Changes in the biophysical properties and ultrastructure of lungs, and intrapulmonary fibrin deposition in experimental acute pancreatitis

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SUMMARY Using an experimental model of acute pancreatitis in the rat, we have studied changes in the biophysical properties of lungs and intrapulmonary fibrin deposition in this condition. Acute pancreatitis is associated with a significant decrease in pulmonary compliance ($p < 0.01$) and a significant increase in lung weight ($p < 0.01$) compared with a control sham operation group. These changes are associated with a 24% increase in intrapulmonary ^{125}I fibrinogen deposition ($p < 0.01$), and an 18% increase in ^{125}I fibrinogen deposition per gram of lung tissue ($p < 0.05$) in acute pancreatitis, compared with a control sham operation group. The increased fibrinogen deposition is abolished by treatment with low dose heparin. Using the same animal model changes in pulmonary ultrastructure are shown using scanning electron microscopy. The results indicate that pulmonary abnormalities are associated with intrapulmonary fibrin deposition in experimental acute pancreatitis and these findings may be relevant to the well described respiratory complications of the condition in man.

The importance of various respiratory complications which occur in patients with acute pancreatitis has been repeatedly emphasised¹⁻⁶ since pleural empyema was first described in such a patient in 1932.⁷ Though several theories, including denaturation of surfactant^{8,9} and the development of arterio-venous shunts,¹⁰ have been suggested to explain these pulmonary abnormalities, the mechanism remains incompletely understood and probably involves many factors.

The clinical features are those of adult respiratory distress syndrome,¹¹ a condition which has been attributed to intrapulmonary fibrin deposition.^{12,13} Coagulation disturbances, which occur in acute pancreatitis,^{6,14,15} may contribute to the pulmonary damage in this condition.

A better understanding of the mechanism involved is desirable to facilitate treatment and improve prognosis in this group of patients. Using a model of acute pancreatitis in the rat, we have studied the changes which occur in the biophysical properties of lung, in the ultrastructural pulmonary appearances and in intrapulmonary fibrin

deposition. The latter was measured using ^{125}I fibrinogen injection.

Methods

MATERIALS

Adult Sprague Dawley rats weighing between 250 g and 350 g were fasted for 24 hours, but given free access to water and then anaesthetised using intraperitoneal pentobarbitone (Sagatal 60 mg/ml) diluted to a 10% solution with ethyl alcohol, in a dose of 0.75 ml/100 g body weight. Acute pancreatitis was then induced as described by Chetty *et al.*¹⁶ This method involves forming a closed duodenal loop around a piece of fine plastic tubing which maintains intestinal continuity. Infected human T tube bile is then injected into the loop with sufficient pressure to produce distension of the loop and reflux along the pancreatic duct. The model consistently produces a severe haemorrhagic form of acute pancreatitis.

EXPERIMENT I

Three groups of eight rats were studied. (1) sham gastrotomy – killed at six hours, (2) acute pancreatitis – killed at six hours, (3) acute pancreatitis – killed at 24 hours.

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In the sham operation group, laparotomy was performed under general anaesthesia, the stomach was opened, as for the passage of the plastic tubing, but then simply closed.

Immediately before death, at the predetermined time, blood was taken for serum amylase estimation (Phadebas technique) and rats were killed by administering a massive overdose of intraperitoneal pentobarbitone. After death the lungs were carefully removed and inflated with 10 ml air and then deflated. Inflation/deflation pressures were measured at 0.5 ml intervals using a three way tap and a water manometer as previously described.¹⁷ The lungs were then cleaned and weighed. Pulmonary compliance was calculated from the gradient of the curve obtained by plotting lung volume against intrapulmonary pressure, over the first 5 ml of a slow deflation from a maximum inflation as recommended.¹⁸ In a further two rats in each of the above three groups, immediately after death the lungs were fixed for scanning electron microscopy by inflating them at a constant pressure (3 ml H₂O) with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Blocks of tissue were then cut, completely dehydrated in increasing concentrations of acetone, and then critical point dried. The dried mounted tissue was then sputter coated with a thin layer (20 nm) of gold and examined in an ISI-60 scanning electron microscope (SEM) using an acceleration voltage of 30 kV. Many areas within each lung were examined to determine the overall condition of the alveoli and a few representative photographs were taken.

