Ginseng Treatment Reduces Bacterial Load and Lung Pathology in Chronic Pseudomonas aeruginosa Pneumonia in Rats

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The predominant pathogen in patients with cystic fibrosis (CF) is Pseudomonas aeruginosa, which results in a chronic lung infection associated with progressive pulmonary insufficiency. In a rat model of chronic P. aeruginosa pneumonia mimicking that in patients with CF, we studied whether the inflammation and antibody responses could be changed by treatment with the Chinese herbal medicine ginseng. An aqueous extract of ginseng was injected subcutaneously, and cortisone and saline were used as controls. Two weeks after challenge with P. aeruginosa, the ginseng-treated group showed a significantly improved bacterial clearance from the lungs (P < 0.04), less severe lung pathology (P = 0.05), lower lung abscess incidence (P < 0.01), and fewer mast cell numbers in the lung foci (P < 0.005). Furthermore, lower total immunoglobulin G (IgG) levels (P < 0.01) and higher IgG2a levels (P < 0.025) in serum against P. aeruginosa sonicate and a shift from an acute type to a chronic type of lung inflammation compared to those in the control and cortisone-treated groups were observed. These findings indicate that ginseng treatment of an experimental P. aeruginosa pneumonia in rats promotes a cellular response resembling a TH1-like response. On the basis of these results it is suggested that ginseng may have the potential to be a promising natural medicine, in conjunction with other forms of treatment, for CF patients with chronic P. aeruginosa lung infection.

A distinctive feature of patients with cystic fibrosis (CF) is the chronic Pseudomonas aeruginosa lung infection which leads to a slowly progressive damage of the lung parenchyma and eventually respiratory insufficiency (7, 27). The chronic P. aeruginosa lung infection is characterized by a significant antibody response and the infiltration of numerous polymorphonuclear leukocytes (PMNs) resembling a TH2-like response (15). This situation results in the inability of the host to clear the bacteria efficiently, and the elastase from PMNs plays an important role in the damage of the lung tissues of CF patients (15, 16, 29). In the present study we investigated the effects of ginseng treatment, for CF patients with chronic P. aeruginosa pneumonia, on the cellular and humoral immune response and enhance bacterial clearance in a rat model (13, 28). In brief, 1 ml of the P. aeruginosa bacterial culture was mixed with 9 ml of seaweed alginate (60% guluronic acid content), and the mixture was forced once with air through a cannula into a solution of 0.1 M CaCl2 in 0.1 M Tris-HCl buffer (pH 7.0). The suspension was adjusted to yield 106 CFU/ml, and the yield was confirmed by colony counts. Treatment protocol. Rats were divided into three groups, each comprising 20 animals.

(i) Group 1. Panax ginseng C. A. Meyer (ginseng) (11) powder was provided by Millington Limited, Jilin, People’s Republic of China. A water extract of ginseng was prepared by the following method. A total of 2.5 g of ginseng powder was mixed with 100 ml of distilled water at room temperature for 20 min, and then the mixture was heated at 90°C for 30 min and then filtered through sterile filter paper twice before use (final concentration, 25 mg of the equivalent of dry powder per ml). The concentration of protein in the ginseng extract was measured by a Bio-Rad method, and the result was 3.5 mg/ml. The endotoxin-like activity was measured by a Limulus amoebocyte lysate assay (2), and the level was 60 ng/ml, which is 1,660 times lower than the dosage of lipopolysaccharide (LPS) that we used in another study (20). The ginseng solution was injected subcutaneously by using a dosage of 25 mg/kg of body weight once a day for 10 days. The dosage was decided on the basis of a ginseng dosage pilot study.

(ii) Group 2. Hydrocortisone (cortisone; Upjohn s.a., Puurs, Belgium), a commercial product with a concentration of 50 mg/ml which is commonly used as an anti-inflammatory agent, was used as a control drug in the study. Cortisone was administered subcutaneously at a dosage of 25 mg/kg of body weight once a day for 10 days. The dosage was determined on the basis of the dosage used in the treatment of CF patients with P. aeruginosa infection (5).

(iii) Group 3. Group 3 was the control group. Sterile saline (0.9%) was injected subcutaneously at 1 ml/kg of body weight once a day for 10 days. In all groups the injections were started on the same day as challenge with P. aeruginosa alginate beads.

The anti-P. aeruginosa activities of the drugs were screened by an agar disk diffusion test. Blood agar plates (State Serum Institute, Copenhagen, Denmark) were seeded with P. aeruginosa PAO 579 to obtain semiconfluent growth, 10-mm-diameter paper disks containing 25 μl of undiluted solution of each drug were placed on the seeded plates, and the plates were incubated overnight at 37°C. Antibacterial activity was recorded as the inhibition zone around the disks after incubation.

