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Effect and mechanism of Oleanolic acid and Ursolic acid on small intestine peristalsis

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ABSTRACT

Objective To explore the effects and its mechanism of oleanolic acid and ursolic acid on small intestine smooth muscle contractility in vivo and in vitro. Methods Ink propulsion method was used to observe oleanolic acid and ursolic acid in normal mice effects of small intestine motor. Small intestine smooth muscle specimens were established in a guinea pig in vitro. BL-420F biological systems was used to observe the effect of oleanolic acid and ursolic acid on isolated intestinal smooth muscle contraction amplitude, and atropine and diphenhydramine on its role. Results Oleanolic acid and ursolic acid increased the rate of ink propulsion dose-dependently. Both of them could increase contraction amplitude of isolated small intestinal smooth muscle of guinea pigs, and oleanolic acid has a stronger effect ($P<0.05$). Atropine and diphenhydramine could decrease the shrinkage effect of oleanolic acid and ursolic acid on intestinal smooth muscle. Conclusions Oleanolic acid and ursolic acid could promote intestinal peristalsis, expescially oleanolic acid. Its mechanism may be related to emotional cholinergic M receptor and histamine H1 receptor.

KEYWORDS

Oleanolic acid; Ursolic acid; Small intestinal smooth muscle; Atropine; Diphenhydramine.

INTRODUCTION

Oleanolic acid and ursolic acid, three pentacyclic terpenoids, are free or combined glycosides widely in plant kingdom. They share similar structural and has anti-inflammatory, antitumor, antiviral, antioxidant and other biological activity[1]. The current clinical use is anti-inflammatory, hepatoprotective, anticancer, antibacterial, antiviral, blood and other medicinal effects. But whether there is a role in the treatment of anorectal disease, literature has not yet been reported. In this paper, we observed whether oleanolic acid and ursolic acid influenced intestinal peristalsis, and then to investigate its possible mechanism.

Drugs and reagents

Oleanolic acid and ursolic acid were purchased from China control of pharmaceutical and biological products, the batch number is 110742-200513 and 110742-200514, purity quotient 99.9%. Sodium carboxymethyl cellulose was commercially pure analysis. Oleanolic acid and ursolic acid were treated with 1% sodium carboxymethyl cellulose solution configuration. Diphenhydramine Hydrochloride Injection and Atropine Sulfate Injection were purchased in Tianjin Jinyao amino acid Co., Ltd. Magnesium sulfate analysis of pure were purchased in Tianjin Olympic technology company, with pure water for 10% solution. Krebs solution (mmol/L): sodium chloride 114.0, potassium chloride 4.7, magnesium chloride 1.2, calcium chloride 2.5, sodium dihydrogen phosphate 1.8sodium bicarbonate18.0 glucose 11.5, pH= 7.4.

Animals

SPF Kunming mice, 18-22g, both male and female, were purchased from Beijing Fukang biological Polytron Technologies Inc,China, certificate No.: SCXK< Jin >2009-0008. The SPF level in guinea pigs, weight 250-300g, male and female, purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd, certificate number: SCXK< Beijing >2012-0001.

Instrument

BL-420F biological function experimental system. The HW-400E constant temperature trough and smooth muscle tension transducer, Chengdu taimeng Technology Co., ltd..

Small intestine movement experiment

90 mice, after fasting for 24 h, were randomly divided into 9 groups (10 rats in each group),namely, blank control group, atropine group, Magnesium sulfate group, oleanolic acid group in low/middle/ high dose (0.2, 0.4, 0.8 g/kg), ursolic acid group in low/middle/ high dose (0.2, 0.4, 0.8 g/kg). In addition to the blank group gavaged with saline, mice in other group were given administered drugs by po (0.1ml/10g). After 15 minutes, all mice were given India ink by po. (0.1ml/10g),and then they were sacrificed by cervical dislocation 20 minutes later. Immediately, we removed the intestines without traction,flat on the glass plate, measured length from the pylorus to the most distal of ink after straightening (A/cm), then the measurement from the pylorus to ileocecal distance for the total length of the small intestine (B /cm), A / B was the intestinal propulsive rate (%).

The isolated ileum smooth muscle experiment[2]

Guinea pigs were fasted for 24h but normal drinking water. We stuck a blow on the head to death, quickly took out the terminal ileum (from the ileocecal valve about 10cm) about 20cm, and rinsing in modified Kirschner, removal of mesangial or fat attached, cutting the growth of about 2 cm number of segment. We installed and adjusted isolated intestinal smooth muscle of experimental instruments (constant temperature trough and smooth muscle tension transducer). One end of intestine fixed on the ventilation hook, was suspend with 20ml isothermal Krebs solution, which was supply gas 95%O_2 and 5%CO_2, 2-3 bubble / min. The other end of intestine was connected with the tension sensor and tighten the thread, making the specimen loading was 1 g. We opened BL-420F biological function experimental system, and began to experiment after balance of 0.5 h to be stable baseline. The drugs were calculated with the final concentration in the bath. The experimental group and the dosing sequence is as follows: ①0.05 g/ml oleanolic acid ②0.05 g/ml of ursolic acid ③diphenhydramine hydrochloride (0.1 mg/ml) + oleanolic acid (0.05 g/ml) ④diphenhydramine hydrochloride (0.1 mg/ml) + ursolic acid (0.05 g/ml) ⑤ atropine (0.04 mg/ml) + oleanolic acid (0.05 g/ml) ⑥ atropine (0.04 mg/ml) + ursolic acid (0.05 g/ml). During the test, at first, we traced normal movement curve of bowel,then recorded waveform bowel movement before and after administration of 5 min. After each administration was completed, bowel was rinse immediately with preheating Krebs solution to 3 times, until back to normal, then the next administration.

