Effect of Ethanol Extracts of Three Chinese Medicinal Plants with Laxative Properties on Ion Transport of the Rat Intestinal Epithelia

Jong-Chang TSAI,[a] Shuli TSAI,[b] and Weng-Cheng CHANG*,[b,c]

[a] *Department of Physical Education, National Changhua University of Education; Changhua, 500 Taiwan;* [b] *Department of Physiology, China Medical University; and* [c] *Department of Sports Medicine, China Medical University; 91 Hsueh-Shih Road, Taichung, 404 Taiwan.* Received April 25, 2003; accepted October 10, 2003

The effects of ethanol extracts of three Chinese medicinal plants Dahuang (*Rheum palmatum* L.), Badou (*Croton tiglium* L.), and Huomaren (*Cannabis sativa* L.), on ion transport of the rat intestinal epithelia were studied. Rat intestinal epithelia mounted in an Ussing chamber attached with voltage/current clamp were used for measuring changes of the short-circuit current across the epithelia. The intestinal epithelia were activated with current raised by serosal administration of forskolin 5 μM. Ethanol extracts of the three plants all augmented the current additively when each was added after forskolin. In subsequent experiments, ouabain and bumetanide were added prior to ethanol extracts of these medicinal plants to determine their effect on Na^+ and Cl^- movement. The results suggest that ethanol extracts of the three medicinal plants may affect the Cl^- movement more directly than Na^+ movement in the intestinal epithelial cells. The results provide evidence for the pharmacologic mechanism of the three Chinese medicinal plants on the intestinal tract.

Key words Chinese medicinal plant; ion transport; intestinal epithelia; laxative; Ussing chamber

According to the Chinese medical literature, Dahuang (*Rheum palmatum* LINN., family Polygonaceae), Badou (*Croton tiglium* LINN., family Euphorbiaceae), and Huomaren (*Cannabis sativa* LINN., family Cannabaceae) have commonly been used as laxative agents for a long period.[1] Despite the considerable use of these plants, little is known about their actions on cellular events.

Laxatives act to promote water secretion and facilitate passage of bowel contents. In the intestinal epithelia, water secretion and absorption are fundamental aspects of daily activities.[2,3] Water movement across the epithelia is generally driven by an osmotic gradient created by active ion transport.[4] Typically, the epithelial cells absorb water by active Na^+ uptake which is pumped by Na^+–K^+ ATPase on the serosal side, and secrete water by actively pumping out Cl^- which is powered by the Na^+–K^+–$2Cl^-$ cotransporter on the serosal side.[5,6] The Na^+ and Cl^- transport changes and results in water movement in the intestinal epithelia. This is seen in many cases of diarrhea and is the pharmacologic mechanism of antidiarrheal agents.[7—10]

To detect ion transport in epithelia, the Ussing chamber attached with voltage/current clamp for measuring short-circuit currents across epithelial tissues is a reliable method and has been used for decades. Movement of charged ions across the epithelia generally creates a potential difference between the epithelia.[11] When the potential difference is clamped to zero, the short-circuit current is recorded using the voltage-clamp equipment. Fluctuation of the short-circuit current can be monitored and reflects changes of ion across the epithelia.[12,13]

Using this method, our previous work revealed that the ethanol extracts of three Chinese medicinal plants had antidiarrheal properties, namely, Qinpi (*Fraxini cortex*), Kushen (*Sophora flavescens*, AITON), and Huanglian (*Coptis teeta*, WALLICH) affect Na^+ or Cl^- movement in the rat intestinal epithelia.[14] Since laxatives and antidiarrheal agents all act primarily on the intestinal tissue, the present study was aimed at discerning the effects of extracts from three Chinese medicinal plants with laxative characteristics on ion transport of the rat intestinal epithelia. The results indicate that the ethanol extracts of the three medicinal plants may affect the Na^+–K^+–$2Cl^-$ cotransporter more directly than Na^+–K^+ ATPase on the serosal side of the intestinal epithelial cells. It provides evidence that the three Chinese medicinal plants have laxative properties.

MATERIALS AND METHODS

Chinese Medicinal Plants and Extract Preparation Dried Dahuang (*Rheum palmatum* L.), Badou (*Croton tiglium* L.), and Huomaren (*Cannabis sativa* L.) were purchased from the Chinese pharmacy of the China Medical College Hospital. Twenty grams of each dried material was dissolved in 200 ml of ethanol and boiled for 30 min. The supernatants were then collected and concentrated with a vacuum evaporator (EYELA N-N SERIAS, Japan) until the volume was reduced to 5 ml and were stored at −20 °C in a refrigerator.