EXPERIMENT 2

Pulmonary fibrin deposition was studied using ¹²⁵I human fibrinogen injection in four groups of eight Sprague Dawley rats. This technique has previously been validated by Saldeen.¹⁹ (1) Sham gastrotomy, (2) acute pancreatitis, (3) acute pancreatitis plus heparin (50 IU/kg subcutaneously), (4) acute pancreatitis plus dextran (2 ml intravenously).

Acute pancreatitis was induced as previously reported¹⁶ and immediately after operation 0.05 mg of iodinated (¹²⁵I) human fibrinogen injection (approximately 7 μ Ci) in 0.2 ml sterile water was injected into a femoral vein. In group 3 this was followed by 50 IU/kg heparin injected subcutaneously and in group 4 by 2 ml dextran 70 injected into the opposite femoral vein. All rats were killed after six hours by the method previously described and the lungs were immediately removed, blotted dry, and weighed before counting in an LKB-Wallac 80 000 gamma scintillation counter. Organ counting was performed relative to an ¹²⁵I standard (approximately 7 μ Ci ¹²⁵I) against which

the injection had been counted. Statistical significance of the difference between the mean values of each group was calculated by Student's *t* test.

Results

EXPERIMENT 1

Mean serum amylase concentrations were $14\,140 \pm 3600$ IU/l (SD) in control group (I) compared with $106\,400 \pm 79\,629$ IU/l ($p < 0.01$) and $169\,400 \pm 59\,826$ IU/l ($p < 0.001$) in pancreatitis groups (II) and (III) respectively.

The specific pulmonary compliance (compliance/lung weight) was 0.79 ± 0.08 ml/cmH₂O/g in the sham operation group (I), compared with 0.64 ± 0.11 ml/cmH₂O/g ($p < 0.01$) and 0.69 ± 0.09 ml/cmH₂O/g ($p < 0.01$) in the pancreatitis groups (II) and (III) respectively (Fig. 1).

Lung weight (as a percentage of total body weight) was $0.34 \pm 0.03\%$ in control group (I) compared with $0.49 \pm 0.13\%$ ($p < 0.01$) and $0.42 \pm 0.07\%$ ($p < 0.01$) in pancreatitis groups (II) and (III) respectively (Fig. 2).

Figures 3 and 4 show representative scanning electron micrographs of lung from the sham operation group and the pancreatitis group killed at

Fig. 1 Specific pulmonary compliance in control sham operation group (I) and pancreatitis groups at six hours (II) and 24 hours (III) – (mean $\pm$ SD).

Fig. 2 Lung weight expressed as % of total body weight in control sham operation group (I) and pancreatitis groups at six hours (II) and 24 hours (III) (mean $\pm$ SD).

six hours and at original magnification of 2000.

In the pancreatitis groups, gross distortion of alveolar architecture was seen. The type I pneumocytes which form the alveolar wall were contracted and the interalveolar septa had a crenated, thickened and irregular surface (Fig. 4) compared with the sham operated animals (Fig. 3). Though the mechanisms of these changes await

further clarification, they were diffusely present throughout all sections of the lungs examined.

EXPERIMENT 2

The percentage of ^{125}I fibrinogen deposited in the lungs of the control group (1) was $1.38 \pm 0.17\%$ compared with $1.72 \pm 0.25\%$ ($p < 0.01$) in the pancreatitis group (2). This 24% increase was abolished by treatment with a low dose of heparin (group 3) ($1.33 \pm 0.15\%$, $p < 0.01$) and by intravenous dextran 70 (group 4) ($1.13 \pm 0.1\%$, $p < 0.001$) (Fig. 5).

