Effect of Bile on Liver Function Tests in Experimental E. Coli Peritonitis in the Rat

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Summary

Hepatic dysfunction is a frequent finding in sepsis and peritonitis. In the present study, hepatic function in experimental peritonitis in the rat was determined by measuring serum levels of bilirubin, alkaline phosphatase (ALP), glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT), together with antipyrine (AP) clearance as a determinant of microsomal function. Peritonitis was induced by intraperitoneal injection of 3×10^8 colony-forming units of E. coli together with either 1.0 ml bile or saline. E. coli + bile peritonitis rats had significantly elevated levels of bilirubin, ALP, GOT and GPT as compared with both controls and rats with peritonitis induced by E. coli alone. The derangements gradually increased with time over the 10-hour period studied. In contrast, no reduction of AP clearance was observed in the peritonitis models. On the contrary, AP clearance was enhanced at 10 hours after induction of peritonitis by E. coli alone. In conclusion, hepatic dysfunction as revealed by routine laboratory tests is seen early in experimental peritonitis in the rat, but this is not accompanied by a reduced AP clearance rate.

Key words

Experimental peritonitis – Liver function – Antipyrine clearance

Introduction

Liver dysfunction is a frequent finding in bacterial sepsis, ranging from 0.6% among patients with bacteremia (15), to an almost obligatory finding in patients with septic shock and multiple organ failure (4). Usually, the hepatic failure that occurs in late sepsis is preceded by milder hepatocellular dysfunction in the early period of sepsis (2). Whether similar derangements occur in peritonitis with different pathophysiological mechanisms has not been systematically studied. We therefore evaluated the liver function in two different experimental peritonitis models in the rat, using standard liver function tests and antipyrine (AP) clearance. AP clearance, which determines hepatic function quantitatively, evaluates mainly the hepatic microsomal function (16).

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Materials and methods

Animals

Forty-two male Sprague-Dawley rats, weighing approximately 300 g, were used. The animals had free access to water and food pellets.

Induction of peritonitis

Escherichia coli peritonitis (n = 12) was induced by the intraperitoneal injection of 1.0 ml E. coli, 3×10^8 colony-forming units (CFU)/ml, serotype 046:K1:H31. The bacteria were kept frozen at −80 ° and injected immediately after thawing, i.e. in their log phase. E. coli + bile peritonitis (n = 12) was induced by injection of 3×10^8 CFU E. coli as above together with 1.0 ml sterile human gallbladder bile derived from elective, routine cholecystectomies. Bile alone, without bacteria, was injected intraperitoneally in 12 rats. Controls (n = 6) received 1.0 ml sterile saline intraperitoneally.

Liver function tests

Liver function tests, i.e. serum concentrations of bilirubin, alkaline phosphatase, GOT and GPT, were done at 5 hours ("early") and at 10 hours ("late") after induction of peritonitis. Bilirubin was determined in accordance with the method of *Kuffer* et al. (8) and the other enzymes were determined using the methods of the Committee on Enzymes of the Scandinavian Society of Clinical Chemistry and Clinical Physiology (13, 14). AP clearance was determined from the AP plasma decay in "early" (n = 6/group) or "late" peritonitis (n = 6/group) by a single sample method assay (11). Four mg of AP (Sigma Chemical Co., St. Louis, MO, USA) were suspended in 1.0 ml sterile saline and injected intravenously (hindlimb) at the time of the induction of peritonitis (for "early" peritonitis sampling) or at 5 hours after the intraperitoneal bacterial challenge ("late" peritonitis). Five hours after AP injection, 500 µl blood was withdrawn by intracardial puncture into heparinized tubes, simultaneous with blood sampling for the above-mentioned liver function tests. The blood samples for AP analysis were centrifuged at 2,000 rpm for 10 minutes, after which the plasma portion was removed and frozen at −20 °C. AP was analysed by high performance liquid chromatography (HPLC) as described earlier (11).

Statistics

Liver function tests and AP clearance values between the groups were compared using Student's t-test.

