

Hepatic damage during acute pancreatitis in the rat

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Abstract

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We studied the alterations in the metabolism of liver mitochondria in rats with acute pancreatitis. Male Wistar rats were allocated to a control group (group I) and to five other groups corresponding to 2, 4, 12, 24 and 48 h after the induction of acute pancreatitis by the injection of 5% sodium taurocholate into the pancreatic duct. Sham-operated animals were submitted to the same surgical steps except for the induction of acute pancreatitis. Mitochondrial oxidation and phosphorylation were measured polarographically by determining oxygen consumption without ADP (basal respiration, state 4) and in the presence of ADP (activated respiration, state 3). Serum amylase, transaminases (ALT and AST) and protein were also determined. Ascitic fluid, contents of amylase, trypsin and total protein were also determined and arterial blood pressure was measured in all groups. In ascitic fluid, trypsin and amylase increased reaching a maximum at 2 and 4h, respectively. Serum amylase increased at 2 h reaching a maximum at 4 h. Serum transaminase levels increased at 12 and 24 h. After 2 h (and also 4 h) there was an increase in state 4 respiration (45.65 ± 1.79 vs 28.96 ± 1.50) and a decrease in respiration control rate (3.53 ± 0.09 vs 4.45 ± 0.08) and in the ADP/O ratio (1.77 ± 0.02 vs 1.91 ± 0.01) compared to controls ($P < 0.05$). These results indicate a disruption of mitochondrial function, which recovered after 12 h. In the 48-h groups there was mitochondrial damage similar to that occurring in ischemic lesion. Beat-to-beat analysis (30 min) showed that arterial blood pressure remained normal up to 24 h (111 ± 3 mmHg) while a significant decrease occurred in the 48-h group (91 ± 4 mmHg). These data suggest biphasic damage in mitochondrial function in acute pancreatitis: an initial uncoupled phase, possibly secondary to enzyme activity, followed by a temporary recovery and then a late and final dysfunction, associated with arterial hypotension, possibly related to ischemic damage.

Key words

- Acute pancreatitis
- Hepatic lesion
- Mitochondria

Introduction

Acute pancreatitis is known to produce morphologic and functional changes in the liver and is associated with a high mortality rate (1-3). Many experimental studies suggest that hepatic cells can be injured during acute pancreatitis due to pancreatic enzymes or kinins expected to enter the portal system (4). It has been shown that the pancreatitis-associated ascitic fluid depresses rat liver cell oxygen uptake (5-7).

In an attempt to characterize these alterations, an experimental model was developed to investigate mitochondrial liver metabolism in rats with acute pancreatitis and the results were correlated with hemodynamic data, ascitic fluid composition and histological findings.

Material and Methods

Male Wistar rats weighing 220-270 g were used in all experiments. The animals were kept at 22-24°C on a 12-h light-dark cycle, and fasted for 16 h, with free access to water. Rats were allocated to a control group (without any operation) (group I, N = 14), and to one of five other (experimental) groups according to the time elapsed after acute pancreatitis (AP) induction: 2 h (group 2, N = 12), 4 h (group 4, N = 6), 12 h (group 12, N = 8), 24 h (group 24, N = 7), and 48 h (group 48, N = 7).

Acute pancreatitis was induced after laparotomy under ketamine chloride anesthesia (0.2 ml/100 g body weight). The main pancreatic duct was cannulated transduodenally with a flexible catheter (Intracath, P50, Becton-Dickinson, Parsippany, NJ) and 0.5 ml of 5% sodium taurocholate in saline was infused at a constant rate. In order to direct the solution to the pancreatic duct a clamp was applied to the proximal part of the hepatic duct during injection (8,9). Sham-operated groups were submitted to the same surgical steps as the experimental group except

for the induction of acute pancreatitis and the animals were sacrificed 2, 4, 12, 24 and 48 h after the surgical procedure.

The animals were killed by a blow to the head followed by exsanguination. Blood samples were collected from animals of all groups to measure serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Technicon RA-1000 System), amylase (10) and total protein (11). Ascitic fluid was removed from the abdominal cavity with a syringe and placed in glass vessels and the volume was determined. The contents of free active trypsin and total trypsin after activation of trypsinogen by enterokinase (12,13), amylase and total protein were also determined.

