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EXPERIMENTAL

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Time course of lung injury in rat acute pancreatitis

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Abstract *Objective:* Lung injury is a severe complication of acute pancreatitis that increases the mortality rate of the disease. The pathophysiology of acute pancreatitis has been studied in several experimental models, but the kinetics of pulmonary complications in relation to the pancreatic disease is not completely understood. We then studied the severity of acute pancreatitis-associated lung injury over 18 h in rats that had taurocholic acid injection in the pancreatic duct and determined whether blood collected from rats with pancreatitis is toxic enough to induce injury in normal lungs. *Design and setting:* Prospective, randomized, and controlled animal study in an animal research laboratory in a university hospital. *Interventions:* We isolated lungs from rats with acute pancreatitis 2, 6, and 18 h after taurocholic acid injection in the biliopancreatic duct and perfused them with blood collected from the same rats. Additionally, blood collected from rats with acute pancreatitis (time-points: 2 and 6 h) was perfused in normal lungs. *Measurements and results:* Taurocholic acid injection induced a severe pancreatic injury that started as early as 2 h after the injection and persisted without recovery over the 18-h study period. In contrast, the pulmonary injury was transient, appearing at the 6-h time point with recovery by the end of the study. Pulmonary injury was moderate and evidenced mostly during lung reperfusion. Interestingly, blood collected at the 2-h time point in pancreatic rats induced pulmonary injury in normal lungs while blood collected at the 6-h time-point was not toxic. *Conclusions:* While pancreatic injury persists over the full experimental period, pulmonary injury is transient in our experimental model. The recovery of lung injury by 18 h might be explained by a decrease in the overall toxicity of pancreatic blood over time.

Keywords Rats · Lung injury · Perfused lungs · Acute pancreatitis

Introduction

During acute pancreatitis the incidence of pulmonary complications varies from 15% to 55%, and their severity ranges from mild hypoxemia without clinical or radiological abnormalities to severe acute respiratory distress syndrome [1, 2, 3]. The early phase of the pancreatic disease is associated with a systemic inflammation while the second is characterized by the appearance of local complications such as fat necrosis, pseudocyst formation, and pancreatic abscesses. Two peaks of pulmonary complications during the early phase of acute pancreatitis have been observed by Berry et al. [4]. The first one is observed on hospital admission, and radiological abnormalities have been found in 15% of patients while by day 5 new radiological abnormalities are reported in additional 71% patients of the study. However, the kinetics of pulmonary complications in relation to the pancreatic disease is not well established.

Moreover, the mechanisms of propagation of the disease from pancreas to lungs is largely unknown. Mediators released by the inflamed pancreas, the peripancreatic tissues, or ascites have been administered in healthy rodents to reproduce the lung injury observed in severe acute pancreatitis. For example, trypsin [5, 6, 7] and elastase [5] can reproduce lung injury in healthy rodents. Moreover, ascites collected from rats with acute pancreatitis and injected to normal rats also reproduce the injury [8, 9]. Another way to investigate the propagation of the pancreatic disease to lungs might be to perfuse normal lungs with blood collected from rats with acute pancreatitis. By collecting blood from pancreatic rats at various time points we might be able to determine which phase of the pancreatic disease is the more susceptible to induce injury in healthy lungs.

We then studied the severity of acute pancreatitis-associated lung injury over 18 h in rats that had taurocholic acid injection in the pancreatic duct and determined whether blood collected from rats with pancreatitis is toxic enough to induce injury in normal lungs.

Methods

Induction of acute pancreatitis

Sprague-Dawley rats (350–400 g) were anesthetized with isoflurane, intubated, and mechanically ventilated (FIO_2 0.4) with a rodent ventilator (tidal volume 7 ml/kg; positive end-expiratory pressure, PEEP, 2.5 cm H_2O; respiratory rate 70–80/bpm). After laparotomy the pancreatic duct was cannulated with a PE-10 tubing. A clip was placed on the duct close to the liver and taurocholic acid (4%, 0.5 ml sodium salts; Sigma) was infused at a constant rate. The clip was then withdrawn and the tubing was left in place, the lower extremity being secured into the duodenum to allow a normal bile secretion. Before abdomen closure rats had saline solution (5 ml) into the peritoneum. Rats received buprenorphine and were looked after until lung isolation (2, 6, or 18 h later; Fig. 1). Sham rats were similarly anesthetized and ventilated and after laparotomy received intraperitoneal fluid and buprenorphine injection. All pro-

