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The effect of therapeutic doses of paracetamol on liver function in the rat perfused liver

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Abstract—The isolated liver perfusion technique was used to study the effect of therapeutic doses of paracetamol on hepatic gluconeogenesis and bromosulphthalein clearance from the perfusate and biliary excretion of the dye in the rat. Six groups of rats were studied; those in the three experimental groups were given 0·02 g kg^{-1} paracetamol daily for ninety days. The livers of animals in the control group and in one of the experimental groups were perfused with a medium containing pyruvate. The animals in the second experimental and control group were perfused with a medium containing bromosulphthalein (10 mg/100 mL). The livers of the third experimental and control group were subjected to histological examination. The rate of glucose formation and glucose concentrations were decreased, while, lactate levels and lactate: pyruvate ratios were increased in paracetamol-treated rats. The mean concentration of bromosulphthalein in the perfusate and biliary excretion of the dye were decreased. Macro and micro vesicular fatty change was present in the livers of paracetamol-treated rats. This study demonstrates that chronic administration of therapeutic doses of paracetamol to rats adversely affects liver function, as evidenced by impaired gluconeogenesis and bromosulphthalein clearance from the perfusate, and excretion of the dye into the bile, and provides histological evidence of hepatic damage in rats.

Paracetamol, a medication available over the counter without a prescription, is a major component of many formulations available for the relief of headaches, fever, coughs and cold, and it is the most widely used analgesic/antipyretic agent because of its overall efficacy and safety. In contrast to aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) it does not cause gastrointestinal bleeding, and there is no association of its use with the development of Reye's syndrome. The major problem caused by paracetamol is hepatotoxicity, which may be fatal after large doses of the drug (Black 1984; Hall et al 1986 a, b; O'Dell et al 1986; Monteagudo & Folb 1987). In-vivo studies of glucose metabolism and bromosulphthalein clearance have shown that impaired gluconeogenesis and bromosulphthalein clearance often occurs after liver damage due to paracetamol overdose (Davis et al 1975; Record et al 1975).

Earlier studies have shown that in chronic alcoholics, paracetamol hepatotoxicity occurs with therapeutic or near therapeutic doses of paracetamol (McLain et al 1980; Johnson et al 1981; Seeff et al 1986). A recent report showed that in two patients with a high daily alcohol consumption, liver damage exhibiting clinical and morphological features of paracetamol hepatotoxicity occurred; although the patients took only therapeutic doses of paracetamol (Floren et al 1987). This study shows that the isolated perfusion model has proved to be suitable and useful in providing information on the effects of therapeutic doses of paracetamol on liver function (gluconeogenesis and bromosulphthalein clearance) in livers not damaged by alcohol.

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Materials and methods

The protocol was approved by the Ethics Committee, University of Natal Medical School. The study conformed with accepted principles in the care and use of animals for experimental purposes set out by The Medical Research Council, South Africa.

Sixty six male Wistar rats, 200–220 g, of the University of Natal inbred strain, were kept in stainless steel cages with plastic floors. Each rat was individually earmarked and randomly assigned to one of six groups, three control and three experimental, with eleven rats per group. All the animals were fed on a rat standard diet. Water was freely available. The animals in the experimental groups were given 0·02 g kg^{-1} paracetamol and the control group was given an equal amount of distilled water by

Table 1. Lactate dehydrogenase (LDH) activity in the perfusate at different times after surgery.

Time after surgery (min)	LDH activity (nmol h^{-1} mL^{-1})
10	2·45±0·92
20	1·05±0·04
30	1·04±0·08
40	1·05±0·03
50	1·05±0·03
60	1·04±0·04
70	1·05±0·02
80	1·05±0·08
90	1·05±0·08

gavage daily for ninety days. All the animals were dosed in the morning before the experiments. The livers of animals in the control group and one experimental group were perfused for 75 min with a medium containing pyruvate (500 μM), a gluconeogenic precursor. The livers of the second control and experimental groups were perfused with a medium containing bromosulphthalein (10 mg/100 mL) for 60 min. The livers of animals in the third experimental and control groups were subjected to histological examination. The animals were randomly chosen for perfusion.

Operative technique. Rats were anaesthetized by intraperitoneal injection with a fresh solution of sodium pentobarbitone (60 mg kg^{-1}) and then 0·1 mL (5000 int. units) heparin was administered via the saphenous vein for the liver study. Animals for the histopathology study were killed by decapitation.

