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Bronchodilatory effects of the aqueous extract of *Gynostemma pentaphyllum* and gypenosides III and VIII in anaesthetized guinea-pigs

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Abstract

The bronchodilatory activity of the aqueous extract of *Gynostemma pentaphyllum* Makino leaves was investigated in anaesthetized guinea-pigs and compared with two of its isolated gypenosides (III and VIII). The results showed that the intravenous administration of the decoction of *G. pentaphyllum* (2.5, 5 or 10 mg kg^{-1}) decreased bronchial resistance in basal conditions and significantly ($P<0.01$) reduced (68% inhibition) the bronchoconstrictor action of histamine. Furthermore, the extract antagonized (80% inhibition) the bronchoconstrictor response induced by the antigen in sensitized guinea-pigs. Gypenosides III (0.7 mg kg^{-1}, i.v.) and VIII (0.3 mg kg^{-1}, i.v.) caused a similar protective effect in both experimental models used; however, the duration and the intensity of the action was less than that of the extract containing corresponding quantities of gypenosides III and VIII. This study confirmed the validity of the traditional use of this plant in the treatment of asthma and other respiratory disorders.

Introduction

Gynostemma pentaphyllum Makino (Cucurbitaceae) is a perennial liana which grows wild in Southern China, Japan, India and Korea. In China it is called Jiaogulan and has been used in traditional medicine to treat bronchitis and asthma, whilst in Japan the plant is known as Amachazuru. Phytochemical studies of this plant have identified flavonoids (Fang & Zeng 1989) and approximately 90 dammarane-type glycosides, mainly named gypenosides (Gy), which are closely related to the ginseng saponins. Indeed, gypenosides III, IV, VIII, XII and malonyl gypenosides III and VIII are identical to ginsenosides Rb1, Rb3, Rd, F2, and malonyl ginsenosides Rb1 and Rd (Takemoto et al 1983; Kuwahara et al 1989; Piacente & Pizza 1995). Due to the similarity to the expensive ginseng root, *G. pentaphyllum* has attracted much interest as a medicinal plant.

Pharmacological studies of *G. pentaphyllum* and/or the isolated saponins have shown a variety of interesting activities, such as antitumour, cholesterol-lowering, immuno-potentiating, anti-ulcer, antioxidant, antihypertensive and antithrombotic activities (Cour et al 1995; Lin et al 2000).

We reported recently that the aqueous extract of *G. pentaphyllum* possessed relaxant properties in tracheal preparations from guinea-pigs and significant anticoronaryspastic, anti-arrhythmic and antihypertensive properties similar to those exhibited by verapamil (Circosta et al 2005; Occhiuto et al 2005).

Our aim in this study was to confirm the use of this plant as an anti-asthmatic in folk medicine; the comparative effects of the aqueous extract of *G. pentaphyllum* and of two of its gypenosides on pulmonary ventilation pressure (inspiratory and expiratory pressure) were determined using bronchoconstricted guinea-pigs. Histamine or antigen produces bronchoconstriction and a decrease in pulmonary ventilation pressure has been shown to be a criterion for bronchodilation.

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Materials and Methods

Plant material

Authentic dried leaves of *Gynostemma pentaphyllum* Makino were obtained from the Chinese Academy of Agriculture Sciences (Beijing, China) in September 2004. After verification by a botanist, a voucher specimen was deposited in the Pharmaco-Biological Department, University of Messina, Italy.

The pure Gy III (Rb1) and Gy VIII (Rd) were obtained from the Laboratoires Labser-vice Analytica s.r.l. (Anzola Emilia, Bologna, Italy).

Extract preparation

The extract was prepared by boiling 100 g powdered dried leaves with 1000 mL distilled water for 30 min. After filtration (Whatman N° 4 filter paper) the extract was lyophilized and kept in the dark at 4 °C until used. A 15% yield was obtained.

Immediately before use the residue was dissolved in normal saline to the desired concentrations.

The extract tested was characterized by a sufficient content of gypenosides III (0.9%) and VIII (0.4%) determined by TLC and HPLC (Kuwahara et al 1989).