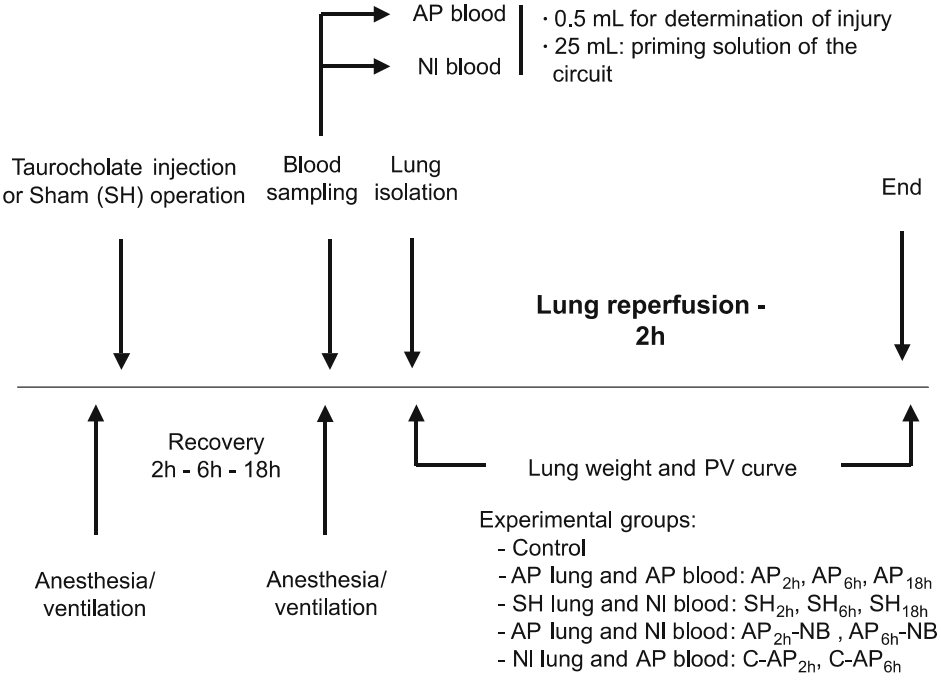

Fig. 1 Experimental protocol. To investigate the time course of acute pancreatitis-induced lung injury lungs were reperfused either 2 h (AP_{2h}, $n = 7$), 6 h (AP_{6h}, $n = 9$), or 18 h (AP_{18h}, $n = 7$) after taurocholic injection. Sham operated rats were studied at similar time points: 2 h (SH_{2h}, $n = 5$), 6 h (SH_{6h}, $n = 6$), or 18 h (SH_{18h}, $n = 6$). To assess the effect of blood collected from rats with acute pancreatitis on pulmonary injury lungs isolated from normal rats (control, C) were reperfused with blood collected from rats 2 h (C-AP_{2h}, $n = 8$) or 6 h (C-AP_{6h}, $n = 8$) after induction of pancreatitis. Conversely, lungs isolated from rats with acute pancreatitis were reperfused with blood collected from normal rats: AP_{2h}-NB ($n = 10$) or AP_{6h}-NB ($n = 10$). Lungs immediately isolated from normal rats were not subjected to laparotomy and served as control group ($n = 8$). *AP* Acute pancreatitis; *Nl* normal; *PV* lung pressure-volume

cedures were performed according to the guidelines stated in the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." The experimental protocol was reviewed and approved by the Ethics Committee for Animal Research at the University Medical Center and by the Veterinary Office in Geneva, Switzerland.

Lung isolation

One hour before lung reperfusion rats were anesthetized, tracheotomized, ventilated, and monitored as previously described. In addition, the femoral artery was cannulated for blood sampling, continuous blood pressure monitoring, and fluid replacement. Rats were anticoagulated and 25 ml blood was gently withdrawn via the arterial cannula and replaced by an equal volume of 6% hydroxyethyl starch to maintain a mean systemic blood pressure above 50 mm Hg to minimize lung ischemia (normovolemic hemodilution). This blood volume was reconcentrated (hematocrit close to 30%) and used as a priming volume into the circuit. After a median sternotomy a polyethylene catheter was placed into the main pulmonary artery via the right ventricular outflow track. Catheters were also placed into the left ventricle for collection of effluent blood, and in the left atrium for continuous left atrial pressure (P_{la}) recording. Following pulmonary artery cannulation lungs were flushed with 30 ml cold (10°C) hydroxyethyl starch solution (perfusion pressure 30 cm H_2O) to minimize warm ischemia until ex vivo reperfusion.

In vitro lung reperfusion

In a humidified Plexiglas chamber (37°C) the heart-lung block was suspended to an isometric force displacement transducer that continuously measures lung weight. Lungs were ventilated with room air mixed with 5% CO_2 at a respiratory rate of 30/min, a tidal volume of 7 ml/kg, and a PEEP of 2.7 cm H_2O. Sighs were applied every 5 min (21 ml/kg). The circuit was primed with blood volume collected from the same or from a different rat (see protocol in Fig. 1). Lungs were then perfused via the pulmonary artery cannula from an arterial reservoir placed at a fixed height to induce a mean pulmonary arterial pressure (P_{pa}) close to 17.5 mm Hg. Pulmonary blood flow (PBF) was continuously measured by a transit-time flowmeter. Effluent blood was drained through the left ventricle cannula whose distal extremity was placed at a height sufficient to obtain a P_{la} of 7.5 ± 0.5 mm Hg.

