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Effect of Ethanol Extracts of Three Chinese Medicinal Plants With Anti-diarrheal Properties on Ion Transport of the Rat Intestinal Epithelia

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Abstract. Effects of ethanol extracts of three Chinese medicinal plants, namely, *Qinpi* (*Fraxini cortex*), *Kushen* (*Sophora flavescens*, AITON), and *Huanglian* (*Coptis teeta*, WALLICH), on ion transport of the rat intestinal epithelia were determined in this study. Rat intestinal epithelia mounted in an Ussing chamber attached to a voltage/current clamp were used for measuring changes in the short circuit current across the epithelia. Activation of the intestinal epithelia by serosal administration of 5 μM forskolin resulted in an increase in basal short circuit current. The ethanol extracts of each of the three plants partially reduced the current stimulated by forskolin. In the following experiments, ouabain and bumetanide were added prior to adding the ethanol extract of these plants for revealing their effect on Na^+ and Cl^- movement. The results suggest that the ethanol extract of the *Qinpi* would affect Cl^- transport. On the contrary, the ethanol extract of *Kushen* would affect Na^+ transport rather than Cl^- movement. This study provides evidences that reveal the pharmacological mechanism of the Chinese plants with anti-diarrheal properties.

Keywords: Chinese medicinal plant, ion transport, intestinal epithelia, anti-diarrhea, Ussing chamber

Introduction

According to the Chinese medical literature, *Qinpi* (*Fraxini cortex*), *Kushen* (*Sophora flavescens*, AITON), and *Huanglian* (*Coptis teeta*, WALLICH) were used for healing certain types of diarrhea (1). Although these plants have been in use for a long period, little is known about their pharmacological mechanisms in detail.

The main etiological cause of diarrhea has been documented to be excess water secretion in the intestinal epithelia (2 – 4). Water movement in the epithelia is driven by an osmotic gradient created by active ion transport. Typically, the epithelial cells absorb water by active Na^+ uptake that is powered by the Na^+-K^+ ATPase on the serosal side and secrete water by the active pumping of Cl^- out of the cells generated by the Na^+-K^+-$2Cl^-$ cotransporter on the serosal side (5 – 7). Changes in Na^+ or Cl^- transport in the epithelial cells are commonly seen in many cases of diarrhea induced by virus or bacteria (8 – 11).

For the detection of ion transport in epithelia, the Ussing chamber attached with voltage/current clamp has been used for measuring short circuit current across epithelial tissues for decades. Since ions are charged, movements of cation or anion across the epithelia generally create a potential difference between the mucosal and serosal sides of the epithelia (12). When the potential difference is clamped to zero by using the voltage-clamp equipment, a short circuit current generated by the ion movements across the epithelia can be recorded. Fluctuation of the short circuit current, therefore, reflects changes of ion transport across the epithelia (13).

The present study is aimed at discerning the effects of extracts from the three Chinese medicinal plants

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mentioned above on ion transport of the rat intestinal epithelia by using the Ussing chamber and the voltage-clamp techniques. The results indicate that these plants' extract can affect either Na^+ or Cl^- movement, respectively, in the rat intestinal epithelia.

Materials and Methods

Preparation of the extracts for Chinese plants

Dried *Qinpi*, *Kushen*, and *Huanglian* were purchased from the Chinese pharmacy of the China Medical College Hospital. Twenty grams of each dried material was dissolved in 200 ml ethanol and heated to boil for 30 min. The supernatants were then collected and concentrated by a vacuum evaporator (EYELA N-N Serias; Tokyo Rikakikai, Tokyo) until the volume was reduced to 5 ml and were stored in a −20°C refrigerator.

Animals and tissue preparation

Healthy male Sprague-Dawley (SD) rats were obtained from Laboratory Animal Breeding and Research Center of the National Science Council, Taipei, Taiwan. The rats were maintained under temperature control of 23°C and kept on a 12-h light-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 Kreb's 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 (13).