Qualitative and semi-quantitative chromatographic analysis of the extract confirmed the presence of discrete amounts of flavonoids as well.

Animals

Male guinea-pigs (350–380 g) were housed under standard laboratory conditions with free access to food and water. Animal care, environmental conditions and use followed the guidelines of the Council of European Communities. The experimental procedures were approved by the Bioethical Committee of the Italian National Health Institute.

Histamine-induced bronchoconstriction in guinea-pigs

Bronchodilatation was studied according to the method described by Konzert & Roessler (1940) with some modification (Occhiuto et al 1999). The guinea-pigs were anaesthetized by intraperitoneal injection of ethyl urethane (1.2 g kg^{-1}). The trachea was intubated and the right external jugular vein cannulated with a polyethylene catheter for drug administration. Considering the respiratory rate of the guinea-pigs, the animals were mechanically ventilated, by pump-ventilation (Harvard), at a frequency of 50 breaths min^{-1} with a respiratory volume of 10 mL/breath. The ventilation pressure (inspiratory and expiratory pressures) in the circuit pump-guinea-pig was measured with a bronchospasm transducer 7020 connected to a recorder Gemini 7070 (both items, Ugo Basile, Comerio, Italy). Bronchoconstriction (an increase in ventilation pressure) was induced by a submaximal dose of histamine (20 $\mu g\,kg^{-1}$, i.v.) predetermined after the cumulative administration of histamine. The aqueous extracts of *G. pentaphyllum* (2.5, 5 or 10 mg kg^{-1}), the pure gypenosides (Gy III 0.7 mg kg^{-1}, Gy VIII 0.3 mg kg^{-1}), at doses corresponding to the approximate content in 66 mg dried leaves (=10 mg of plant extract), or saline (control) were injected into the jugular vein 20 min before starting the infusion of histamine. Maximal changes in pulmonary ventilation pressure (ΔPVP) were expressed as a percentage increase of the basal PVP and the protective effect against histamine-induced bronchoconstriction was expressed as percentage inhibition of ΔPVP values measured in control animals.

Each experimental group consisted of five animals.

Antigen-induced bronchoconstriction in guinea-pigs

The guinea-pigs were divided in groups of five. All animals were sensitized with a nebulized Oleaceae allergen (phenolic extract 0.4% Sarm-Rome) by two successive inhalations (50 μL each) to obtain the maximal bronchospasm upon challenge (Quattrone et al 1990). After 48 h the animals, fasted for 24 h but with water freely available, were anaesthetized with ethyl urethane (1.2 g kg^{-1}, i.p.). They were placed under assisted respiration as described above and administered the Oleaceae allergen (100 μL) by intratracheal instillation. The control group of animals was administered the vehicle (saline). The other groups were treated with various concentrations of plant extract (2.5, 5 or 10 mg kg^{-1}, i.v.), gypenosides (Gy III 0.7 mg kg^{-1}, i.v., or Gy VIII 0.3 mg kg^{-1}, i.v.) or the reference drug (sodium cromoglicate 10 mg kg^{-1}, i.v.), 20 min before intratracheal instillation of the Oleaceae allergen.

Antigen-induced bronchospasm was assessed with or without drug administration. Changes in PVP and percentage inhibition were determined as described above.

Statistical analysis

The results were expressed as mean ± s.d. Differences among different experimental groups were tested for significance using one-way analysis of variance followed by Dunnett's test, taking $P < 0.05$ or $P < 0.01$ as significant.

Results

Effects on bronchial resistance in basal conditions

Intravenous administration of aqueous extract from *G. pentaphyllum* at all dosages assayed (2.5, 5 or 10 mg kg^{-1}) produced a slight decrease in pulmonary ventilation pressure. However, this effect was only statistically significant with 10 mg kg^{-1} plant extract (24.1%). Gypenoside III (0.7 mg kg^{-1}, i.v.) caused a similar effect on bronchial resistance; however, the duration and the intensity of this effect was less than that of the extract containing corresponding quantities of gypenoside III

Table 1 Effects of *Gynostemma pentaphyllum* extract, gypenoside (Gy) III and Gy VIII on pulmonary ventilation pressure (PVP) on basal conditions in guinea-pigs