Liver perfusion/perfusion medium. Isolated livers were perfused in-situ with synthetic recycling medium (Krebs & Henseleit 1932; Hems et al 1966).

Measurement of gluconeogenesis in the perfused liver. The time course of the experiments was as follows. Initial samples of the perfusion medium were taken before initiating perfusion. At this point, the medium was again sampled (5 min). Five minutes later a time zero sample was taken and the perfusion was continued for the next 75 min with sampling of medium at 15 min intervals for the measurement of glucose, lactate and pyruvate.

Bromosulphthalein clearance. The test dye, bromosulphthalein, was added in a concentration of 10 mg/100 mL to the perfusate reservoir and the perfusion continued for 60 min. Samples were then withdrawn from the perfusate reservoir and bile reservoir at 10-min intervals for analysis.

Statistical analysis. Statistical analysis of results was carried out using the Mann-Whitney rank sum test with $P < 0·05$ accepted as the limit of significance for both gluconeogenesis and bromosulphthalein clearance and biliary excretion of the dye.

Microscopy. The liver tissue was fixed for 24 h in 10% buffered-formalin solution, embedded in paraffin wax and 5 μm thick sections were cut. All sections were stained with haematoxylin and eosin; sections were cut at superficial and deep levels in each block of tissue.

Results

After 90 days, the experimental rats appeared healthy with no visible differences from the controls. All the rats gained weight. The livers appeared normal macroscopically and the difference between the wet weights were statistically non-significant between groups. Liver function and viability throughout the perfusion was tested by the lactate dehydrogenase (LDH) activity in the perfusion medium and by histological examination. Except for a rapid increase immediately after surgery, most likely due to damage of some cells during the course of surgery, LDH remained constant for up to 1·5 h from the beginning of the perfusion (Table 1). The histological observation of tissue performed at 90 min after surgery showed preserved hepatic structure without hepatocellular damage.

Table 2. Time-course of the production of lactate and glucose by perfused livers of control rats under aerobic conditions.

Time (min)	15	30	45	60	75
Concn of metabolite (mmol L^{-1})					
Lactate	1·84±0·06	2·23±0·20	2·48±0·16	2·79±0·28	3·07±0·19
Pyruvate	0·235±0·01	0·182±0·02	0·191±0·01	0·190±0·01	0·206±0·01
Glucose	2·54±0·04	3·01±0·13	3·48±0·31	3·96±0·56	4·29±0·76
Lactate:pyruvate ratios	9	12	13	15	15
Metabolic rates (mmol min^{-1} (g wet weight of liver)$^{-1}$)					
Glucose formation	0·07	0·07	0·07	0·07	
Lactate formation	0·03	0·02	0·02	0·04	

The results are expressed as mean + s.e.m. (n = 9). Pyruvate 500 μM was added to the perfusion medium. The initial volume of the medium was 150 mL, which decreased every 15 min by 10 mL (removed for analysis). The livers had a mean wet weight of 6·08 g.

Table 3. Time-course of the production of lactate and glucose by perfused livers of experimental rats under aerobic conditions.

Time (min)	15	30	45	60	75
Concn of metabolite (mmol L^{-1})					
Lactate	2·71±0·06	3·56±0·20	3·91±0·16	4·08±0·28	4·34±0·19
Pyruvate	0·267±0·01	0·215±0·02	0·139±0·01	0·156±0·01	0·138±0·01
Glucose	1·02±0·04	1·14±0·13	1·35±0·31	1·89±0·56	1·95±0·76
Lactate:pyruvate ratios	10	17	28	26	31
Metabolic rates (mmol min^{-1} (g wet weight of liver)$^{-1}$)					
Glucose formation	0·02	0·03	0·07	0·20	
Lactate formation	0·09	0·06	0·03	0·04	

The results are expressed as mean + s.e.m. (n = 10). Pyruvate 500 μM was added to the perfusion medium. The initial volume of the medium was 150 mL which decreased every 15 min by 10 mL (removed for analysis). The livers had a mean wet weight of 6·01 g.

Table 4. Time-course of A. bromosulphthalein clearance ($\mu g\ mL^{-1}\ min^{-1}$) and B. biliary excretion of bromosulphthalein ($mg\ mL^{-1}$) by perfused livers from control and experimental animals.