Liver mitochondria were prepared as described in Ref. 7. Mitochondrial oxygen consumption was measured polarographically (14) using a Gilson 5/6H Oxygraph (Gilson Medical Electronics Inc., Middleton, WI) in a closed reaction vessel fitted with a Clark oxygen electrode (Yellow Springs Instruments Co., Yellow Springs, OH) at 28°C. The incubation medium consisted of 120 mM KCl, 2 mM sodium phosphate, 10 µM rotenone, and 1 mM EGTA, and was buffered at pH 7.1 with 5 mM Tris-HCl. Mitochondria were energized with potassium succinate as substrate at a final concentration of 10 mM. After a brief equilibration period, state 3 (activated state, S3) respiration was induced by the addition of 280 nmol adenosine diphosphate (ADP, Sigma Chemical Co., St. Louis, MO). After all the added ADP was phosphorylated to adenosine triphosphate (ATP), state 4 (basal state, S4) respiration was measured. The ratio of oxygen consumption in the presence of ADP to that in its absence (respiratory control rate, RCR) and the ADP/O ratio were calculated as indices of oxidative and phosphorylative mitochondrial function (15). RCR = oxygen consumption in state 3/oxygen consumption in state 4.

ADP/O = mol of ATP formed from ADP

per atom of oxygen consumed. State 3 and state 4 respiratory rates are reported as nanogram atoms of oxygen per milligram mitochondrial protein per minute. Mitochondria protein content was determined by the method of Lowry et al. (11).

The femoral artery was cannulated 24 h before the induction of acute pancreatitis. Arterial blood pressure (ABP) was recorded in conscious, freely moving rats with the arterial cannula connected to a pressure transducer (Statham P23Db, Hato Rey, PR) coupled to an amplifier (General Purpose Stemtech Inc., Winston, VA). Pulsatile arterial pressure was sampled at 120 Hz under basal conditions and 2, 4, 24 and 48 h after AP with an analog-to-digital converter and processed with a computer on a beat-to-beat basis (AT-CODAS), providing values of mean arterial pressure.

Liver and pancreas from four rats in each group were fixed in 10% formaldehyde and embedded in paraffin, and 5-6- μ m sections were stained with hematoxylin-eosin (H+E).

Statistical analysis

The results are reported as means \pm SD. The significance of the differences between the mean values was evaluated by analysis of variance (ANOVA).

Results

Blood and ascitic fluid

The animals showed increased serum transaminase levels 12 and 24 h after induction of acute pancreatitis (Table 1). Serum amylase increased rapidly within the first 2 h, reached a maximum at 4 h, and then returned to basal levels at 48 h. Total serum protein content decreased 12 and 24 h after the induction of pancreatitis.

Free active trypsin, total trypsin and amylase in the ascitic fluid reached a maximum 2 h (4 h for amylase) after the induction of

acute pancreatitis and then decreased gradually. The protein levels increased 24 and 48 h after induction of AP. The largest volume of ascitic fluid was observed 12 h after the induction of pancreatitis (Table 2).

Oxidation and phosphorylation of liver mitochondria

Table 3 shows the results of liver mitochondrial function. After 2 and 4 h there was an increase in the oxygen consumption rate by liver mitochondria in S3 and S4, and a decrease in RCR and in the ADP/O ratio. At 12 and 24 h, RCR increased to normal levels while the other parameters remained unchanged. After 48 h of acute pancreatitis a significant reduction in RCR, state 3 respiration and ADP/O ratio was observed.

Arterial blood pressure

Blood pressure remained within normal levels at 2 h and 4 h (111 ± 3 mmHg) but significant hypotension was observed in all

Table 1 - Changes in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase and total protein in experimental acute pancreatitis (AP).

Data are reported as means \pm SD. *P<0.05 compared to control (analysis of variance).