Treatment	Dose (mg kg^{-1}, i.v.)	% decrease of the PVP respect to basal values	Duration (s) of the action
Control	–	0	0
Plant extract	2.5	8.5 ± 2.2	14.2 ± 2.5
	5	11.0 ± 1.9	15.7 ± 2.0
	10	24.1 ± 2.8[a]	33.7 ± 3.5[b]
Gy III	0.7	12.3 ± 1.8[a]	21.0 ± 2.7[b]
Gy VIII	0.3	8.2 ± 1.5	10.3 ± 1.2

Data are expressed as mean ± s.d., n = 5. [a]$P < 0.05$ compared with basal values. [b]$P < 0.01$ compared with control.

Table 2 Effects of *Gynostemma pentaphyllum* extract, gypenoside (Gy) III and Gy VIII on histamine-induced bronchoconstriction in guinea-pigs

Treatment	Dose (mg kg^{-1}, i.v.)	Pulmonary ventilation pressure (PVP %)	Inhibition (%)
Control	–	90.10 ± 8.20	–
Plant extract	2.5	30.00 ± 4.25*	66
	5	29.10 ± 3.40*	67
	10	28.20 ± 3.15*	68
Gy III	0.7	37.00 ± 4.35*	58
Gy VIII	0.3	50.30 ± 5.00*	44

Maximal increase PVP is expressed as a percentage of the basal PVP. The protective effect was expressed as percentage inhibition of ΔPVP values measured in control animals. Data are expressed as mean ± s.d., n = 5. *$P < 0.01$ compared with control.

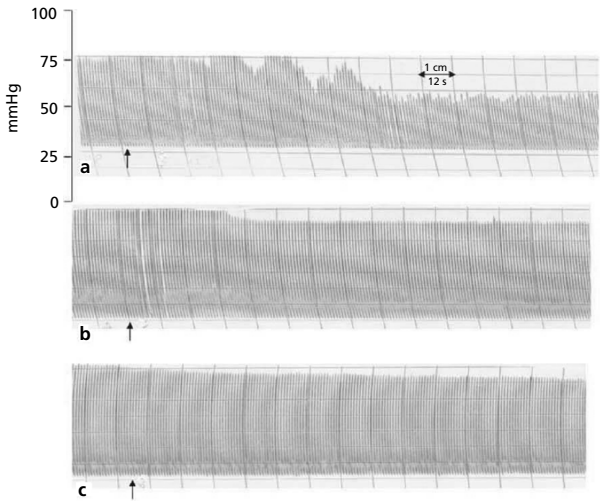

Figure 1 Tracing showing basal pulmonary ventilation pressure (inspiratory pressure and expiratory pressure) in the anaesthetized guinea-pig. a. Effect of plant extract (↑, 10 mg kg^{-1}, i.v.). b. Effect of Gy III (↑, 0.7 mg kg^{-1}, i.v.). c. Effect of Gy VIII (↑, 0.3 mg kg^{-1}, i.v.).

(Table 1, Figure 1). Gy VIII (0.3 mg kg^{-1}, i.v.) produced a slight and insignificant decrease in PVP (8.2%).

Effects on histamine-induced bronchoconstriction

The effects of the drugs on histamine-induced bronchoconstriction were compared with controls (Table 2). In the control group, intravenous administration of histamine significantly increased the pulmonary ventilation pressure (90.10% ± 8.20) whereas in the presence of either plant extract or gypenosides, histamine produced a slight increase in PVP (28–50%). The maximum percentage inhibition of histamine-induced effects on PVP was 68% and 58% for extract and Gy III, respectively. It was clear that both the plant extract and gypenosides caused bronchodilation.

