The Antiulcer Action of Sophora and the Active Constituent in Sophora. II. The Antiulcer Action of Vexibinol

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The inhibitory effect of vexibinol, one of the flavanols found in Sophora, on gastric ulcers induced by HCl–ethanol has been reported previously. In the present study, the effect of vexibinol was examined in various experimental ulcer models in order to determine the mechanism of its antiulcer action. The results indicated that the oral administration of vexibinol at 25—50 mg/kg significantly inhibited the development of ulcers induced by HCl–ethanol, 0.6 N HCl, 0.2 N NaOH, absolute ethanol and 1% NH_3. In addition, an intraduodenal administration of vexibinol at 300 mg/kg significantly inhibited Shay's ulcer. Further, intraduodenal administration at 300 mg/kg significantly inhibited acid secretion caused by 2-deoxy-D-glucose. These results suggest that vexibinol has not only gastric mucosal protective action but also an inhibitory effect on the secretion of gastric acids.

Keywords Sophorae Radix; *Sophora flavescens*; vexibinol; flavanol; antiulcer effect

It has previously been reported[1] that vexibinol (VX),[2] one of the flavanols found in relatively large quantities in Sophora, inhibits gastric ulcers induced by HCl–ethanol at a dosage less than those required for spizofurone,[3] teprenone,[4] and sofalcone,[5] which are antiulcer drugs known to activate gastric mucosal protective factors. In the present study, in order to examine further the characteristics of antiulcer action of VX, the effect of VX was examined in various experimental ulcer models (Fig. 1).

Materials and Methods

Experimental Animals Vexibinol was extracted and purified according to the method described in the previous report.[1] Wistar male rats (200—250 g, Oriental Bioservice) and ddY male mice (25 g, Oriental Bioservice) were fasted for 24 h (48 h in the case of Shay's ulcer experiments) prior to experiments.

Experimental Chemicals The following chemicals were used as reference drugs: spizofurone (Maone, Takeda), cimetidine (Sigma), ranitidine (Zantac, Glaxo Sankyo), pirenzepine (Gastrozepine, Japan Boehringer Ingelheim) and atropine (atropine sulfate, Merck). Each drug was suspended in 5% arabic gum solution or dissolved in distilled water, and administered orally or intraduodenally at a dosage of 1 ml/200 g for rats and 0.2 ml/20 g for mice. Animals in control groups received an oral, intraperitoneal, or intraduodenal administration of 5% arabic gum solution.

Chemicals used for production of gastric ulcers and for stimulation of gastric acid secretion were indomethacin (Sigma), *N*-ethylmaleimide (Wako Pure Chemicals), histamine (histamine hydrochloride, Sigma), serotonin (serotonin creatine sulfate, Wako Pure Chemicals), carbachol (Sigma), 2-deoxy-D-glucose (Wako Pure Chemicals) and pentagastrin (ICI-Sumitomo). They were suspended in 1% carboxymethylcellulose (CMC) or dissolved in physiological saline and were orally administered at 1 ml/200 g, or subcutaneously or intravenously administered at 0.2 ml/200 g.

Methods for Development of Various Experimental Ulcer Models 1) Ulcers Induced by HCl–Ethanol: Ulcers were developed according to the methods of Mizui and Doteuchi.[6] Briefly, after each rat (250 g) was fasted for 24 h, HCl–ethanol (60% ethanol + 150 mM HCl) at the amount of 1.5 ml/rat was orally administered. One hour thereafter, animals were killed with ether and the stomach was excised. Then 10 ml of 2% formalin was infused into the stomach and the stomach was soaked in 2% formalin for 10 min. It was then cut open along the greater curvature and the length of each lesion in the glandular portion was summed (total length of ulcers). Test drugs were administered orally or intraperitoneally 1 h prior to the administration of HCl–ethanol.

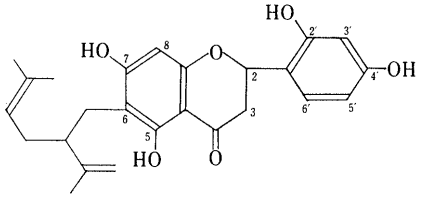

Fig. 1. Chemical Structure of Vexibinol

The antiulcer activity of VX was also examined in rats pretreated with either indomethacin or *N*-ethylmaleimide. Either indomethacin or *N*-ethylmaleimide was subcutaneously injected at 5 and 10 mg/kg, respectively, 1 h prior to the administration of test drugs.

