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Comparison of two Chinese medical herbs, Huangbai and Qianniuzi, on influence of short circuit current across the rat intestinal epithelia

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Abstract

Huangbai (*Phellodendron spec.*) and Qianniuzi (*Pharbitis spec.*) are two traditional Chinese medical herbs used for *anti-diarrheal and laxative agents*, respectively. Ethanol and water extracts of these two herbs were prepared and effects of the extracts on ion transport of the rat intestinal epithelia were studied. For measuring changes of the short circuit current across the epithelia, the rat intestinal epithelia were mounted in the Ussing chamber and attached with voltage/current clamp. The intestinal epithelia were firstly activated by serosal administration of 5 μM forskolin. As current raised and being stable, extracts of these herbs were added, respectively, and changes in the short circuit current were recorded. Ethanol extract of Huangbai attenuated the current increment; on the contrary, ethanol extract of Qianniuzi augmented the current increment additionally. Water extracts of the two herbs showed minor effects on the current in comparison to ethanol extracts. The results provide evidences to reveal the pharmacological mechanism of the two Chinese medical herbs on the intestinal tissue.

Keywords: Chinese medical herb; Ion transport; Intestinal epithelia; Short circuit current; *Phellodendron spec.*; *Pharbitis spec.*

1. Introduction

Huangbai and Qianniuzi are two traditional Chinese medical herbs used for treatment of dysentery and edema, respectively. Huangbai is cortex of *Phellodendron amurense* Rupr. or *Phellodendron chinense* C.K. Schneider (family Rutaceae). Qianniuzi is mature seed of *Pharbitis nil* (L.) Choisy or *Pharbitis purpurea* Lunell (family Convolvulaceae). According to the Chinese medical literature (Yen, 1994), Huangbai is categorized as heat relief medicine and is effective in curing dysentery, diarrhea, and other syndromes. Qianniuzi is categorized as laxative agent and is effective for excluding body fluid. It is used in treatments of edema and ascites or used as diuretic agent. In regard to the impact on the alimentary tract, these two herbs seem to exert totally different effect on the water movement across the intestinal epithelia.

Diarrhea occurs when excess water secreted in the intestinal tract (Fondacaro, 1986). On the other hand, the laxatives act to promote water secretion in the digestive tract and facilitate passage of bowel contents (Izzo et al., 1998). Water movement across the epithelia is generally driven by osmotic gradient created by active ion transport (Powell et al., 1972). Typically, the epithelial cells would absorb water by active Na^+ uptake which is pumped by the Na^+-K^+ ATPase on serosal side, and secrete water by actively pumping Cl^- out which is powered by the Na^+-K^+-$2Cl^-$ *co-transporter* on serosal side (Schultz and Frizzell, 1972; Hawker et al., 1978). Increase of Cl^- transport that leads to water secretion is commonly seen in many cases of diarrhea elicited by virus or bacteria (Davidson et al., 1977; McEwan et al., 1990; Osman et al., 1998).

To detect ion transport in epithelia, the Ussing chamber attached with voltage/current clamp for measuring short circuit current across epithelial tissues is a reliable method and is used for decades. As the ions are charged, movement of ions across the epithelia generally creates potential difference across the epithelia (Kurkchubasche et al., 1998). When the potential difference is clamped to zero, a short circuit current resemble of the ion current across the epithelia could be recorded. Fluctuation of the short circuit current can be monitored and reflects changes of ion across the epithelia (Field et al., 1971).

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Since excess Cl^- and water movement from serosal side to lumen is the major event of diarrhea and main action of laxatives, we presumed that the two Chinese medical herbs might act on the intestinal tissue and affect ion transport in the intestinal epithelia. This present study, hence, was aimed to study the effects of extracts from the two herbs with anti-diarrheal and laxative characteristic on ion transport of the rat intestinal epithelia. Using the Ussing chamber and voltage clamp method, the rat ileal epithelia were activated by forskolin to stimulate Cl^- secretion (Sharp and Hynie, 1971), and then the extracts were added to discern their effects on the short circuit current across the rat ileal epithelia. The result shows that the two herbs extract act oppositely on influencing the short circuit current across the forskolin-activated intestinal epithelia. It provides evidences to reveal the pharmacological effect of the two Chinese medical herbs.