Tissue Preparation Healthy male Sprague–Dawley (SD) rats were obtained from the 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 2-cm segments 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 along the mesenteric border, and the serosal and muscular layers were peeled away under a binocular microscope.[12]

Ussing Chamber and Recording of Short-Circuit Current The epithelia were mounted between the Ussing chambers (CHM6, W.P.I., Sarasota, FL, U.S.A.). The buffers were oxygenated continuously and maintained at 37 °C. An automatic voltage clamp (DVC1000, W.P.I.), was corrected for fluid resistance between the potential difference-sensing Ag/AgCl electrodes. A second pair of Ag/AgCl electrodes monitored the short-circuit current across the tissue continu-

* To whom correspondence should be addressed. e-mail: wcchang@mail.cmu.edu.tw

Table 1. Changes in the Basal Short-Circuit Current across Rat Ileal Epithelia before and after the Addition of Forskolin and in Presence of the Ethanol Extracts of Three Medicinal Plants

	n	Basal short-circuit current	10 min after addition of forskolin (*a*)	10 min after addition of extract (*b*)	*b*−*a*
Dahuang (*Rheum palmatum* L.)	6	45.2±11.2	95.5±16.6	109.8±16.6	+14.3±3.1
Badou (*Croton tiglium* L.)	6	42.0±11.3	98.7±8.1	113.3±11.0	+14.7±3.0
Huomaren (*Cannabis sativa* L.)	6	47.0±3.4	99.7±9.4	112.9±8.7	+13.2±2.9

Values are expressed as mean±S.D. $\mu A/cm^2$ for 6 individual measurements.

ously. The bathing solution in the serosal and mucosal chambers was 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. These chemicals were purchased from Merck (Darmstadt, Germany). The buffer was gassed with 95% O_2–5% CO_2 before filling the chambers.[13)]

Experimental Procedure To show the effects of the extracts on short-circuit current of the rat ileal epithelia, forskolin (Sigma, St. Louis, MO, U.S.A.) was first added on serosal side (final concentration 5 μM) of the tissue. The current rose and was stable after 1—3 min. Ethanol extract of the medicinal plant (25 μl) was then added on serosal side about 20 min after forskolin. Changes in the short-circuit current were recorded continuously during the experiment. To discern whether the movement of sodium or chloride was involved in the effects of the extracts on short-circuit current across the epithelia, ouabain (an inhibitor of Na^+–K^+ ATPase, purchased from Sigma) and bumetanide (an inhibitor of Na^+–K^+–$2Cl^-$ cotransporter, purchased from Sigma) were added, respectively, (final concentration 100 μM) 20 min after forskolin. In both cases, there was a drop in the current and it was stable after 1—3 min. Ethanol extract of each plant (25 μl) was then added on the serosal side about 10 min after ouabain or bumetanide.

Statistical Analysis Data are expressed as mean±S.D. Comparisons between two groups were performed using Student's *t*-test. A difference of $p<0.05$ was considered statistically significant.

RESULTS

Dahuang The ethanol extract of Dahuang further raised the short-circuit current across the forskolin-activated ileal epithelia. The increase in current was 14.3±3.1 $\mu A/cm^2$ (Table 1). When adding ouabain before the ethanol extract of Dahuang, the current increment was attenuated to 7.7±2.0 $\mu A/cm^2$, which is significantly different from when Dahuang extract was added alone ($p=0.02$). When adding bumetanide before the ethanol extract of Dahuang, the current increment was reduced to 2.7±1.9 $\mu A/cm^2$, and the difference is significant ($p=0.00001$). The values with ouabain and bumetanide treatments were significantly different from each other ($p=0.001$) (Fig. 1).

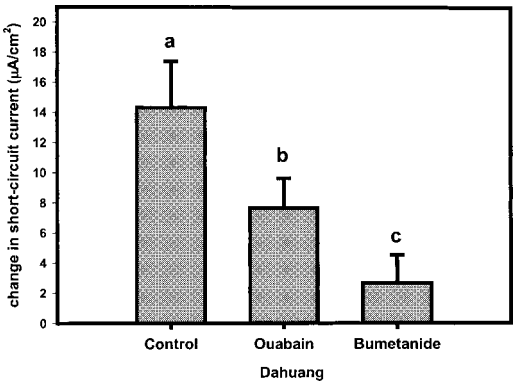

Fig. 1. Effect of the Ethanol Extract of Dahuang on the Short-Circuit Current Measured across Rat Intestinal Epithelia

Dahuang increased the short-circuit current of the forskolin-activated ileal epithelia (control). The current augmentation was attenuated by adding ouabain before the Dahuang extract (ouabain). Addition of bumtanide nearly blocked the effect of Dahuang on increment in the short-circuit current (bumetanide). Values are mean±S.D. for 6 individual measurements. Different letters indicate statistically significant different ($p<0.05$) determined using Student's *t*-test.