Statistical Analysis

Values were expressed as mean±SEM. All statistical analyses were performed using the SPSS software, version 16.0. Comparisons between multiple independent groups were conducted using one-way ANOVA followed by post hoc test. Group differences resulting in p-values of less than 0.05 were considered to be statistically significant.

RESULTS

Effect of oleanolic acid and ursolic acid on small intestinal peristalsis of mice

TABLE 1 : Effect of oleanolic acid and ursolic acid on intestinal peristalsis of normal mice ($\overline{X} \pm S$)

Groups	Dose（g/kg）	Advance rate (%)
blank control group	--	44.95±4.04
atropine group,	0.05	30.24±1.34▲
Magnesium sulfate group	0.001	68.41±2.88▲▼
oleanolic acid group in low dose (0.2 g/kg)	0.2	46.19±3.69▼◆
oleanolic acid group in middle dose (0.4 g/kg),	0.4	57.02±2.30▼
oleanolic acid group in high dose (0.8 g/kg)	0.8	72.28±4.52▲▼
ursolic c acid group in low dose (0.2 g/kg)	0.2	38.40±3.89◆
ursolic acid group in middle dose (0.4g/kg)	0.4	42.55±3.47◆
ursolic acid group in high dose (0.8 g/kg)	0.8	61.09±2.22▲▼

Notes: Compared with blank control group, ▲P<0.05; Compared with atropine group,▼P<0.05; Compared with the Magnesium sulfate group, ◆P<0.05

As shown in TABLE 1, compared with the control group, High dose of oleanolic acid and ursolic acid can obviously promote the ink mobile, increase the advance rate（P<0.05）,but most importantly, the promoting effects were dose dependent, and the same concentration of oleanolic acid stronger than ursolic acid.

Effect of oleanolic acid and ursolic acid on isolated intestinal smooth muscle

TABLE 2 : Effect of oleanolic acid and ursolic acid on isolated small intestinal smooth muscle contraction of guinea pig ($\overline{X} \pm S$)

Groups	Ranges /g
blank control group	3.14±0.34
oleanolic acid group	6.40±0.27▲
diphenhydramine hydrochloride + oleanolic acid	3. 95±0.35▼
atropine + oleanolic acid	3.76±0.35▼
ursolic acid group	5.89±0.28▲
diphenhydramine hydrochloride + ursolic acid	3. 59±0.38◆
atropine + ursolic acid	3.65±0.29◆

Notes: Compared with blank control group, ▲P<0.05; Compared with oleanolic acid group,▼P<0.05; Compared with ursolic acid group, ◆P<0.05

As shown in TABLE 2, compared with the control group, Oleanolic acid and ursolic acid can obviously improve the intestinal smooth muscle contraction (P<0.05), after adding diphenhydramine or atropine, contraction of small intestine smooth muscle decreased, and difference was significant（P<0.05). High dose of oleanolic acid and ursolic acid can obviously promote the ink mobile, increase the advance rate（P<0.05）,but most importantly, the promoting effects were dose dependent, and the same concentration of oleanolic acid stronger than ursolic acid.

DISCUSSIONS

According to incomplete statistics, Oleanolic acid is widely distributed in 190 species of plants about 60 families in nature, for example, swertia whole herb, oldenlandia diffusa, glossy privet fruit etc. Ursolic acid is distributed in 62 plant

species in 27 families, mainly in medicinal plants such as fruit of glossy privet, hawthorn, loosestrife, Xia Kucao, plantain, licorice root, forsythia and Kudingcha[3]. Oleanolic acid and Ursolic acid, belong to pentacyclic terpenoids,are the main functions of these natural product composition, with many similar pharmacological activity, exparticularly in anti-inflammatory, hepatoprotective, anti tumor and immune regulation etc. The structure of Oleanolic acid and ursolic acid is similar, and the only difference is that the methyl heterogeneous in rings three terpene E ring[4]. The body experiment initially descripted, Oleanolic acid and ursolic acid could promote peristalsis of small intestine smooth muscle, and had dose dependent effect, but Oleanolic acid stronger than ursolic acid, probably due to efficacy of small differences caused by the two different positions of the substituents and different spatial structure.

Intestinal smooth muscle contraction is chemical energy to mechanical energy with a variety of neurotransmitter participation. At present, we all know the main excitatory neurotransmitter is acetylcholine (Ach) in the gastrointestinal system[5]. Ach can make depolarization in the smooth muscle cell membrane, stimulate excitatory junction potential produced by the gastrointestinal tract wall of cholinergic nerve, promote gastrointestinal peristalsis[6]. Atropine, as a competitive antagonist, combined with M cholinergic receptor, blocked the effect of Ach on the contraction of intestinal tract, decreased the amplitude and frequency of gastrointestinal motility. This study showed, with the action of atropine, oleanolic acid and ursolic acid decreased intestinal contraction amplitude, which descripted oleanolic acid and ursolic acid may promote intestinal peristalsis by activating cholinergic M receptor. In addition, there was a large number of histamine receptors in gastrointestinal smooth muscle, among them, activation of H1 receptor can promote gastrointestinal smooth muscle contraction, and significantly increase the gastrointestinal blood flow[7]. In this experiment, Diphenhydramine, H1 receptor antagonist, decreased the contraction amplitude of small intestine smooth muscle, which told us that Oleanolic acid and ursolic acid promoted intestinal peristalsis function may be related to the histamine H1 receptor. If there are other receptors involved, we still need further confirmed.

CONCLUSIONS

In conclusion, Oleanolic acid and ursolic acid could promote intestinal peristalsis, expescially Oleanolic acid,which may be related to emotional cholinergic M receptor and histamine H1 receptor.

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