The effect of dilution of HCl–ethanol by the gastric contents following the VX treatment was also examined according to the method reported by Kurebayashi *et al.*[7] and Okabe *et al.*[8] Briefly, the abdomen was cut open under ether anesthesia 45 min after test drug administration. A small opening was made in the fundus region through which the gastric content was removed. The opening was then closed, the abdomen was sutured back and the experiment was continued as described above.

Furthermore, in place of HCl–ethanol as a necrosis-inducing substance, 0.6 N HCl, 0.2 N NaOH, absolute ethanol[9] and 1% NH_3[10] were also employed using the same experimental procedure.

2) Stress Induced Ulceration Caused by Water Restraint: Mice were used in this study according to a modification[11] of the method described by Takagi and Okabe.[12] Briefly, each mouse (25 g) was fasted for 24 h, placed in a vinyl chloride pipe and immersed in a water bath at 22 °C for 7 h. The animal was killed with ether, then the stomach was excised and treated with formalin, and the ulcer index was determined. The severity of the lesions found in the glandular portion was scored as follows: 0, no lesions in the mucosal membrane; 1, bleeding; 2, bleeding and lesions; 3, heavy bleeding and lesions. Test drugs were orally administered 10 min prior to the stress treatment.

3) Shay's Ulcers: The experiment was performed according to the method of Shay *et al.*[13] Briefly, rats (250 g) were fasted for 24 h, then the pylorus was ligated under ether anesthesia. The stomach was excised 13 h thereafter, and following formalin treatment, the ulcer index was determined. The ulcer index corresponds to the severity of lesions found in the glandular portion: 0, no lesions; 1, bleeding or light lesions; 2, moderate lesions; 3, severe lesions; 4, holes. Test drugs were intraduodenally administered immediately after the pyloric ligation.

4) Indomethacin-Induced Ulcers: Rats (200 g), fasted for 24 h, received a subcutaneous injection of 30 mg/kg of indomethacin suspended in 1% CMC. They were killed with ether 7 h thereafter, and the stomach was excised. The stomach was treated in the same manner as described for the HCl–ethanol ulcer and the ulcer index was obtained. Test drugs were orally administered 30 min prior to the indomethacin treatment.

5) Histamine Induced Ulcers: Rats (200 g), fasted for 2 h, received an intraperitoneal injection of 300 mg/kg histamine dissolved in physiological saline. Four hours thereafter, the rats were killed with ether and the stomach was excised. The severity of the lesions in the glandular portion was scored into one of 5 categories and the ulcer index was obtained. Test drugs were orally administered 30 min prior to the administration of histamine.

6) Serotonin Induced Ulcers: Rats (200 g), fasted for 24 h, received a

subcutaneous injection of 20 mg/kg serotonin dissolved in physiological saline. They were killed with ether 18 h thereafter, and the stomach was excised. The severity of the lesions in the glandular portion was scored into one of 5 categories and the ulcer index was obtained. Test drugs were orally administered 30 min prior to the serotonin injection.

7) Phenylbutazone Induced Ulcers: Rats (220 g), fasted for 24 h, received an oral administration of 250 mg/kg phenylbutazone suspended in 5% arabic gum. Seven hours thereafter, rats were killed with ether and the stomach was excised. The stomach was then treated in the manner described in the case of HCl–ethanol induced ulcer and the total length of lesions was obtained. Test drugs were orally administered 30 min prior to the administration of phenylbutazone.

The Measurement of Basal Acid Secretion Pylorus ligation was performed according to Shay *et al.*[13] were used. Briefly, following the fasting of rats (250 g) for 24 h, the pylorus ws ligated under ether anesthesia. Rats were killed with ether 4 h thereafter, the stomach was excised and the gastric acid was obtained. Following centrifugation of gastric solution (3000 rpm, 10 min), the volume of the gastric solution, the pH, the activity, the pepsin activity and the amount of hexosamine were determined. The pH was measured by using pH test papers (Toyo Test Paper, Toyo Filter Paper). The acidity was determined by titrating on 0.05 N NaOH using phenolphthalein as an indicator, and the amount of gastric acid secretion was calculated from the product of the amount of gastric solution and the acidity. The pepsin activity was determined by using Anson's method as described by Miwa *et al.*,[14] and the amount of pepsin secretion was calculated from the product of the amount of gastric solution and the pepsin activity.