Challenge procedures and blood samples. At the time of challenge, all rats were anesthetized subcutaneously with a 1:1 mixture of etomidate (Janssen, Birkerød, Denmark) and midazolam (Roche, Hvidovre, Denmark) at a dose of 1.5 ml/kg of body weight and were tracheotomized (13). Intratracheal challenge with 0.1 ml of P. aeruginosa (105 CFU/ml) in alginate beads was performed as described previously (13). The incision was sutured with silk, and the wounds healed without any complications. Fourteen days after challenge, all rats were

**MATERIALS AND METHODS**

Animals. Sixty female Lewis rats (Charles River, Würtzburg, Germany) that were 7 weeks old and that had a body weight of approximately 150 g were used.

Challenge strain. P. aeruginosa PAO 579 (kindly provided by J. R. W. Govan, Department of Bacteriology, Medical School, University of Edinburgh, Edinburgh, United Kingdom), which stably maintains a mucoid phenotype and which is International Antigenic Typing System type O:2/5, was used in our study.

Immobilization of P. aeruginosa in seaweed alginate beads. Immobilization of P. aeruginosa in seaweed alginate beads was done as described previously (14, 28). In brief, 1 ml of the P. aeruginosa bacterial culture was mixed with 9 ml of seaweed alginate (60% guluronic acid content), and the mixture was forced once with air through a cannula into a solution of 0.1 M CaCl2 in 0.1 M Tris-HCl buffer (pH 7.0). The suspension was adjusted to yield 106 CFU/ml, and the yield was confirmed by colony counts.

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TABLE 1. Median numbers of CFU of *P. aeruginosa* in rat lungs 14 days after intratracheal challenge

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. (of animals)</th>
<th>Median (range) bacterial count (CFU/lung)</th>
<th>No. (%) of rats with CFU &lt;100 CFU*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)</td>
<td></td>
<td>8.5 x 10^3 (1.3 x 10^3–6.3 x 10^3)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Ginseng (10)</td>
<td></td>
<td>1.5 x 10^3 (0–2.2 x 10^3)</td>
<td>7 (70)%</td>
</tr>
<tr>
<td>Cortisone (10)</td>
<td></td>
<td>5.6 x 10^3 (0.4 x 10^3–6.7 x 10^3)</td>
<td>2 (20)</td>
</tr>
</tbody>
</table>

* A three-by-two chi-square analysis for <100 CFU showed a significant difference between the groups (P < 0.05; \( \chi^2 = 7.178 \)).

* P < 0.04 compared to the control group.

* P < 0.025 compared to the cortisone-treated group.

* P < 0.02 compared to the cortisone-treated group.

significantly lower than those in the two control groups (*P < 0.04*), and 7 of 10 rats in the ginseng-treated group were found to be infected with fewer than 100 CFU. This number was significantly higher than those for the control or cortisone-treated group (*P < 0.025*). The difference in the percentage of rats in all three groups with fewer than 100 CFU was significant (*P < 0.05*).

Pathology. (i) Macroscopic lung pathology. Abscesses, atelectases, hemorrhages, and fibrinous adhesion to the thoracic wall or diaphragm could be found in all groups of rats 2 weeks after challenge. However, the lung pathology in the ginseng-treated group was significantly milder (Table 2) compared to those in the control and the cortisone-treated groups (*P < 0.02 and 0.01, respectively*). The differences between the groups for rats with a score of 1 and 2 and those with a score of 4 were significant (*P < 0.005 and P < 0.001, respectively*).

The incidence of lung abscesses in the ginseng-treated group was also significantly lower than those in the other two groups (*P < 0.01 and P < 0.001, respectively*).

(ii) Histopathological changes in the lungs. Significantly milder pathology was found microscopically in the ginseng-treated group (Table 3) compared to those in the cortisone-treated group (*P < 0.04*) and the control group (*P < 0.05*). Acute inflammation in lung tissues was seldom found in the ginseng-treated group. Furthermore, the mast cell number in the ginseng-treated group was significantly lower than those in the other two groups (*P < 0.04 and P < 0.004, respectively*). The differences between the groups for rats with a score of 1 and those with a score of 3 were significant (*P < 0.005*).

RESULTS

Disk diffusion examination on blood agar plates. None of the three agents studied was found to have anti- *P. aeruginosa* activity.

Mortality. Only one rat (in the cortisone-treated group) died during the study; no deaths were found among rats in the two other groups.