2. Materials and methods

2.1. The Chinese medical herbs and extracts preparation

Huangbai and Qianniuzi were purchased from the Chinese pharmacy of the China Medical College Hospital. Twenty grams of each dried material was dissolved in 200 ml ethanol or *de-ionized* water and heated to boil for 30 min, respectively. The supernatants were then collected and concentrated by vacuum evaporator until the volume was reduced to 5 ml and were stored in −20 °C refrigerator.

2.2. Animals and tissue preparation

Healthy male Sprague–Dawley (SD) rats were obtained from Laboratory Animal Breeding and Research Center of National Science Council, Taipei, Taiwan. The rats were maintained under temperature control of 23 °C and kept on a 12-h light:12-h dark cycle, with diet and water supplied ad libitum. Rats weighing 250–300 g were selected and killed by exposure to ether. In each rat, several segments of 2 cm ileum free of Peyer's patches were immediately removed and washed in *Krebs'* solution. Intact and flat sheets of the ileal epithelia were prepared by cutting open along the mesenteric border, and the serosal and muscular layers were peeled away under a binocular microscope (Kurkchubasche et al., 1998).

2.3. The Ussing chamber

The epithelia were mounted between the Ussing chambers (CHM6, W.P.I., Sarasota, FL, USA). The buffers were oxygenated continuously and maintained at 37 °C. An automatic voltage clamp (DVC1000, W.P.I., Sarasota, FL, USA), corrected for fluid resistance between the potential difference sensing Ag/AgCl electrodes. A second pair of Ag/AgCl electrodes monitors the short circuit current across the tissue continuously. The bathing solution in mucosal chamber is standard Kerbs'-HCO_3^--Ringer composed of (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 11.1; pH 7.4. The serosal buffer is same as mucosal solution only the glucose is excluded. These chemicals were purchased from Merck (Darmstadt, FRG). Both buffers were gassed with 95% O_2–5% CO_2 before filling the chambers.

2.4. Experimental procedure

To discern the effects of the extracts on short circuit current of the rat ileal epithelia, forskolin (Sigma, St. Louis, MO, USA) was firstly added on serosal side (final concentration 5 μM) of the tissue. The current rise and is stable after 1–3 min. Twenty-five microliter ethanol or water extracts of each herb was then added on serosal side 20 min after forskolin. Change of the short circuit current was recorded continuously during the experiment.

2.5. Statistical analysis

Data are expressed as mean ± S.D. (standard deviation). Comparisons between two groups were performed by using Student's *t*-test, respectively. A difference of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Ethanol extract

Rat ileal epithelia mounted in the Ussing chamber were activated by forskolin to stimulate Cl^- secretion, and the short circuit current raised about 40–50 μA/cm^2 1–3 min after forskolin stimulation. The raised current was stable for at least 45 min. The current generally fluctuated no more than 5 μA/cm^2 (Table 1, Control). Ethanol *extracts* of Huangbai and Qianniuzi showed opposite effect on the short circuit current across the forskolin-activated ileal epithelia. Ethanol extract of Huangbai added 10 min after forskolin attenuated the current increment raised by forskolin. The current decrement is 7.0 ± 2.4 μA/cm^2, which is significantly different from control ($P = 0.0001$, Table 1). On the contrary, Ethanol extract of Qianniuzi additionally augmented the current increment raised by forskolin. The current increment raised by the Qianniuzi is 18.3 ± 4.1 μA/cm^2, which is significantly *different* from control as well ($P = 0.00003$, Table 1).

