Early hepatic dysfunction in a large animal model of nonhypotensive sepsis

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S SULLIVAN, M TROSTER, A LINTON. Early hepatic dysfunction in a large animal model of nonhypotensive sepsis. Can J Gastroenterol 1991;5(6):219-223. Sepsis was induced in 12 sheep by cecal devascularization and perforation. Intravenous crystalloid was administered to maintain the pulmonary capillary wedge pressure at preseptic levels. By 24 h there was a significant drop in albumin and alkaline phosphatase with increases in aspartate aminotransferase (AST), lactate dehydrogenase and bilirubin. By 48 h the alkaline phosphatase had returned to presepsis levels, the bilirubin continued to rise, the AST and lactate dehydrogenase had plateaued while the albumin remained low. These biochemical alterations were confirmed by examining data from 25 sheep used in similar sepsis experiments. These data revealed that marked elevations in AST were associated with a higher central venous pressure and worse renal impairment, but there was no relationship with any other hemodynamic parameter, PO_2, pH or serum lactate. Histologically these biochemical alterations were associated with extensive microvesicular fatty change at 24 h and centrilobular necrosis at 48 h. Electron microscopy revealed mitochondrial degeneration, hyperplasia of the smooth endoplasmic reticulum and intracellular cholestasis.

Key Words: *Cholestasis, Hepatic dysfunction, Liver, Multiorgan failure, Sepsis*

Début de dysfonctionnement hépatique dans un grand modèle animal de septicémie non hypotensive

RESUME: On a provoqué une septicémie chez 12 moutons en effectuant une dévascularisation et perforation du caecum. Des cristalloïdes ont été administrés par voie intraveineuse afin de maintenir la pression capillaire pulmonaire au niveau antérieur à la septicémie. En 24 heures, on a noté une diminution significative des concentrations d'albumine et de phosphatases alcalines ainsi qu'une élévation de l'aspartate aminotransférase (AST), de la lacticodéshydrogénase (LDH) et de la bilirubine. Au bout de 48 heures, les valeurs des phosphatases alcalines étaient retournées à leur niveau initial, la bilirubine continuait à augmenter et les AST et LDH étaient stationnaires tandis que l'albumine restait faible. Les résultats portant sur 25 moutons ayant servi à des expériences du même type ont confirmé ces modifications biochimiques. Ces

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HEPATIC DYSFUNCTION OCCURring during the course of systemic sepsis is well recognized (1-6). If hypotension does not occur the major abnormalities are increases in alkaline phosphatase and bilirubin with the occasional development of clinical jaundice. Persisting hyperbilirubinemia is associated with poor prognosis (2). The pathogenesis of these abnormalities remains an enigma, but may be due to an endotoxin induced reduction in bile flow (5). Liver histology is usually mildly abnormal and shows nonspecific changes (1,2). In contrast to the cholestatic changes of normotensive sepsis, hypotension and hypoxia can produce marked elevations in serum transaminases, associated with centrilobular hepatic necrosis (8).

In the present study the authors document the early biochemical and histological changes associated with nonhypotensive sepsis induced by experimental peritonitis in sheep.

MATERIALS AND METHODS

The experimental protocol has been described previously (7-10). Twelve sheep aged between nine and 18 months, weighing between 10 and 50 kg were used. Twenty-four hours prior to initial surgery solid food was withheld, but the sheep were allowed free access to water. Under halothane anesthesia and after endotracheal intuba-

données ont de plus révélé que les hausses marquées d'AST étaient associées à une pression veineuse centrale plus élevée et à une insuffisance hépatique plus grave, mais qu'il n'existait aucun lien avec d'autres paramètres hémodynamiques, PO_2, pH ou lactates sériques. Sur le plan histologique, ces modifications biochimiques s'accompagnaient d'une dégénérescence graisseuse microvésiculaire étendue à 24 heures et d'une nécrose centro-lobulaire à 48 heures. Un examen au microscope électronique a révélé une dégénérescence mitochondriale, une hyperplasie du réticulum endoplasmatique agranulaire et une cholestase intracellulaire.

tion the aorta was catheterized with a silastic catheter, the pulmonary artery with a triple lumen flow directed right heart catheter, and the urinary bladder with a self retaining Foley catheter. The sheep were allowed to recover in a metabolic cage with free access to food and water for at least 24 h before induction of sepsis. Intravenous Ringers' lactate was administered at a rate of 8 L/24 h to volume load the animals.