Bacteriology. *P. aeruginosa* could be cultured from the lungs of most surviving rats 2 weeks after challenge (Table 1). However, the bacterial count in the ginseng-treated group was signific-
Serum antibody responses. (i) Antibody responses to *P. aeruginosa* sonicate. Two weeks after challenge, the level of anti-*P. aeruginosa* sonicate IgG antibody in the sera of rats in the ginseng-treated group was significantly lower (Table 5) than that in the control group (*P* < 0.01) and the cortisone-treated group (*P* < 0.005). The IgM level in the cortisone-treated group was increased notably compared to that in the control group (*P* < 0.05) and that in the ginseng-treated group (*P* < 0.04). The IgM level in the ginseng-treated group tended to be lower than that in the control group, but the difference was not significant (*P* = 0.06). No significant difference in serum IgA level was found among the groups.

The IgG2a level in the ginseng-treated group was significantly higher (*P* < 0.025) than that in the control group (Table 6), although a significantly lower total IgG level was found in the ginseng-treated group (*P* < 0.04). The IgM level in the ginseng-treated group tended to be lower than that in the control group, but the difference was not significant (*P* = 0.06). No significant difference in serum IgA level was found among the groups.

Table: Serum antibody responses to *P. aeruginosa* sonicate 14 days after challenge with *P. aeruginosa* in rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Median ELISA unit (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Control (20)</td>
<td>1.35 (0.38–4.35)</td>
</tr>
<tr>
<td>Ginseng (20)</td>
<td>0.59 (0.00–2.88)</td>
</tr>
<tr>
<td>Cortisone (19)</td>
<td>1.66 (0.52–4.34)</td>
</tr>
</tbody>
</table>

(ii) Antibody responses to *P. aeruginosa* alginate. No significant difference in levels of antibody to *P. aeruginosa* alginate in serum were found 2 weeks after intratracheal challenge (Table 7).
ciliated with antigen-specific IgG1 production, the secretion of IL-4 and IL-10, as well as the proliferation of B cells and mast cells, which favor humoral immunity (3, 4, 6, 12, 18, 21, 22, 25). In CF patients, the P. aeruginosa lung infection provokes a rapid antibody response; in turn, the immune complexes are formed in the airways, which attract PMNs to the lungs, leading to damage of the lung tissue (8). High incidences of lung abscess and acute inflammation, high IgG levels against P. aeruginosa sonicate in serum, more mast cells in the inflammatory foci, and poor bacterial clearance were found in the control and the cortisone-treated groups in our present study, indicating a TH2-like response. In contrast, significantly lower serum IgG levels but higher IgG2a levels against P. aeruginosa, lower incidences of lung abscess and acute inflammation, fewer mast cells in lung foci, and much better clearance of bacteria from the lungs were found in the ginseng-treated group, indicating a TH1-like response. The higher number of mast cells and stronger IgG response in the control and the cortisone-treated groups might be related to the secretion of IL-10, a TH2 cytokine which plays a role in stimulating the proliferation and differentiation of mast cells and B cells. Mast cells play a role in the lung defense mechanism by releasing several neutrophil chemoattractants like tumor necrosis factor alpha and initiating neutrophil influx to the lung foci (1). In the present study, it was noticed that more mast cells were followed by more PMNs in the lung foci in the control group, whereas the opposite situation was found in the ginseng-treated group.

The mechanism of the curative effects of ginseng in the rat model of P. aeruginosa pneumonia is still unknown. However, from our results we believe that the effect of ginseng in lowering the serum IgG level and enhancing the shift of pulmonary inflammation from PMN to MN infiltration might be one of the reasons. On the other hand, ginseng can significantly activate the phagocytic activities of PMNs (10) and other phagocytes (30), increase natural killer cell activity and the level of lysozyme in serum, and increase the lymphocyte responses to concanavalin A and LPS (30, 31). These properties of ginseng might also be associated with the changes seen in the ginseng-treated group of our study. Cortisone is an anti-inflammatory agent commonly used to treat various diseases, including CF (5, 26). In our study, subcutaneous administration of cortisone showed no effect on P. aeruginosa pneumonia. On the other hand, mast cells are involved in the TH2 response, and they were found most frequently along the acute inflammatory foci in our study. Therefore, it might be an indication to apply antihistamine agents in the treatment of chronic P. aeruginosa lung infection.

In conclusion, ginseng appears to be a potentially promising remedy for the treatment of chronic P. aeruginosa lung infection in CF patients. Further studies to clarify the mechanisms involved in this model are warranted.

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REFERENCES