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.) corrected for fluid resistance between the potential difference sensing Ag/AgCl electrodes. A second pair of Ag/AgCl electrodes continuously monitored the short circuit current across the tissue. The bathing solution in the serosal and mucosal chamber was composed of 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 11.1 mM glucose, pH 7.4. These chemicals were purchased from Merck (Darmstadt, FRG). Both buffers were gassed with 95% O_2 – 5% CO_2 before filling the chambers (12).

Experimental procedure

To show the effects of the extracts on short circuit current of the rat ileal epithelia, forskolin (Sigma, St. Louis, MO, USA) was first added on the serosal side (final concentration of 5 μM) of the tissue. The current increased and was stable after 3 – 5 min. At 10 min after the addition of the forskolin, 25 μl ethanol extract of each medicinal plant was then added on the serosal side. Change in the short circuit current was recorded continuously during the experiment. Because ethanol extracts of the medicinal plants were used for the experiments, the effect of ethanol on the short circuit current of the rat ileal epithelia was discerned in another experiment. The result showed the short circuit current showed little change in three intestinal tissues after the addition of 25 μl ethanol in the mucosal buffer (10 ml). Hence the vehicle (ethanol) effect was small. To discern whether the movement of sodium or chloride is involved in the effects of extract on the short circuit current across the epithelia, ouabain (inhibitor of Na^+-K^+ ATPase) (Sigma) or bumetanide (inhibitor of Na^+-K^+-$2Cl^-$ cotransporter) (Sigma) was added at the final concentration of 100 μM 10 min after forskolin addition. In both cases, there was a drop in the current and was stable after 1 – 3 min. Twenty-five microliters of the ethanol extract of each medicinal plant was then added on the serosal side at about 20 more min after ouabain or bumetanide addition.

Statistical analyses

Data are expressed as the mean ± S.D. Comparisons among groups were performed by using one-way ANOVA. A difference of $P<0.05$ was considered statistically significant.

Results

Qinpi

Administration of the ethanol extract of *Qinpi* reduced the short circuit current of the forskolin-activated ileal epithelia. The decrease in current was 9.0 ± 2.0 μA (Table 1). Figure 1 shows that if ouabain was added before the ethanol extract of *Qinpi*, the decrease in current was 7.2 ± 2.8 μA (Table 2), which is not significantly different from that of adding *Qinpi* extract only. It seems that the effect of *Qinpi* extract on reducing the short circuit current is not influenced by the prior addition of ouabain. However, if bumetanide was added before the ethanol extract of *Qinpi*, the current reduction was attenuated to 3.0 ± 2.0 μA (Table 3); this value is significantly different from that of adding *Qinpi* extract only ($P = 0.002$). This shows that adding bumetanide before *Qinpi* extract diminishes the effect of *Qinpi* on reducing the short circuit current of the forskolin-activated ileal epithelia.

Table 1. Changes in basal short circuit current induced by the ethanol extracts of three medicinal plants administered to rat ileal epithelia pretreated with forskolin

	Basal short circuit current	10 min after the addition of forskolin (a)	10 min after the addition of extract (b)	b – a
Qinpi (*Fraxini cortex*)	35.5 ± 11.4	88.5 ± 31.4	80.4 ± 30.5	–9.0 ± 2.0
Kushen (*Sophora flavescens*)	52.3 ± 1.7	79.3 ± 5.2	73.3 ± 5.4	–5.7 ± 1.7
Huanglian (*Coptis teeta*)	58.7 ± 21.7	109.2 ± 23.7	95.7 ± 25.8	–13.5 ± 8.4

Values represent means ± S.D. $\mu A/cm^2$ for 6 individual measurements.