Control measurements of hemodynamic, renal and hepatic function were obtained 4 h before induction of sepsis. Under general anesthesia a mid-line laparotomy was performed. The cecum and ileocecal junction were identified and all of the cecum to within 5 cm of the ileocecal valve was devascularized. A quantity of fecal material was allowed to remain in the cecum and the distal cecum was ligated with #2 silk. A local omentectomy was performed to prevent localization of infection. A 2 cm perforation was made in the cecal tip and fecal material was allowed to spill into the perianal cavity. The abdomen was closed with a running suture. Animals were allowed to recover in a metabolic cage. Over the ensuing 12 h all animals began to show clinical evidence of systemic infection as evidenced by increasing respiratory rate and development of fever, lethargy and anorexia.

Postoperatively, fluid administration was guided by the pulmonary capillary wedge pressure. Sufficient intravenous crystalloid was infused to maintain the balloon occluded wedge pressure at presepsis levels.

The pulmonary capillary wedge pressure, mean arterial pressure, central venous pressure, heart rate, respiratory rate, thermodilution cardiac output and systemic vascular resistance index were measured prior to surgery and repeated daily for the duration of the experiment. Blood was obtained from the arterial line for daily measurements of blood gases and from the venous line for hemoglobin, white blood cell count, albumin, creatinine, urea, total bilirubin, aspartate aminotransferase (AST), and lactate dehydrogenase. Blood was also drawn for aerobic and anaerobic culture in broth and subculture on blood and chocolate agar. Urine was collected for measurement of creatinine and calculation of creatinine clearance.

In six sheep liver biopsies were performed at the time of induction of sepsis. In three Trucut liver biopsies were performed at 24 h and in another three at 48 h. The tissue for light microscopy was immediately fixed in 10% formalin and subsequently processed by the usual paraffin embedding techniques. All sections were stained with hematoxylin and eosin, Chromotrope-Aniline blue (for collagen) and colloidal iron (for albumin). The specimens for electron microscopy were fixed in 3% cold glutaraldehyde and post fixed in 1% osmium tetroxide. The tissues were dehydrated in a graded series of alcohols and embedded in Epon-Aralvite. Sections of 80 nm were cut on the Reichert Om-2 ultramicrotome and stained with alcoholic uranyl acetate and Reynold's lead acetate. The sections were examined on a Philips 410 electron microscope.

This model of sepsis proved uniformly fatal, no animal surviving beyond 72 h. Sheep that appeared agonal were immediately killed by intravenous injection of pentobarbital. During the studies sheep were cared for by qualified technicians. Postoperative anesthesia consisted of meperidine given intravenously at 6 to 8 h intervals. Sheep showing any signs of distress were immediately killed as previously described. The study protocol was approved by the local ethics committee governing medical research and the care of experimental animals.

The data are expressed as the mean ± standard deviation. Analysis of variance was used to examine the effect of time on each of the dependent variables. Data from the present group of sheep were compared by two sample Student's *t* test with data from sheep used in similar sepsis experiments (9, 13,14).

TABLE 1
Hemodynamic parameters at baseline, and 24 and 48 h after induction of sepsis

Parameter	Baseline	24 h	48 h
Number	12	12	10
Mean arterial pressure (mmHg)	108±13	104±12	115±8
Heart rate (beats/min)	87±24	149±31*	152±21*
Cardiac index (L/min/m^2)	5.0±1.1	5.3±1.8	6.7±3.3*
Systemic vascular resistance index (d·s·cm^{-5}/m^2)	1727±356	1636±441	1452±413*
Pulmonary capillary wedge pressure (mmHg)	12±4	13±7	13±4
Central venous pressure (cm H_2O)	3±3	5±6	3±3

**Change from baseline significant, P<0.05*

RESULTS

In all sheep the presence of polymicrobial sepsis was confirmed at 24 h by blood culture. The most frequently grown organisms included *Serratia marcescens*, *Enterobacter cloacae*, *Pseudomonas* species, *Bacteroides* species and various strains of *Escherichia coli*. Autopsy examination revealed marked abdominal distension with distended

loops of bowel and large quantities of free peritoneal fluid. An inflammatory mass was always present in the right lower quadrant.