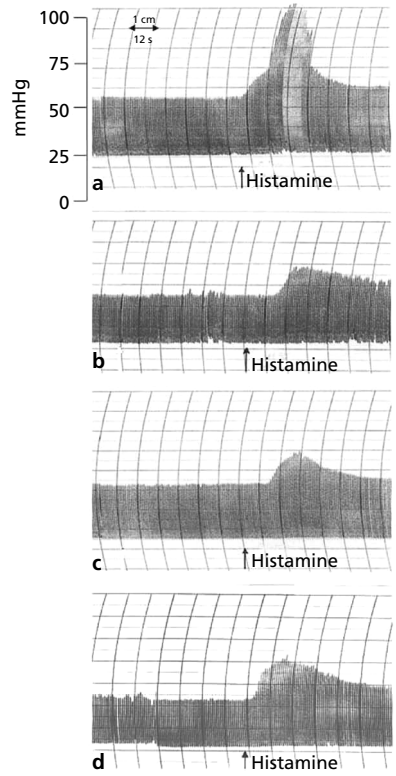

Figure 2 Tracing showing the pulmonary ventilation pressure in the anaesthetized guinea-pig. a. Effect of histamine (↑, 20 μg kg^{-1}, i.v.) in the control. b. Effect of histamine administered 20 min after the plant extract (10 mg kg^{-1}, i.v.) and in the presence of (c) Gy III (0.7 mg kg^{-1},i.v.) and (d) Gy VIII (0.3 mg kg^{-1}, i.v.).

Figure 2a shows a representative tracing of pulmonary ventilation pressure showing an increase in PVP after administration of histamine (20 μg kg^{-1}, i.v.) in control. The inhibition of PVP increased in the presence of the plant extract (10 mg kg^{-1}), Gy III (0.7 mg kg^{-1}) and Gy VIII (0.3 mg kg^{-1}) as reflected in Figure 2b, c and d, respectively.

Table 3 Effects of *Gynostemma pentaphyllum* extract, gypenoside (Gy) III, Gy VIII and sodium cromoglicate on antigen-induced bronchoconstriction in guinea-pigs

Treatment	Dose ($mg\,kg^{-1}$, i.v.)	Pulmonary ventilation pressure (PVP, %)	Inhibition (%)
Control	–	85 ± 5.40	–
Plant extract	2.5	34 ± 3.00*	60
	5	33 ± 2.90*	61
	10	17 ± 2.20*	80
Gy III	0.7	32 ± 2.50*	62
Gy VIII	0.3	35 ± 2.00*	59
Sodium cromoglicate	10	15 ± 1.50*	82

Maximal increase in PVP is expressed as a percentage of the basal PVP. The protective effect was expressed as percentage inhibition of ΔPVP values measured in control animals. Data are expressed as mean ± s.d., n = 5. *$P < 0.05$ compared with control.

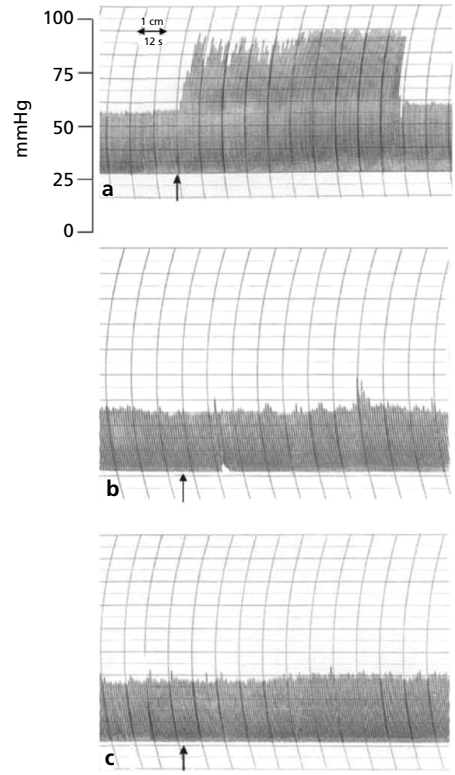

Figure 3 Effect of plant extract and sodium cromoglicate on the antigen-induced increase of the PVP in sensitized guinea-pigs. Effect of the antigen challenge in control (a) and in the presence of (b) the plant extract ($10\,mg\,kg^{-1}$) or (c) sodium cromoglicate ($10\,mg\,kg^{-1}$).