3.2. Water extract

Water extracts of the two herbs showed minor effects on the short circuit current in comparison to the ethanol extracts. Water extract of Huangbai and Qianniuzi showed opposite effect on the short circuit current across the

Table 1
Changes of basal short circuit current across the rat ileal epithelia before, after adding forskolin, and after adding ethanol extracts of the two medical herbs

	n	Basal short circuit current	10 min after adding forskolin (*A*)	10 min after adding extract (*B*)	*B*−*A*
Control	6	57.3 ± 23.6	105.7 ± 7.6	107.3 ± 7.2[c]	+3.0 ± 3.6
Huangbai[a]	6	47.3 ± 6.3	103.2 ± 6.8	96.2 ± 7.7	−7.0 ± 2.4*
Qianniuzi[b]	6	40.8 ± 10.8	95.3 ± 28.3	113.7 ± 27.7	+18.3 ± 4.1*

Values represent means ± S.D. μA/cm^2 for six individual measurements.
[a] *Phellodendron amurense* or *Phellodendron chinense*.
[b] *Pharbitis nil* or *Pharbitis purpurea*.
[c] Adding 25 μl ethanol.
* $P < 0.001$ vs. control.

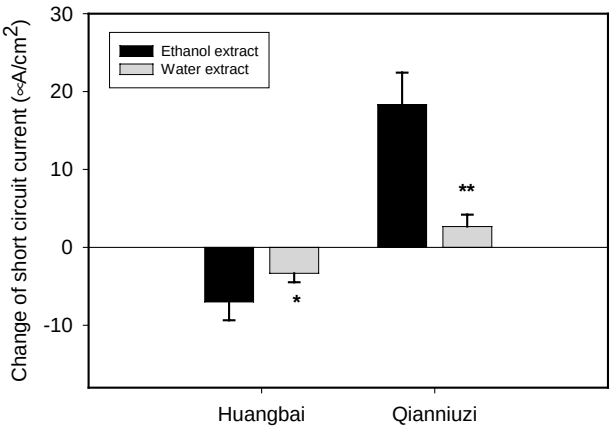

Fig. 1. Comparison of the water and ethanol extracts of Huangbai and Qianniuzi on the short circuit current across the forskolin-activated rat intestinal epithelia. Water extracts of the two herbs showed minor effects in comparison to the ethanol extracts. Values represent means ± S.D. for six individual measurements in ethanol extract and three individual measurements in water extract. Statistically significant difference ($P < 0.05$) was determined by Student's *t*-test. * $P < 0.05$, ** $P < 0.001$ vs. ethanol extract.

forskolin-activated ileal epithelia. Water extract of Huangbai added 10 min after forskolin showed effect of attenuation on the current increment caused by forskolin. Nevertheless, the current attenuation is diminished to 3.3 ± 1.2 μA/cm^2, and the value is significantly different from the current decrement attenuated by ethanol extract of Huangbai ($P = 0.04$, Fig. 1). Water extract of Qianniuzi added 10 min after forskolin showed augmenting effect on the current increment caused by forskolin. The current increment, however, is reduced to 2.6 ± 1.5 μA/cm^2, and the value is significantly different from the current increment augmented by ethanol extract of Qianniuzi ($P = 0.0004$, Fig. 1).

4. Discussion

In this present study, effects of ethanol and water extracts of Huangbai and Qianniuzi on influence of ion transport are compared. The results indicate that these two Chinese medical herbs exert contrary effects on the short circuit current across forskolin-activated rat intestinal epithelia. This is significant since these two traditional Chinese medical herbs were used for treatments of different syndromes; namely, one is presumed to be anti-diarrheal and the other is assumed to be laxative for inducing diarrhea. The short circuit current reflects ion transport across the intestinal epithelia. Ethanol and water extracts of Huangbai show attenuating effect on the short circuit current, which is similar to other anti-diarrheal agents. Gabriel et al. (1999) reported latex of the herb *Croton lechleri* is commonly used to treat various kinds of secretory diarrheas in southern America. The SP-303 derived from the latex is confirmed to reduce the short circuit current across Caco-2 epithelia monolayers. Another potential anti-diarrheal agent, namely, the short-chain fatty acids produced in colon by bacterial fermentation (Rabbani et al., 1999), likewise generate inhibiting effect on the short circuit current of the intestinal epithelia induced water and electrolyte secretion by cholera toxin (Vidyasagar and Ramakrishna, 2002). The short-chain fatty acids are proven to stimulate Na^+ and Cl^- absorption, hence, hinder Cl^- and water secretion. Besides, boiled rice is incorporated into oral rehydration therapy for patients with secretory diarrhea. Extract from boiled rice inhibit transepithelial cAMP-stimulated Cl^- current in T84 cells and inhibit forskolin-induced current as well (Mathews et al., 1999).