Results

At 4 to 6 hours after induction of peritonitis, the animals showed signs of illness, including piloerection, hyperpnea and low motoric activity. The septic state was fully

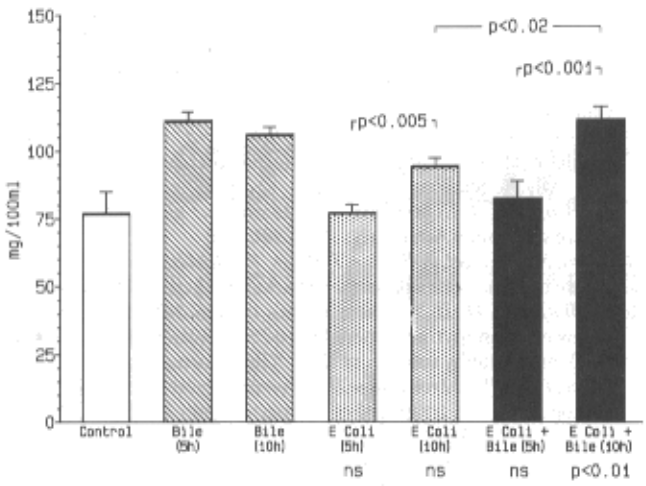

Fig 1 Serum levels of bilirubin in control rats and in rats with peritonitis induced by bile, E. coli or E. coli + bile at 5 or 10 hrs prior to blood sampling. Means + SEM are shown. There were 6 rats in each group. P indicates the probability level of random difference between groups of rats with peritonitis or between controls and the respective peritonitis groups. ns = not significant

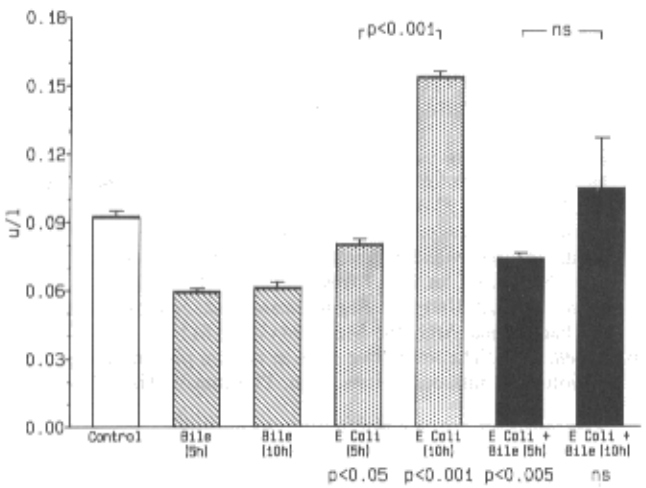

Fig. 2 Serum levels of alkaline phospatase in control rats and in rats with peritonitis induced by bile, E. coli or E. coli + bile at 5 or at 10 hrs prior to blood sampling. Means + SEM are shown. There were 6 rats in each group. P indicates the probability level of random difference between groups of rats with peritonitis or between controls and the respective peritonitis groups. ns = not significant

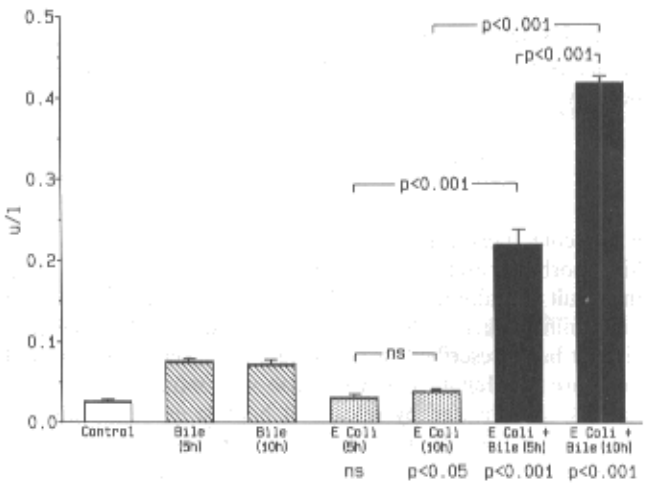

Fig. 3 Serum levels of glutamic-oxaloacetic transaminase (GOT) in control rats and in rats with peritonitis induced by bile, E. coli or E. coli + bile at 5 or 10 hrs prior to blood sampling. Means + SEM are shown. There were 6 rats in each group. P indicates the probability level of random difference between groups of rats with peritonitis or between controls and the respective peritonitis groups. ns = not significant