Table 2. Effect of oubain on the changes in basal short circuit current induced by the ethanol extracts of three medicinal plants administered to rat ileal epithelia pretreated with forskolin

	Basal short circuit current	10 min after the addition of forskolin	10 min after the addition of ouabain (a)	10 min after the addition of extract (b)	b – a
Qinpi (*Fraxini cortex*)	58.0 ± 3.9	112.2 ± 9.7	83.2 ± 10.2	76.0 ± 10.1	–7.2 ± 2.8
Kushen (*Sophora flavescens*)	55.7 ± 8.0	104.2 ± 6.9	74.3 ± 14.8	71.5 ± 3.8	–2.8 ± 1.2
Huanglian (*Coptis teeta*)	49.0 ± 10.3	99.8 ± 13.3	75.2 ± 16.2	66.7 ± 16.1	–8.5 ± 2.2

Values represent means ± S.D. $\mu A/cm^2$ for 6 individual measurements.

Table 3. Effect of bumetanide on the changes in basal short circuit current induced by the ethanol extracts of three medicinal plants administered to rat ileal epithelia pretreated with forskolin

	Basal short circuit current	10 min after the addition of forskolin	10 min after the addition of bumetanide (a)	10 min after the addition of extract (b)	b – a
Qinpi (*Fraxini cortex*)	52.7 ± 13.7	113.2 ± 22.7	76.8 ± 23.9	73.8 ± 25.6	–3.0 ± 2.6
Kushen (*Sophora flavescens*)	49.3 ± 10.0	102.7 ± 11.7	64.7 ± 11.8	59.7 ± 13.2	–5.0 ± 2.1
Huanglian (*Coptis teeta*)	53.6 ± 6.9	104.8 ± 8.6	77.7 ± 11.9	70.7 ± 11.9	–7.0 ± 1.5

Values represent means ± S.D. $\mu A/cm^2$ for 6 individual measurements.

Kushen

The extract of *Kushen* likewise could partially reduce the short circuit current of the forskolin-activated ileal epithelia. The decrease in current was 5.7 ± 1.8 μA (Table 1). Figure 2 shows that if ouabain was added before the ethanol extract of *Kushen*, the decrease in current was attenuated to 2.8 ± 1.2 μA (Table 2), and the value is significantly different ($P = 0.03$) from that of adding *Kushen* extract only (control). This indicates that adding ouabain before the *Kushen* extract blocks the effect of *Kushen* on reducing the short circuit current. On the contrary, the addition of bumetanide before the ethanol extract of *Kushen* had few effects on the reduction of current by the extract. This is because the decrease in current remains at 5.0 ± 2.1 μA (Table 3); the value is not significantly different from that of adding *Kushen* extract alone.

Huanglian

The ethanol extract of *Huanglian* reduced the short circuit current of the forskolin-activated ileal epithelia as well. The decrease of short circuit current by the ethanol extract of *Huanglian* in the forskolin-activated intestinal epithelia was 13.5 ± 8.4 μA (Table 1). Figure 3 shows that adding ouabain and bumetanide before the *Huanglian* extract both attenuate the effect of *Huanglian* on reducing the short circuit current. If ouabain was added before the ethanol extract of *Huanglian*, the decrease in current was 8.5 ± 2.2 μA (Table 2). Although this value is lower than that of adding *Huanglian* extract only, they are not significantly different from each other ($P = 0.236$). Adding bumetanide before the ethanol extract of *Huanglian* attenuated the current decrement to 7.0 ± 1.5 μA (Table 3). This value is also not significantly different ($P = 0.101$) compared to the current

