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Anti-ulcer effect of Magnoliae Cortex and its active constituents

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Abstract

The effect of natural medicines used as stomachics and that of Chinese medicinal compounds used in gastrointestinal disorders on the protective action of gastric mucosal membrane were examined in HCl-ethanol induced ulcer in rats. Hange-kôboku-tô (Ban-Xia-Hou-Pu-Tang), a Chinese medicinal compound, was found to be effective in HCl-ethanol induced ulcer. The effect of Magnoliae Cortex, one of the active ingredients in Hange-kôboku-tô, was further analyzed by examining the actions of each of the fractions of Magnoliae Cortex acetone extract using column chromatography in pharmacological experiments. Magnolol and hônokiol, constituents in Magnoliae Cortex, at 200 mg/kg significantly inhibited HCl-ethanol induced ulcers and 0.2 N NaOH induced ulcers. However, they did not affect ulcers induced by water-stress and by pylorus ligation.

Kew words anti-ulcer effect, magnolol, hônokiol, Magnoliae Cortex, Hange-kôboku-tô (Ban-Xia-Hou-Pu-Tang)

Abbreviations IR, infrared ; mp, melting point ; MS, mass Spectrum ; NMR, nuclear magnetic resonance ; Hange-kôboku-tô (Ban-Xia-Hou-Pu-Tang), 半夏厚朴湯

Introduction

There are many Chinese medicinal compounds used for gastrointestinal disorders and natural medicines used as stomachics, which are effective in clinical usage. However, very few of them have been examined for their effectiveness in pharmacological experiments. We have been analyzing Chinese medicinal compounds and natural medicines in basic pharmacological experiments in order to substantiate their clinical actions. Recently, the extracts of Hange-kôboku-tô (Ban-Xia-Hou-Pu-Tang) and its ingredients, Magnoliae Cortex, Zingiberis Rhizoma and Perillae Herba, were found to be effective in the HCl-ethanol induced ulcer model which has frequently been used to determine the activation of gastric mucosal membrane protective factors. In this report, active constitutents in Magnoliae Cortex and their mode of action were examined.

Materials and Methods

Hange-kôboku-tô extract was obtained by mixing the daily dosage of Pinelliae Tuber (*Pinellia ternata* BREITENBACH, China), Hoelen (*Poria cocos* WOLF, China), Magnoliae Cortex (*Magnolia obovata* THUNB., Japan), Perillae Herba (*Perilla frutescens* BRITTON, Japan) and Zingiberis Rhizoma (*Zingiber officinale* ROSCOE, China) at 5 : 5 : 3 : 2 : 3 ratio[1)] and simmered in 500 ml of water for 45 min to half of the original volume and then centrifuged at 3000 rpm for 15 min. Each natural medicine was obtained from local markets in Osaka. The supernatant was freeze-dried and used as Hange-kôboku-tô extract. Fifty percent-methanol extracts of the individual ingredients were obtained by soaking them in 3 volumes of 50%-methanol at cold temperature for 2 days, filtered and concentrated under reduced pressure at below 50℃. They were then dried

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and kept in a desicator. Since non-polar fractions of Magnoliae Cortex 50%-methanol extract were found to be the active fractions, the active constituents of Magnoliae Cortex were sought in acetone extract in which non-polar substances can be isolated. The method for the isolation of the acetone extract of Magnoliae Cortex was the same as that for the 50%-methanol extracts. Fractionation of the active constituents of Magnoliae Cortex and the isolation and purification of magnolol and hônokiol were carried out as shown in Fig. 1. Each of the individual compounds was identified based on melting point (mp), infrared (IR), nuclear magnetic resonance (NMR) and mass Spectrum (MS).[2] Teprenone (Eisai), sofalcone (Taisho Pharmaceutical Co.) and atropine-H_2SO_4 (Merck Co.) were used as reference drugs in the following experimental ulcer models. Each of the drugs was suspended in 5%-acacia gum and control animals received only the 5%-acacia gum solution.

