Parenteral administration of trypsin triggers lung emphysema

E. Reichart*, P. Boerkmann*, F. Plenat**

ABSTRACT: Eight weeks after a single intravenous injection of trypsin, more than half of 26 treated rats showed pulmonary emphysema, as demonstrated by a significant increase of the mean linear intercept (MLI=107 μm) in comparison with 11 controls (69±15 μm) (mean±sd). As observed 56 days after the injection, the intraperitoneal administration of trypsin (24 rats) also leads to lung emphysema (MLI=101–106 μm), as does endotracheal instillation of elastase (13 rats), (MLI=108 μm).

The intraperitoneal administration of trypsin in animals constitutes a model close to human pathology with which lung alterations in acute pancreatitis may be studied. Having no elastolytic properties, trypsin cannot directly induce emphysema. The observation of a pulmonary leucostasis in eight rats sacrificed early after the trypsin injection suggested that leucocyte trapping and activation are important for the genesis of this trypsin-triggered emphysema.


Emphysema is characterized pathologically by abnormal enlargement of airspaces distal to the terminal bronchioles with destruction of alveolar walls [1]. The mechanisms of these dramatic alterations in humans are not precisely known. However, two observations have indicated the importance of proteolysis in human emphysema. The first reported that an inheritable deficiency of α1-protease inhibitor (α1-PI) is often associated with an early onset, hereditary, panlobular emphysema [2]. The second showed that the intratracheal injection of crude papain could produce a lesion in mammals which is comparable to emphysema [3]. These two observations indicate that the maintenance of normal lung structure requires a balance between proteases and their inhibitors and that excessive proteolytic activity in the lungs, because of increased protease release or decreased inhibition, results in emphysema. Only proteases that degrade elastin can produce emphysema in experimental animals [4]. Attempts to use non-elastolytic proteases have been uniformly unsuccessful.

The intravenous injection of trypsin is a well-established procedure, used to examine the role of proteases bringing about early lung injury after acute haemorrhagic pancreatitis [5–7]. Recent studies in our laboratory unexpectedly showed that pulmonary emphysema develops in rats eight weeks after one intravascular infusion of this enzyme [8–10]. Having no elastolytic activity, trypsin cannot directly produce emphysema in experimental animals. As a pulmonary leucostasis and a decreased blood α1-PI activity were observed during the acute phase of the experiments, it is probable that this trypsin-triggered emphysema was mainly due to the release of leucocyte elastase [8, 9].

Intraperitoneal administration of trypsin may constitute a model with which lung alterations after acute pancreatitis can be studied. Intraperitoneal injection of trypsin is a route for trypsin administration that is more practical than intravascular infusion. Furthermore, in acute pancreatitis, an enzyme-rich fluid often exsudes from the pancreas into the peritoneal cavity. Thus, intraperitoneal injection of trypsin may constitute a model close to human pathology, with which lung alterations in acute pancreatitis can be investigated. In the present study, we have compared the early and long-term effects on the lung of intraperitoneal injection of trypsin with those observed when this enzyme is intravenously injected.

Materials and methods

Animals

Male Wistar rats, randomly bred, were obtained from IFFA-CREDO breeding laboratory (France); they weighed 347±44 g (mean±sd) at the start of the experiment.

To investigate the long-term effects of two different doses (D or 1/2D) of intravenously (TIV) or intraperitoneally (TIP) injected trypsin, a set of 50 rats (table 1) was divided into several groups and compared with 11 saline-treated rats (controls) or 13 rats rendered emphysematous by an endotracheal instillation of elastase (ELAS).
To assess the early lung effects of trypsin when administered intraperitoneally a second set of eight rats were injected at the dose (D) of 45 U benzyl arginine ethyl ester (BAEE)-kg⁻¹ BW over 2 h (BAEE used as substrate). As our previous experiments showed that intraperitoneal administration of normal and sterile saline does not induce any lung alteration, this control was not performed again.

The lung characteristics of these treated or control animals were compared with those of a wide population of rats of the same strain (87 healthy rats maintained in the laboratory and studied over several years).

Enzyme administration, lung preparations and data analysis

Trypsin intravenous injections and elastase intratracheal instillations were carried out under general anesthesia with intraperitoneal sodium pentobarbital (38 mg·kg⁻¹·h⁻¹). The t-test was used to determine whether the statistical differences between the groups were significant.

Results

Anatomical observations

The architecture of terminal airspaces in the lungs of the eight rats sacrificed early after the trypsin injection was always maintained. However, granulocytes could be observed, in each case, sequestrated within the pulmonary microcirculatory bed and in four cases within the alveoli also (fig. 1). In three rats, oedema was localized in the connective tissue surrounding small bronchioles and blood vessels.
Alveolar oedema and/or fibrin thrombi in the capillaries were never detected. No alterations were noticed in the lung of the 11 control rats which were perfused with sterile saline for 2 h.

As the structural alterations in the lung of the rats sacrificed on the 56th day were slight, it was usually impossible to unequivocally show loss of alveolar parenchyma by light microscopy alone, without the help of quantitative techniques. Dilatation of terminal airspaces and effacement of alveolar septa were widely and uniformly spread, without a pulmonary anteroposterior lesional gradient. The airways were always unaltered and neither an inflammatory exsudate nor polymorphonuclear leucocyte (PMN) intravascular trapping could be detected.