The Measurement of Stimulus Induced Acid Secretion The experiment was carried out according to the method described by Kitagawa *et al.*[15] and Sanae *et al.*[4] Briefly, following the fasting of rats (250 g) for 24 h, the trachea was cannulated under urethane (1.2 g/kg, i.p.) anesthesia and the abdomen was cut open. The pylorus and cardia were ligated and a small opening was made in the glandular portion. A three-way stopper was inserted into the opening through which 4 ml of physiological saline at 37 °C was introduced several times to wash out the inside of the stomach. The acid secretion was allowed to stabilize for 1 h, physiological saline at 37 °C was infused into the stomach every 30 min through the three way stopper, and the recovered perfusate was titrated with 0.1 N NaOH using phenolphthalein as an indicator to determine the amount of acid secretion. Drugs to stimulate the acid secretion, histamine, carbachol and pentagastrin, were injected subcutaneously and 2-deoxy-D-glucose was injected intravenously through the tail vein 1.5 h after the start of the experiment. Test drugs were injected intraduodenally 15 min prior to the administration of acid secretion stimulants.

Statistical Treatment Statistical significance was calculated by using the Mann-Whitney U-test in the experiments with the water restraint induced stress ulcer, Shay's ulcer, histamine induced ulcers and serotonin induced ulcers. Student's *t*-test was used in other experiments.

Results

Effects of VX in Various Experimental Ulcer Models 1) The Effect on Ulcers Induced by Necrosis Inducing Substances VX given orally at 25, 50 and 100 mg/kg significantly inhibited ulcers induced by HCl–ethanol, in a dose-dependent manner. The extents of inhibition by VX at 10, 25, 50 and 100 mg/kg were 31.3, 71.9, 74.6 and 98.3%, respectively. VX given intraperitoneally at 50 and 100 mg/kg did not inhibit the ulceration but tended to exacerbate it (Table I). The treatment with indomethacin significantly increased the ulceration induced by HCl–ethanol. VX still significantly inhibited the HCl–ethanol induced ulcers in the presence of indomethacin, but the inhibitory action of VX was slightly less than that of VX in the absence of indomethacin (Fig. 2). VX also significantly inhibited the ulceration in the animals pretreated with *N*-ethylmaleimide (Fig. 2).

VX at 50 mg/kg still significantly inhibited the ulceration induced by HCl–ethanol in the animals whose gastric

Table I. Effects of Vexibinol and Spizofurone on HCl–Ethanol Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg)	*n*	Total length of ulcers (mm)	Inhibition (%)
p.o. treatment				
Control		7	121.1 ± 14.3	
Vexibinol	10	6	83.2 ± 15.7	31.3
	25	6	34.1 ± 10.8[a]	71.9
	50	6	30.8 ± 5.6[a]	74.6
	100	6	2.0 ± 2.0[a]	92.3
Spizofurone	100	5	8.0 ± 3.5[a]	93.4
i.p. treatment				
Control		7	63.9 ± 7.7	
Vexibinol	50	7	69.1 ± 17.6	−8.1
	100	7	95.1 ± 25.9	−48.8
Spizofurone	100	7	22.9 ± 3.5[a]	64.2

Gastric ulcers were induced by oral administration of 1.5 ml of 60% ethanol in 150 mM HCl (HCl–ethanol). Test drugs were administered orally (*p.o.*) or intraperitoneally (i.p.) 1 h before HCl–ethanol treatment. Animals were killed 1 h after HCl–ethanol treatment. *a*) Significantly different from the control group at $p<0.01$.

