Inhibition of Immediate Allergic Reactions by Ethanol Extract from Plumbago zeylanica Stems

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The antiallergic properties of the 70% ethanol extract from Plumbago zeylanica stems (EPZ) were investigated in the present study. The extract (500, 1000 mg/kg, p.o.) dose-dependently inhibited systemic anaphylactic shock induced by compound 48/80 in mice, reduced homologous passive cutaneous anaphylaxis and skin reactions induced by histamine or serotonin in rats, significant differences were observed at the dose of 1000 mg/kg. In vitro, EPZ (5, 20, 50 μg/ml) concentration-dependently reduced histamine release from rat peritoneal mast cells caused by compound 48/80 and antigen. EPZ (50 μg/ml) markedly increased intracellular cAMP content of rat mast cells. These findings demonstrate that EPZ inhibits mast cell-dependent immediate allergic reactions, which is probably mediated by reducing the release of mediators such as histamine from mast cells via elevating intracellular cAMP level and weakening the inflammatory action of mediators.

Key words Plumbago zeylanica; anaphylactic shock; mast cell; passive cutaneous anaphylaxis; histamine release; cAMP

Plumbago zeylanica L. (Plumbaginaceae) is a tropical shrub. In China, it mainly grows in the southern provinces, including Guangdong, Guangxi, Fujian, Sichuan and Yunnan. The roots and leaves of P. zeylanica are widely used as medicinal herbs in China and India. In traditional Chinese medicine, P. zeylanica is believed to have functions of removing wind, dispersing stagnant blood, removing toxic factors and killing intestinal parasites, and clinically used to treat rheumatism, intestinal parasites, anemia due to stagnant blood, external and internal trauma, toxic swelling and malignant furunculous scabies. In India, it is usually used to treat fever or malaria. Pharmacological studies have indicated that P. zeylanica extract has antiplasmodial, antimicrobial, antifungal, antiinflammatory, antihyperglycemic, hypolipidaemic and antiatherosclerotic activities.

Recently, we investigated the antiallergic properties of several medicinal herbs and herbal products that are frequently used in Hong Kong to treat rhinitis, and found that P. zeylanica was one of the active herbs. In this paper, we described the inhibitory effects of the ethanol extract from P. zeylanica stems on experimental immediate allergic reactions.

MATERIALS AND METHODS

Preparation of Plant Extract Samples of P. zeylanica were purchased from the herbal market in Hong Kong, China in October 2001. Its identity is confirmed as the stem of P. zeylanica L. by anatomical and TLC analysis as well as comparison with authentic specimens kept at the Herbarium, Department of Biology, The Chinese University of Hong Kong. A voucher specimen (But 0106) is deposited in the Museum of Institute of Chinese Medicine, The Chinese University of Hong Kong. Two hundred grams of P. zeylanica stems were ground and refluxed with 70% ethanol (1.5 l) three times for 1 h. After filtration with filter paper, the clear supernatants were concentrated under reduced pressure at 45 °C in a vacuum rotary evaporator, and lyophilized to give a dry extract (11.8 g). The extract (EPZ) was freshly dissolved in distilled water or PBS (NaCl 154 mM, KCl 2.7 mM, CaCl2 0.9 mM, Na2HPO4 4 mM and KH2PO4 2.7 mM) before use.

Chemicals and Reagents Compound 48/80, ovalbumin (Chicken egg, Grade V), 1-α-phosphatidyl-L-serine and 5-hydroxytryptamine hydrochloride (serotonin) were purchased from Sigma (St. Louis, MO, U.S.A.). Inactive bacterial suspension of Bordetella pertussis, o-phthalaldehyde (OPT), prednisolone and histamine dihydrochloride were purchased from Wako (Osaka, Japan); disodium cromoglycate (DSCG) was obtained from Biomol (PA, U.S.A.). 125I-cAMP radiomunoassay kit was purchased from Shanghai University of Traditional Chinese Medicine. Other reagents used were of analytical grade.

Animals Male ICR mice (weighing 18—20 g) and male SD rats (weighing 180—220 g) from the animal centers of The Chinese University of Hong Kong and China Pharmaceutical University were used. They were maintained on a standard pellet diet with free access to water, and housed in an air-conditioned room at 23±2 °C with lighting from 8:00 to 20:00.

Systemic Anaphylactic Shock Induced by Compound 48/80 in Mice The experiment was carried out according to the method described by Shin et al. Mice were given an intraperitoneal injection of 10 mg/kg of compound 48/80. Mortality during 1 h after the induction of systemic anaphylactic reaction was monitored. EPZ was orally administered 1 h before the injection of compound 48/80.

Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats Rat anti-ovalbumin serum containing IgE was prepared as earlier described. In brief, rats were immunized with 0.5 ml of suspension containing 1 mg of ovalbumin, 10 mg of aluminum hydroxide gel (s.c.) and 1 ml of inactive bacterial suspension of Bordetella pertussis (2×1010 cells/ml, i.p.), simultaneously. Seven days later, rats were immunized again following the same procedure cited above. Fourteen days later, rats were anesthetized with ketamine and xylazine, and blood was withdrawn from the carotid arteries, then rat anti-ovalbumin serum was separated. The anti-ovalbumin IgE antibody was determined by PCA in rats, and the titer (1 : 32) was expressed as the highest dilution causing a lesion more than 5 mm in diameter.

The antiserum diluted 4-fold with saline (50 μl) was intra-
dermally injected into 2 sites on the shaved dorsal skin of rats. After 48 h, rats were challenged with 0.5 ml of saline containing 2 mg of ovalbumin and 5 mg of Evans blue via tail vein. After 30 min, rats were sacrificed. The skin surrounding spots was removed and incubated with 1% KOH for 24 h. The dye that leaked into spots was extracted with a mixture of acetone and phosphoric acid, and was determined colorimetrically.\(^{10}\) EPZ was orally administered 1 h before the challenge of antigen. DSCG was intravenously injected just before the challenge.

**Histamine Release Induced by Compound 48/80 and Ovalbumin from Rat Peritoneal Mast Cells** The experiments were conducted according to the methods of Kubo et al.\(^{11,12}\) Intact rats and sensitized rats, which were immunized 24 h before test by intraperitoneal injection of 1 ml of rat anti-ovalbumin serum prepared in rat PCA, were used for compound 48/80 test and ovalbumin test, respectively. Mixed peritoneal cells were collected by peritoneal lavage and were purified by centrifugation through Ficoll density gradient. Then, purified mast cells were washed and resuspended in PBS containing 5.6 mm glucose and 0.1% bovine serum albumin. Mast cell preparations were about 92% pure as assessed by toluidine blue staining. Cell viability was confirmed to be around 90% before and after experiments by the trypan blue exclusion test.

Purified rat peritoneal mast cells (2×10\(^6\) cells/ml) were preincubated at 37 °C for 10 min. Then EPZ and DSCG dissolved in PBS were added 5 min before activation by compound 48/80 (0.5 μg/ml) or ovalbumin (1 mg/ml) plus Lα-phosphatidylyl-1-serine (100 μg/ml). The reaction was stopped 10 min later by chilling test tubes in ice water. The supernatant and cell pellets were then separated by centrifugation. In the cell pellets, 0.05% Triton-100 was added to liberate the residual histamine. After addition of 0.036% OPT methanol solution, histamine content in supernatant (Supernatant) and cell pellets (Cell pellet) were determined spectrofluorometrically (Em 360 nm, Ex 450 nm). For estimating the spontaneous release of histamine (Spontaneous), exactly the same procedure without adding samples and activators was followed. The release percentage of histamine was calculated by the following equation.

\[
\text{histamine release} \% = \frac{\text{supernatant} - \text{spontaneous}}{\text{supernatant} + \text{cell pellet}} \times 100\%
\]

**cAMP Assay of Rat Peritoneal Mast Cells** The cAMP level was determined according to the method reported previously.\(^{13}\) In brief, purified mast cells were resuspended in prewarmed (37 °C) PBS containing 5.6 mm glucose and 0.1% bovine serum albumin. An aliquot of cells (2×10\(^5\) cells/50 μl) were added to tubes containing 50 μl of prewarmed EPZ (50 μg/ml) for different periods of incubation at 37 °C. The reaction was terminated by addition of 0.9 ml of assay buffer for cAMP quantification using \(^{125}\)I-cAMP radioimmunoassay (RIA) kit.

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**Table 1. Effect of EPZ on Anaphylactic Shock Induced by Compound 48/80 in Mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>EPZ</td>
<td>200</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>10</td>
<td>40</td>
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Mice were orally given EPZ 1 h prior to intraperitoneal injection of compound 48/80 (10 mg/kg). Mortality rate (%) within 1 h after the injection of compound 48/80 was represented as number of dead mice/number of total experimental mice.

**Skin Reactions Induced by Histamine and Serotonin in Rats** Histamine- and serotonin-induced cutaneous reactions were carried out as previously described.\(^{14}\) Briefly, 50 μl of histamine (2 μg) and serotonin (0.02 μg) were intradermally injected into the spots on the shaved dorsal skin of rats, respectively. Then, 1 ml of physiological saline containing 5 mg of Evans blue was immediately injected into the tail vein. EPZ and prednisolone were orally administered 1 h before challenge. Animals were sacrificed by bleeding 30 min after the induction of reactions, and the skin surrounding each reaction spot was cut off, from which the extravasated dye was extracted and assayed by means of the method described in rat PCA.

**Statistics** The data are presented as mean±S.D. Statistical significance between groups was tested by ANOVA and Dunnett’s test. A probability value less than 0.05 was considered as significant.

**RESULTS**

**Effect on Systemic Anaphylactic Shock Induced by Compound 48/80 in Mice** As shown in Table 1, an intraperitoneal injection of compound 48/80 (10 mg/kg) resulted in a fatal shock in all mice, and EPZ pretreatment (500, 1000 mg/kg, p.o.) dose-dependently reduced the mortality rate.

