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Short Communication

Studies on the expectorant, antitussive and antiasthmatic properties of asterosaponin extracted from *Luidia quinaria*

Chenghua Guo[1,2]*, Xuexi Tang[1], Xinwei Dong[2] and Yan Yang[1]

[1]College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China.
[2]Scientific and Technological College of Chemistry and Biology, Yantai University, Yantai 264005, China

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The aim of this study was to analyze the expectorant, antitussive and antiasthmatic effects of asterosaponin from *Luidia quinaria* through secretion of phenol red from mouse tracheas, frequency of cough caused by ammonia in mice and asthma induced by histamine in guinea pig, respectively. Results showed that asterosaponin extracted from *L. quinaria* at doses of 20 mg/kg and 40 mg/kg could significantly increase secretion of phenol red from mouse tracheas, prolonged the latent period of asthma induced by histamine, and decreased the frequency of cough caused by ammonia. In conclusion, asterosaponin from *L. quinaria* has obvious antitussive, antiasthmatic and expectorant effects.

Key words: *Luidia quinaria*, asterosaponin, antitussive, antiasthmatic, expectorant.

INTRODUCTION

There has been extensive interest in the search for new biologically active compounds from natural sources over the last decade. Great attention has been paid to natural products from marine organisms such as echinoderms, sponges and ascidians and considerable interest currently centers on their remarkable diversity of chemical structures and pharmacological activities (Kunetsova et al., 1982; Findlay et al., 1987; Kitagawa and Kobayashi 1993; Krebs 1986).

Substances with potent pharmacological effect and low toxicity are the most possible candidates for future clinical application. Steroidal glycosides are the predominant metabolites of starfishes and are responsible for their toxicity. Structurally, these saponins can be divided into 3 classes, asterosaponins, steroidal cyclic glycosides and glycosides of polyhydroxylated sterols. A broad variety of biological activities of starfish asterosaponins have been reported, among which are cytotoxic, hemolytic, antibacterial, antineoplastic, antifungal, antiviral properties, anti-inflammatory effects and ichthyotoxic effect as well (Hashimoto et al. 1960; Faulkner et al., 1995; Guo et al., 2000; Choi et al., 1999; Ivanchina et al., 2000; Andersson et al., 1989). However, pharmacological effects of asterosaponins such as expectorant, antitussive, antiasthmatic and other chronic bronchitis and respiratory diseases have not been reported.

In the present study, asterosaponins was isolated from starfish *L. quinaria* and its structure elucidated on the basis of extensive spectroscopic experiments and chemical correlations. The bioactivities of asterosaponins such as expectorant, antitussive and antiasthmatic were evaluated according to national new drug pharmacological experiment standards. The results will provide pharmacological groundwork for application of asterosaponins in chronic bronchitic diseases.

MATERIALS AND METHODS

Chemicals and materials

Asterosaponin was extracted from starfish *L. quinaria*. Positive control drugs such as acute bronchitis syrup, NH_4Cl tablet, codeine phosphate and aminophylline tablets were obtained from pharmaceutical factory in China. Phenol red, ammonia, histamine, acetylcholine and other chemicals were at analytical grade and were purchased from chemical company in Yantai.

Animals

Kunming mice (18-22g) and guinea pigs (200-250 g) were obtained

*Corresponding author. E-mail: gch@ytu.edu.cn. Tel.:+86-0535-6903551. Fax +86-0535-6902063

Table 1. Effects of asterosaponin on phenol red secretion from mouse tracheas.

Groups	Doses	O.D
Control	10 ml/kg	0.042 ± 0.012
Acute bronchitis syrup	15 ml/kg	0.044 ± 0.017
Asterosaponin	20 mg/kg	0.063 ± 0.011*
Asterosaponin	40 mg/kg	0.081±0.014**
NH_4Cl	1000mg/kg	0.052 ± 0.020

*P < 0.05, **P < 0.01 compared with control; n=10

from pharmacological laboratories of Yantai university, barrier housed in a clean environment with access to water and food *ad libitum* and kept in a temperature-controlled environment maintained on a 12 h cycle. The colony was free of pathogens. The experiment was done according to the experimental animal committee regulations.

Assay of expectorant effects

5 groups of Kunming mice (n = 10 for each group) were used in this assay. The control group was treated with saline. Acute bronchitis syrup (15 ml/kg), asterosaponin (20 mg/kg), asterosaponin (40 mg/kg) and NH_4Cl (1000 mg/kg) were administrated intragatrically to mice of experimental groups respectively for 7 consecutive days except NH_4Cl group, which was only treated on the 7th day. Phenol red was prepared in saline with final concentration of 2.5% and injected intraperitoneally (0.2 ml/10 g) 60 min after last administration. Mice were then sacrificed by cervical decapitation 30 min after phenol red injection. Trachea was then dissected out and placed in 1 ml saline. After 10 min, the solution was mixed with 0.1 ml NaOH (1mol/L) and absorbance at 570 nm was measured on BIORAD Model 550 microplate reader.

