Effects of extracts of mixed Allii Bakeri Bulbus and Trichosanthis Fructus on gastric lesions

Fumihide TAKANO, Fumihiko YOSHIZAKI*, Miki AHIKO, Hitoshi SATOH
Toshio ANDO and Shuji HISAMICHI

Department of Pharmacognosy, Tohoku College of Pharmacy

(Received November 7, 1990. Accepted January 23, 1991.)

Abstract

Anti-gastric lesion activities of a mixture of *Allium bakeri* (bulbs) and *Trichosanthes kirilowii* var. *japonica* (fruits) (1 : 1) were examined in rats using 50% ethanolic extract.

Decreases of gastric secretion and acid output in pylorus-ligation and suppressions of gastric lesions induced by water-immersion stress and HCl·ethanol were exhibited as effects of Trichosanthes fruits without influence of simultaneous presence of bulbs of *Allium bakeri*.

Aggravations of Shay' ulcer and HCl·aspirin induced gastric lesion caused by bulbs of *Allium bakeri* disappeared owing to the simultaneous presence of Trichosanthes fruits.

These results intimate one of the meanings of combination of these crude drugs in some Chinese medicinal prescriptions.

Key words *Allium bakeri* REGEL, *Trichosanthes kirilowii* MAXIMOWICZ var. *japonica* KITAMURA, 50% ethanolic extract, rat, gastric secretion, anti-gastric lesion.

Abbreviations ABE, 50% ethanol extract of *Allium bakeri* (bulb); ATE, 50% ethanol extract of mixed *Allium bakeri* (bulb) and *Trichosanthes kirilowii* var. *japonica* (fruit); TKE, 50% ethanol extract of *Trichosanthes kirilowii* var. *japonica* (fruit).

Introduction

In previous papers, we reported the aggravation of pylorus ligation-induced gastric ulcer in rats by using a 50% ethanolic extract of the bulb of *Allium bakeri* REGEL (Allii Bakeri Bulbus) (ABE)[1] and the protective effect of 50% ethanolic extract of the fruit of *Trichosanthes kirilowii* MAXIMOWICZ var. *japonica* KITAMURA (Trichosanthis Fructus) (TKE) on several experimental gastric lesions in rats.[2]

Both crude drugs are found together in some Chinese medicinal prescriptions, such as Karo-Gaihaku-Hakusyu-to, Karo-Gaihaku-Hange-to, and the meaning of the combination is ambiguous. Therefore, in this paper we investigated the anti-gastric lesion activities of the mixture of these two crude drugs (1 to 1, 50% ethanol extract, ATE) in rats and compared the activities with them individually.

Subjects and Methods

Materials : Bulbs of *Allium bakeri* (from Gunma prefecture, Japan) and fruits of *Trichosanthes kirilowii* var. *japonica* (from Miyazaki prefecture, Japan) were purchased from Nakaikohshindo (Kobe). They (individual 100g or mixture of each of 50 g) were extracted three times with 50% ethanol (1 *l*) at 90°C for 2hr. After removal of the solvent *in vacuo* and drying of the extracts, colored powders (ABE, 65.8 g ; TKE, 32.8 g ; ATE, 48.5 g ; respectively) were obtained. They were dissolved in bidistilled water and a constant injection volume of 1.0 ml/200 g was used. The vehicle alone was administered to the control group in the same manner.

*〒 981 仙台市青葉区小松島 4-4-1
東北薬科大学生薬学教室 吉崎文彦
4-4-1 Komatsushima, Aoba-ku, Sendai 981, Japan

Journal of Medical and Pharmaceutical Society for WAKAN-YAKU **8**, 23－28, 1991

Animals : Male Wistar rats weighing between 160-230 g were used. They were housed in cages with mesh bottoms to prevent coprophagia and kept at 23±1℃. The animals were starved for 24 or 48 hr before use but allowed free access to water till the beginning of the experiment.

Gastric secretion in pylorus-ligated rats : Rats were killed by an overdose of ether after 7hr pylorus ligation. The gastric juice was collected and the volume was measured. Acid concentration was estimated by titration of the gastric juice with 0.05 N NaOH using a pH meter to endpoint pH 7.0. ABE and ATE were intraduodenally administered immediately after pylorus-ligation.

Water immersion stress - induced gastric lesions : According to the manner of Takagi *et al.*,[3,4] rats were immobilized in a stress-cage and immersed vertically in a water bath at 23±1℃ to the level of the xiphoid process for 7 hr. ABE and ATE were given orally immediately before stress treatment. The rats were killed by a blow on the head 7 hr later. Subsequently, their stomachs were removed and inflated by injecting 10 ml of 2% buffered formalin. Each preparation was then incised along the greater curvature and examined for the presence of mucosal lesions. The length (mm) of each lesion was measured under a microscope (×7), summed per stomach, and used as the lesion index.