Hemodynamics: Hemodynamics at baseline, 24 h and 48 h are shown in Table 1. The mean arterial pressure, pulmonary capillary wedge pressure and central venous pressure were unchanged while heart rate and cardiac index increased and systemic vascular resistance index decreased significantly over 48 h.

Hematology: The hemoglobin remained unchanged (baseline 98±110 g/L; 24 h 105±122 g/L; 48 h 98±15 g/L). The white blood cell count dropped significantly (baseline 7.7±4.0 $x10^9$/L; 24 h 3.1±1.3; 4 8 h 2.3±1.0).

Renal parameters: After the induction of sepsis the blood urea nitrogen and creatinine gradually rose while the creatinine clearance fell (Table 2). These changes were significant at 48 h.

Hepatic biochemistry: The changes in hepatic biochemistry are shown in Table 2. By 24 h the albumin and alkaline phosphatase had dropped, while the AST and lactate dehydrogenase had risen. At 48 h the alkaline phosphatase had returned to presepsis levels, the albumin remained low and the AST and lactate dehydrogenase had plateaued. Identical changes were seen in 25 sheep from other sepsis experiments (Table 3).

Four of the sheep in this experiment had elevations in AST greater than 300 IU/L at 24 h. Therefore, their hemodynamic and biochemical data were combined with data from 25 septic sheep of other experiments. Fourteen sheep with ASTs greater than 300 IU/L (mean±SD, 447±259) were compared with 23 sheep with ASTs less than 300 IU/L (mean±SD, 173±150). There were no significant differences in mean arterial pressure, heart rate, cardiac index, or systemic vascular resistance index. The sheep with high AST had higher central venous pressures (6.5±15.8 mmHg versus 3.3±1.7 mmHg) P=0.02. There were no differences in PO_2, pH or lactate. Similarly, there were no differences in albumin, bilirubin or alkaline phosphatase. The sheep with high AST had higher levels of urea (5.3±1.2 versus 4.3±1.2 mmol/L) and creatinine (126±73 versus 96±49 µmol/L) but these changes were not significant.

TABLE 2
Renal and hepatic parameters at baseline, and 24 and 48 h after induction of sepsis

Parameter	Baseline	24 h	48 h
Number	12	12	10
Blood urea nitrogen (mmol/L)	5.0±2	6.5±2.2	8.2±3.6*
Creatinine (µmol/L)	73±11	130±78*	176±72*
Creatinine clearance (mL/min)	162±47	113±51	80±30*
Albumin (g/L)	30±3	22±3*	22±3*
Bilirubin (µmol/L)	3±1	8±4*	20±10*
Alkaline phosphatase (IU/L)	84±50	48±25*	86±72
Lactate dehydrogenase (IU/L)	456±81	572±135*	685±208*
Aspartate aminotransferase (IU/L)	153±52	241±97*	219±101

Change from baseline significant, P<0.05

TABLE 3
Hepatic parameters at baseline, and 24 and 48 h in 25 sheep from other sepsis experiments

Parameter	Baseline	24 h	48 h
Number	25	25	14
Albumin (g/L)	31±4	23±4*	25±4*
Bilirubin (µmol/L)	4±3	11±9*	17±9*
Alkaline phosphatase (IU/L)	155±55	86±33*	112±78*
Lactate dehydrogenase (IU/L)	493±84	627±254*	682±311*
Aspartate aminotransferase (IU/L)	132±48	291±244*	249±98*

Change from baseline significant, P<0.05

Light microscopy: After 24 h of sepsis, the liver showed quite extensive microvesicular fatty change and intracellular edema (Figure 1). There was Kupffer cell hyperplasia and leukocytes in the sinusoids. Isolated liver cell necrosis was noted with formation of Councilman bodies. After 48 h of sepsis, the changes consisted of centrilobular necrosis of the liver lobule and deposits of albumin in the sinusoids.

Electron microscopy: Electron microscopy results of liver biopsy are shown in Figure 2. At 24 h the liver cells showed lipid droplets in the hepatocytes. The mitochondria were increased in size and number with degenerative changes including loss of cristae, intramitochondrial crystalloids and myelin figure formation. Giant mitochondria were occasionally seen. The smooth endoplasmic reticulum was hyperplastic and in some instances the rough endoplasmic reticulum also showed increased prominence with dilation and stacking. After 48 h of sepsis the number of lipid droplets increased. Intracellular cholestasis was seen with deposits of bile pigment and collagen deposition in the space of Disse. Increased numbers of lysosomes and peroxisomes were also noted.