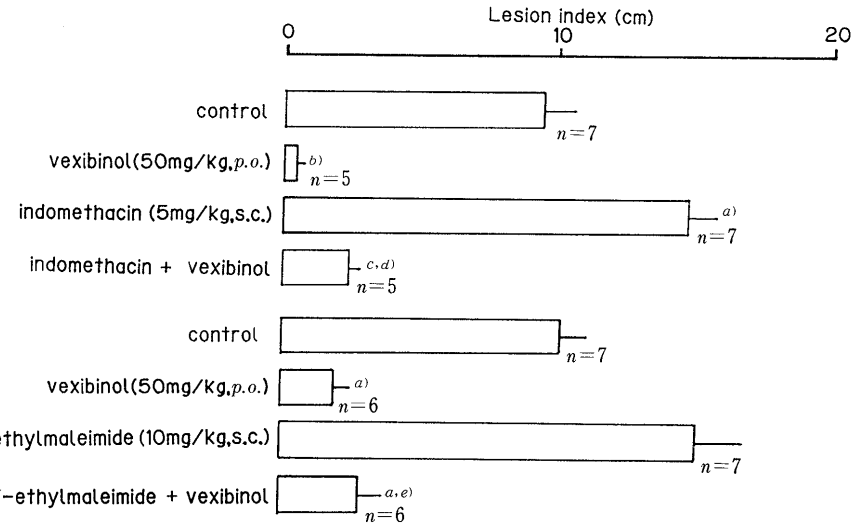

Fig. 2. Influence of Pretreatment with Indomethacin and *N*-Ethylmaleimide on the Cytoprotective Effect of Vexibinol against HCl–Ethanol Induced Gastric Lesions in Rats

Gastric lesions were induced by the oral administration of 1.5 ml of 60% ethanol in 150 mM HCl (HCl–ethanol). Vexibinol was administered orally 1 h before HCl–ethanol treatment. Indomethacin or *N*-ethylmaleimide was administered s.c. 2 h before HCl–ethanol treatment. Animals were killed 1 h after HCl–ethanol treatment. *a*) $p<0.05$, *b*) $p<0.01$ compared with the control. *c*) $p<0.05$ compared with vexibinol. *d*) $p<0.01$ compared with indomethacin. *e*) $p<0.01$ compared with *N*-ethylmaleimide.

Table II. Influence of Removal of the Gastric Contents on the Cytoprotective Effect of Vexibinol against HCl–Ethanol Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg)	n	Total length of ulcers (mm)	Inhibition (%)
Control		6	81.7±15.2	
Vexibinol	25	5	53.0±10.5	35.1
	50	5	35.1± 9.6[a]	57.1

Gastric ulcers were induced by oral administration of 1.5 ml of 60% ethanol (v/v) in 150 mM HCl (HCl–ethanol). Test drug was administered orally 1 h before HCl–ethanol treatment. Gastric contents of rats were removed 45 min after test drug administration. Animals were killed 1 h after HCl–ethanol treatment. It was evident that the protection effect of vexibinol was not caused by dilution of HCl–ethanol with gastric contents. *a*) Significantly different from the control group at $p<0.05$.

Table III. Effects of Vexibinol and Spizofurone on Necrotizing Agent-Induced Gastric Ulcers in Rats

Treatment	Dose (mg/kg)	n	Total length of ulcers (mm)	Inhibition (%)
Absolute ethanol ulcer				
Control		8	63.3±12.0	
Vexibinol	25	6	11.0± 3.4[b]	82.6
	50	6	4.7± 2.3[b]	92.6
Spizofurone	100	6	1.8± 1.0[b]	97.2
0.6 N HCl ulcer				
Control		7	134.3± 9.9	
Vexibinol	25	6	75.8±13.9	43.6
	50	6	43.7± 5.5[b]	67.5
Spizofurone	100	6	24.5± 1.5[b]	81.8
0.2 N NaOH ulcer				
Control		8	120.3±15.3	
Vexibinol	25	6	19.6± 9.0[b]	83.7
	50	6	10.4± 7.3[b]	91.4
Spizofurone	100	6	12.7± 3.4[b]	89.4
1% NH_3 ulcer				
Control		8	113.3±12.3	
Vexibinol	25	5	67.0±14.0[a]	40.9
	50	6	50.8±10.2[b]	55.2
Spizofurone	100	6	24.3± 8.3[b]	78.6

Gastric ulcers were induced by oral administration of 1.5 ml of each necrotizing agent (NA). Test drugs were administered orally 1 h before NA treatment. Animals were killed 1 h after NA treatment. Significantly different from the control at *a*) $p<0.05$, *b*) $p<0.01$.

contents were removed. The inhibition by VX at 25 mg/kg, however, was not statistically significant (Table II).