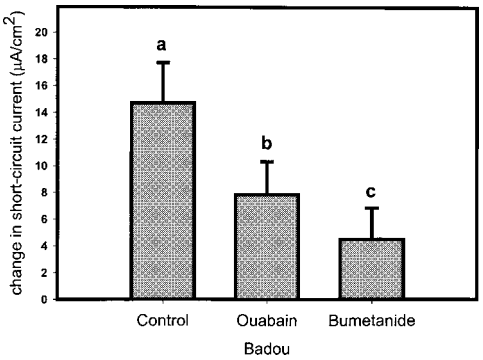

Fig. 2. Effect of the Ethanol Extract of Badou on the Short-Circuit Current Measured across Rat Intestinal Epithelia

Badou increased the short-circuit current of the forskolin-activated ileal epithelia (control). The current augmentation was reduced by adding ouabain before the Badou extract (ouabain). Addition of bumtanide impeded the effect of Badou on the increment in the short-circuit current (bumetanide). Values are mean±S.D. for 6 individual measurements. Different letters indicate statistically significant different ($p<0.05$) determined using Student's *t*-test.

Badou The ethanol extract of Badou also increased the short-circuit current across the forskolin-activated epithelia to 14.7±3.0 $\mu A/cm^2$ (Table 1). When ouabain was added before the ethanol extract of Badou, the current was 7.8±2.5 $\mu A/cm^2$. The value was significantly different from that when Badou extract was added alone ($p=0.001$). Adding bumetanide before the ethanol extract of Badou attenuated the current increment to 4.5±2.3 $\mu A/cm^2$, which was significantly different from that with Badou extract alone ($p=0.00006$). The values of ouabain and bumetanide treatment were also significantly different from each other ($p=0.038$) (Fig. 2).

Huomaren The ethanol extract of Huomaren likewise increased the short-circuit current across the forskolin-activated epithelia to 13.2±2.9 $\mu A/cm^2$ (Table 1). Adding ouabain before the ethanol extract of Huomaren attenuated the current increment to 8.2±1.7 $\mu A/cm^2$, which was significantly different from that when Huomaren extract was added

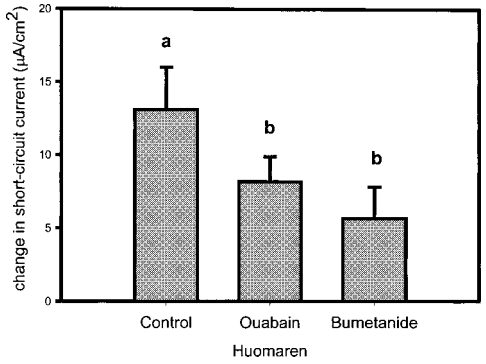

Fig. 3. Effect of the Ethanol Extract of Huomaren on the Short-Circuit Current Measured across Rat Intestinal Epithelia

Huomaren increased the short-circuit current of the forskolin-activated ileal epithelia (control). The current augmentation was diminished by the addition of ouabain before the Huomaren extract (ouabain). Addition of bumtanide partially blocked the effect of Huomaren on augmenting the short-circuit current (bumetanide). Values are mean± S.D. for 6 individual measurements. Different letters indicate statistically significant different ($p<0.05$) determined using Student's *t*-test.

alone ($p=0.005$). Adding bumetanide before the ethanol extract of Huomaren attenuated the current increment to 5.7± 2.2 μA/cm^2, which was significantly different ($p=0.0005$) compared with treatment of Huomaren extract alone. The values of ouabain and bumetanide treatment, however, did not differ significantly different from each other ($p=0.051$) (Fig. 3).

DISCUSSION

Dahuang, Badou, and Huomaren are categorized as laxatives in the Chinese medical literature.[1] They are mainly used to induce diarrhea and facilitate defecation. Our data reveal that ethanol extracts of these three plants all augment the short-circuit current of the rat ileal epithelia activated by forskolin. Forskolin stimulates the production of cellular cyclic AMP and leads to Cl^- movement, thus increasing the short-circuit current across the epithelia.[15] Our results confirm that extracts of the three plants influence ion transport across the rat ileal epithelia. Moreover, the results are contrary to our previous study on three Chinese medicinal plants with antidiarrhea properties. Ethanol extracts of Qinpi (*Fraxini cortex*), Kushen (*Sphora flavescens*, AITON), and Huanglian (*Coptis teeta*, WALLICH) reduced the short-circuit current of the forskolin-activated rat ileal epithelia.[14] It appears that extracts of these Chinese medicinal plants with laxative and antidiarrheal properties exert completely opposite effects on the short-circuit current of the forskolin-activated rat ileal epithelia.