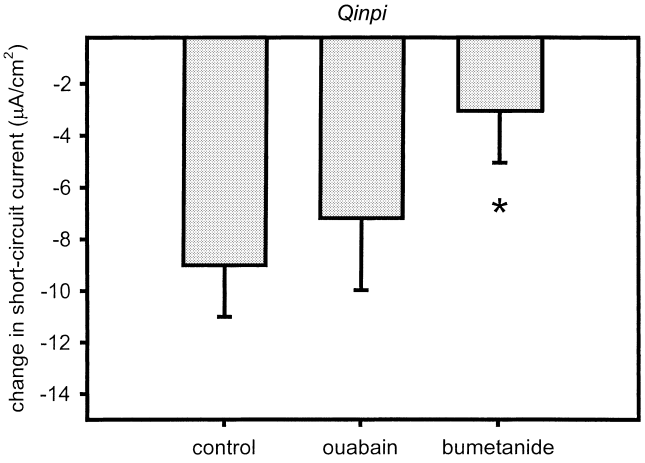

Fig. 1. Effect of oubain and bumetanide on the decrease in short circuit current induced by the ethanol extract of *Qinpi*. The current were measured across the forskolin-activated rat intestinal epithelia. Control: with *Qinpi* extract on the forskolin-activated ileal epithelia. Ouabain: with ouabain (final concentration of 100 μM) before the *Qinpi* extract on the forskolin-activated ileal epithelia. Bumetanide: with bumetanide (final concentration of 100 μM) before the *Qinpi* extract on the forskolin-activated ileal epithelia. Values represent means ± S.D. for 6 individual measurements. Asterisk indicates a statistically significant difference $P<0.05$, as compared to the control by one way ANOVA.

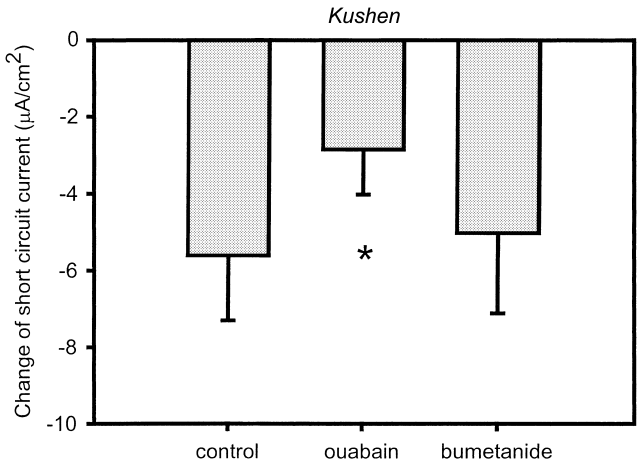

Fig. 2. Effect of ouabain and bumetanide on the decrease in short circuit current induced by the ethanol extract of *Kushen*. The current was measured across the forskolin-activated rat intestinal epithelia. Control: with *Kushen* extract on the forskolin-activated ileal epithelia. Ouabain: with ouabain (final concentration of 100 μM) before the *Kushen* extract on the forskolin-activated ileal epithelia. Bumetanide: with bumetanide (final concentration of 100 μM) before the *Kushen* extract on the forskolin-activated ileal epithelia. Values represent means ± S.D. for 6 individual measurements. Asterisk indicates a statistically significant difference $P<0.05$, as compared to the control by one way ANOVA.

reduction generated by treatment of *Huanglian* extract alone.

