Protection of mice by a protease inhibitor, aprotinin, against lethal Sendai virus pneumonia

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Proteolytic activation of Sendai virus in the lungs of mice is necessary to cause pneumopathogenicity. Using Sendai virus-infected lung block cultures, protease inhibitors were tested for their antiviral effect by examining inhibition of proteolytic activation. Among the inhibitors tested, a serine protease, aprotinin, was shown to be most effective. In vivo protection experiments demonstrated that aprotinin, when administered intranasally, could confer protection on mice against lethal Sendai virus pneumonia through the same mechanism as observed in the in vitro system. The present study provides an experimental basis for the use of protease inhibitors as antiviral drugs.
Fig. 1. *In vitro* suppressive effect of aprotinin on Sendai virus replication in mouse lung block cultures. Sendai virus-infected mouse lung blocks were cultured either in the presence (△, △) or absence (○, ○) of aprotinin at a concentration of 2500 U/ml. Amounts of whole (open symbols) and infectious virus (closed symbols) in the culture medium were determined.

Among the inhibitors, leupeptin (200 μg/ml) was as effective as aprotinin but no inhibitory effect was observed with either soybean trypsin inhibitor (1000 and 2000 μg/ml) or TLCK (20 μg/ml).

We also tested the *in vivo* effect of aprotinin. When aprotinin was administered intranasally six times after virus inoculation, production of infectious virus in the lung was reduced markedly by 12 h after infection (Fig. 2a). Whole virus titres at this time, however, were the same as those in the control mice, suggesting that the suppressive mechanism of aprotinin observed in vitro

Table 1. Effects of various protease inhibitors on Sendai virus production in mouse lung block cultures

<table>
<thead>
<tr>
<th>Protease inhibitors</th>
<th>Concentration (μg/ml)</th>
<th>Virus production (CIU/ml)*</th>
<th>Ratio of Infectious/whole virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>4.1 × 10^4</td>
<td>4.4 × 10^4</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>2500†</td>
<td>3.2 × 10^3</td>
<td>4.8 × 10^4</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>200</td>
<td>1.8 × 10^3</td>
<td>1.8 × 10^4</td>
</tr>
<tr>
<td>Soybean trypsin</td>
<td>1000</td>
<td>9.3 × 10^3</td>
<td>1.3 × 10^4</td>
</tr>
<tr>
<td>inhibitor</td>
<td>2000</td>
<td>8.6 × 10^3</td>
<td>9.0 × 10^3</td>
</tr>
<tr>
<td>TLCK</td>
<td>20</td>
<td>3.2 × 10^4</td>
<td>5.1 × 10^4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Virus titres in the culture fluids 3 days after infection.
† Units/ml, instead of μg/ml.
‡ Unable to be determined because of the severe cytotoxicity of the reagent.
was also operating in vivo. Since production of infectious virus was suppressed at 12 h, viral spread in the lung was limited thereafter and, as a consequence, virus production 24 h after infection was significantly lower in the aprotinin-treated mice than in the control mice. Intranasal administration of aprotinin six times within 12 h of virus inoculation while the control mice received phosphate-buffered saline (PBS) instead of aprotinin. When infected with 2.5 × 10^4 CIU per mouse, all of the 10 aprotinin-treated mice survived, whereas six out of 10 mice in the control group died of viral pneumonia (Fig. 3b and a, respectively). Similarly, when infected with 2.5 × 10^5 CIU per mouse, 10 out of 11 aprotinin-treated mice, but none of the 10 control mice, survived the infection (Fig. 3d and c). The protective effect of aprotinin was still observed when the mice were infected with 1.3 × 10^6 CIU per mouse (Fig. 3e and f). The differences in the mortality ratios between the aprotinin-treated and the control mice were statistically significant (P < 0.005, P < 0.001 and P < 0.05 for the mice infected with 2.5 × 10^4 CIU, 2.5 × 10^5 CIU and 1.3 × 10^6 CIU per mouse, respectively).

The present results clearly demonstrate that intranasal administration of aprotinin suppressed the activation of Sendai virus in mouse lung and thereby conferred protection against lethal viral pneumonia. This phenomenon is thought to be a direct reflection of the inhibitory effect of aprotinin on the proteolytic cleavage of the F glycoprotein in the bronchial epithelium. It has been reported previously that aprotinin suppressed the cleavage of Sendai virus F protein in embryonated eggs (Zhirnov et al., 1985). In influenza virus-infected mice, aprotinin suppressed proteolytic cleavage of virus haemagglutinin and interfered with multiple step replication of the virus in mouse lungs (Zhirnov et al., 1984). Tashiro et al. (1987) also reported that another protease inhibitor, leupeptin, suppressed a bacterial protease(s) which mediates cleavage of influenza virus haemagglutinin and that consequently the mice were protected from lethal virus pneumonia. Many other viruses also require proteolytic cleavage of their structural proteins for viral maturation. Replication of poliovirus and some retroviruses was inhibited by a cysteine protease inhibitor, cystatin (Korant et al., 1985) and an aspartyl protease inhibitor, pepstatin A (Yuasa et al., 1975; Katoh et al., 1987), respectively, and both of these suppressed the virus-encoded proteases.

Aprotinin is a well known serine protease inhibitor, consisting of 58 amino acids with an Mr of about 6500. It has a broad inhibitory spectrum including trypsin, chymotrypsin, plasmin and kallikrein, and is widely used for therapeutic purposes in patients with acute pancreatitis. The present study showed that aprotinin needs to be administered at relatively short intervals to exhibit its inhibitory effect because of its rapid clearance or inactivation in the lung. However, frequent intranasal administration of the drug rather decreased its protective effect (data not shown), probably by injuring physical

**Fig. 3. Protection of mice against lethal Sendai virus pneumonia with aprotinin.** Mice infected with 2.5 × 10^4 CIU (a and b), 2.5 × 10^5 CIU (c and d) and 1.3 × 10^6 CIU (e and f) received intranasal administrations of either aprotinin (b, d and f) or PBS as a control (a, c and e) six times within 12 h of virus inoculation. The body weight of each mouse was plotted daily. Closed triangles indicate dead mice.
protective mechanisms such as ciliary movement of the bronchial epithelium. A directly toxic effect of aprotinin was not likely to be the reason because no toxicity was observed in mouse lung block cultures.

It was shown in the present study that higher concentrations of aprotinin were required to inhibit the cleavage of Sendai virus F protein both in vitro and in vivo. This is not surprising, because aprotinin probably needs to be taken up by the bronchial epithelium in an active form to interact with the protease. The rationale for this statement originated from the observation by Tashiro & Homma (1983a) that non-infectious Sendai virus with uncleaved F protein did not become infectious when inoculated into mouse respiratory tracts. The result suggests that the F protein-cleaving enzyme was present inside the bronchial epithelium but not on the cell surface. It may be reasonable, therefore, to assume that a protease inhibitor with higher accessibility to the intracellular target would be more effective. To that end, we are currently screening synthetic protease inhibitors with lower $M_5$ which may be taken up more easily by the cells. Our present study provides an experimental basis for the use of protease inhibitors as antiviral drugs.

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References


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