The effects of these three medicinal plants on Na^+ and Cl^- transport were further studied by adding ouabain or bumetanide before the addition of the extracts. The results with Dahuang, Badou, and Huomaren were similar. Treatment with bumetanide prior to the extracts of these plants diminished the current increment caused by the three extracts alone. Bumetanide blocks the Na^+–K^+–2Cl^- cotransporter and hence retards Cl^- movement toward the lumen. Accordingly, we speculate that the ethanol extracts of Dahuang, Badou, and Huomaren may affect the Na^+–K^+–2Cl^- cotransporter in the intestinal epithelial cells, facilitate Cl^- movement from the serosal side to the lumen, and hence raise the current across the epithelia. On the other hand, adding ouabain attenuated the current increment caused by the three extracts alone; however, the decrement was less than that with bumetanide treatment. The Na^+–K^+–2Cl^- cotransporter is commonly attributed to secondary active transport, which must act coupled through the primary active transport, namely, the Na^+–K^+ ATPase, in many epithelia.[16] The current decrease with ouabain treatment may be due to the fact that ouabain inhibits the Na^+–K^+ ATPase and then prevents Na^+ from moving out of the cytosol to the serosal side to create an Na^+ gradient for the Na^+–K^+–2Cl^- cotransporter to exert Cl^- movement. The Cl^- movement from the serosal side to the lumen is hindered and hence the current cannot be elevated further by the extracts of these plants.

The contents of the three plants have been described. Dahuang extract may contain rhein, aloe-emodin, sennoside, and others.[1] Rhein was reported to act on a human intestinal cell line to induce ion secretion, apoptosis, and indirect chemotaxis of polymorphonuclear leukocytes.[17] Sennosides increase paracellular permeability of small molecules and stimulate chloride secretion.[18] Aloe-emodin anthrone and rhein anthrone promote intestinal water secretion.[19] Badou contains mainly croton oil that consists of phorbol, crotonic acid, and others.[1] Phorbol ester is well known for its action on protein kinase C and thus serial cellular events including Ca^{2+} and ion transport.[20] The extract of Huomaren may contain trigonelline, betaine, phytin, and others.[1]

It should be noted that adding ethanol extracts of these plants has little effect on the short-circuit current of rat ileal epithelia not activated by forskolin (data not shown). This implies that extracts of these plants may not act directly on the Na^+–K^+–2Cl^- cotransporter. Changes in the short-circuit current in the epithelia reflect disturbance of the ion transport and consequently the water movement in the tissue. There are many potential stimulators of ion transport and water secretion in the intestinal tissue, notably inflammatory molecules and neuropeptides. They act on the signaling molecules on the cell membrane and subsequently on the ion transport apparatus. Cholera toxin, for example, stimulates the G protein on the membrane of intestinal epithelial cells, and elicits signal transduction leading to Cl^- movement from the serosa to mucosa and hence water secretion into the intestinal lumen.[21—23] In addition, intrinsic factors produced by immune or nervous tissues may participate in the molecular events leading to diarrhea.[24] Lipopolysaccharide (LPS) of the Gram-negative bacteria may stimulate the immunocytes or neural cells in the intestinal wall to produce nitric oxide or prostaglandin, and these two molecules are involved in various cases of diarrhea.[25—29] Proinflammatory cytokines, such as interleukin (IL)-1 and IL-3, stimulate Cl^- secretion,[30,31] while the antiinflammatory cytokines IL-4, IL-10, and IL-13 promote intestinal uptake of Na^+ and Cl^-.[32,33] In conclusion, this present study confirms that ethanol extracts of Dahuang, Badou, and Huomaren may have more direct effects on Cl^- movement than on Na^+ movement in the rat intestinal epithelia. Nevertheless, further study is needed to discriminate the detailed actions in the intestinal epithelia elicited by these Chinese medicinal plants.

Acknowledgments This study was supported by a grant to J. C. Tsai and W. C. Chang from the National Science

Council, Taiwan (NSC89-2316-B-039-002), and from the China Medical University (CMC90-M-01).

