Inhibitory effects of cryptoporus polysaccharide on airway constriction, eosinophil release, and chemotaxis in guinea pigs

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KEY WORDS polysaccharide; ovalbumin; bronchoconstriction; respiratory function tests; eosinophil; peroxidase; platelet activating factor; chemotaxis; asthma; guinea pig

ABSTRACT

AIM: To study effects of cryptoporus polysaccharide (CP) on antigen-induced bronchoconstriction, eosinophil peroxidase (EPO) release in vivo, and on platelet activating factor (PAF)-induced eosinophil chemotaxis in vitro in guinea pig. METHODS: The asthma model of guinea pig was formed with ovalbumin (OVA). The changes of lung resistance ($R_L$) and dynamic lung compliance ($C_{dyn}$), EPO level in bronchoalveolar lavage fluids (BALF) and eosinophil migration were determined. RESULTS: Pretreatment of CP at doses of 3, 9, and 27 mg/kg by intragastric gavage (ig), qd for 10 d, inhibited early asthma response in a dose-dependent manner. Inhibitory rates of mean increase value from 1 to 30 min of $R_L$ were 34.8 %, 74.4 % ($P<0.05$), and 79.6 % ($P<0.05$), respectively. Inhibitory rate of mean reduction value of $C_{dyn}$ were 22.9 %, 40.5 % ($P<0.01$), and 66.5 % ($P<0.01$), respectively. Pretreatment of CP at doses of 3, 9, and 27 mg/kg also inhibited late asthma response, and the reduction of EPO level in BALF were 3.1 %, 16.9 % ($P<0.01$), and 20.1 % ($P<0.01$), respectively. The inhibitory rates of CP at concentrations of 0.13, 1.3, 13, 130 nmol/L to eosinophil migration induced by PAF were 6.8 %, 17.2 % ($P<0.05$), 29.6 % ($P<0.01$), and 35.9 % ($P<0.01$). CONCLUSION: CP protects lung against increase of $R_L$ and reduction of $C_{dyn}$, decreases EPO level in the asthma model, and inhibits eosinophil chemotaxis induced by PAF. The results suggest that CP may be a novel antiinflammatory agent for the treatment of asthma and allergic diseases.

INTRODUCTION

Cryptoporus volvatus grows wildly in China and its fruiting body has been used for asthma and bronchi-
Materials and methods

Materials and drugs Hartley guinea pigs of either sex, six month weighing 345 g±32 g were from Center of Medical School of Zhejiang University (Grade II, Certificate No 20010014 conferred by Zhejiang Medical Laboratory Animal Administration Committee). Cryptoporus polysaccharide (TSBIO Science & Technology Co, Ltd, purity: 98 %, molecular weight: 15 000 daltons), Triton X-100 and Tris (Shanghai Sangon Biological Engineering Technology and Services Co, Ltd), Urethane and O-phenylenediamino dihydrochloride (OPD, Shanghai Chemical Reagent Company), Heparin sodium (Xuzhou Biochemical Pharmaceutical Factory), Sephadex G-200 (Pharmacia), RPMI 1640 MEDIUM (Hyclone), L-α-phosphatidylcholine, β-acetyl-γ-o-alkyl (platelet activating factor, PAF), ovalbumin (grade II), and PERCOLL (Sigma Chemical Company) were commercially available.

Sensitizing procedures The guinea pigs were sensitized im with 10 g/L ovalbumin 1 mL. The animals were used 24 d after aerosol antigen challenged.

Treatment procedures and the lung function measurement CP at doses of 3, 9, and 27 mg/kg qd (in our preliminary experiments, guinea pigs were administrated with CP at doses of 1, 3, 9, 27, and 54 mg/kg to determine dose-effect) were given ig on d 14 after sensitization for 10 d, salbutamol as positive control drug 4 mg/kg by ig 1 h before antigen challenge. Guinea pig was anesthetized with urethane (1 g/kg, intraperitoneal, ip) at d 24. The trachea was cannulated and placed in a whole body plethysmography for measurement of lung resistance (\(R_L\)) and dynamic lung compliance (\(C_{dyn}\)). After 5 min for stabilizing preparation, increase of \(R_L\) and reduction of \(C_{dyn}\) of airway constric-
Chemotaxis was determined by counting eosinophils that had migrated completely through the filter in three high-power fields (HPF: 200×) per well randomly\[7\]. In the following experiment the bottom wells were filled with PAF at the concentration of 400 nmol/L because it caused the maximum chemotaxis effect, and the upper wells were filled with eosinophils 30 µL that had been treated with 15 µL CP at concentrations of 0.13, 1.3, 13, and 130 nmol/L, respectively. The following procedures were same as the former chemotaxis assays.