Assessment of lung function

Airway pressure (P_{aw}), tidal volume, and airflow resistance were measured. Continuous breath by breath dynamic lung compliance (C_{dyn}) and expiratory airway resistance (R_{aw}) were assessed by the following formulas: C_{dyn} = tidal volume/(peak P_{aw} – PEEP) and R_{aw} = (peak P_{aw} – PEEP)/maximal expiratory air flow. Inspiratory and expiratory quasistatic pressure-volume curves were performed before lung reperfusion and at the end of the 2-h reperfusion period by inflating and deflating lungs at a constant rate (0.3 ml/s) from PEEP level to a volume of 7 ml or 25 cm H_2O (whichever occurred first) using an automated infusion pump. The volumes obtained at an inspiratory and expiratory P_{aw} of 10 cm H_2O were recorded (V_{10insp} and V_{10exp}, respectively). Pulmonary vascular resistance (PVR) was calculated by dividing $P_{pa} - P_{la}$ by PBF. Every 30 min a blood sample was collected to measure blood gas, glucose and electrolyte concentrations, and hematocrit. Total red and white blood cell count and percentage of polymorphonuclear cells (PMNs) were determined with an automated cell counter. Amylase concentration was measured using an automatized analyzer.

Wet-to-dry lung weight ratio

The inferior lobe of each lung was weighed (wet weight), dried at 60°C for 2 days, and weighed again to determinate the wet-to-dry lung weight ratio.

Experimental groups

To investigate the time course of acute pancreatitis-induced lung injury in our experimental model lungs were reperfused either 2 h (AP_{2h}, $n = 7$), 6 h (AP_{6h}, $n = 9$), or 18 h (AP_{18h}, $n = 7$) after taurocholic injection. Sham-operated (SH) rats were studied at similar time points: 2 h (SH_{2h}, $n = 5$), 6 h (SH_{6h}, $n = 6$), or 18 h (SH_{18h}, $n = 6$) (Fig. 1). To assess the effect of blood collected from rats with acute pancreatitis on lung edema and pulmonary mechanics lungs isolated from normal rats (C) were reperfused with blood collected from rats 2 h (C-AP_{2h}, $n = 8$) or 6 h (C-AP_{6h}, $n = 8$) after induction of pancreatitis. Conversely, lungs isolated from rats with acute pancreatitis were reperfused with blood collected from normal rats: AP_{2h} + NB ($n = 10$) or AP_{6h} + NB ($n = 10$). Finally, lungs immediately isolated from normal rats served as a control group (control, $n = 8$).

Statistical analysis

Group data are presented as mean ± SE values. Statistical comparison among the treatment groups at baseline or at the end of the study was performed by a one-way analysis of variance followed by Newman–Keuls multiple comparison test if the analysis of variance resulted in a p value less

than 0.05, and comparisons involving time used a multivariate analysis of variance for repeated measurements.

Results

Taurocholic acid induced pancreatic injury

Induction of acute pancreatitis by taurocholic acid injection was well tolerated, and all animals rapidly recovered from anesthesia. Rats had an increase in serum amylase concentration as early as 2 h after taurocholic acid injection, and high serum concentrations were maintained 6 h and 18 h after pancreatitis induction (Fig. 2). Hemoconcentration, another marker of the disease, was absent in the AP_{2h} group but occurred in the AP_{6h} and AP_{18h} groups (Fig. 3A). Hemoconcentration was associated with a marked increase in the percentage of circulating PMN cells (Fig. 3B). Alteration in blood gases or in the alveolar-arterial O_2 (A-aDO_2) pressure gradient was statistically significant only in the AP_{6h} group (Fig. 3C).

Following lung isolation and before the start of reperfusion the initial weight of heart-lung blocks (see the15-min time point on Fig. 4A, B) did not differ between the experimental groups. Serum amylase concentrations, PMN cells, and pressure A-aDO_2 gradient in the AP_{2h}-NB and AP_{6h}-NB groups were similar to those in the respective AP groups, and in the C-AP_{2h} and C-AP_{6h} groups similar to the control group (data not shown). In summary, taurocholic acid injection induced a severe pancreatic injury that started as early as 2 h after the injection while systemic complications (hemoconcentration and increased circulating PMNs) occurred at 6 h without recovery by 18 h. Lung injury was mild and transient; the A-aDO_2 gradient increased only in the AP_{6h} group. At the time of lung isolation all experimental groups had similar heart-lung weights. Laparotomy by itself increased circulating PMNs (Fig. 3B) without any effect on hematocrit, serum amylase, or the A-aDO_2 pressure gradient.

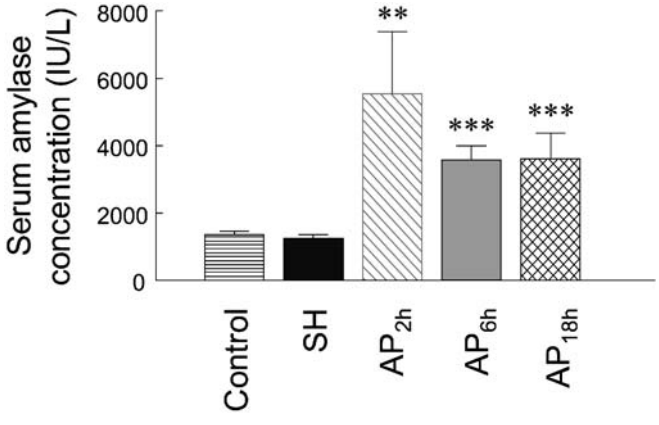

Fig. 2 Serum amylase concentrations in control rats, sham-operated rats (*SH*), and rats that had taurocholic acid injection in the pancreatic duct 2 h (*AP_{2h}*), 6 h (*AP_{6h}*), and 18 h (*AP_{18h}*) before blood collection; see Fig. 1 for experimental groups. $^{**}p<0.01$, $^{***}p<0.001$ vs. control or SH groups (one-way analysis of variance followed by Newman–Keuls test)