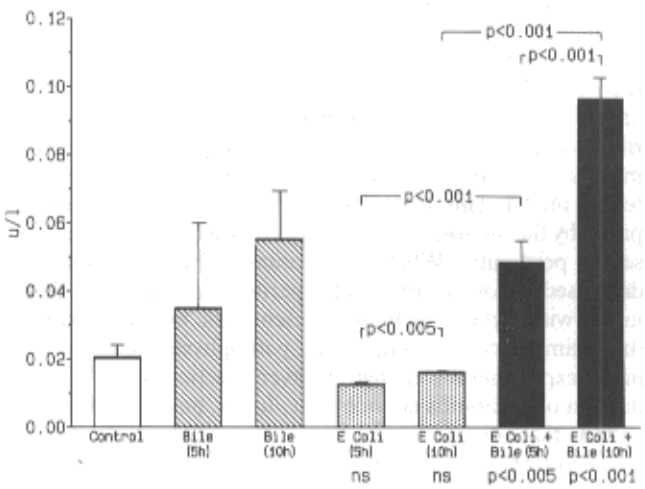

Fig. 4 Serum levels of glutamic-pyruvic transaminase (GPT) in control rats and in rats with peritonitis induced by bile, E. coli or E. coli + bile at 5 or at 10 hrs prior to blood sampling. Means + SEM are shown. There were 6 rats in each group. P indicates the probability level of random difference between groups of rats with peritonitis or between controls and the respective peritonitis groups. ns = not significant

developed after 10 hours. As can be seen in Figs. 1–4, this development of peritonitis was accompanied by derangements in the liver function tests. Most evident was the elevation of the serum GOT levels. The changes increased with time in the peritonitis models. E. coli + bile peritonitis was associated with more deranged values than E. coli peritonitis. The intraperitoneal injection of bile alone was also followed by deranged liver function tests, though not to the same extent as in E. coli + bile peritonitis.

Table 1 Antipyrine clearance (ml/min) in experimental bile, E. coli and E. coli + bile peritonitis

Control	0.90 ± 0.06
Bile (0–5 h)	0.94 ± 0.01
E. coli (0–5 h)	0.97 ± 0.04
E. coli + bile (0–5 h)	0.96 ± 0.15
Bile (0–10 h)	0.96 ± 0.04
E. coli (0–10 h)	1.18 ± 0.05*
E. coli + bile (0–10 h)	0.99 ± 0.08

Values are means ± SEM of six animals
* indicated $p < 0.05$ compared with control

In contrast, the AP clearance was not inhibited by the peritonitis. On the contrary, in the E. coli peritonitis model, AP clearance increased at 10 hours after induction of peritonitis as compared with controls (Table 1).

Discussion

Bile peritonitis, which is seen in association with acute, perforated cholecystitis, is still associated with high morbidity and mortality rates (6, 9, 12). Mortality is often the result of multiple organ failure, in which hepatic failure is a predominant factor. Progressive hepatic dysfunction has previously been described in septic rats after cecal ligation and puncture (2). Hepatic injury was also seen in our rat peritonitis models, as indicated by release of hepatic enzymes into the blood. E. coli + bile peritonitis was thereby accompanied by more severely deranged values than E. coli peritonitis. The hepatic dysfunction was progressive, as was seen by comparing the values early and late in the peritonitis course. We also studied the liver microsomal function in the peritonitis animals by means of AP clearance. AP clearance is often used in studies of hepatic function, mainly in studies on the drug-detoxifying capacity of the liver (16). The drug is eliminated by the liver (1) by the mono-oxygenase enzymes (5). Its plasma clearance is thus a quantitative measure of the detoxifying capacity of the liver, which is of importance for example for drug elimination rates. Antipyrine is closely correlated to other "vital hepatic functions" also of a non-microsomal nature such as galactose elimination capacity and the capacity of urea-N-synthesis (7). Interestingly, we found that the antipyrine clearance was not inhibited in either of the peritonitis models. It was, on the contrary, exaggerated in the E. coli peritonitis model. This indicates an increased detoxification capacity by the mono-oxygenase enzymes early in the course of a severe peritonitis. Whether quantitative liver functions are decreased following prolonged peritonitis cannot be determined with certainty from the present data, but presumably is the ultimate course. The exact pathophysiological mechanisms explaining the changes in liver function noted after induction of peritonitis are not clear. It has, however, been suggested that endotoxin plays an important role in the development of multiple organ failure in septic patients, possibly mediated by cytokines (3, 10).

In conclusion, the two peritonitis models in the rat are accompanied by hepatic dysfunction as reflected by derangement of routine liver function tests. In contrast, during the early phase of peritonitis, the activity of detoxifying mono-oxygenase enzymes in the liver seems well preserved, and even enhanced, as evaluated by antipyrine clearance.

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