	ALT (IU)	AST (IU)	Amylase (U/ml)	Total protein (g%)
Control (N = 4)	33 \pm 1	153 \pm 10	7.5 \pm 0.1	8.2 \pm 0.2
AP 2 h (N = 8)	48 \pm 4	200 \pm 19	17.1 \pm 1.9*	8.0 \pm 0.2
SHAM 2 h (N = 3)	43 \pm 3	142 \pm 5	7.8 \pm 0.1	7.8 \pm 0.1
AP 4 h (N = 5)	68 \pm 3	241 \pm 7	29.7 \pm 1.3*	7.9 \pm 0.4
SHAM 4 h (N = 2)	44 \pm 8	141 \pm 11	7.6 \pm 0.1	7.3 \pm 0.1
AP 12 h (N = 5)	145 \pm 17*	472 \pm 47*	17.5 \pm 2.1*	6.7 \pm 0.2*
SHAM 12 h (N = 2)	68 \pm 2	138 \pm 3	9.1 \pm 0.1	7.9 \pm 0.1
AP 24 h (N = 6)	133 \pm 31*	590 \pm 110*	17.9 \pm 2.6*	6.2 \pm 0.2*
SHAM 24 h (N = 2)	67 \pm 8	140 \pm 18	8.8 \pm 0.2	7.8 \pm 0.4
AP 48 h (N = 4)	63 \pm 6	177 \pm 32	8.6 \pm 0.9	7.7 \pm 0.3
SHAM 48 h (N = 2)	65 \pm 6	165 \pm 9	8.4 \pm 0.8	7.7 \pm 0.4

animals at 24 and 48 h (91 ± 4 mmHg) (Figure 1).

Histological analysis

Acute pancreatitis with acinar necrosis, hemorrhage and edema was observed in all animals. Sinusoidal liver congestion and slight inflammation were observed at 2, 4 and 12 h. Necrosis and degenerative changes of hepatocytes were observed at 24 and 48 h (Figure 2).

Discussion

Hepatic dysfunction is associated with AP and has been described by many authors (1-3,16-25). Potent hepatotoxic substances have been found in the pancreatitis-associated ascitic fluid whose toxicity on isolated rat liver mitochondria function has been described (5-7). The effect on mitochondrial function was mainly due to uncoupling of oxidative phosphorylation. However, a slight enhancement of ATP synthesis in liver mito-

Table 2 - Analysis of pancreatitis-associated ascitic fluid in rats.

Data are reported as means \pm SD. *P<0.05 compared to other groups without asterisk; **P<0.05 compared to 2, 4, 12, 24 h (analysis of variance). U/ml Trypsin = μ mol of substrate released per ml per min. U/ml Amylase = mg of substrate released per min per ml. AP = Acute pancreatitis.

	Volume (ml)	Activated trypsin (10^{-3} U/ml)	Total trypsin (10^{-3} U/ml)	Amylase (U/ml)	Total protein (g%)
AP 2 h (N = 7)	3.9 \pm 0.3	5.65 \pm 0.87*	42.38 \pm 9.1*	269 \pm 25*	3.2 \pm 0.2
AP 4 h (N = 5)	4.4 \pm 1.3	4.37 \pm 0.63*	29.22 \pm 5.0*	304 \pm 16*	2.0 \pm 0.2
AP 12 h (N = 6)	9.6 \pm 3.4*	2.00 \pm 0.25	4.77 \pm 0.7	124 \pm 13	3.0 \pm 0.2
AP 24 h (N = 7)	4.0 \pm 0.9	0.95 \pm 0.21	5.22 \pm 0.9	81 \pm 16	4.2 \pm 0.2*
AP 48 h (N = 5)	2.4 \pm 0.9	0.32 \pm 0.10	2.27 \pm 0.8	4 \pm 1**	4.5 \pm 0.2*

Table 3 - Oxidation and phosphorylation activities of mitochondria in experimental acute pancreatitis.

Data are reported as means \pm SD. S3 and S4 = Oxygen consumption in ng atoms of O_2 min^{-1} mg protein $^{-1}$; RCR = S3/S4. *P<0.05 compared to control and sham groups (analysis of variance); **P<0.05 compared to control, AP 2, 4, 12, 24 h and sham groups (analysis of variance).