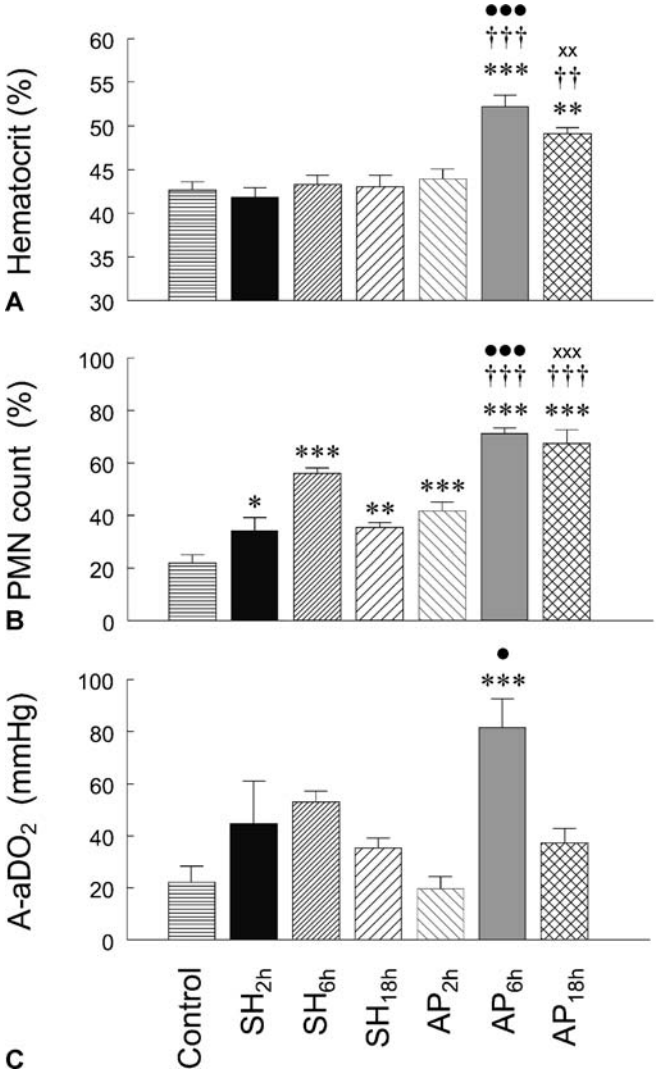

Fig. 3 Blood hematocrit (%, **A**), circulating polymorphonuclear (*PMN*) cells (%, **B**), and alveolar-arterial O_2 pressure gradient (*A-aDO_2*, **C**) in control rats, sham-operated (*SH*) rats, and rats with acute pancreatitis (*AP*). Rats had sham operation or taurocholic acid injection 2, 6, or 18 h before blood collection; see Fig. 1 for experimental groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control; $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ vs. AP_{2h} group; $^{\bullet}p<0.05$, $^{\bullet\bullet\bullet}p<0.001$ vs. SH_{6h} group; $^{xx}p<0.01$, $^{xxx}p<0.001$ vs. SH_{18h} group (one-way analysis of variance followed by Newman–Keuls test).

Reperfusion of lungs with blood collected from the same rat

We first perfused lungs isolated from normal, sham, and pancreatic rats with blood collected from the same rats.