REFERENCES

1) Yen C. H., "The Pharmacology of Chinese Plants,", 1st ed., Chin-Yin Publishing, Taipei, 1994 (in Chinese).
2) Powell D. W., Binder H. J., Curran P. F., *Am. J. Physiol.*, **223**, 531—537 (1972).
3) Fondacaro J. D., *Am. J. Physiol.*, **250**, G1—G8 (1986).
4) Rabbani G. H., Greenough W. B., "Textbook of Secretory Diarrhea," ed. by Lebenthal E., Duffey M. E., Raven Press, New York, 1990, pp. 233—255.
5) Schultz S. G., Frizzell R. A., *Gastroenterology*, **63**, 161—170 (1972).
6) Hawker P. C., Mashiter K. E., Turnberg L. A., *Gastroenterology*, **74**, 1241—1247 (1978).
7) Davidson G. P., Gall D. G., Pertic M., Butler D. G., Hamilton J. R., *J. Clin. Invest.*, **60**, 1402—1409 (1977).
8) McEwan G. T., Schousboe B., Skadhauge E., *Pflugers Arch.*, **417**, 174—179 (1990).
9) Osman N. E., Westrom B., Karlsson B., *Scand. J. Gastroenterol.*, **33**, 1170—1174 (1998).
10) Starkey W. G., Candy D. C., Thornber D., Collins J., Spencer A. J., Osborne M. P., Stephen J., *J. Pediatr. Gastroenterol. Nutr.*, **10**, 361—370 (1990).
11) Field M., Fromm D., Mccoll I., *Am. J. Physiol.*, **220**, 1388—1396 (1971).
12) Kurkchubasche A. G., Cardona M., Watkins S. C., Smith S. D., Albanese C. T., Simmons R. L., Rowe M. I., Ford H. R., *Shock*, **9**, 121—127 (1998).
13) Gabriel S. E., Davenport S. E., Steagall R. J., Vimal V., Carlson T., Rozhon E. J., *Am. J. Physiol.*, **276**, G58—G63 (1999).
14) Tsai J. C., Tsai S., Chang W. C., *J. Pharmacol. Sci.*, in press (2004).
15) Sharp G. W. G., Hynie S., *Nature* (London), **229**, 266—269 (1971).
16) Frizzell R. A., Welsh M. J., Smith P. L., *Soc. Gen. Physiol. Ser.*, **36**, 137—145 (1981).
17) Raimondi F., Santoro P., Maiuri L., Londei M., Annunziata S., Ciccimarra F., Rubino A., *J. Pediatr. Gastroenterol. Nutr.*, **34**, 529—534 (2002).
18) Leng-Peschlow E., *J. Pharm. Pharmacol.*, **45**, 951—954 (1993).
19) Yagi T., Yamauchi K., Kuwano S., *J. Pharm. Pharmacol.*, **49**, 22—25 (1997).
20) Epple H. J., Fromm M., Riecken E. O., Schulzke J. D., *Scand. J. Gastroenterol.*, **36**, 731—737 (2001).
21) Kimberg D. V., Field M., Johnson J., Henderson A., Gershon E., *J. Clin. Invest.*, **50**, 1218—1230 (1971).
22) Petritsch W., Eherer A. J., Holzer-Petsche U., Hinterleitner T. A., Beubler E., Krejs G. J., *Gut*, **33**, 1174—1178 (1992).
23) Jodal M., Holmgren S., Lundgren O., Sjoqvist A., *Gastroenterology*, **105**, 1286—1293 (1993).
24) Lundgren O., Jodal M., *Comp. Biochem. Physiol.*, **118A**, 319—329 (1997).
25) Brunsson I., Sjotlqvist A., Jodal M., Lundgren O., *Acta Physiol. Scand.*, **130**, 633—642 (1987).
26) Pugin J., Schurer-Maly C. C., Leturcq D., Moriarty A., Ulevitch R. J., Tobias P. S., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 2744—2748 (1993).
27) Konturek P. H., *Scand. J. Gastroenterol.*, **33**, 691—700 (1998).
28) Izzo A. A., Mascolo N., Capasso F., *Digest. Dis. Sci.*, **43**, 1605—1620 (1998).
29) Tyler K., Keith J. C., Jr., *Lab. Invest.*, **80**, 1269—1280 (2000).
30) Chiossone D. C., Simon P. L., Smith P. L., *Eur. J. Pharmacol.*, **180**, 217—228 (1990).
31) Theodorou V., Eutamene H., Fioramonti J., Junien J. L., Bueno L., *Gastroenterology*, **106**, 1493—1500 (1994).
32) Madsen K. L., Tavernini M. M., Mosmann T. R., Fedorak R. N., *Gastroenterology*, **111**, 936—944 (1996).
33) Zund G., Madara J. L., Dzus A. L., Awtrey C. S., Colgan S. P., *J. Biol. Chem.*, **271**, 7460—7464 (1996).