### RESULTS

#### Effect of CP on airway constriction in antigen challenged guinea pigs

There was no significant difference in basal $R_L$ and $C_{dyn}$ between each group. Inhaled antigen caused bronchoconstriction that peaked within 60 s. Pretreatment with CP at dose of 3, 9, and 27 mg/kg (ig) inhibited early asthma response of sensitized guinea pigs induced by aerosol OVA in a dose-dependent manner (Tab 1, 2). Regarding $R_L$ of control group as 100 %, inhibitory rate of mean increase value from 1 to 30 min of $R_L$ was 34.8 %, 74.4 % ($P<0.05$), and 79.6 % ($P<0.05$), respectively. Regarding $C_{dyn}$ of control group as 100 %, inhibitory rate of mean reduction value of $C_{dyn}$ was 22.9 %, 40.5 % ($P<0.01$), and 66.5 % ($P<0.01$), respectively. Salbutamol 4 mg/kg (ig), as a positive control drug, the inhibitory rate of mean increase value from 1 to 30 min of $R_L$ and $C_{dyn}$ was 66.8 % ($P<0.05$), and 64.1 % ($P<0.01$), respectively.

#### Effect of CP on EPO level in antigen challenged guinea pigs

Pretreatment with CP at dose of 3, 9, and 27 mg/kg (ig) inhibited early asthma response of sensitized guinea pigs induced by aerosol OVA in a dose-dependent manner (Tab 1, 2).

### Statistical analysis

Data were expressed as Mean±SD, and analyzed by Dunnett’s test.

#### Tab 1. Inhibition of cryptoporus polysaccharide (CP) ig on ovalbumin induced increase of lung resistance ($R_L$) of guinea pigs.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0 min</th>
<th>1 min</th>
<th>2 min</th>
<th>3 min</th>
<th>4 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>25 min</th>
<th>30 min</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>100</td>
<td>165±18</td>
<td>241±47</td>
<td>224±54</td>
<td>219±59</td>
<td>158±16</td>
<td>143±14</td>
<td>141±12</td>
<td>109±8</td>
<td>104±11</td>
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</tr>
<tr>
<td>CP 3 mg/kg</td>
<td>9</td>
<td>100</td>
<td>151±22</td>
<td>203±24</td>
<td>176±22</td>
<td>167±17</td>
<td>156±14</td>
<td>140±20</td>
<td>128±14</td>
<td>124±14</td>
<td>132±17</td>
<td>118±13</td>
</tr>
<tr>
<td>CP 9 mg/kg</td>
<td>6</td>
<td>100</td>
<td>120±10</td>
<td>139±18</td>
<td>124±9 b</td>
<td>126±5</td>
<td>122±3</td>
<td>135±13</td>
<td>107±13</td>
<td>110±10</td>
<td>107±5</td>
<td>105±4</td>
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<tr>
<td>CP 27 mg/kg</td>
<td>7</td>
<td>100</td>
<td>117±6 b</td>
<td>122±6 b</td>
<td>118±7 b</td>
<td>121±5</td>
<td>124±6</td>
<td>113±9 b</td>
<td>117±7</td>
<td>111±8</td>
<td>106±6</td>
<td>107±4</td>
</tr>
<tr>
<td>Salbutamol 4 mg/kg</td>
<td>8</td>
<td>100</td>
<td>125±11</td>
<td>126±11 b</td>
<td>127±11 b</td>
<td>131±16 b</td>
<td>129±18</td>
<td>119±14</td>
<td>116±14</td>
<td>131±17</td>
<td>122±20</td>
<td></td>
</tr>
</tbody>
</table>