1) HCl-ethanol induced gastric mucosal membrane lesions : Wistar male rats weighing approximately 200 g were divided into groups of 5—6 animals. After 24 hr fasting, they were orally administered test drugs. One hour thereafter, each rat received oral administration of 1 ml of 150 mM HCl-60% ethanol. Each animal was then killed by ether 1 hr after the administration of the necrotizing agent, and the stomach was excised and fixed in 2%-formalin solution. Lesion index was calculated by adding the length (cm) of the lesions in the fundus region. Student's t-test was used for the statistical analysis.[3]

2) 0.2 N NaOH induced gastric mucosal membrane lesions : The examination was the same method as in 1) except that 0.2 N NaOH was used as the necrotizing agent instead of HCl-ethanol.[4]

3) Pylorus ligation induced gastric ulcers : The method used was the same as previously reported.[5] Briefly, Wistar male rats weighing approximately 200 g were divided into groups of 5—6 animals. After 48 hr of fasting, the rats were anesthetized with ether and the abdomen was opened. The pylorus region of the stomach was ligated, followed by an administration of test drugs into the duodenum. Thirteen hours thereafter, the stomach was excised and fixed in 2%-formalin solution. The ulcer index was determined based on the ulcer formation in the forestomach. Mann-Whitney's U-test was used for statistical analysis.

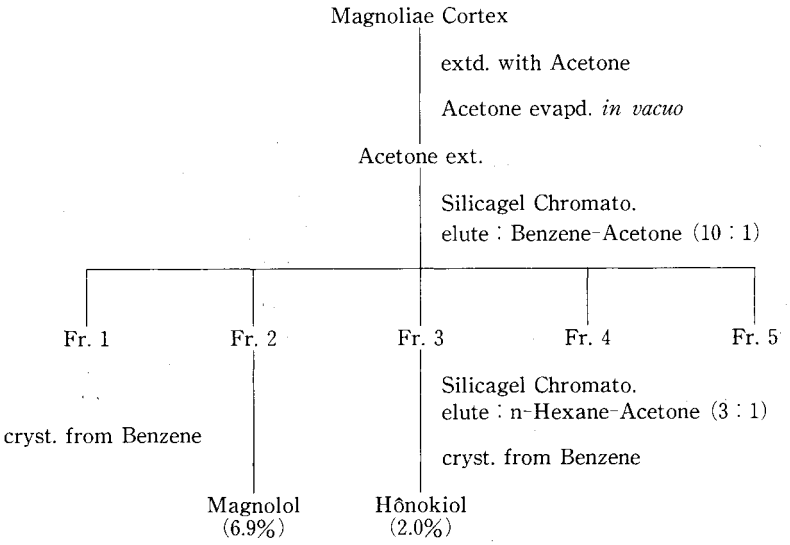

Fig. 1 Flow diagram of extraction and fractionation of Magnoliae Cortex.

4) Water immersion stress induced gastric ulcers : The method used was the same as previously reported.[6] Briefly, male ddY mice weighing approximately 20 g were fasted for 24 hr. Test drugs were then administered orally, and each animal was put into a vinyl chloride pipe (10 cm long, 2 cm i.d.), covered at the top and bottom with metal mesh, and immersed in water (22℃) to the neck. After 5 hr, the animals were sacrificed and the stomachs were excised. Each stomach was then fixed in 2%-formalin solution. The ulcer index was determined based on the degree of erosions in the fundus region. Mann-Whitney's U-test was used for the statistical analysis.

Results

HCl-ethanol induced gastric mucosal membrane lesions

As shown in Table I, the extract of Hange-kôboku-tô at 3000 mg/kg significantly inhibited the gastric mucosal lesions. Specifically, the lesions were inhibited by 91.6% as compared to the control. In order to further determine the active ingredients in Hange-kôboku-tô, the effects of the individual ingredients were examined on the gastric mucosal lesions. As shown in Table II, Magnoliae Cortex, Zingiberis Rhizoma and Perillae Herba (1000 mg/kg, *p.o.*, each) inhibited the gastric lesions by 99.4%, 80.2% and 98.9%, respectively.

To further examine the active constituents in Magnoliae Cortex, the Magnoliae Cortex acetone extract was fractionated into Fr. 1 to Fr. 5 as shown in Fig. 1. The dosage shown in Table III for each of the fractions was determined based on

Table I Effect of Hange-kôboku-tô on HCl-ethanol induced gastric lesions in rats.