Treatment with absolute ethanol, 0.6 N HCl, 0.2 N NaOH and 1% NH_3 induced large number of string- and belt shaped ulcers in the glandular portion similar to HCl–ethanol-induced ulcers. VX inhibited the formation of these ulcers, in a dose-dependent manner. An inhibitory rate of more than 90% was observed against the ulcers induced by absolute ethanol and 0.2% NaOH upon oral administration of VX at 50 mg/kg. The inhibitory action of VX in the 0.6 N HCl- and 1% NH_3-induced ulcers was slightly less (Table III).

2) The Effect of VX on Stress Induced Ulceration Caused by Water Restraint The stress induced by 7 h of water restraint caused several dot shaped ulcerations accompanied by bleeding in the glandular portion. VX inhibited this ulceration, in a dose-dependent manner, and at 300 mg/kg VX showed a significant inhibition. Spizofurone administered orally at 300 mg/kg also significantly inhibited the ulceration (Table IV).

Table IV. Effects of Vexibinol and Spizofurone on Stress-Induced Ulceration Caused by Water Restraint in Mice

Treatment	Dose (mg/kg)	n	Unlcer index (Mean±S.E.)	Inhibition (%)
Control		10	2.3±0.2	
Vexibinol	100	9	1.8±0.2	21.7
	300	10	0.7±0.3[a]	69.6
Spizofurone	300	10	0.4±0.3[a]	82.6

Gastric ulcers were induced by immersing animals in a water bath (22 °C). Test drugs were administered orally 30 min before stress treatment. Animals were killed 7 h after stress treatment. *a*) Significantly different from the control group at $p<0.01$.

Table V. Effects of Vexibinol and Atropine on Gastric Ulcers in Pylorus-Ligated Rats (Shay Ulcer)

Treatment	Dose (mg/kg)	n	Ulcer index (Mean±S.E.)	Inhibition (%)
Control		7	3.4±0.4	
Vexibinol	100	7	2.3±0.5	32.4
	300	7	1.6±0.6[a]	52.9
Atropine	30	7	1.4±0.5[a]	58.8

Test drugs were administered intraduodenally, immediately after pylorus ligation. Animals were killed 13 h after pylorus ligation. *a*) Significantly different from the control at $p<0.05$.

Table VI. Effects of Vexibinol on Various Experimental Gastric Ulcer Models in Rats

Treatment	Dose (mg/kg)	n	Total length of ulcers (mm)	Ulcer index (Mean±S.E.)	Inhibition (%)
1) Indomethacin-induced ulcer					
Control		8	14.1±4.4	—	
Vexibinol	100	7	10.4±2.1	—	26.2
	300	7	11.9±1.7	—	16.1
Ranitidine	50	7	0.3±0.2[a]	—	98.0
2) Histamine-induced ulcer					
Control		7	—	3.3±0.4	
Vexibinol	100	7	—	2.7±0.4	18.2
	300	6	—	3.2±0.5	3.0
Spizofurone	100	6	—	3.3±0.2	0.0
3) Serotonin-induced ulcer					
Control		9		2.1±0.3	
Vexibinol	100	7		2.6±0.4	23.8
	300	6		3.0±0.3[a]	−42.9
Spizofurone	300	6		2.5±0.3	19.0
4) Phenylbutazone-induced ulcer					
Control		9	13.9±3.8	—	
Vexibinol	100	7	5.4±2.2	—	72.7
	300	7	0.3±0.2[a]	—	97.8
Spizofurone	300	7	7.9±5.3	—	43.2

1) Gastric ulcers were induced by subcutaneous injection of indomethacin (30 mg/kg). 2) Gastric ulcers were induced by intraperitoneal administration of histamine (300 mg/kg). 3) Gastric ulcers were induced by subcutaneous injection of serotonin (20 mg/kg). 4) Gastric ulcers were induced by oral administration of phenylbutazone (250 mg/kg). Test drugs were administered orally 30 min before ulcer-inducing substance treatment. Animals were killed 4 h (2), 7 h (1, 4) and 18 h (3) after ulcer-inducing substance treatment. Significantly different from the control at *a*) $p<0.05$, *b*) $p<0.01$.

3) The Effect of VX on Shay's Ulcers Thirteen hours after the pylorus ligation, ulcers accompanied by bleeding were observed in the fundus region, and some of them penetrated the stomach wall. VX administered intraduodenally inhibited the ulceration, in a dose-dependent manner, and at 300 mg/kg it showed significant inhibition.