	RCR	S3	S4	ADP/O
Control (N = 14)	4.45 \pm 0.08	127.84 \pm 5.81	28.96 \pm 1.50	1.91 \pm 0.01
AP 2 h (N = 12)	3.53 \pm 0.09*	160.10 \pm 5.57*	45.65 \pm 1.79*	1.77 \pm 0.02*
SHAM 2 h (N = 3)	4.55 \pm 0.28	120.71 \pm 9.25	26.70 \pm 2.22	1.95 \pm 0.02
AP 4 h (N = 6)	3.51 \pm 0.19*	153.62 \pm 8.07*	44.45 \pm 3.30*	1.78 \pm 0.04*
SHAM 4 h (N = 3)	4.12 \pm 0.07	119.90 \pm 4.54	27.20 \pm 1.22	1.91 \pm 0.03
AP 12 h (N = 8)	4.26 \pm 0.05	149.45 \pm 3.87*	35.12 \pm 0.87*	1.85 \pm 0.02*
SHAM 12 h (N = 3)	4.28 \pm 0.28	106.50 \pm 5.63	25.08 \pm 2.96	1.90 \pm 0.01
AP 24 h (N = 7)	4.24 \pm 0.23	144.77 \pm 8.38*	33.95 \pm 2.98*	1.77 \pm 0.02*
SHAM 24 h (N = 3)	4.06 \pm 0.06	117.67 \pm 0.78	28.99 \pm 0.23	1.94 \pm 0.03
AP 48 h (N = 7)	3.15 \pm 0.23*	84.99 \pm 8.98**	26.50 \pm 1.58	1.81 \pm 0.04*
SHAM 48 h (N = 2)	4.19 \pm 0.16	114.19 \pm 8.99	27.65 \pm 1.48	1.93 \pm 0.04

chondria 24 h after the induction of AP has been reported by others (21,22). These investigators also observed a significant reduction in mitochondrial function over a period of 2 days after the induction of AP (21). However, the early periods of acute pancreatitis were not evaluated in these studies.

In the present study we observed increased levels of trypsin and amylase in the ascitic fluid in the early periods (2 and 4 h) after induction of acute pancreatitis (Table 2), suggesting that systemic absorption of toxic substances from the ascitic fluid may occur early after induction of AP, causing liver damage.

Our results demonstrate disruption of mitochondrial function early after induction of acute pancreatitis (Table 3). In the early

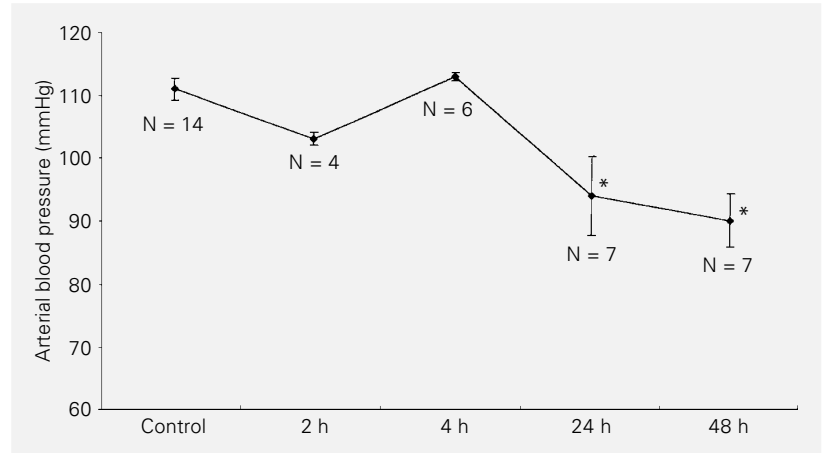


Figure 1 - Arterial blood pressure in acute experimental pancreatitis. Data reported as means \pm SD for N animals in each group. * $P < 0.05$ compared to control (analysis of variance).