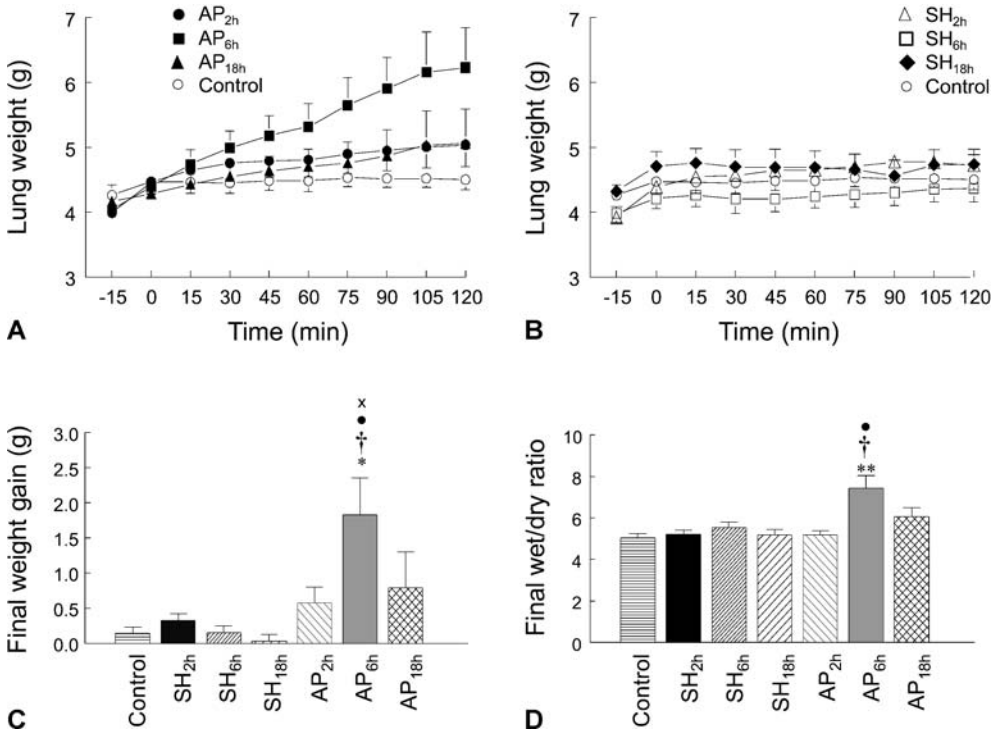

Fig. 4 Lung weight recording during reperfusion (**A**, **B**), final weight gain (**C**), and final wet/dry ratio (**D**) in AP_{2h} ($n=7$), AP_{6h} ($n=9$), or AP_{18h} ($n=7$), SH_{2h} ($n=5$), SH_{6h} ($n=6$), SH_{18h} ($n=6$), or control ($n=8$) groups; see Fig. 1 for experimental groups. $^{*}p<0.05$, $^{**}p<0.01$ vs. control group; $^{\dagger}p<0.05$ vs. AP_{2h} group; $^{\bullet}p<0.05$ vs. SH_{6h} group; $^{x}p<0.05$ vs. AP_{18} group (one-way analysis of variance followed by Newman–Keuls test)

During the 2-h reperfusion period P_{pa}, P_{la}, and PEEP were maintained steady ($\pm$ 10% from baseline values) as described above. PBF, hematocrit, and pH did not change significantly over time and did not differ between groups. When lungs isolated from rats with acute pancreatitis were reperfused with blood collected from the same rat lung weight increased steadily over time (approx. 1 g/h) only in the AP_{6h} group (Fig. 4A). No weight increase was observed in control and SH groups (Fig. 4B). The final weight gain (Fig. 4C) and the final wet/dry ratio (Fig. 4D) in the AP_{6h} group were significantly higher than in control, SH_{6h}, and AP_{2h} groups. The continuous increase in weight over time during reperfusion was associated with a progressive and significant decrease in C_{dyn} ($p < 0.05$ vs. SH_{6h}; see Electronic Supplementary Material, ESM, S.F1A) and a significant increase in R_{aw} (ESM S.F1C). Furthermore, at the end of lung reperfusion, the quasistatic pressure-volume relationship was significantly altered in this group in comparison to the SH_{6h} group (Fig. 5B). The inspiratory (V_{10insp}) and expiratory (V_{10exp}) lung volumes obtained at 10 cm H_2O pressure were significantly reduced in the PA_{6h} group, showing an important reduction in static compliance (Table 1). No difference in lung pressure-volume curves had been observed between groups at the start of reperfusion.

Table 1 Lung volumes at 10 cm airway pressure at the beginning (15 min) and at the end (120 min) of the reperfusion period

Experimental groups	Number of rats	V_{10insp} (ml)		V_{10exp} (ml)	
		15 min	120 min	15 min	120 min
Control	8	6.04 ± 0.40	6.04 ± 0.47	6.96 ± 0.31	7.07 ± 0.42
SH_{2h}	5	4.48 ± 0.34	5.29 ± 0.18	5.22 ± 0.40	6.06 ± 0.33
SH_{6h}	6	5.43 ± 0.27	4.95 ± 0.26	6.72 ± 0.41	5.77 ± 0.39
SH_{18h}	6	5.66 ± 0.50	5.07 ± 0.44	6.52 ± 0.64	5.93 ± 0.39
AP_{2h}	7	5.62 ± 0.31	5.02 ± 0.18	6.78 ± 0.26	7.02 ± 0.40
AP_{6h}	9	5.25 ± 0.40	$2.97 \pm 0.76^{**,\dagger}$	5.87 ± 0.40	$4.23 \pm 0.71^{*}$
AP_{18h}	7	5.32 ± 0.33	4.30 ± 0.72	6.08 ± 0.37	5.28 ± 0.71

Inspiratory (V_{10insp}) and expiratory (V_{10exp}) lung volume at 10 cmH_2O airway pressure; $^{*}P<0.05$; $^{**}P<0.01$ *versus* control; $\dagger P<0.05$ *versus* SH_{6h}. See Fig. 1 for experimental groups.