The percentage of ^{125}I fibrinogen deposited per gram of lung tissue was $0.9 \pm 0.09\%/g$ in the control group (1) compared with $1.07 \pm 0.18\%/g$ in the pancreatitis group (2) ($p < 0.05$). This 18% increase was abolished by treatment with heparin in group 3 ($0.77 \pm 0.06\%/g$, $p < 0.01$). Treatment with dextran 70 (group 4) did not significantly decrease ^{125}I fibrinogen deposition per gram of lung tissue ($0.91 \pm 0.08\%/g$, $p > 0.05$) (Fig. 6).

Three rats in group four died soon after administering the dextran 70. This was attributed to dextran anaphylaxis a problem previously reported with Sprague Dawley rats.²⁰ Because of their deaths the results with dextran are anomalous and difficult to interpret. They do, however, show a favourable trend and, therefore we feel they are worth reporting.

Discussion

The raised serum amylase concentrations in groups (II) and (III) in the first experiment confirm that the model used produces acute pancreatitis. Histological confirmation of this is seen in Figure 7 which

Fig. 3 Scanning electron micrograph of rat lung from sham operated animal (original magnification $\times 2000$).

Fig. 4 Scanning electron micrograph of rat lung from pancreatitis group killed at six hours (original magnification $\times 2000$).

Fig. 5 Percentage of ^{125}I fibrinogen taken up by lungs in sham operation group (1), acute pancreatitis group (2) and pancreatitis groups treated with heparin (3) and dextran 70 (4) (mean $\pm$ SD).

Fig. 6 Percentage of ^{125}I fibrinogen taken up per gram of lung weight in sham operation group (1), acute pancreatitis group (2) and pancreatitis groups treated with heparin (3) and dextran 70 (4) (mean $\pm$ SD).

Fig. 7 Histological appearance of pancreatic tissue from rat subjected to pancreatitis model and killed at six hours (Haematoxylin + eosin, original magnification $\times 120$).

shows haemorrhage with intraductal and periductal abscess formation; and the appearances are consistent with acute haemorrhagic pancreatitis. A significant decrease in pulmonary compliance and an increase in pulmonary weight occurred at both six and 24 hours compared with the controls, with maximum changes occurring at six hours.

As pulmonary compliance depends on both the intrinsic elastic properties of the alveolar wall and surfactant, the decrease could be due to a reduction in the concentration of the latter factor alone.^{8,9} The ultrastructural changes shown by SEM, however, suggest that the alveolar wall is morphologically altered in acute pancreatitis and particularly that the abnormalities occur diffusely throughout affected lungs. Though these changes are difficult to quantify the alveoli and pneumocytes undoubtedly appear contracted in the pancreatitis groups compared with the control group.

The results of the second experiment indicate that the changes are associated with intrapulmonary fibrin deposition, in that at 6 hours (when the changes in biophysical properties are maximal) there was a significant increase in the uptake of ¹²⁵I fibrinogen in lungs in the pancreatitis group compared with the control group. This finding gives further credence to the view that acute pancreatitis produces the same pulmonary abnormalities that occur in adult respiratory distress syndrome.^{6,11-13} How fibrin deposition produces the changes reported is open to debate, but it seems likely that the harmful effects are due to peptides released during the conversion of fibrinogen to fibrin²¹ or to the products of fibrin breakdown themselves.²² We

have previously shown that the degree of hypoxia in patients with acute pancreatitis correlates with plasma fibrinogen concentrations and also is associated with complicated disease.⁶ Our results suggest that these patients might benefit from early treatment with low dose heparin or dextran. Though the experimental results with dextran were conflicting because of the anaphylactic problem, they do show a trend towards decreasing intrapulmonary fibrin deposition and are therefore worthy of note – particularly as dextran anaphylaxis in humans is relatively uncommon. Further studies are necessary to clarify this.

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