DISCUSSION

The cause of the hepatic dysfunction that occurs in septic states is unclear. Studies in humans are difficult because of the complexities of the clinical situation and the uncertainty caused by the necessary concomitant use of blood transfusions and potential hepatotoxins such as antibiotics and parenteral nutrition.

In septic humans the major hepatic biochemical abnormality is elevation in conjugated bilirubin with mild to moderate increases in alkaline phosphatase and transaminases (1,2,6). Occasionally there may be marked elevations in alkaline phosphatase (15). If hypoxia or severe hypotension develops there may be marked elevations in transaminases (8).

In patients dying of septic shock,

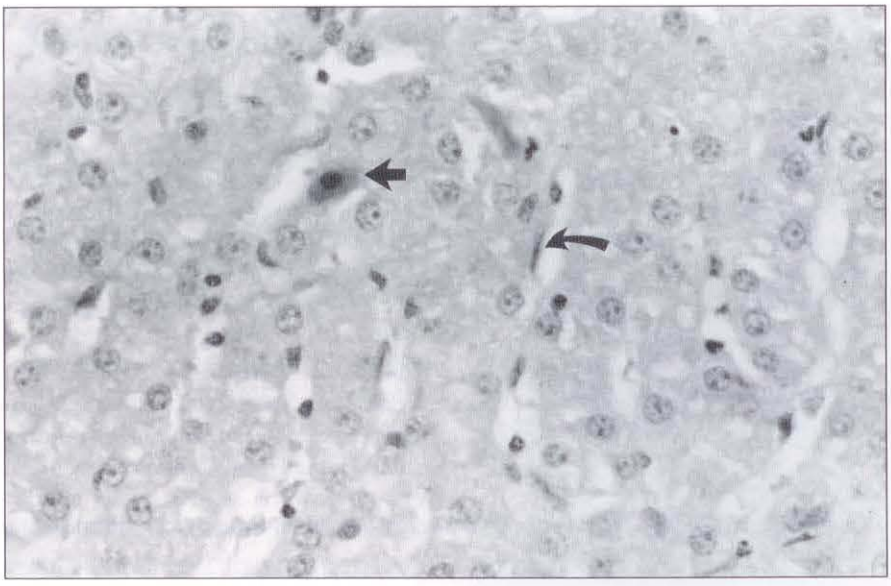

Figure 1) *Light microscopy of liver after 24 h of sepsis showing diffuse microvasicular fatty change, degenerating hepatocytes with early formation of Councilman body (short arrow), and hyperplastic Kupffer cells (curved arrow). Hematoxylin and eosin x 170*

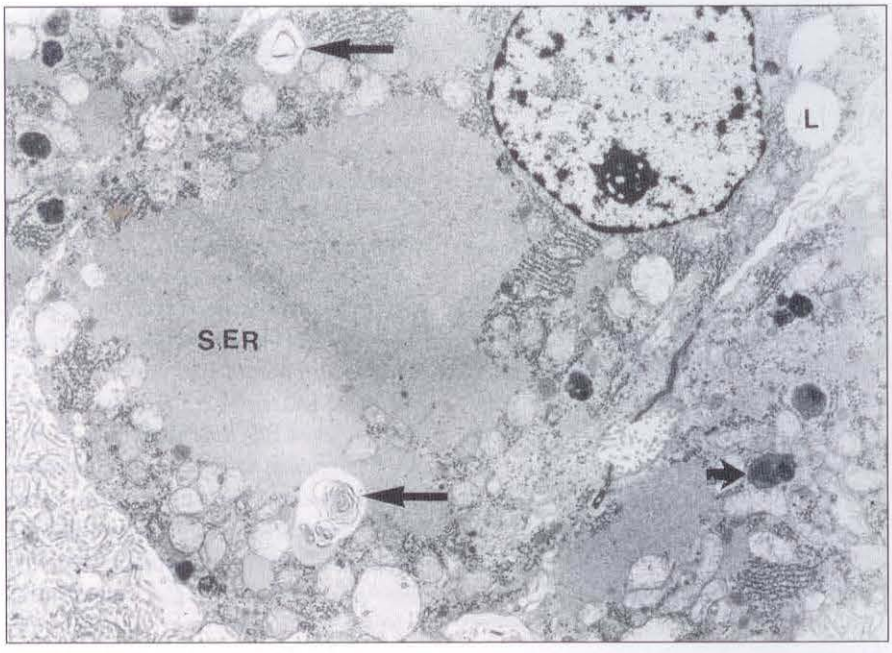

Figure 2) *Electron microscopy of liver after 24 h of sepsis showing lipid deposits (L), hypertrophied, smooth endoplasmic reticulum (SER), myelin figures (long arrows) and bile deposits (short arrows). x 6700*