	Time (min)					
A.	10	20	30	40	50	60
Control (11)	8·4±0·07	7·1±0·02	5·9±0·01	5·1±0·01	2·2±0·04	0·7±0·08
Experimental (11)	7·2±0·01	6·9±0·02	3·1±0·04	2·9±0·09	3·1±0·07	3·0±0·08
B.	10	20	30	40	50	60
Control (11)	0·14±0·04	0·18±0·06	0·27±0·12	0·38±0·22	0·46±0·28	0·71±0·35
Experimental (11)	0·19±0·09	0·23±0·08	0·28±0·15	0·33±0·14	0·37±0·12	0·38±0·17

The results are expressed as mean±s.d. Bromosulphthalein was added, at a dosage of 10 mg/100 mL, to the perfusion medium.

Similar results were obtained in control and drug-treated groups. Only the clear, yellowish coloured livers were considered as well perfused (n=41); those of the red colour were discarded (two in the first control group and one from the second experimental group).

Gluconeogenesis. The livers of control rats showed appreciable amounts of glucose and lactate during 75 min perfusion with pyruvate as substrate (Tables 2, 3). The glucose and lactate concentration increased linearly with time. The mean glucose concentration was 4·29±0·28 mmol at the end of perfusion and the average rate of glucose formation was 0·07 mmol min^{-1} (g wet weight liver)$^{-1}$. The lactate concentration was 3·07±0·14 mmol L^{-1} after 75 min of perfusion and the mean rate of lactate formation was 0·04 mmol min^{-1} (g wet weight liver)$^{-1}$. About 294 μmol pyruvate was utilized so that the lactate:pyruvate ratio averaged 15.

In the experimental rats the glucose concentration increased linearly for 30 min and then remained constant until the end of the experiment. The mean glucose concentration obtained from the perfusate was 1·95±0·25 mmol L^{-1} at the end of perfusion and the average rate of glucose formation was 0·03 mmol min^{-1} (g wet weight liver)$^{-1}$. The lactate concentration increased linearly with time. The mean lactate concentration derived from the perfusate at the end of the perfusion was 4·34±0·51 mmol L^{-1} and the rate of lactate formation was 0·06 mmol min^{-1} (g wet weight of liver)$^{-1}$. The experimental livers utilized about 412 μmol pyruvate so that the lactate:pyruvate ratio averaged 30.

The rate of glucose formation and glucose concentration were decreased; lactate levels and lactate:pyruvate ratios were increased in the experimental groups compared with control ($P<0·05$).

Bromosulphthalein clearance. Paracetamol, given at a daily dose of 0·02 g kg^{-1}, led to a significant decrease in the rate of removal of bromosulphthalein from the perfusate (Table 4). This is demonstrated by the comparison of the observed rate of disappearance of bromosulphthalein in drug-treated livers with controls. Sixty minutes after bromosulphthalein administration a decrease in dye clearance was seen. The values were 8·4±0·07 at 10 min and 0·7±0·03 $\mu g\ mL^{-1}\ min^{-1}$ at 60 min in the control group compared with 7·2±0·6 at 10 min and 6·0±0·2 $\mu g\ mL^{-1}\ min^{-1}$ at 60 min in the experimental animals. Excretion rate of bile was decreased in the paracetamol-treated rats. The concentration of bromosulphthalein in the bile of untreated livers during 60 min collection ranged from 1·4±0·5 at 10 min to 7·1±1·2 mg mL^{-1} at 60 min in the control animals compared with 1·9±0·8 at 10 min to 3·8±0·8 mg mL^{-1} at 60 min in the drug-treated animals. At 60 min the concentration of the dye both in the perfusate and in the bile were significantly different compared with controls ($P<0·05$).

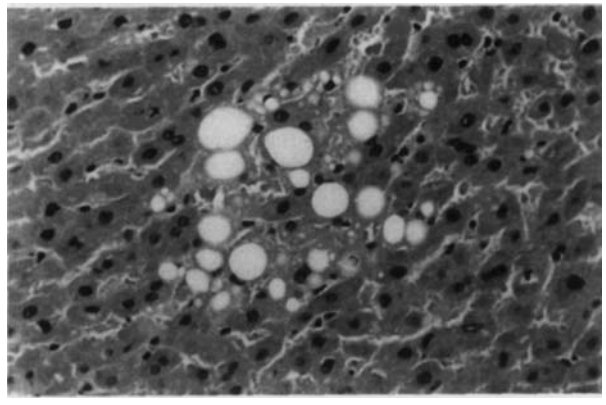

FIG. 1. Liver of paracetamol-treated rats showing fatty change ×450.