Assay of cough caused by ammonia

Male and female mice were randomized into 4 groups with 10 animals for each group. One group of animals treated with saline was kept as control. Codeine phosphate group was treated with codeine phosphate (5 mg/kg) intragastrically for 3 consecutive days and the other 2 groups were treated with different doses of asterosaponin intragastrically (20 mg/kg, 40 mg/kg) for 3 consecutive days respectively. On the 3rd day, 60 min after drug administration, mice were exposed to irritant agent, ammonia, for 5 s using an ultrasonic nebulizer (PARIMASTER) via whole body exposure. Latent period of cough (S, from exposure to first cough) and times of cough during 2 min period were recorded.

Assay of asthma induced by histamine

Guinea pigs with latent period of asthma within 150 s were chosen for this study. Guinea pigs were randomly divided into 4 groups with 10 animals for each group. The control animals were pretreated with vehicle (saline 0.9%). Animals of the other 3 groups were pretreated with aminophylline (125 mg/kg), asterosaponin (20 mg/kg) and asterosaponin (40 mg/kg) intragastrically for 3 consecutive days, respectively. One hour after last administration, guinea pigs were exposed to irritant agent, histamine and acetylcholine mixture, for 20 s using an ultrasonic nebulizer (PARIMASTER) via whole body exposure. Latent periods of asthma were recorded.

Statistical analysis

Data were expressed as mean ± S.D. Differences between groups were analyzed by students' unpaired t-test for individual comparisons and by one-way analysis of variance (ANOVA). Differences were considered significant when $P < 0.05$ and very significant when $P < 0.01$.

RESULTS AND DISCUSSION

Expectorant effect of asterosaponin

Asterosaponin significantly increased phenol red secretion from mice tracheas by 50 and 90% of control at doses of 20 and 40 mg/kg respectively (Table 1). Our results indicated that asterosaponin had a strong expectorant effect and provided pharmacological data-base for further application of asterosaponin in chronic bronchitis and other respiratory diseases therapeutics.

Antitussive effects of asterosaponin

As shown in Table 2, the antitussive effects of asterosaponin on mice was examined by the frequency of cough caused by ammonia and the latent period of cough after administration. Asterosaponin at dose of 20 mg/kg could prolong the latent period of cough by 12% and significantly decreased coughing time by 15% within 2 min when compared to control ($P < 0.05$). Asterosaponin at dose of 20 mg/kg could prolong the latent period of cough by 26% and dramatically decrease cough time by 33% within 2 min when compared to control ($P < 0.01$). This suggested that asterosaponin has a strong anti-tussive effect.

Antiasthmatic effects of asterosaponin

We also tested the effects of asterosaponin on the latent period of asthma caused by histamine (Table 3). Our results show that asterosaponin could significantly prolong the latent period of asthma induced by histamine and this prolongation was dose-dependent. The latent period of asthma was significantly increased by 29% ($P < 0.05$) and 49% ($P < 0.01$) at dose of 20 and 40 mg/kg respectively when compared with the control.

Conclusion

Chronic respiratory system diseases, although not life-threatening, can cause general debility, weakness and social embarrassment, which contribute to deterioration in quality of life. More recent epidemiological surveys have provided data that currently available antitussives for both acute and chronic cough are not very effective and that there is an unmet need of more effective

Table 2. Effects of asterosaponin on cough caused by ammonia.

Groups	Doses (ml/kg)	Latent period of cough		Cough times	
		Prior drugs	After drugs	Prior drugs	After drugs
Control	NS	24.5 ± 3.4	25.1± 2.8	21.7 ± 3.1	20.3 ± 6.4
Codeine	5 mg/kg	24.0 ± 3.7	28.4 ± 2.3	21.0 ± 3.6	13.1 ± 5.9*
Phosphate	20 mg/kg	24.8 ± 3.0	28.0 ± 3.0	21.8 ± 3.7	17.2 ± 4.2*
Asterosaponin	40 mg/kg	25.0 ± 2.6	31.7 ± 5.0	25.0 ± 2.7	13.7 ± 3.5**

Values presented as the mean ± S.D. (n=10).
*P < 0.05, **P < 0.01 compared with control.

Table 3. Effects of asterosaponin on latent period of asthma caused by histamine.

Groups	Doses	Latent period of asthma(s)
Control	NS	54.1±14.9
Aminophylline	125 mg/kg	83.3±12.1**
Asterosaponin	20 mg/kg	69.8 ± 8.2*
Asterosaponin	40 mg/kg	80.4±14.9**

*P<0.05, **P<0.01 compared with control; n=10.

antitussives.

Our studies showed that asterosaponin extracted from *L. quinaria* had excellent antitussive, antiasthmatic and expectorant properties. Although further studies are needed to better evaluate its activities and its mechanism(s), our data suggest that asterosaponin might be a candidate for the therapeutics of chronic bronchitis and other respiratory diseases.

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