L, range 4.6 to 10.2) were similar to paracetamol alone (7.9 mmol/L, range 5.6 to 9.8; normal range 9.4 to 14.5 mmol/L). Propylene glycol was also given to four mice without paracetamol and the blood Normotest, plasma aspartate aminotransferase and glucose all remained within the normal range.

On macroscopic examination of the liver the median score of liver damage after paracetamol was 3 (range 1.5 to 5) and this was almost totally abolished by simultaneous administration of propylene glycol (median 0, range 0 to 0.5, $P < 0.01$). A less beneficial effect was observed when propylene glycol was delayed for 3 hr (1.25, range 0.5 to 2.5, $P < 0.01$). There was a significant correlation between the liver damage score and the plasma aspartate aminotransferase values ($r = 0.8068$, $P < 0.001$; $N = 51$) in mice given paracetamol. Median liver weight was not significantly affected by paracetamol administration in those mice surviving for 24 hr (0.98 g, range 0.73 to 1.19) as compared to normal mice (1.12 g, range 0.90 to 1.18). However, as a result of the haemorrhagic necrosis there was considerable pooling of blood in the liver with paracetamol-induced liver injury and this contributed to the liver weight. Liver weight was significantly lower than normal in mice given paracetamol followed by propylene glycol at 0 hr (0.86 g, range 0.69 to 1.17, $P < 0.01$) and at 3 hr (0.92 g, range 0.81 to 1.31, $P < 0.05$), with no visible pooling of blood in the liver. Similar effects were found when the liver to body weight ratios were considered. Mortality, in keeping with the apparent protective effects, was considerably reduced in both groups given propylene glycol and paracetamol (0/10, 0%) as compared to paracetamol alone (8/19, 42%).

There was no significant difference in the concentration of paracetamol in blood, as determined from the total ^{14}C count which would include paracetamol metabolites, in samples taken from mice 30 min after paracetamol administration with (median 1133 μmol/L, range 985 to 1164) and without propylene glycol (779 μmol/L, range

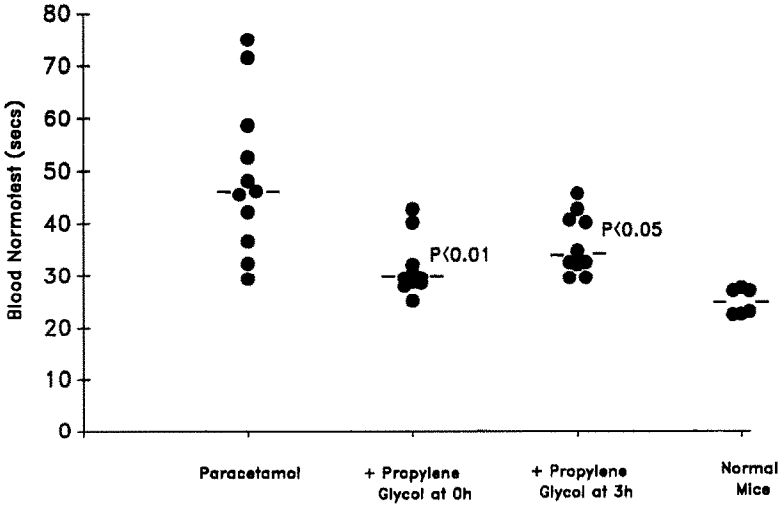

Fig. 1. The effect of propylene glycol on Normotest blood clotting time in mice at 24 hr after paracetamol administration. The horizontal bars correspond to the median value. The significance level is in comparison to paracetamol alone.