Treatment	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (Mean±S.E.)	Inhibition (%)
Control	—	6	9.24±1.51	—
Hange-kôboku-tô	1500	5	5.79±2.73	37.3
	3000	5	0.78±0.24**	91.6
Sofalcone	300	5	0.53±0.38**	94.3

Gastric lesions were induced by oral administration of 1 ml of HCl-ethanol (60% ethanol+150 mM HCl). Each drug was given orally 1 hr before HCl-ethanol administration. Animals were sacrificed 1 hr after HCl-ethanol administration. Significantly different from control at $^{**}p<0.01$.

Table II Effects 50%-methanol ext. of Pinelliae Tuber, Magnoliae Cortex, Zingiberis Rhizoma, Hoelen and Perillae Herba on HCl-ethanol induced gastric lesions in rats.

Treatment	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (Mean±S.E.)	Inhibition (%)
Control	—	6	10.13±1.20	—
Pinelliae Tuber	1000	5	10.71±2.05	0.0
Magnoliae Cortex	1000	5	0.06±0.04**	99.4
Zingiberis Rhizoma	1000	5	2.01±0.48**	80.2
Hoelen	1000	5	4.97±2.03*	50.9
Perillae Herba	1000	5	0.11±0.04**	98.0
Sofalcone	300	5	0.05±0.05**	99.5

Gastric lesions were induced by oral administration of 1 ml of HCl-ethanol (60% ethanol+150 mM HCl). Each drug was given orally 1 hr before HCl-ethanol administration. Animals were sacrificed 1 hr after HCl-ethanol administration. Significantly different from control at $^{*}p<0.05$, $^{**}p<0.01$.

Table III Effects of fractions of Magnoliae Cortex acetone ext. on HCl-ethanol induced gastric lesions in rats.

Treatment	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (Mean±S.E.)	Inhibition (%)
Control	—	6	8.65±1.57	—
Magnoliae Cortex				
Fr. 1	130	5	9.56±2.61	0.0
Fr. 2	310	4	0.00±0.00**	100.0
Fr. 3	60	5	2.00±0.75*	76.9
Fr. 4	110	5	3.86±1.48	55.4
Fr. 5	280	5	11.73±1.27	0.0
Sofalcone	300	5	1.60±0.50**	81.5

Gastric lesions were induced by oral administration of 1 ml of HCl-ethanol (60% ethanol+150 mM HCl). Each drug was given orally 1 hr before HCl-ethanol administration. Animals were sacrificed 1 hr after HCl-ethanol administration. Significantly different from control at $*p<0.05$, $**p<0.01$.

Table IV Effects of magnolol and hônokiol on HCl-ethanol induced gastric lesions in rats.

Treatment	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (Mean±S.E.)	Inhibition (%)
Control	—	6	9.24±1.51	—
Magnolol	100	5	6.27±2.43	32.2
	200	5	3.70±1.09*	60.0
Hônokiol	100	5	2.17±0.66**	76.5
	200	5	0.84±0.31**	90.9
Sofalcone	300	5	0.53±0.38**	94.3

Gastric lesions were induced by oral administration of 1 ml of HCl-ethanol (60% ethanol+150mM HCl). Each drug was given orally 1 hr before HCl-ethanol administration. Animals were sacrificed 1 hr after HCl-ethanol administration. Significantly different from control at $*p<0.05$, $**p<0.01$.

Table V Effects of magnolol and hônokiol on 0.2 N NaOH induced gastric lesions in rats.

Treatment	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (Mean±S.E.)	Inhibition (%)
Control	—	5	9.43±1.17	—
Magnolol	100	5	0.30±0.07**	96.8
	200	5	0.23±0.21**	97.6
Hônokiol	100	5	0.33±0.10**	96.5
	200	5	0.00±0.00**	100.0
Sofalcone	300	5	0.47±0.29**	95.0

Gastric lesions were induced by oral administration of 1 ml of 0.2 N NaOH. Each drug was given orally 1 hr before 0.2 N NaOH administration. Animals were sacrificed 1 hr after 0.2 N NaOH administration. Significantly ditterent from control at $**p<0.01$.