One control rat showed some degree of focal peribronchiolar enlargement of the alveoli.

Morphometric data

Whatever the dose, intraperitoneal administration of trypsin resulted in a significant (p<0.001) MLI increase (ΔMLI) (table 2). A similar ΔMLI was observed in the lungs of the rats receiving trypsin by the vascular route (p<0.001) at a dose of 45 or 22.5 U·kg⁻¹. No statistical differences were found between TIP and TIV rats, or between the trypsin-treated rats and ELAS rats. The value of the MLI did not depend on the type of parenteral administration.

Statistical definition of pathology

Using the value established in a large population of our strain of male Wistar rats (n=87) (MLI=77±12 μm) we defined the upper limit of normal MLI value (mean±2 SD) to be 102 μm. Each rat used in this experiment and characterized by a value above the limit was considered as abnormal, or emphysematous if it was treated with an enzyme.

No control rat in this study was abnormal, demonstrating the quality of the rats used and the innocuousness of the saline treatment in this experiment.

The sensitivity of this set of rats to enzymes was relatively low; only 54% of the ELAS rats were emphysematous. Interestingly, a similar percentage of "abnormal" rats was found in the trypsin-treated groups. Intraperitoneal injection, whatever the dose, had an effect similar to that of intravenous infusion of trypsin. As the AMLI of the TIP and TIV treated rats was as abnormal as that of the ELAS rats, we concluded that TIP and TIV rats were emphysematous.

![Fig. 1. – At the 4th hour after injecting trypsin intraperitoneally at a dose of 45 U·kg⁻¹ BW·h⁻¹ over 2 h, numerous granulocytes are sequestred within the microcirculatory bed (>). A few polymorphonuclear leucocytes are also present within the terminal airspaces (†→).](image1)

![Fig. 2. – Histological section from the right diaphragmatic lobe of a TIV-D rat on the 56th day after the trypsin infusion. Terminal airspaces are enlarged and some interalveolar walls appear to have "dissolved". TIV-D: full dose of trypsin injected intravenously (haematoxylin and eosin, magnification x60).](image2)

![Fig. 3. – Histological section from the right diaphragmatic lobe of a TIP-D rat on the 56th day after an intraperitoneal injection shows enlarged terminal airspaces with simplification of lung structures. TIP-D: full dose of trypsin injected intraperitoneally.](image3)
The average value of MLI of all the trypsin-treated groups (TIV dose or half-dose, TIP dose or half-dose) increased significantly (***, p<0.001) compared with controls (saline-treated rats). This modification was of the same magnitude as elastase-treated rats (ELAS). For abbreviations see legend to table 1.

**Table 2.** Mean linear intercept (MLI) in trypsin-treated rats in comparison with elastase- or saline-treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rats</th>
<th>MLI:sd</th>
<th>p</th>
<th>ΔMLI % control value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11</td>
<td>69.3±15.1</td>
<td>--</td>
<td>100</td>
</tr>
<tr>
<td>ELAS</td>
<td>13</td>
<td>108.3±28.6</td>
<td>***</td>
<td>156</td>
</tr>
<tr>
<td>TIV-1/2D</td>
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<td>105.8±22.5</td>
<td>***</td>
<td>154</td>
</tr>
<tr>
<td>TIV-D</td>
<td>14</td>
<td>105.9±25.6</td>
<td>***</td>
<td>154</td>
</tr>
<tr>
<td>TIP-1/2D</td>
<td>11</td>
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<td>***</td>
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<tr>
<td>TIP-D</td>
<td>13</td>
<td>101.2±18.8</td>
<td>***</td>
<td>146</td>
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</tbody>
</table>

The average value of MLI of all the trypsin-treated groups (TIV dose or half-dose, TIP dose or half-dose) increased significantly (***, p<0.001) in comparison with controls (saline-treated rats). This modification was of the same magnitude as elastase-treated rats (ELAS). For abbreviations see legend to table 1.

**Discussion**

The intravenous injection of trypsin into mammals is a well-established model to investigate the role of proteases bringing about early lung vascular injury in acute pancreatitis [5-7]. In sheep, plasmatic α1-PI activity is at its lowest about 150 min after the beginning of a trypsin infusion at the rate of 4.5 mg·kg·BW·h" during 2 h [5]. Leucocytes are sequestered within the lungs and pulmonary lymph flow and transvascular protein clearance are increased [5]. The sequestration of leucocytes could be due to the protease activation of the complement system in the blood [7] as well as to fibrin degradation products generated by fibrinolysis, which is activated by intravenously injected trypsin [6].

Using similar experimental conditions, we showed previously that the infusion of trypsin also induces an early pulmonary leukostasis and oedema in rats. The observed α1-PI activity was about 94% of the initial value 150 min after the start of the infusion of trypsin in our rats [10], whereas, at this time, PI activity was 79% of the initial value in sheep [5]. This discrepancy may be explained by the high inhibition capacity of the rat plasmatic α1-PI [13]. Alpha1-PI activity may, however, be inhibited in rats [14] for two hours when using very large doses of trypsin. Such doses are incompatible with the long-term survival of animals.