Shay ulcer : The pyloric ligation was carried out according to the method of Shay *et al.*.[5] ATE was orally given immediately after pylorus ligation. Animals were killed 18 hr later by an overdose of ether. Their stomachs were removed, inflated with 2% buffered formalin, incised along the greater curvature and examined for lesions developed in the fore-stomach. The longitudinal and abscissal length of each lesion was measured and the multiplied products were summed per stomach and used for an ulcer index. The index was established as follows : ulcer area 0 mm^2, 0 ; 1-6 mm^2, 1 ; 7-12mm^2, 2 ; 13-18 mm^2, 3 ; 19-24 mm^2, 4 ; >24 mm^2 or perforation, 5.

HCl · ethanol - induced gastric mucosal lesions : This model was carried out according to Mizui and Doteuchi,[6] and rats were administered 1 ml of HCl·ethanol (150 mM HCl in 60% ethanol) orally. ABE and ATE were given orally 30 min beforehand. The rats were killed by a blow on the head 1hr later, and the stomachs were removed and inflated by injecting 10 ml of 2% buffered formalin. Each preparation was then incised along the greater curvature and examined for the presence of mucosal lesions. The length (mm) of each lesion was measured under a microscope (×7), summed per stomach, and used as the lesion index. In a separate experiment, indomethacin (Nacalai tesque) suspended in saline with a trace of Tween 80 (Nacalai tesque) was given subcutaneously at a dose of 5 mg/kg 1hr before HCl·ethanol was administered.

HCl · aspirin-induced gastric mucosal lesions ; According to Guth *et al.*,[7] rats were given 1 ml of HCl·aspirin (aspirin 150 mg/kg in 150 mM HCl) orally. ABE, TKE and ATE were administered orally 30 min before the giving of HCl·aspirin. The rats were killed by a blow on the head 1hr later, and the lesion index was prepared in the manner described above. In a separate experiment, indomethacin was given and the lesion index was obtained by the same procedure mentioned above.

Statistics : Data are presented as the mean±S. E.. Statistical analysis was performed utilizing the Student's t-test. Values of $p < 0.05$ were regarded as significant.

Results

Gastric secretion in pylorus-ligated rats

The volume of gastric secretion and acid concentration after 7 hr pylorus-ligation are revealed in Table I. Intraduodenal administration of ATE significantly decreased the gastric acid secretion and inhibited acid concentration at dose of 5000 mg/kg. No significant differences from the control groups were found at other doses.

Water immersion stress-induced gastric lesions

As exhibited in Table II, ATE at doses of 1000 and 5000 mg/kg given by peroral administration revealed significant inhibition of the gastric lesions, while there was no significant difference

Table I Effects of ABE and ATE on gastric acid secretion in pylorus-ligated rats.

Substance	Dose (mg/kg, *i.d.*)	No. of rats	Volume (ml)	Acidity (mEq/l)	Acid output (μ Eq/hr)
Control		9	5.7±0.4	90.0± 9.6	74.0±10.8
ABE	500	8	5.3±0.6	81.8± 6.9	58.4± 7.4
	1000	7	6.1±0.6	85.4± 6.0	75.9±10.2
	3000	8	6.7±0.4	84.9± 2.5	80.9± 6.4
Control		8	7.6±0.4	111.2±17.0	131.7±15.4
ATE	500	8	6.8±0.5	107.6± 8.2	110.3±13.2
	1000	9	7.6±0.4	106.6±13.6	120.7±20.2
	5000	8	0.5±0.1[a)]	62.4±10.4[a)]	8.5± 1.8[a)]

All values represent the mean±S.E..
a) Significantly different from control, $p<0.01$.

Table II Effects of ABE and ATE on water-immersion stress-induced gastric lesions in rats.

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		9	12.5±0.9	
ABE	500	9	12.0±2.0	4.3
	1000	9	11.3±1.4	9.7
	3000	9	14.2±1.9	-18.8
Control		9	16.2±1.1	
ATE	500	9	13.2±1.5	18.3
	1000	9	11.7±1.3[a)]	27.4
	5000	9	9.4±1.6[b)]	41.9

All values represent the mean±S.E..
Significantly different from control, a) $p<0.05$, b) $p<0.01$.

Table III Effect of ATE on gastric ulceration in pylorus-ligated rats.

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Ulcer index	Inhibition (%)
Control		6	3.3±0.3	
ATE	500	7	3.0±0.2	9.1
	1000	8	1.6±0.5[a)]	51.5
	5000	8	1.1±0.4[b)]	66.7

All values represent the mean±S.E..
Significantly different from control, a) $p<0.05$, b) $p<0.01$.

from the control value in the case of ABE.

Shay ulcer

Effect of ATE on pyloric ligation-induced gastric ulcer is shown in Table III. Peroral given ATE significantly inhibited the development of ulcer at doses of 1000 and 5000 mg/kg.