Ethanol and water extracts of Qianniuzi augment the short circuit current across forskolin-activated epithelia, which is analogous to other substances that induce diarrhea. Cholera toxin, taken for example, stimulates the G protein on membrane of the intestinal epithelial cells, elicits signal transduction leading to Cl^- movement from serosa to mucosa and, hence, increases the short circuit current across the intestinal epithelia (Kimberg et al., 1971; Petritsch et al., 1992). In bacterial infection of digestive tract, lipopolysaccharide (LPS) of the Gram-negative bacteria may stimulate the immunocyte or the neural cell in the intestinal wall to produce nitric oxide or prostaglandin (Konturek, 1998), and these two molecules affect ion transport in the intestinal epithelia as they modulate the short circuit current across the epithelia (Brunsson et al., 1987; Rao et al., 1994; Izzo et al., 1998). In addition, intrinsic factors produced by immune or nervous tissues are frequently observed in inflammatory bowel diseases to elicit molecular events leading to diarrhea (Lundgren and Jodal, 1997). Pro-inflammatory cytokines,

such as interleukin (IL)-1and IL-3 stimulate Cl^- secretion (Chiossone et al., 1990; Theodorou et al., 1994), while anti-inflammatory cytokines IL-4, IL-10, and IL-13 promote intestinal uptake of Na^+ and Cl^- (Madsen et al., 1996; Zund et al., 1996), which is similar to other anti-diarrheal agents.

Accordingly, the result confirms that extracts of the two herbs are active on influencing ion transport across the rat ileal epithelia. Nevertheless, it should be noted that adding ethanol extracts of these herbs on mucosal side did not affect the current as adding these extracts in the serosal side (data not shown). They influence the current in the forskolin-activated rat ileal epithelia when adding in the serosal side. In this regard, these herbs extract might not act on channel or transporter on the apical membrane of the ileal epithelial cells. In addition, these extracts showed insignificant effect on the short circuit current of rest rat ileal epithelia (data not shown). Therefore, they may not act directly on the Na^+-K^+ ATPase or Na^+-K^+-$2Cl^-$ *co-transporter* located on the basolateral membrane of the epithelial cells neither. They probably act via the signaling molecules in the cell that affect ion transport apparatus.

The minor effect of water extracts in comparison to ethanol extracts indicate that functional substances of Huangbai and Qianniuzi on affecting ion transport are less soluble in water than in ethanol. Content of the two herbs have been described by Yen (1994). Huangbai extract contains berberine, magnoflorine, phellodendrine, palmatine, and others. According to the Merck Index (1996), berberine and palmatine are less soluble in water. The berberine is alkaloid found in many plants and frequently stated as antibacterial agent (Yen, 1994). Moreover, the berberine is reported to inhibit secretory action of the intestinal epithelial cells by affecting K^+ channels (Taylor et al., 1999) and may block Ca^{2+} influx in smooth muscle cells of Guinea pig colon (Cao et al., 2001). Accordingly, the berberine in Huangbai extract might be active and be the candidate component in reducing the short circuit current across the forskolin-activated rat intestinal epithelia. The other components may be active as well, however, effects of these compounds are currently unknown. Qianniuzi extract contain mainly oil constituents, namely, pharbitin, pharbitic acid, tiglic acid, nilic acid, and others (Yen, 1994). These components should be more soluble in ethanol than in water. Functions of these compounds are seldom reported.

In conclusion, *the present* study proves that ethanol extracts of Huangbai and Qianniuzi may affect ion and consequently water movement across the rat intestinal epithelia. Effects of these two extracts are opposed and this provides clues on revealing the different pharmacological effects of these two herbs. Further study will be needed to discriminate the detailed actions on the intestinal epithelia elicited by these two traditional Chinese medicines.

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