post mortem liver histology shows changes of venous congestion, ischemic necrosis, fatty change, Kupffer cell hyperplasia and intrahepatic cholestasis. Occasionally cholestasis may be evident at the cholangiolar level (2).

In experimental endotoxic shock the early morphological changes in the liver include injury to sinusoidal epithelium, sinusoidal congestion with degranulated leukocytes, fibrin and platelet aggregates, focal mid zonal necrosis, Kupffer disruption, microvesicular fat, swollen mitochondria and proliferation of smooth endoplasmic reticulum (16-20).

In the present study the authors used a large animal model of systemic sepsis which mimics the changes in septic humans undergoing active resuscitation (21). It produces a normotensive state characterized by high cardiac output, low peripheral resistance and the gradual development of renal dysfunction. The hepatic biochemical changes consisted of an early and plateauing increase in AST and lactate dehydrogenase with progressive increases in bilirubin. The early drop in albumin is not due to impaired hepatic synthesis, but rather to increased vascular endothelial permeability and loss of albumin from the vascular space (10,11).

While a portion of the increase in bilirubin may represent increased bilirubin load from hemolysis this is likely a small component as there were no significant changes in hemoglobin. It is likely that the primary explanation for hyperbilirubinemia is decreased bile flow which has been shown in previous experiments to follow infusion of endotoxin or bacteria. At what level this defect occurs is not clear, but experimentally endotoxin has been shown to decrease bile flow at the canalicular level possibly by inhibition of H^+K^+-ATPase (22).

Elevated AST and lactate dehydrogenase are biochemical evidence of hepatocellular necrosis. Histologically this was characterized at 24 h by focal hepatocellular necrosis with occasional Councilman bodies while at 48 h there was extensive centrilobular necrosis. As there was no hypotension or hypoxemia and no arterial hemodynamic differences between the sheep with or without high AST levels, it is likely that this necrosis represents ischemia occurring as a result of sinusoidal events, perhaps from sinusoidal congestion and collagen deposition in the space of Disse. It is possible that hepatocellular necrosis could arise from centrilobular venous congestion. The sheep with very high ASTs had higher central venous pressures than those with normal ASTs; however, the differences were small and unlikely, by themselves, to be of clinical importance. Finally, 'toxic' events are also a possibility, particularly in view of the marked microvesicular fatty change and mitochondrial degeneration.

Usually the hepatic dysfunction of the normotensive sepsis syndrome in humans is characterized by cholestasis. Therefore, the early drop in alkaline phosphatase was unexpected as it has not been previously described in human or animal studies. By 48 h alkaline phosphatase had returned to presepsis levels; however, as all animals were dead by 72 h it is unknown whether the levels would have continued to rise if the animals had survived. The cause of this early drop and subsequent rise in alkaline phosphatase is unknown. Alkaline phosphatase is manufactured by the liver in response to a variety of insults. The marked hypertrophy of the smooth endoplasmic reticulum may represent the liver's synthetic response to the sepsis. However, why the alkaline phosphatase dropped initially is unknown.

Generally, physicians are more impressed by increases in liver enzymes than decreases. Occasionally, low levels of liver enzymes may aid in diagnosis. Perhaps an unexplained drop in alkaline phosphatase in a febrile, critically ill patient might be an early manifestation of the sepsis syndrome, just as increases in cardiac output and decreases in systemic vascular resistance may be early harbingers.

The clinical significance of these changes is unclear. Generally, the poor prognosis associated with liver dysfunction in the setting of systemic infection is more a reflection of the severity of the sepsis syndrome than of associated liver failure. This is supported by the frequent occurrence of liver dysfunction in septic patients who also have renal failure, respiratory distress syndrome, or encephalopathy associated with sepsis (23-25).

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