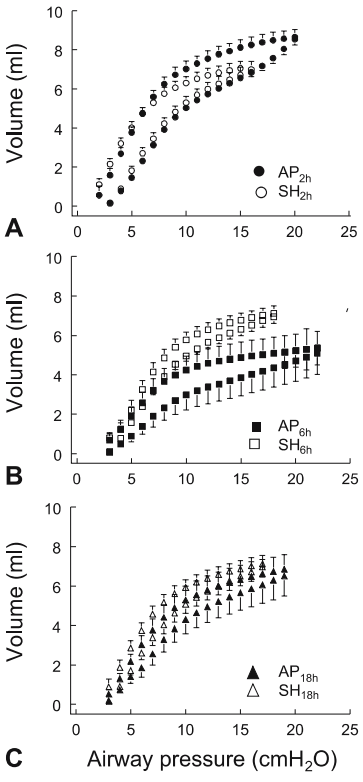

Fig. 5 Inspiratory and expiratory pressure- volume curves in isolated lungs from sham rats (*SH*, *open symbols*) and from rats with pancreatitis (*AP*, *closed symbols*). Lung reperfusion was performed 2 h (**A**), 6 h (**B**), or 18 h (**C**) after sham operation or taurocholic acid injection. AP_{2h} ($n = 7$), SH_{2h} ($n = 9$), AP_{6h} ($n = 7$), SH_{6h} ($n = 5$), AP_{18h} ($n = 6$), SH_{18h} ($n = 6$); see Fig. 1 for experimental groups

Reperfusion of pancreatic lungs with normal blood

Reperfusing lungs isolated from pancreatic rats (time point: 6 h) with normal blood (PA_{6h}-NB group) did not modify the time course of dynamic lung mechanics, quasistatic pressure-volume relationship, or final wet-to-dry lung weight ratio in comparison to the AP_{6h} group (ESM S.F2; Fig. 6 and Table 2). Moreover, the increase in lung weight was not attenuated (Fig. 6B, D). Furthermore, in comparison to all other experimental groups the pulmonary vascular resistances were significantly reduced throughout the reperfusion period ($p < 0.01$, data not shown). Pancreatic lungs reperfused with normal blood (time point 2 h, PA_{2h}-NB group) did not differ from time-matched pancreatic lungs reperfused with their own blood except for a small initial increase in lung weight during the first minutes of reperfusion (Fig. 6A). In summary, reperfusing pancreatic lungs with normal blood did not attenuate lung injury.

Reperfusion of normal lungs with pancreatic blood

Reperfusing normal lungs with pancreatic blood collected from rats 6 h after taurocholic acid injection (C-AP_{6h} group) produced no alteration in lung weight, dynamic or static mechanics in comparison to the control group (ESM S.F2; Fig. 6). The only abnormality was a reduced R_{aw} during the whole reperfusion period (ESM S.F2D). In contrast, reperfusing normal lungs with pancreatic blood collected 2 h after taurocholic acid injection (C-AP_{2h} group) was associated with a rapid initial increase in lung weight (Fig. 6A), and the final weight gain was significantly higher than in control group (Fig. 6C). A transient increase in C_{dyn} was also observed in the first hour of reperfusion, but C_{dyn} returned to baseline value by the end of the reperfusion period (ESM S.F2A). This was associated with a moderate but significant alteration in the inspiratory pressure-volume relationship, as documented by a significantly reduced V_{10insp} (Table 2). In summary, blood collected 2 h after taurocholic acid injection induces moderate injury in normal lungs while blood collected 6 h after the injection did not.

Discussion

Our study shows that taurocholic acid injection induces a severe pancreatic injury that started as early as 2 h after the injection and persisted without recovery over the 18-h study period. In contrast, the pulmonary injury is transient, appearing at 6 h with a complete recovery by the end of the study. Pulmonary injury was moderate and evidenced mostly during lung reperfusion. Interestingly, blood collected at 2 h in pancreatic rats induced pulmonary injury in normal lungs while blood collected at the 6-h time point was not toxic. Thus while pancreatic injury persisted over the full experimental

Table 2 Lung volumes at 10 cm airway pressure at the beginning (15 min) and at the end (120 min) of the reperfusion period

Experimental groups	Number of rats	V_{10insp} (ml)		V_{10exp} (ml)	
		15 min	120 min	15 min	120 min
AP_{2h} + NB	10	5.84 ± 0.28	5.60 ± 0.33	6.35 ± 0.30	7.41 ± 0.19
C-AP_{2h}	8	5.68 ± 0.29	4.07 ± 0.24**,††	6.43 ± 0.34	6.08 ± 0.50
AP_{6h} + NB	8	5.87 ± 0.15	2.88 ± 0.58**	6.77 ± 0.21	4.29 ± 0.50**
C-AP_{6h}	8	5.39 ± 0.24	$4.89 \pm 0.40^{\in,\$}$	6.10 ± 0.24	5.72 ± 0.43

Inspiratory (V_{10insp}) and expiratory (V_{10exp}) lung volume at 10 cmH_2O airway pressure; **$P < 0.01$ *versus* control (see Table 1); ††$P < 0.01$ *versus* AP_{2h} + NB; $^{\in}P < 0.05$ *versus* AP_{6h} + NB; $^{\$}P < 0.05$ *versus* AP_{6h} (see Table 1). See Fig. 1 for experimental groups.