Table VI Effects of magnolol and hônokiol on pylorus ligation induced gastric ulcers in rats.

Treatment	Dose (mg/kg, *i.d.*)	No. of rats	Ulcer index (Mean±S.E.)	Inhibition (%)
Control	—	6	5.00±0.00	—
Megnolol	100	5	4.40±0.40	12.0
	200	5	3.00±0.63	40.0
Hônokiol	100	5	4.40±0.40	12.0
	200	5	3.00±0.45*	40.0

Each drug was given intraduodenally immediately after pylorus ligation. Animals were sacrificed 13 hr after pylorus ligation. Significantly different from control at $*p<0.05$.

Table VII Effects of magnolol and hônokiol on water immersion stress induced gastric ulcers in mice.

Treatment	Dose (mg/kg, *p.o.*)	No. of mice	Lesion index (Mean±S.E.)	Inhibition (%)
Control	—	12	2.92±0.08	—
Magnolol	100	10	3.50±0.22	0.0
	200	10	3.40±0.27	0.0
Hônokiol	100	10	3.80±0.13	0.0
	200	10	3.80±0.13	0.0
Atropine-H_2SO_4	30	10	1.50±0.17**	48.6

Gastric ulcers were induced by immersing into the water bath (22°C) up to the xiphoid process. Each drug was given orally 10 min before stress. Animals were sacrificed after 5 hr stress later. Significantly different from control at $**p<0.01$.

yields of the individual fractions. Fr. 2 and Fr. 3, whose inhibition was 100% and 76.9%, respectively, significantly inhibited the gastric lesions. Fr. 4, whose inhibition was 55.4%, had no significant inhibitory effect on the lesions. Fr. 1 and Fr. 5 had no effect at all.

As shown in Fig. 1, Fr. 2 and Fr. 3 were further fractionated, and magnolol and hônokiol were obtained. Table IV shows that magnolol at 200 mg/kg inhibited the gastric lesions by 60.0% and hônokiol at 100 and 200 mg/kg inhibited by 76.5 and 90.9%, respectively.

Effects of magnolol and hônokiol on 0.2 N NaOH induced gastric mucosal membrane lesions

As shown in Table V, the oral administration of either magnolol or hônokiol at 100 mg/kg and 200 mg/kg abolished the gastric lesions.

Effects of magnolol and hônokiol on pylorus ligation induced gastric ulcers

Table VI shows that magnolol and hônokiol at 100 mg/kg did not have any significant inhibitory action. At 200 mg/kg, both drugs inhibited the ulcerations by only 40%.

Effects of magnolol and hônokiol on water immersion stress induced gastric ulcers

Table VII shows that the oral administration of either magnolol or hônokiol at 100 mg/kg and 200 mg/kg did not have any inhibitory effect on the gastric ulcers.

Discussion and Conclusions

It has been reported that magnocurarine, which is an alkaloid constituent in Magnoliae Cortex, possesses muscle relaxant and antitremor actions.[7] Magnoliae Cortex extract was shown

to have sedative effects[8] and magnolol was reported to have antiulcer action.[9] It has been thought that Chinese medicinal compounds and natural medicines activate the protective mechanism in the body, thereby curing disease and maintaining health. A similar concept was expressed in "Shen-nong-ben-Cao-jin." Based on the notion that the effects of natural medicines used as stomachics and Chinese medicinal compounds used for gastrointestinal disorders have to be examined on the body's natural protective mechanisms, the effect of Hange-kôboku-tô was examined on the HCl-ethanol induced ulcers which is often used as the experimental model for the analysis of cellular protective action. The results in the present experiment indicated that magnolol and hônokiol are the active constituents in Magnoliae Cortex. Even though the antiulcer effects of magnolol and hônokiol have already been reported using non-oral routes of drug administration,[9] it is important to use the oral administration method in line with the clinical usage of Chinese medicinal compounds and natural medicines.