Atropine administered intraduodenally at 30 mg/kg also significantly inhibited the ulceration (Table V).

4) The Effect of VX on Indomethacin-Induced Ulcers Treatment with indomethacin induced a large number of dot-shaped and string-shaped ulcers in the glandular portion. VX given orally at 100 and 300 mg/kg did not show any significant inhibitory effect. Ranitidine given orally at 50 mg/kg significantly inhibited the ulceration (Table VI).

5) The Effect of VX on Histamine Induced Ulcers Histamine induced a large number of string shaped and belt-shaped ulcers in the glandular portion. Oral administration of VX at 100 and 300 mg/kg did not have any significant inhibitory effect. Spizofurone given orally at 100 mg/kg also did not show any significant effect (Table VI).

6) The Effect of VX on Serotonin-Induced Ulcers Treatment with serotonin induced a large number of string-shaped and belt-shaped ulcerations in the glanduar portion. VX treatment worsened the ulceration significantly at 300 mg/kg VX. Spizofurone administered orally at 300 mg/kg did not have any significant effect (Table VI).

7) The Effect of VX on Phenylbutazone Induced Ulcers Treatment with phenylbutazone induced a large number of string shaped ulcers in the glandular portion. At 300 mg/kg, VX showed a significant inhibitory effect. Oral administration of spizofurone at 300 mg/kg appeared to be inhibitory, but the effect was without statistical significance (Table VI).

The Effect of VX on Basal Acid Secretion VX administered intraduodenally inhibited the amount of gastric solution, acid secretion and pepsin secretion significantly at 300 mg/kg. VX, however, did not have any significant effect on pH. Cimetidine given intraduodenally at 100 mg/kg significantly increased the pH, but had no significant effect on the amount of gastric solution and pepsin secretion, though there appeared to be an inhibitory tendency (Table VII).

The Effect of VX on Acid Secretion Induced by Stimulants A subcutaneous injection of histamine at 5 mg/kg gradually increased the acid secretion to a peak at 1 h after the injection and it gradually declined thereafter. VX given intraduodenally at 300 mg/kg showed no significant effect at any time. Cimetidine administered intraduodenally at 100 mg/kg significantly inhibited the acid secretion and the effect continued for 2.5 h (Fig. 3).

A subcutaneous injection of carbachol at 0.03 mg/kg rapidly increased the acid secretion, which reached the peak level after 30 min and gradually decreased thereafter. VX given intraduodenally at 300 mg/kg inhibited the increase in the acid secretion at 30 min after the carbachol injection but had no significant effect at any other time. Pirenzepine administered intraduodenally at 100 mg/kg significantly inhibited the increase in the acid secretion, and the in-

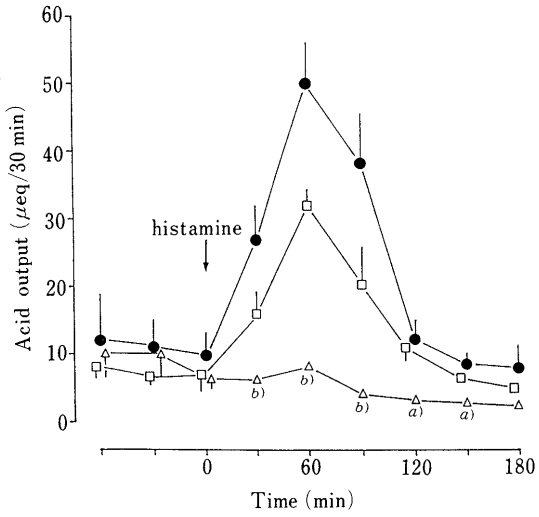

Fig. 3. Effects of Vexibinol and Cimetidine on Gastric Acid Secretion Induced by Histamine in Anesthetized Rats

Acid secretion was stimulated by subcutaneous administration of histamine (5 mg/kg). Test drugs were administered intraduodenally 15 min before histamine treatment. Each point represents the mean ± S.E. Significantly different from the control at *a*) $p<0.05$, *b*) $p<0.01$.
—●—, control ($n=7$); —□—, vexibinol (300 mg/kg, $n=5$), —△—, cimetidine (100 mg/kg, $n=4$).