Effects on antigen-induced bronchoconstriction

In sensitized guinea-pigs, intratracheal administration of antigen resulted in the typical bronchospastic response characterized by a marked increase of the PVP (85%) (Table 3).

Intravenous administration of aqueous extract at all doses tested significantly protected ($P < 0.05$) against antigen-induced bronchospasm. In particular a dose of $10\,mg\,kg^{-1}$ showed an inhibitory effect on the increase of the PVP, the intensity of which was similar to that shown by sodium cromoglicate, a well-known drug used in the prophylaxis of asthma (80% and 82% of inhibition, respectively). Gypenosides at doses corresponding with those contained in $10\,mg\,kg^{-1}$ *G. pentaphyllum* extract were able to exert bronchodilator effects, but the intensity of these effects was less than that of the extract.

A significant inhibition on the increase of antigen-induced PVP in the presence of plant extract and sodium cromoglicate is shown in Figure 3.

Discussion

Bronchial asthma is characterized by the spasm and hyperresponsiveness of the airway smooth muscle (Boushey et al 1980; Barnes 1989). Histamine causes bronchial muscle contraction (by H_1-receptors) which greatly increases airway resistance. Antigen-induced bronchoconstriction in sensitized guinea-pigs is a widely used model of experimental asthma and the response is mediated by a variety of agents such as histamine, leukotrienes, bronchoconstrictor prostaglandins and platelet-activating factor among others (Tavares-Murta et al 1993). In addition, it is known that to induce bronchoconstriction, agents such as histamine and antigen are thought to cause an increase of the free intracellular Ca^{2+} by increasing Ca^{2+} release from cellular depots and/or by increasing Ca^{2+} influx. In fact, the calcium channel blocker nifedipine blocked the histamine and antigen-induced bronchospasm in guinea-pigs and rats.

The intravenous administration of the aqueous extract from *G. pentaphyllum* leaves, at doses corresponding to human therapeutic oral doses, and of the pure gypenosides III and VIII, decreased bronchial resistance in basal conditions and produced a protective effect against histamine and antigen-induced bronchoconstriction in the anaesthetized guinea-pigs. These effects indicated that *G. pentaphyllum* extract possessed significant bronchodilatory properties and suggested that Gy III and Gy VIII were two of its active components responsible for these properties. Supporting results were demonstrated previously, where relaxation of the tracheal preparation was evident in the presence of the plant extract and gypenosides (Occhiuto et al 2005). These results indicated that the effect of the plant extract was more pronounced as compared with the pure gypenosides and that the plant extract exhibited a lesser effect in the histamine-induced bronchoconstriction experimental model as compared with the antigen-induced bronchoconstriction model. These properties of the plant extract implied that more than one active principle and more than one mechanism of action might have been responsible for bronchodilation.

Phytochemical analysis of *G. pentaphyllum* extract confirmed the presence of gypenosides III (0.9%) and VIII (0.4%), and flavonoids. It is known that some

gypenosides exert an inhibitory effect on Ca^{2+} uptake (by Ca^{2+}-channel blockade) and Ca^{2+} mobilization (from extracellular membrane or intracellular sources) (Xiong & Sun 1989). Many studies have reported the anti-allergic properties of flavonoids and their derivatives such as sodium cromoglicate, a chromone known for its clinical use in the therapy of asthma (Gabor 1986). The anti-allergic action of flavonoids seems to be mediated through the inhibition of Ca^{2+} influx and protein kinase C activation (Kimata et al 2000). It is possible that the presence of flavonoids in *G. pentaphyllum* extract may partially explain its effect against antigen-induced bronchospasm. Some of the possible mechanisms include histaminic receptor antagonism, interference with mediator release implicated in allergic reactions or calcium mobilization.

Although the mechanism of bronchodilator action of *G. pentaphyllum* extract and of the gypenosides has not been investigated, it is likely that the action may be mediated through their ability to interfere with calcium overload, which represents the common signal transduction pathway used by all bronchoconstrictive stimuli. Studies are necessary to elucidate these interesting findings. However, the bronchodilatory property of *G. pentaphyllum* was consistent with the use of this plant as an anti-asthmatic in folk medicine.

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