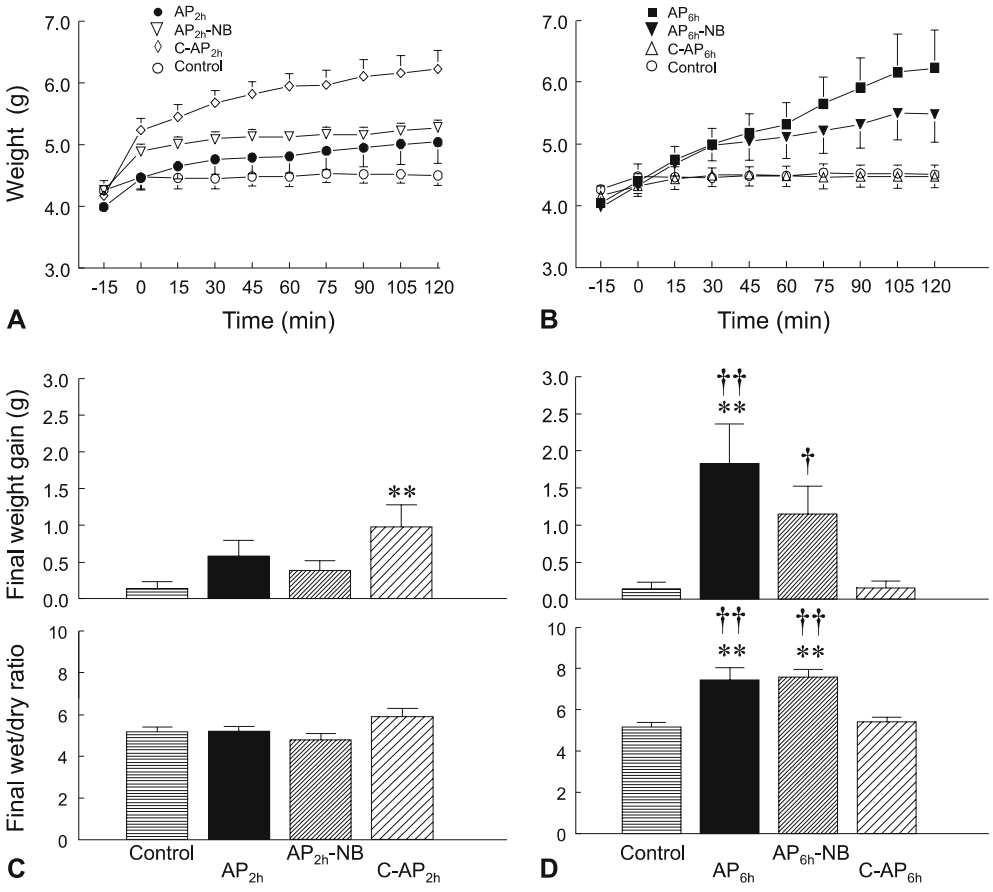

Fig. 6 Weight during the 2-h reperfusion period (**A**, **B**). Experimental groups are: AP_{2h} ($n=7$), AP_{2h}-NB ($n=10$), C-AP_{2h} ($n=8$), control ($n=8$), AP_{6h} ($n=9$), AP_{6h}-NB ($n=10$), C-AP_{6h} ($n=8$); see Fig. 1 for experimental groups. Final weight gain and final weight/dry ratio (**C**, **D**) are showed in the same experimental groups. $^{**}p<0.01$ vs. control group; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. the C-AP_{6h} group

period, pulmonary injury was only transient (Fig. 7). The recovery of lung injury by 18 h might be explained by a decrease in the overall toxicity of pancreatic blood over time.

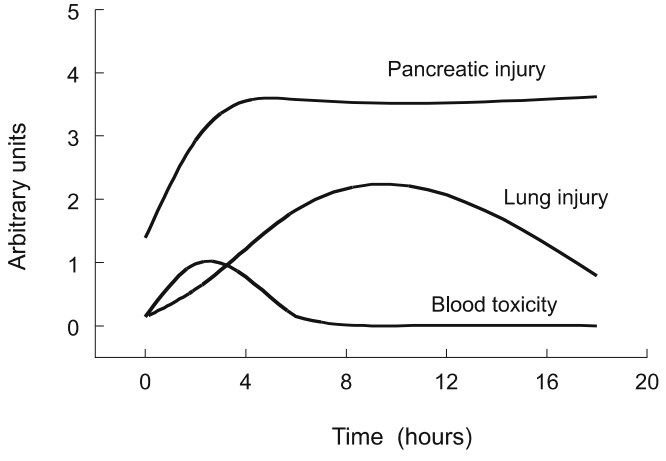

Fig. 7 Schematic representation of pancreatic injury (estimated by serum amylase concentrations), lung injury (lung weight gain), and blood toxicity (estimated by the lung weight gain induced by pancreatic blood perfusion in normal lungs)

Experimental models of acute pancreatitis

Several experimental models of acute pancreatitis with variable severity and mortality rates have been described in the literature to investigate the pathophysiology of the disease. Intraperitoneal administration of supramaximal doses of cerulein (a cholecystokinin analog) induced a mild edematous pancreatitis [10] while administration of a cholin-deficient/ethionine-supplemented diet to young female mice induced a necrotizing and hemorrhagic pancreatitis, with death occurring within 5 days [11]. Intraductal injection of taurocholic acid induced a severe disease with a high mortality within hours of injection [12]. Pancreatic lesions with bacterial infiltration were observed as early as day 1. By day 3 hemorrhagic necrosis, fat necrosis, cell infiltration, and intraparenchyma edema were observed in rats that survived. Six hours after taurocholic acid administration Pereda et al. [13] also observed acinar necrosis, interstitial edema, and infiltrate of inflammatory cells. Another model that combines glycodeoxycholic acid injection and cerulein perfusion showed increase in myeloperoxidase activity and pancreatic edema after 3 h, and the abnormalities persisted by 24 h [6].