HCl·ethanol-induced gastric mucosal lesions

Pretreatment with ABE at 30 min before HCl·ethanol peroral administration revealed no significant activity against the caused gastric lesions at doses of 500 and 1000 mg/kg (Table IV). However, pretreatment with ATE significantly inhibited the lesions produced as shown in Table IV. Indomethacin pretreatment did not so much influence the protective effect of ATE (Table V).

HCl·aspirin-induced gastric mucosal lesions

Table IV Effects of ABE and ATE on HCl·ethanol-induced gastric lesions in rats.

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		8	101.7± 9.9	
ABE	500	7	105.6±12.4	-3.9
	1000	8	103.4± 9.8	-1.7
	3000	8	7.3± 4.2[a)]	92.8
Control		9	85.1± 3.7	
ATE	100	10	53.4± 7.9[a)]	37.2
	500	9	16.2± 2.7[a)]	81.0
	1000	10	6.4± 1.5[a)]	92.5
	5000	10	0.0± 0.0[a)]	100.0

All values represent the mean±S.E..
a) Significantly different from control, $p<0.01$.

Table V Effect of ATE on HCl·ethanol-induced gastric lesions in rats pretreated with indomethacin.

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		9	80.3 ±1.6	
ATE	100	9	55.5 ±8.1[a)]	31.0
	500	10	54.6 ±4.3[a)]	32.1
	1000	10	15.6 ±2.2[a)]	80.6
	5000	10	0.01±0.01[a)]	99.9

All values represent the mean±S.E..
a) Significantly different from control, $p<0.01$.

Table VI Effects of ABE, TKE and ATE on HCl·aspirin-induced gastric lesions in rats.

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		8	52.2±3.1	
ABE	500	8	74.6±4.2[a)]	-43.0
	1000	8	84.7±6.1[a)]	-62.1
	3000	8	81.7±5.0[a)]	-56.4
Control		13	72.7±5.3	
TKE	100	10	62.2±5.0	13.8
	500	10	29.2±5.6[b)]	59.5
	1000	10	22.6±5.8[b)]	68.7
Control		9	95.8±4.9	
ATE	500	10	70.8±6.3[b)]	26.1
	1000	9	59.1±3.8[b)]	38.3
	5000	10	53.0±3.4[b)]	44.7

All values represent the mean±S.E..
Significantly different from control, a) $p<0.05$, b) $p<0.01$.

As displayed in Table VI, pretreatment with ABE at 30 min before HCl·aspirin administration significantly aggravated the induced lesions at doses of 500, 1000 and 3000 mg/kg. On the contrary, TKE pretreatment significantly decreased the lesion index at doses of 500 and 1000 mg/kg (Table VI). ATE pretreatment also significantly protected the rats from developing lesions at doses of 500, 1000 and 5000 mg/kg (Table VI). This significant protection was revealed in spite of pretreatment with indomethacin as exhibited in Table VII.

Table VII Effect of ATE on HCl·aspirin-induced gastric lesions in rats pretreated with indomethacin.

Substance	Dose (mg/kg, *p.o.*)	No. of rats	Lesion index (mm)	Inhibition (%)
Control		10	123.6±3.8	
	500	10	102.3±3.0	17.2
	1000	9	87.7±2.2[a)]	29.0
	5000	9	62.9±3.2[a)]	49.1

All values represent the mean±S.E..
a) Significantly different from control, $p<0.01$.

Discussion

ATE scarcely affected gastric acid secretion at doses of 500 and 1000 mg/kg. ABE also had little effect on the acid secretion while TKE strongly decreases it[2)] at the same dosage. The quantity of TKE contained in 1000 mg of ATE is about one-third, 333 mg which was calculated based on the amounts of yielded extracts of individual crude drugs. The dose of 100 mg/kg TKE has no significant influence on the acid secretion[2)] and 333 mg/kg of TKE in 1000 mg/kg of ATE may have no remarkable activity. A larger dosage of 5000 mg/kg of ATE significantly depressed the gastric acid secretion. In this condition, about 1663 mg of TKE exists in 5000 mg of ATE and this dosage seems to be enough for potent activity. Accordingly, the activities of ATE on gastric secretion at a dose of 5000 mg/kg are presumed to be due to the activities of TKE in ATE without the influence of ABE in ATE.

As reported previously,[2)] TKE prevents the gastric lesion induced by stress at doses of 500 and 1000 mg/kg, whereas ABE showed no effect on it as revealed in this experiment. And yet, ATE exhibited significant inhibition of the lesion at high doses of 1000 and 5000 mg/kg. As the activity at a dose of 5000 mg/kg (corresponding to 1663 mg of TKE) was less than that of 1000 mg/kg TKE, the possibility of a negative influence of ABE on activity of TKE is not to be discarded. But it seems reasonable to assume that the action of TKE intact appeared as an action of ATE.