No study had been performed on the long-term effects on the lung of a trypsin perfusion in animals. Unexpectedly, eight weeks after trypsin infusions, lung emphysema was observed in our rats and demonstrated by a ΔMLI of about 490% in comparison with the saline-treated controls. On histological examination, alveolar wall ruptures were clearly observed in 20 out of the 24 trypsin-treated rats [10]. Furthermore, when compared with a larger population of healthy rats of the same strain, 92% of the trypsin-treated rats had a statistically "abnormal" value of MLI [10]. It is surprising that emphysema appears in rats eight weeks after a perfusion of trypsin alone. Having no elastolytic properties, trypsin cannot directly cause emphysema. Trace amounts of elastase might possibly exist in the injected trypsin solution. Even if that were the case, the small amount of elastase could not by itself induce emphysema. Indeed, large doses of elastase injected into the blood are known to produce no observable pulmonary histological changes [15]. Moreover, when injected into the blood stream, traces of elastase would be diluted and dispersed in the whole organism. It is, thus, unlikely that emphysema in our experiment is due to a contamination by an exogenous elastase.

The observation of a pulmonary leukostasis in the rats sacrificed early suggests that leucocyte trapping and activation are important for the genesis of this trypsin-triggered emphysema [8-10]. It can be assumed that extravasated polymorphonuclear leucocytes inevitably disintegrate, releasing a potent serine protease, and also secrete free oxygen radicals, when stimulated. Thus, it is very likely that the emphysema following a trypsin overload is due to a local excess of endogenous elastases and free radicals in the lung. The decrease in α1-PI activity in our rats may be due to the proteolytic effect of trypsin. It may be admitted that antiprotease also decreases in the lung since lung antiprotease is considered as coming from the circulation. Experimental emphysema is usually obtained by using exogenous and heterologous elastases in very large supraphysiological doses introduced into the airways. This model undoubtedly creates a local enzyme excess but does not precisely mimic what happens in pathology. In human pathology, the enzymes or the cells producing enzymes reach the pulmonary interstitium, alveolar walls and alveolar spaces by passing through the capillary walls. In our experiment, the trypsin injected operates in the same way to produce emphysema. Moreover, our experiment demonstrates that endogenous elastases are effectively sufficient for producing an emphysema, since no exogenous elastase was given to our rats.

In the present experiment, emphysema in the lung of TIV, TIP and ELAS rats sacrificed on the 56th day is evidenced by the statistically significant increase of MLI. This increase in the TIV and ELAS rats is smaller than that observed in our previous works as is the percentage of "abnormal" subjects in these two groups of treated rats. It can thus be assumed that the set of rats used in this experiment is less sensitive to intravenously injected trypsin or intratracheally instilled elastase than the former one. Studying polyserites induced by very large doses of intraperitoneally injected trypsin, WARTER et al. [16, 17] also observed that the sensitivity of rats to this enzyme varies from one subject to another. At the present time, this varying sensitivity to trypsin administrations remains unexplained.

In the present experiment, as in the preceding one, the MLI increase in the groups of trypsin- and elastastreated rats is of the same magnitude [9, 10]. This suggests that the intensity of the induced pulmonary lesions does not directly depend on the enzyme which
is used but on unknown, indirect mechanisms, which should be studied.

Peritoneal and pleural exudates are often observed during the acute attacks of pancreatitis in humans. High concentrations of pancreatic enzymes are detected in plasma and activity of proteases are evidenced by alpha-macroglobulin variations [18, 19]. Intraperitoneal injection of trypsin may, thus, constitute a model close to human pathology with which lung alterations in acute pancreatitis may be studied. Furthermore, the intraperitoneal route may make it possible to avoid potential circulatory perturbations occurring when trypsin is injected intravenously (speed of injection, vascular resistance variations). In this study, we show that 56 days after trypsin injection the lung alterations are of the same intensity whatever the route of administration of this enzyme. Moreover, the percentage of "abnormal" subjects in the two groups of treated rats is similar. It can thus be assumed that the intraperitoneally injected trypsin reaches the lung through the blood stream. However, a transdiaphragmatic route is possible [16, 17] but not supported in our experiments by the absence of anteroposterior lesional gradient of histological pulmonary changes.

In conclusion, we confirm that emphysema develops after one single and parenteral administration of trypsin. This experiment demonstrates that an extrapulmonary excess of a protease having no elastolytic activity leads to emphysema. Moreover, in this model of acute pancreatitis, polymorphonuclear leucocytes sequestrated into the lung micromicrocirculatory bed and terminal airspaces may induce a local elastolysis since emphysema was observed. Further investigations are necessary to determine the factors that attract PMN into the alveolar structures and also the conditions of a possible neutrophil elastase burden in the lung. To the best of our knowledge, only one case of pancreatitis-related emphysema is described in the literature [20]. As indicated by our results it would be worth investigating for possible emphysema in nonsmoking and non-alcoholic patients recovering from an acute attack of pancreatitis.

References