Paracetamol and conventional antimalarial drugs induced hepatotoxicity and its protection by methionine in rats

Ervilla E Dass* & K K Shah
Department of Pharmacology, Medical College, The Maharaja Sayajirao University of Baroda, Vadodara 390 001, India

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Hepatotoxicity, induced in rats, by treatment with high doses of paracetamol and chloroquine was confirmed by estimating blood transaminase levels. Hepatoprotective effect was determined by administering combination of methionine (10% of paracetamol /chloroquine, p o) and hepatotoxic drugs quinine. The results were confirmed by histopathological examination of liver. Paracetamol (7g / kg) and chloroquine (970 mg/kg) administration increased significantly the transaminase levels. Methionine alone did not produced any change. Hepatonecrosis induced by paracetamol, chloroquine alone and their combinations and its protection with methionine was revealed by histopathological study whereas the combination of paracetamol and methionine showed no significant histopathological difference when compared to the normal liver section. The results reveal that, methionine significantly prevented the rise in transaminases levels produced by hepatotoxic doses of paracetamol and chloroquine. But, to prevent occasional cases of paracetamol overdosage, it is not advisable to give methionine concurrently with paracetamol to patients who are taking paracetamol therapeutically.

Materials and Methods
Young albino rats of either sex weighing 200-400g body weight were used. They were given free access to laboratory chow and tap water ad libitum. Animals were allowed to acclimate in an environment of normal room temperature.

Rats were kept fasting 16 hours prior to administration of paracetamol. Paracetamol, as a freshly prepared suspension in distilled water was administered by intragastric cannula. The volume administered was maintained constant at 20 ml/kg, since Ferguson has shown that the drug toxicity can be increased with increasing volume of distilled water as vehicle. The rats were kept in a cage after administration of paracetamol and provided food and water ad libitum.

To study the hepatotoxicity induced by paracetamol, chloroquine, quinine and its protection by methionine the rats were divided into following experimental groups:

(1) Control group of 6 rats was taken to determine the normal liver function tests;
(2) paracetamol (7 gm/kg) was administered as a single oral dose in a group of 9 rats;
(3) methionine (10% of paracetamol dose) per se was studied in a group of 6 rats;
(4) combination of paracetamol (7 g/kg) with methionine (10% of paracetamol dose) was given in group of 9 rats;
(5) chloroquine (970 mg/kg) was administered as a single oral dose in a group of 6 rats;
(6) combination of paracetamol (7 g/kg) with chloroquine (970 mg/kg) was administered at the
blood was collected and analysed for standard liver function tests (8) quinine (3) combination of paracetamol (7g/kg), chloroquine (970 mg/kg) and methionine (10% of paracetamol dose) was given in a group of 6 rats; (7) Following drugs administration, after 24 hr, the blood was collected and analysed for standard liver function tests (SGOT, SGPT) according to Reitman and Frankel method by using autospan semiautomatic analyser. The livers were removed after blood collection, and prepared for histopathological examination in 10% formal saline.

Statistical analysis

Results are expressed as means ± S.E. in terms of international units litre⁻¹. For comparison between two groups unpaired Student’s t test was employed. P < 0.05 was considered significant.

Results and Discussion

The results are presented in Table 1 and Figs 1–6.

The evidence of hepatic necrosis from the liver sections of paracetamol and chloroquine alone treated rats appears to be early damage indicated by hydropic vacuolation, marked congestion and necrosis of hepatocytes. Whereas liver sections from the combination of paracetamol and methionine and from paracetamol, chloroquine and methionine showed no significant difference as compared to normal liver sections.

Oral methionine given between 10 and 24 hours after ingestion of paracetamol seems more protective than iv N-acetylcysteine. The treatment protocol for oral methionine is simple and therapy is completed within 12 hr, as compared with 3 days for oral N-acetylcysteine treatment and 2 hr for iv N-acetylcysteine. The side effects are unimportant and vomiting is mild when compared with that occurring after oral N-acetylcysteine treatment.

To be effective, methionine must be given within 8-10 hr of paracetamol ingestion. However some liver damage may still occur in highly susceptible patients of alcoholics poisoning in whom the treatment is delayed. Administration of methionine later than 10-12 hr after overdosage of paracetamol is not only useless but also dangerous, since it cannot be metabolised by a damaged liver and may aggravate hepatotoxicity.