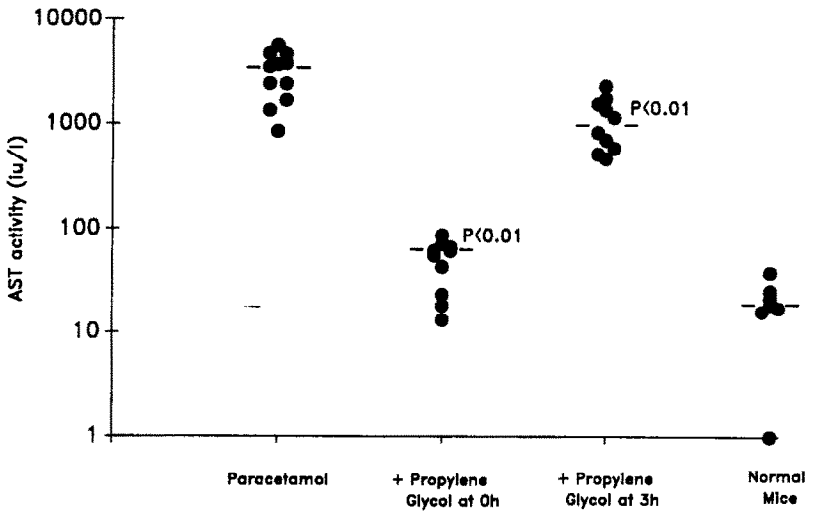

Fig. 2. The effect of propylene glycol on plasma aspartate aminotransferase (AST) activity in mice at 24 hr after paracetamol administration. The horizontal bars correspond to the median value. The significance level is in comparison to paracetamol alone.

657 to 1429) and at 60 min (1123 μmol/L, range 1047 to 1217 and 957 μmol/L, range 736 to 1289, respectively). At 2 hr the concentration of paracetamol was significantly greater in mice which had received paracetamol and propylene glycol (718 μmol/L, range 565 to 753) compared with mice given paracetamol alone (524 μmol/L, range 290 to 624, $P < 0.05$). There was no significant difference in the total amount of radioactive paracetamol in the livers of mice given paracetamol plus propylene glycol (1.14 μmol/g, range 0.96 to 1.31) compared with those given paracetamol alone (1.22 μmol/g, range 0.90 to 1.49).

Discussion

In these experiments propylene glycol has been shown to prevent the development of severe liver injury when given up to 3 hr after the administered dose of paracetamol. The underlying mechanism of this protection is not known, but there are several possible explanations. The absorption of paracetamol from the gut is unlikely to have been affected as the propylene glycol was given by a different route to the paracetamol and this was confirmed in the experiments with radioactive paracetamol. Blood levels of paracetamol were similar in the presence of propylene glycol over the first hour and were slightly higher at 2 hr, although the liver levels were not significantly different at this time. Thus the effects of propylene glycol on the hepatotoxicity of paracetamol would appear to be in the liver. As propylene glycol is a diol, it may have similar properties to ethanol which when given at the same time as paracetamol prevents formation of the toxic metabolite of paracetamol (*N*-acetyl-*p*-benzoquinoneimine) by inhibition of the hepatic cytochrome P450-dependent mixed-function oxidase system [4, 5]. If propylene glycol does have an inhibitory effect on hepatic cytochrome P450-dependent oxidation, then there are considerable implications for the use of propylene glycol as a solvent for compounds in studies of drug metabolism. The metabolism of propylene glycol to lactate and pyruvate, which are good substrates for glycogen sythesis, as well as for energy forming reactions in the liver, may also help explain the beneficial effects observed [6]. Administration of propylene glycol at the same time as paracetamol prevented the decrease in blood glucose seen after paracetamol alone. However, it is not possible to determine whether this was due to the glycogenic effects of the metabolites of propylene glycol or a consequence of the reduction in liver damage. Similar effects were not seen, however, when propylene glycol was administered at 3 hr. Finally propylene glycol might act as a free radical scavenger in the same way as mannitol [7], a longer chain hydroxyl compound, preventing lipid peroxidation by the reactive metabolite of paracetamol [8].

The dose used in the experiments (4 mL/kg) was based on the volume used (80 μL injected per mouse) to dissolve a potential protective agent under assessment. This would represent a considerable dose for a human, although propylene glycol is reported to have a low toxicity in animals [6] and there was no overt evidence of any adverse effects in the mice at this dose. The efficacy of lower doses was not studied nor were different routes of administration.

This study has shown protective effects of propylene glycol against paracetamol-induced liver injury in mice. Further experiments are required to elucidate the mechanisms involved, which may have wider implications on the use of propylene glycol as a solvent for experimental studies.

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