**Effect on Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats** EPZ (500, 1000 mg/kg), orally administered 1 h before the antigen challenge, dose-dependently inhibited PCA by 20.8% and 30.5%, respectively. Significant difference was observed at the dose of 1000 mg/kg. The reference drug DSCG (2 mg/kg), intravenously injected just before challenge, potently inhibited PCA by 68.8% (Fig. 1).

**Effect on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 or Ovalbumin** Total histamine content in mast cells was approximately 14.4 μg/million cells. The spontaneous release percentages of histamine in compound 48/80 and ovalbumin tests were 10.2% and 8.3%, respectively. As shown in Fig. 2, compound 48/80 (0.5 μg/ml) elicited 68.3% of histamine release from peritoneal mast cells of intact rats. Ovalbumin (1 mg/ml) elicited 39.7% of histamine release from mast cells of sensitized rats. EPZ pretreatment clearly inhibited compound 48/80-induced histamine release at concentrations of 20 and 50 μg/ml, and it also clearly reduced antigen-induced histamine release at concentrations of 5, 20 and 50 μg/ml. DSCG markedly reduced histamine release induced by compound 48/80 and antigen.
was 0.6

leaked into the spots was determined. The amount of dye in the skin without reaction

Evans blue
dorsal skin of rats. After 48 h, rats were challenged with 2 mg of ovalbumin and 5 mg of

m

significantly when mast cells were incubated with EPZ (50

As shown in Fig. 3, intracellular cAMP content increased

Fig. 3. Effect of EPZ on cAMP Content of Rat Peritoneal Mast Cells

P. zeylanica stems (EPZ) inhibited the systemic anaphylaxis caused by compound 48/80 in mice and homolo-
gous passive cutaneous anaphylaxis (PCA) in rats at doses of 500 and 1000 mg/kg. In vitro, EPZ clearly reduced histamine
release from rat peritoneal mast cells by compound 48/80 or antigen at concentrations of 20, 50 µg/ml. Interestingly, al-
though the inhibition of anaphylactic shock and PCA by the extract is only moderate, the inhibition of histamine release
seems to be potent. A possible explanation for this phenomenon is that EPZ exhibits potent in vitro effects by directly act-
ing on a receptor on the surface of mast cells or interfering with the signal transduction pathway mediated by the recep-
tor. The precise reason needs to be investigated.

Compound 48/80, a well-known secretagogue of mast
cells, induces nonspecific anaphylactic reaction via vasoac-

Effect on Cutaneous Reactions Induced by Histamine and Serotonin in Rats

Cutaneous reactions were elicited by an intradermal injection of histamine (2 µg) and serotonin (0.02 µg), respectively. EPZ (500, 1000 mg/kg, p.o.) reduced the reaction due to histamine by 22.9% and 41.5%, and re-
duced the reaction due to serotonin by 19.3% and 26.5%, re-
spectively. Significant differences were observed at the dose of 1000 mg/kg. Prednisolone, a steroidal anti-inflammatory
agent, significantly inhibited the reactions at a dose of 20
mg/kg (Fig. 4).

DISCUSSION

Effect on cAMP Content of Rat Peritoneal Mast Cells

As shown in Fig. 3, intracellular cAMP content increased significantly when mast cells were incubated with EPZ (50
µg/ml). It peaked at 2 min after incubation, and gradually re-
turned to basal level after 10 min.
tive amines such as histamine from mast cells and basophils.13,15) Rat PCA, an animal model of IgE-mediated immediate allergic reactions, is also induced by mediators such as histamine secreted from mast cells. However, the secretion process of mediators in PCA differs from that in compound 48/80-induced anaphylaxis; it results from aggregation of specific IgE receptors (FcεRI) on the surface of mast cells by corresponding antigen. The findings suggested that EPZ inhibited IgE-dependent or -independent degranulation and exocytosis of mast cells, consequent mediator release and anaphylactic responses. Weston et al.18,19) have reported that agents that induce and sustain elevation in intracellular cAMP attenuate the stimulated release of mediators from mast cells and basophils. EPZ 50 μg/ml, incubated with rat mast cells, dramatically increased the intracellular cAMP content. It is therefore speculated that EPZ probably prevented the activation of mast cells and release of mediators by elevating intracellular cAMP level.

EPZ was also shown to inhibit the skin reactions induced by histamine and serotonin in rats at doses of 500 and 1000 mg/kg, which indicated that the extract directly reduced the inflammatory action of mediators.

In conclusion, the ethanol extract from *P. zeylanica* stems shows inhibitory effects on immediate allergic reactions, which is probably mediated by reducing the release of mediators such as histamine from mast cells via elevating intracellular cAMP level and weakening the inflammatory action of mediators. Further work is necessary to clarify the active components and precise mechanism of *P. zeylanica*.

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REFERENCES