#### Tab 2. Inhibition of cryptoporus polysaccharide (CP) ig on ovalbumin induced reduction of dynamic lung compliance ($C_{dyn}$) of guinea pigs.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0 min</th>
<th>1 min</th>
<th>2 min</th>
<th>3 min</th>
<th>4 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>25 min</th>
<th>30 min</th>
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</thead>
<tbody>
<tr>
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<td>100</td>
<td>65±18</td>
<td>49±15</td>
<td>48±9</td>
<td>48±3</td>
<td>52±1</td>
<td>72±11</td>
<td>78±12</td>
<td>79±10</td>
<td>96±9</td>
<td>100±10</td>
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<tr>
<td>CP 3 mg/kg</td>
<td>9</td>
<td>100</td>
<td>67±7</td>
<td>56±7</td>
<td>67±8</td>
<td>67±8</td>
<td>71±8</td>
<td>80±8</td>
<td>82±9</td>
<td>88±9</td>
<td>85±8</td>
<td>96±7</td>
</tr>
<tr>
<td>CP 9 mg/kg</td>
<td>6</td>
<td>100</td>
<td>73±9</td>
<td>67±10</td>
<td>70±9</td>
<td>77±9 b</td>
<td>82±7 a</td>
<td>78±9</td>
<td>92±13</td>
<td>87±14</td>
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<td>95±5</td>
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<tr>
<td>CP 27 mg/kg</td>
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<td>100</td>
<td>81±6</td>
<td>76±8</td>
<td>79±8 b</td>
<td>79±7 b</td>
<td>80±5 a</td>
<td>94±6</td>
<td>97±6</td>
<td>102±6</td>
<td>103±7</td>
<td>105±6</td>
</tr>
<tr>
<td>Salbutamol 4 mg/kg</td>
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<td>100</td>
<td>83±8</td>
<td>82±8 b</td>
<td>89±9 b</td>
<td>91±10 b</td>
<td>86±9 b</td>
<td>90±10</td>
<td>86±10</td>
<td>93±10</td>
<td>93±13</td>
<td>95±12</td>
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</table>
27 mg/kg (ig) inhibited EPO level in BALF of antigen sensitized and challenged guinea pigs by 3.1 %, 16.9 % ($P<0.01$), and 20.1 % ($P<0.01$), respectively (Tab 3). As a positive control, ketotifen 5 mg/kg inhibited EPO release by 19.0 % induced by antigen ($P<0.01$).

**DISCUSSION**

In this study, we first demonstrated that CP inhibited antigen inhalation challenge-induced airway constriction in sensitized guinea pigs. CP had no direct relaxation effect on airway smooth muscle in vitro, but it inhibited Schultz-Dale constriction reaction of airway smooth muscle induced by antigen and leukotriene D$_4$ (LTD$_4$) from normal guinea pigs lung stimulated by calcimycin in vitro$^3$. Recently, the stabilizing effect of CP on mast cell membrane and regulating effect of CP on Th1/Th2 cells balance have been found (unpublished data made by our Lab). Together, these evidences suggest that CP had effects on histamine, leukotrienes, and cytokines produced from mast cells in asthmatic airways.

Eosinophils are the predominant inflammatory cell type found in the airway of asthmatics and these cells have been suggested to play a role in the pathogenesis of asthma. Activated eosinophil are capable of releasing a variety of inflammatory mediators including cationic proteins such as EPO, eosinophil derived neurotoxin and eosinophil cationic protein. The levels of these proteins are increased 24 h after bronchial allergen challenge and there is evidence to suggest that these cationic proteins damage the respiratory epithelium and may contribute to the development of bronchial hyperresponsiveness. Furthermore an increase in the number of activated eosinophils present in the airway has been demonstrated to correlate well with disease severity and a large number of eosinophils and their granule products have been found in and around the asthmatic airway$^8$. In this experiment we choose OPD as the substrate to determine EPO level in BALF$^9$. Consistent with other studies$^{10}$, EPO activity in BALF was increased in sensitized guinea pigs after antigen challenge in our experiments. CP decreased the release of EPO and protected airway and lung tissues.

Chemotaxis is the directed cell migration toward a
chemical stimulus. By this crucial mechanism eosinophils migrate into inflammatory sites. *In vitro*, a number of stimuli have been identified that are potent and effective eosinophil chemoattractants. The most notable of these are PAF\(^{[11]}\) and some members of chemokine family such as eotaxin, RANTES\(^{[12,13]}\). PAF activates tyrosine kinase and PI\(_3\) kinase in eosinophils, then activates mitogen-activated protein kinases to increase intracellular Ca\(^{2+}\) which is related to eosinophils’ movement\(^{[14]}\). CP significantly attenuated PAF-induced chemotaxis in a concentration-dependent manner. CP has an inhibitory effect on asthmatic airway inflammation by acting on eosinophil chemotaxis.

In conclusion, CP markedly inhibited airway constriction and EPO release from eosinophil in asthmatic model of guinea pig and significantly inhibited eosinophil chemotaxis. The results suggest that CP may be a novel antiinflammatory agent for the treatment of asthma and allergic diseases.

REFERENCES