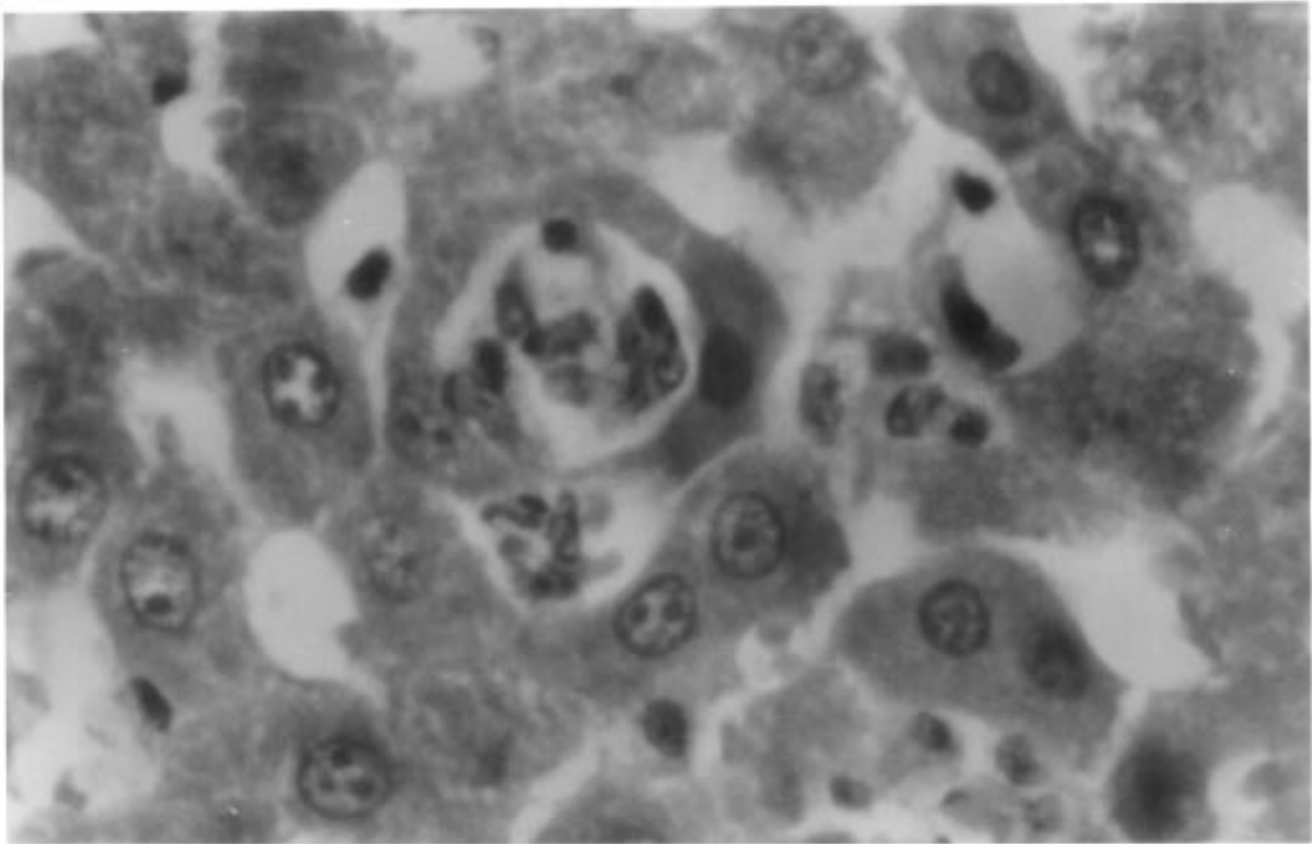


Figure 2 - Liver biopsy 48 h after induction of acute pancreatitis showing lobular focal necrosis and mononuclear inflammatory cell infiltrates. H+E. 400X.

periods an uncoupling effect on mitochondrial function was observed, together with decreases in RCR, an increase in state 4 respiration and a reduction in the ADP/O ratio. In this early phase (2 and 4 h) no significant changes in serum transaminases were observed (Table 1). After the 12-h period, however, an increasing depression of all parameters of mitochondrial respiratory functions with decreased mitochondrial ATP synthesis was observed. Since biological functions depend on a source of high energy phosphate, its decrease may cause severe damage to hepatic cells. Actually, after the 12-h period we observed increased levels of serum AST and ALT indicating hepatic cellular damage. At 48 h after induction of AP, a decrease in RCR, in state 3 respiration and in ADP/O ratio was observed, suggesting a degenerative and necrotizing process characteristic of cellular ischemia (Table 3).

Kitamura et al. (21) also demonstrated significant damage to mitochondrial function 48 h after the induction of AP. In the present study, these alterations were associated with a decrease in arterial blood pressure (Figure 1) and are consistent with the results of previous studies demonstrating depressed liver mitochondrial respiratory

function in response to shock and ischemia (26-28). Cell death may result from mitochondrial respiratory dysfunction.

Thus, the present findings demonstrate a biphasic damage to liver mitochondrial function in acute pancreatitis: an initial phase of uncoupling of oxidative phosphorylation and a late phase with damage to mitochondrial ATP synthesis. In the early phase of AP, the level of pancreatic enzymes in the ascitic fluid is high, suggesting that disruption of mitochondrial function during this period may be related to toxic substances released during this phase (5), followed by a temporary and partial recovery (Table 3). In the late phase, mitochondrial dysfunction is associated with arterial hypotension and probably related to ischemic damage.

Further studies are needed to elucidate the different factors involved in the pathophysiology of AP, in particular the abnormalities of liver mitochondrial function and to determine the appropriate treatment.

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