Surprisingly, such severity was not found in our experimental groups AP_{2h}, AP_{6h}, or AP_{18h}. The absence of mortality in our model might be explained by the fact that we secured the lower extremity of the biliopancreatic tubing into the duodenum after taurocholic acid injection, preventing bile leak into the peritoneum. After taurocholic acid injection the increase in serum amylase concentrations occurred within 2 h without recovery by 18 h after taurocholic acid injection. Hemoconcentration and inflammation, evidenced by an increased circulating PMNs, occurred later and persisted 18 h after taurocholic acid injection, demonstrating the persistent severity of the pancreatic disease.

Characteristics of lung injury over time

Few previous studies investigated the early changes in pulmonary mechanics after the initiation of acute pancreatitis by taurocholic acid. In this model Lichtenstein et al. [12] described alveolar edema by day 1 associated with PMNs infiltration while Milani et al. [14] found a significant increase in pulmonary elastance. In the latter study lung sections showed uneven distribution of ventilation, edema in alveoli, and PMN infiltration [14]. As early as 2 h after bile acid injection, alveoli are filled with fluid, macrophages, red blood cells, and cellular debris [15]. The enlargement of alveolar septa was evident and severe endothelial changes were obvious with disintegration of type I epithelial cells, adherence of platelets to capillary endothelium, and loss of endothelial cell cytoplasm [15]. An increase in pulmonary wet-to-dry ratio was found as early as 1 h after taurocholic acid injection [15]. Six hours after taurocholic acid administration the infiltration of PMNs was significantly increased, and alveola walls were enlarged [13, 16]. Other experimental models have also been used to study the time course and severity of pulmonary injury. In cerulein-injected mice pulmonary injury was characterized by an early but sustained edema (within 12 h) associated with an increased pulmonary microvascular permeability (within 36 h) [17]. In the absence of complication pulmonary injury recovered within 7 days [17].

In our experimental model lung injury was moderate and transient. The A-aDO_2 pressure gradient measured in vivo (before lung isolation) increased 6 h after taurocholic acid injection and returned to baseline value by 18 h. Moreover, before lung perfusion heart-lung weights were similar in SH, C, and AP rats. During reperfusion lung weight steadily increased in the AP_{6h} group in contrast to all other groups whose weight remained within normal values. In the AP_{6h} group alteration in pulmonary mechanics was also evidenced by an increase in R_{aw} associated with a decrease in C_{dyn}. At 6 h pressure-volume curves were also altered by lung reperfusion. Thus pulmonary injury in our experimental model was minor, transient (6 h after taurocholic acid injection), and evidenced mostly by lung reperfusion (Fig. 7).

Toxicity of pancreatic blood on normal lungs

Pulmonary injury has previously been reproduced by injecting either pancreatic enzymes, ascites, or blood collected from pancreatic animals in healthy rodents, but no study has investigated whether ascites or blood toxicity varies over time. Trypsin and trypsinogen perfusion in healthy rats increased pulmonary edema and myeloperoxidase activity [6] while pancreatic elastase induced cytokine-mediated lung injury through the nuclear factor κ B second messenger system [5]. Ascites collected 24 h after taurocholic acid administration in rats importantly aggravated lung injury in rats with mild pancreatitis (single cerulein injection) [18]. Sterile, endotoxin and cytokine-free ascitic fluid collected from rats with glycodeoxycholic acid administration in the biliopancreatic duct was infused in normal rats [9]. Twenty-four hours after ascites injection alveolar leukocytes and protein were significantly increased, and thickening of alveolar septa was observed. Finally, serum with high trypsin activity isolated from rats 6 h after glycodeoxycholic acid and cerulein administration has been injected into normal rats [7]. Increased alveolar edema and increased myeloperoxidase activity characterized the lung injury.

When we reperfused normal lungs with pancreatic blood collected from rats 6 h after taurocholic acid injection, no lung injury was observed. In contrast, reperfusing normal lungs with pancreatic blood collected 2 h after taurocholic acid injection was associated with a significant weight gain, transient increase in C_{dyn}, and alterations in the inspiratory pressure-volume relationship. We then hypothesized that the decreased overall toxicity of pancreatic blood over time would explain the recovery of lung injury during pancreatic disease in our experimental model. As described in human pancreatitis, the production of anti-inflammatory cytokines over time is likely to limit the toxic effects of activated enzymes and proinflammatory mediators [19].

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