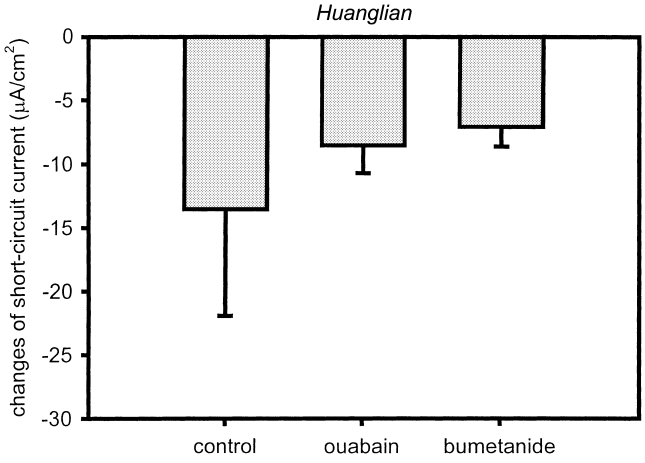

Fig. 3. Effect of ouabain and bumetanide on the decrease in short circuit current induced by the ethanol extract of *Huanglian*. The current was measured across the forskolin-activated rat intestinal epithelia. Control: with *Huanglian* extract on the forskolin-activated ileal epithelia. Ouabain: with ouabain (final concentration of 100 μM) before the *Huanglian* extract on the forskolin-activated ileal epithelia. Bumetanide: with bumetanide (final concentration of 100 μM) before the *Huanglian* extract on the forskolin-activated ileal epithelia. Values represent means ± S.D. for 6 individual measurements.

Discussion

Qinpi, *Kushen*, and *Huanglian* are categorized as heat relief medicines in the Chinese medical literature (1). They are used to cure fever, diarrhea, and various inflammatory diseases. By using the Ussing chamber and voltage-current technique, this present study discerned the effects of these three Chinese medicinal plants with anti-diarrheal properties on ion transport of the rat intestinal epithelia. Our data reveal that the ethanol extracts of *Qinpi*, *Kushen*, and *Huanglian* could all reduce the short circuit current across the forskolin-activated rat ileal epithelia. Forskolin stimulates the production of cellular cyclic AMP and hence Cl^- movement leading to elevation of short circuit current across the epithelia (14). Consequently, the results imply that extracts of the three plants may affect ion transport in the rat ileum epithelia, and this may be critical for their therapeutic effects as anti-diarrheal agents. It should be noted that the results of the present study are completely opposite to our study on other Chinese medicinal plants with laxative properties, namely, *Dahuang* (*Rheum palmatum* Linn.), *Badou* (*Croton tiglium* Linn.), and *Huomaren* (*Cannabis sativa* Linn.). Ethanol extracts of the three medicinal plants with laxative properties exert effects on augmenting the short circuit current of the forskolin-activated rat ileal epithelia (15). It seems that effects of these Chinese medicinal plants with anti-

diarrheal and laxative properties on the short circuit current of the forskolin-activated rat ileal epithelia are totally different.

The effects of these three medicinal plants on Na^+ and Cl^- transport were further studied by adding ouabain or bumetanide before treatment with the extracts. Ouabain inhibits the Na^+-K^+ ATPase and hence blocks Na^+ uptake, while bumetanide inhibits the Na^+-K^+-$2Cl^-$ cotransporter and then retards Cl^- movement. The results of *Qinpi* show that the reducing effect of *Qinpi* extract on the short circuit current is not affected by ouabain, but affected by bumetanide. Since the *Qinpi* extract cannot reduce the current when the Cl^- movement is inhibited by bumetanide, it is deduced that *Qinpi* extract may share a similar effect with the bumetanide; that is, *Qinpi* extract may have an effect on Cl^- movement. On the contrary, the *Qinpi* extract can reduce the current despite the Na^+ movement being inhibited by ouabain; it hence is suggested that the *Qinpi* extract might not affect Na^+ movement, otherwise the current should not be reduced additionally after ouabain treatment.

The result of *Kushen* was contrary to the result of *Qinpi*. Treatment of ouabain prior to *Kushen* extract attenuated the decreasing effect of short circuit current caused by *Kushen* extract only on the forskolin-activated rat ileal epithelia. Nonetheless, *Kushen* extract decreases the short circuit current further despite adding bumetanide before the extract. The results would indicate that the ethanol extract of *Kushen* affects Na^+ movement rather than Cl^- movement. Reduction of the short circuit current by *Kushen* is attributed to its modification of Na^+ transport.