It is also reported that ABE aggravates Shay' ulcer[1)] and TKE protects conversely against it.[2)] The activity of TKE was superior to that of ABE in ATE which contains both, so an undesirable action of ABE was compensated.

It was found that ATE inhibits the gastric lesion formation induced by HCl·ethanol and HCl·aspirin at doses of 500 and 1000 mg/kg, both are no effective dosages on gastric acid secretion. In the case of gastric lesion resulting from HCl·ethanol, since ABE had no activity at doses of 500 and 1000 mg/kg, the activity of TKE (suppressing the lesions at doses of 100, 500 and 1000 mg/kg)[2)] which contained in ATE appears to occur intact. On the other hand, ATE repressed HCl·aspirin-induced gastric lesions, which were aggravated by ABE dose dependently, at doses of 500, 1000 and 5000 mg/kg owing to the presence of TKE, which depressed these lesions, in ATE. This activity of TKE seems intense because it appears in spite of severe interference from ABE. Participation of "cytoprotection" of TKE which is not mediated by endogenous prostaglandins[2,8,9)] may contribute to the intensive activity of TKE. Consequently,

ATE showed significant prevention against an HCl・aspirin-induced gastric lesion pretreated with indomethacin,[10] although it was under the slight negative influence of the indomethacin pretreatment.

Activities of the 50% ethanolic extract of blended Allii Bakeri Bulbus and Trichosanthis Fructus (1 to 1) were examined by using several models of gastric lesions. Harmful effects of Allii Bakeri Bulbus were counteracted by Trichosanthis Fructus and favorable effects of the latter appeared without appreciable influence of the former. These results are available for consideration of the meaning of a combination of crude drugs in Chinese medicinal prescriptions which have been utilized based on experience since ages ago and seems to imply one of the reasons why these two crude drugs coexist in some prescriptions.

和文抄録

薤白と括楼実の1対1混合物の50%エタノールエキスを作り，いくつかの胃損傷モデルに対する作用をそれぞれの生薬単味の作用と比較し，検討した。

胃酸分泌やストレス，塩酸・エタノールによる胃損傷では括楼実の抑制作用が薤白の存在にほとんど影響されることなく混合物において出現し，Shay潰瘍や塩酸・アスピリンによる胃損傷では薤白による悪化作用が混合物において栝楼実の存在により消失していた。これらにより，いくつかの漢方薬における両者の同時配合の意義の一部が暗示された。

References

1) Yoshizaki, F., Takano, F.,Kumagai, E., Sakurai, M. and Hisamichi, S. : Several pharmacological studies on extracts of Allii Bakeri Bulbus. *Shoyakugaku Zasshi* **43**, 211-215, 1989 (in Japanese).

2) Takano, F., Yoshizaki, F., Suzuki, K., Suya, N., Ando, T. and Hisamichi, S.: Anti-ulcer effects of trichosanthes fruits. *Chem. Pharm. Bull.* **38**, 1313-1316, 1990.

3) Takagi, K., Kasuya, Y. and Watanabe, K. : Studies on the drugs for peptic ulcer. A reliable method for producing stress ulcer in rats. *Chem. Pharm. Bull.* **12**, 465-472, 1964.

4) Takagi, K. and Okabe, S. : The effects of drugs on the production and recovery process of the stress ulcer. *Jpn. J. Pharmacol.* **18**, 9-18, 1968.

5) Shay, H., Komarov, S.A., Fels, S.S., Meranze, D., Gruenstein, M. and Siplet, H. : A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology* **5**, 43-61, 1945.

6) Mizui, T. and Doteuchi, M. : Effect of polyamines on acidified ethanol-induced gastric lesions in rats. *Jpn. J. Pharmacol.* **33**, 939-945, 1983.

7) Guth, P.H., Aures, D. and Paulsen, G. : Topical aspirin plus HCl gastric lesions in the rat. Cytoprotective effect of prostaglandin,cimetidine and probanthine. *Gastroenterology* **76**, 88-93, 1979.

8) Robert, A. : Gastric cytoprotection by sodium salicylate. *Prostaglandins*, Supp. **21**, 139-146, 1981.

9) Konturek, S.J., Brozozowski, T., Piastucki, I., Radecki, T., Dembinski, A. and Dembinskakiec, A. : Role of locally generated prostaglandins in adaptive gastric cytoprotection. *Dig. Dis. Sci.* **27**, 967-971, 1982.

10) Okabe, S., Takeuchi, K., Ikenishi, H., Ohmi, F. and Takaoka, A. : Effects of SM powder, a combined stomatic, on gastric secretion and various gastric and duodenal lesions in rats. *Nippon Yakurigaku Zasshi* **81**, 285-294, 1983 (in Japanese).