The present experiments also indicated that magnolol and hônokiol had no effect on the stress induced ulcers and had only a weak effect on the pylorus ligation induced ulcers. Moreover, both magnolol and hônokiol significantly inhibited the 0.2 N NaOH induced and HCl-ethanol induced ulcers which are used for the examination of cellular protective actions. As we have reported that chikusetsusaponin, the saponin isolated from Panacis Japonici Rhizoma, inhibited the HCl-ethanol induced ulcers,[10] these results suggest that magnolol and hônokiol may activate the gastric mucosal membrane protective mechanism and thereby exhibit antiulcer effects.

Recently, the hypothesis was advanced that prostaglandins (PG) are involved in the protective action in gastrointestinal cells,[4] and PG may be related to part of the mechanisms involved in gastrointestinal lesions. In addition, it has been reported that a weak stimulus of 0.25 N HCl increases the level of PG in gastric mucosal membrane which in turn protects the mucosal membrane from stronger stimulants.[11] It is also known that PGE_2 and its derivatives, at 1/100 of the dosage necessary to inhibit gastric acid secretion, protect against gastric mucosal lesions induced by absolute ethanol, strong acid, strong alkalis and boiling water.[4] These results suggest that it is important to determine the level of PG in gastric mucosal membrane in order to examine the protective effects of various drugs.[12] The effects of not only magnolol and hônokiol but also natural medicines and Chinese medicinal compounds used as stomachics are currently being investigated on PG synthesis.

和文抄録

健胃生薬及び胃腸疾患にも用いられる漢方方剤の有効性を，胃粘膜保護作用の有無を知るのにしばしば用いられる病態モデルである HCl-ethanol 潰瘍を用いて screening 試験したところ，漢方方剤'半夏厚朴湯'にも有効性を認めた。半夏厚朴湯構成生薬の中で特に有効であったコウボクに関して詳細に検討するために，コウボクアセトンエキスのカラムクロマト法による分離分画と薬理実験を併用して行ったところ，コウボクに含有される magnolol 及び hônokiol が HCl-ethanol 潰瘍及び0.2 N 水酸化ナトリウム潰瘍を200 mg/kg の経口投与で有意に抑制した。しかし拘束水浸ストレス潰瘍や幽門結紮潰瘍では無効であった。これらのことより magnolol や hônokiol は胃粘膜保護作用により抗潰瘍作用を示すのではないかと思われた。

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2) magnolol : mp : 110.0—110.8℃, MS *m/e* : 266.1308 (M^+), $C_{18}H_{18}O_2$ IR (KBr) : 3160, 1640 cm^{-1}, ^1H-NMR (80 MHz, $CDCl_3$) δ : 3.4 (4H, d, aryl-CH_2-CH=×2), 5.1 (4H, m, -CH=CH_2×2), 5.6 (2H, s, phenolic -OH×2) disappeared with D_2O, 5.95 (2H, m, -CH=CH_2×2), 6.8－7.3 (6 H, m, aromatic H). ^{13}C-NMR (20 MHz, $CDCl_3$) δ : 39.3(t), 115.8(t), 116.8(d), 124.4(s), 129.8(d), 131.4(d), 133.4(s), 137.5(d), 150.9(s).
hônokiol : mp : 87.2—88.0℃, MS *m/e* : 266.1286 (H^+), $C_{18}H_{18}O_2$ IR (KBr) : 3300, 1640cm^{-1}, ^1H-NMR (80MHz, $CDCl_3$) δ : 3.33 (2H, d, aryl-CH_2-CH=), 3.44 (2H, d, aryl-CH_2-CH=), 5.1 (4H, m, -CH=CH_2×2), 5.11 (1 H, s, phenolic -OH) disappeared with D_2O, 5.13 (1H, s,

phenolic -OH) disappeared with D_2O, 5.9 (2H, m, $-CH=CH_2\times2$), 6.8—7.3 (6H, m, aromatic H). ^{13}C-NMR (20 MHz, $CDCl_3$) δ : 35.1(t), 39.3(t), 115.6(t), 115.7(t), 116.5(d), 116.9(d), 126.5(s), 127.9(s), 128.8(d), 128.6(d), 129.7(s), 130.3(d), 131.2(d), 132.4(s), 136.1(d), 137.9(d), 150.8(s), 153.9(s).

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