In the present study, methionine (700 mg/kg i.e. 10% of paracetamol dose) per se did not alter serum transaminases levels but decreases paracetamol induced rise in SGOT and SGPT levels significantly. The incorporation of 10% methionine with paracetamol is found to be optimal as reflected from the pilot experiments which were also carried out using 20% methionine per se which showed no significant difference between 10 and 20% of the corresponding paracetamol dose.

Concurrent administration of paracetamol along with other hepatotoxic drugs is usually seen in many clinical situations. For example, paracetamol alone or in combination with chloroquine is found in malaria. It is a well known that malaria itself and chloroquine can also cause liver damage. Increased serum transaminases levels, due to chloroquine reflected the hepatic injury, which was further precipitated by paracetamol significantly. Methionine remarkably prevented the liver necrosis induced by

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<th>Sr. Groups</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
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<tr>
<td>1 Control (6)</td>
<td>146.75 ± 16.12</td>
<td>55.88 ± 8.01</td>
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<td>2 Paracetamol (9)</td>
<td>910.44 ± 53.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>207.55 ± 24.75&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3 Methionine (6)</td>
<td>157.5 ± 11.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.3 ± 3.47&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>4 Paracetamol + Methionine (10%) (9)</td>
<td>185 ± 24.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.8 ± 5.38&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>5 Paracetamol + Methionine (20%) (9)</td>
<td>179 ± 32.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.7 ± 3.82&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>6 Chloroquine (6)</td>
<td>415.0 ± 35.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>201.83 ± 9.66&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>7 Chloroquine + Paracetamol (6)</td>
<td>1974.67 ± 329.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>525.22 ± 126.99&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>8 Chloroquine + Methionine (6)</td>
<td>200 ± 26.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.17 ± 8.96&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>9 Quinine (4)</td>
<td>164.75 ± 17.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.50 ± 12.61&lt;sup&gt;c&lt;/sup&gt;</td>
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P values: <sup>a</sup><i>P</i> < 0.001, as compared to control. <sup>b</sup><i>P</i> < 0.01, as compared to paracetamol alone. <sup>c</sup><i>P</i> < 0.01, as compared to chloroquine alone. <sup>d</sup><i>P</i> < 0.05, as compared to chloroquine alone. <sup>e</sup><i>P</i> < 0.01 as compared to chloroquine and paracetamol <sup>f</sup><i>P</i> < 0.001 as compared to chloroquine and paracetamol.
Fig. 1 — Liver section from control rats, showing central vein (high power); Fig. 2(a) — Liver section from paracetamol treated rats, marked congestion and necrosis detectable (low power); Fig. 2(b) — Liver section from paracetamol treated rats showing inflammatory reaction of central vein (high power); Fig. 3(a) — Liver section from paracetamol and methionine treated rats, cells apparently normal (low power); Fig. 3(b) — Liver section from paracetamol and methionine treated rats (high power)
combination of hepatotoxic doses of chloroquine and paracetamol which is reflected by non significant rise in transaminases levels. Another antimalarial drug, quinine, which is reported to cause hepatic damage as hypersensitivity reaction at the dose of 650 mg/kg, po did not cause any alteration in serum enzyme levels in the present study. The main events of histological changes reported from paracetamol (7 g/kg po), chloroquine (970 mg/kg po) per se and their combinations were hydropic vacuolation, marked congestion and necrosis of hepatocytes as seen by Boyd, et al. Whereas those treated with paracetamol and methionine (700 mg/kg po), and paracetamol, chloroquine and methionine revealed apparently normal liver histology. This implies that concomitant administration of methionine prevented the hepatonecrotic changes in liver induced by toxic doses of paracetamol and chloroquine.

In pilot experiments, no hepatotoxicity was observed even with 3.5 g/kg dose of paracetamol; which is around 120 times higher than the therapeutic dose for human beings. These findings cannot be extrapolated in clinical situations. The incidence of hepatotoxicity with paracetamol is very low in Indian population. So, to prevent occasional cases of paracetamol overdosage, it is not advisable to give methionine concurrently with paracetamol to patients who are taking paracetamol therapeutically.

References
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