The data of *Huanglian* was different to that of the previous two medicinal plants. Neither ouabain nor bumetanide could significantly attenuate the effect on decreasing the short circuit current by the *Huanglian* extract on the forskolin-activated rat ileal epithelia. It hence is suggested that ethanol extract of *Huanglian* may not affect Na^+ or Cl^- movement. Reduction of the short circuit current by *Huanglian* could be ions other than Na^+ or Cl^-.

Ingredients of the three plants have been studied in many studies (1); however, most of the studies do not provide evidence for supporting their effects on epithelial transport. Instead, these studies indicate that many constituents of the three medicinal plants are effective in anti-inflammation and anti-microorganisms (1). *Qinpi* extract contains fraxetin, esculetin, and other substances (1). The esculetin was reported to inhibit 5'-lipoxygenase and leukotriene biosynthesis (16). *Kushen* contains mainly alkaloids such as matrine and sophoridine (1). *Huanglian* also contains various alkaloids including berberine (1). The berberine alone may exert an anti-secreting action on intestinal epithelial cells by affecting K^+ channels (17) and may block Ca^{2+} influx in the smooth muscle cells of guinea pig colon (18). This could moderately explain why the ethanol extract of *Huanglian* does not affect Na^+ or Cl^- movement in this present study.

It should be noted that addition of ethanol extracts of these plants on the mucosal side did not affect the current like the addition of these extracts on the serosal side did (data not shown). They influence the current in the forskolin-activated rat ileal epithelia when added on the serosal side. In this regard, these plant extracts 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 on the rest of the rat ileal epithelia (data not shown). Therefore, they may not act directly on the Na^+-K^+ ATPase or Na^+-K^+-$2Cl^-$ cotransporter located on the basolateral membrane of the epithelial cells.

The intestinal epithelial cells may respond to many effectors molecules and hence change the ion movements. In those reactions, many cellular components participate in the molecular events that occur outside the cells, through the cell, and finally to the ion transport apparatus on the cell membrane. In cholera-induced diarrhea, the cholera toxin stimulates the G protein on the membrane of the intestinal epithelial cells, elicits signal transduction leading to Cl^- movement from the serosa to the mucosa, and hence water secretion into the intestine lumen (19 – 21). Besides cholera-induced diarrhea, there are other types of diarrhea that result from perturbation of ion and water movements in different ways. Inflammation, taken as an example, is frequently combined with diarrhea in the intestinal tissues. This could be seen in the case of the lipopolysaccharide (LPS) of the Gram-negative bacteria (22, 23). The LPS stimulates the immunocytes or the neural cells in the intestinal wall to produce nitric oxide or prostaglandin, and these two molecules are involved in various cases of diarrhea (24 – 28). Other than nitric oxide or prostaglandin, the immunocytes secrete many cytokines in the inflammatory tissues. Evidences show that pro-inflammatory cytokines, such as interleukin (IL)-1 and IL-3, stimulate Cl^- secretion (29, 30), while anti-inflammatory cytokines IL-4, IL-10, and IL-13 promote intestinal uptake of Na^+ and Cl^- (31, 32).

The effect on ion transport of intestinal epithelia is seen in other medicinal plants with anti-diarrheal properties. TJ-14 extracted from *Hange-Shashin-To*, a compound solution prepared from several Chinese medicinal plants including *Huanglian*, is reported to inhibit diarrhea (33). TJ-14 was also shown to inhibit water secretion caused by cholera toxin (34, 35). SP-303

derived from latex of the plant *Croton lechleri*, from South America, also has an effect on reducing the forskolin-stimulated Cl^- current (36). The present study proves that ethanol extracts of *Qinpi*, *Kushen*, and *Huanglian* can reduce the short circuit current across the forskolin-activated rat ileal epithelia by affecting Na^+, Cl^-, or other ion movements in the rat intestinal epithelia. Nevertheless, these extracts might not act directly on the ion transport apparatus, but likely act through immune or inflammatory pathways. Further study will be needed to distinguish the molecular action in the intestinal epithelia elicited by these Chinese medicinal plants.

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