Microscopy. Histological examination of eight liver biopsy specimens from the eleven paracetamol-treated rats revealed macro and micro vesicular fatty change (Fig. 1). Three liver biopsy specimens from experimental groups and the control group were histologically normal.

Discussion

Paracetamol, a widely used analgesic, is known to cause acute hepatic necrosis (Henry & Volans 1984; Meredith et al 1986), which is sometimes fatal in adults when taken in overdose (Davidson & Eastham 1966; Clark et al 1973; Prescott & Wright 1973; Koch-Weser 1976). The clinical course of patients with liver damage following paracetamol overdose is often complicated by the development of hypoglycaemia 24–72 h after overdose (Record et al 1975). In a study of glucose metabolism in fourteen patients with liver damage due to paracetamol overdose, impaired gluconeogenesis was suggested by mild fasting hypoglycaemia in four patients and raised fasting blood lactate levels (Record et al 1981). It is also recognized that in some patients, chronic ingestion of paracetamol, even in low doses, may produce hepatic necrosis and hepatitis long after the drug has been discontinued (Barker et al 1977; Johnson & Tohman 1977; Bonkowsky et al 1978; Canalese et al 1981; Neuberger & Davis 1981). The recommended maximum dose of paracetamol is 4 g daily.

In this study, paracetamol, at doses corresponding to the therapeutic dose in man, was found to inhibit gluconeogenesis in rat. This was evidenced by decrease in glucose levels, an increase in the concentration of lactate and an increase in the lactate:pyruvate ratios in the perfusion medium of the experimental

animals. The decreased glucose production by the experimental livers which occurred with an increased pyruvate utilization indicates that pyruvate in these animals was used for the production of lactate. In contrast, the rate and amount of glucose production and of pyruvate utilization were increased in the control group indicating that pyruvate was used mainly for the production of glucose. The increased lactate: pyruvate ratio, which reflects the increase in NADH: NAD^+ ratio, suggests that an alteration in the redox potential of the cytosol was responsible for the impaired gluconeogenesis. It is also likely that impaired gluconeogenesis is possibly related to the non-availability of oxaloacetate or the impairment of the activity of key enzymes for gluconeogenesis.

In rats treated with therapeutic doses of paracetamol, a marked bromosulphthalein retention in perfusate and decreased biliary excretion of bromosulphthalein was observed. During the 60-min perfusion, most of the dye was cleared from the perfusate with an increased biliary excretion of the dye in the control animals, whereas in the drug-treated animals after an initial high clearance from perfusate, concentration of the dye remained constant with concomitant decreased biliary excretion of the dye until the end of the experiment. The rate limiting step in the elimination of bromosulphthalein from plasma into the bile of man and experimental animals is probably the conjugation of the dye with hepatic glutathione (Combes 1965).

Previous studies in rats have shown that impaired plasma clearance of bromosulphthalein is associated with low hepatic concentration of glutathione and decreased glutathione transferase activity which results from the paracetamol overdose (Mitchell et al 1973; Davis et al 1975). It is therefore likely, that the impaired bromosulphthalein clearance from the perfusate in this study was related to reduced glutathione levels. In addition to impairment of hepatic uptake and conjugation of the dye, paracetamol markedly inhibits biliary excretion of the dye, and this is manifested both at therapeutic and at hepatotoxic doses of the drug in man (Davis et al 1975). Studies in rats indicate that interaction between paracetamol and bromosulphthalein is at the canalicular membrane level, and appears to be competitive with respect to glutathione levels (Davis et al 1975).

Histological examination of liver biopsy specimens from eight of the eleven paracetamol-treated rats revealed macro and micro vesicular fatty change while all the control animals were normal.

The present study demonstrates that rats, when administered paracetamol chronically in doses equivalent to therapeutic doses in the adult human, develop biochemical and pathological evidence of liver damage.

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