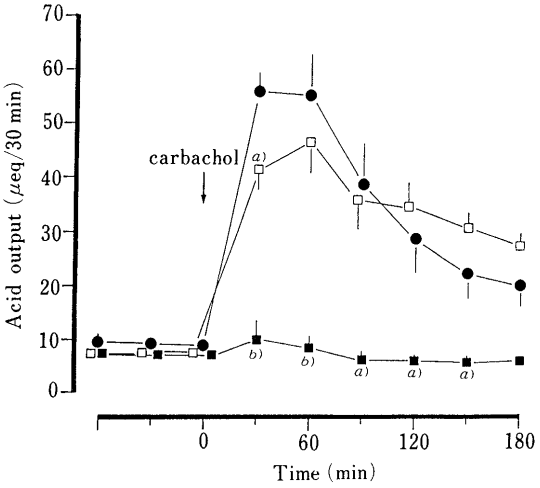

Fig. 4. Effects of Vexibinol and Pirenzepine on Gastric Acid Secretion Induced by Carbachol in Anesthetized Rats

Acid secretion was stimulated by subcutaneous administration of carbachol (0.03 mg/kg). Test drugs were administered intraduodenally 15 min before carbachol treatment. Each point represents the mean ± S.E. Significantly different from the control at *a*) <0.05, *b*) $p<0.01$.
—●—, control ($n=7$); —□—, vexibinol (300 mg/kg, $n=5$); —■—, pirenzepine (100 mg/kg, $n=5$).

TABLE VII. Effects of Vexibinol and Cimetidine on Gastric Secretion in Pylorus-Ligated Rats

Treatment	Dose (mg/kg)	*n*	Volume (ml/rat)	pH	Acidity (meq/l)	Acid output (μEq/4 h)	Pepsin (μg/ml)[a]	Pepsin (μg/4 h)[a]
Control		8	3.5 ± 0.7	1.4 ± 0.1	86.5 ± 7.2	337.2 ± 92.6	544.0 ± 81.0	1.72 ± 0.30
Vexibinol	100	7	1.9 ± 0.3	1.4 ± 0.1	79.5 ± 4.6	159.1 ± 30.3	690.7 ± 86.6	1.21 ± 0.10
	300	7	1.3 ± 0.2[b]	1.5 ± 0.1	75.3 ± 2.3	97.0 ± 14.0[b]	498.3 ± 41.0	0.66 ± 0.10[b]
Cimetidine	100	5	1.5 ± 0.2	2.4 ± 0.2[c]	43.8 ± 5.8[c]	65.1 ± 14.3[b]	788.4 ± 177.8	1.22 ± 0.35

Test drugs were administered intraduodenally after pylorus ligation immediately. Animals were sacrificed 4 h after pylorus ligation. *a*) μg and mg as SIGMA pepsin (1:60000). Significantly different from the control at *b*) $p<0.05$, *c*) $p<0.01$.

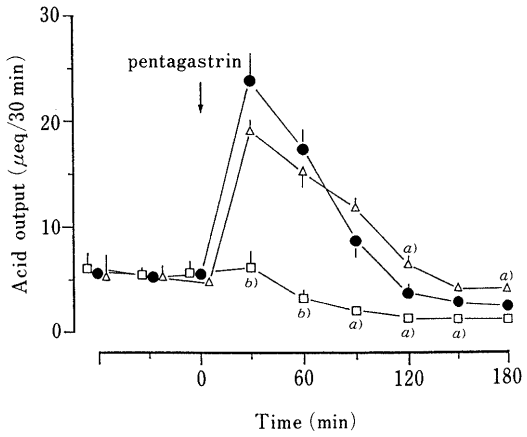

Fig. 5. Effects of Vexibinol and Cimetidine on Gastric Acid Secretion Induced by Pentagastrin in Anesthetized Rats

Acid secretion was stimulated by subcutaneous administration of pentagastrin (0.5 mg/kg). Test drugs were administered intraduodenally 15 min before pentagastrin treatment. Each point represents the mean ± S.E. Significantly different from the control at *a*) $p<0.05$, *b*) $p<0.01$.
—●—, control ($n=7$); —△—, vexibinol (300 mg/kg, $n=6$); —□—, cimetidine (100 mg/kg, $n=6$).

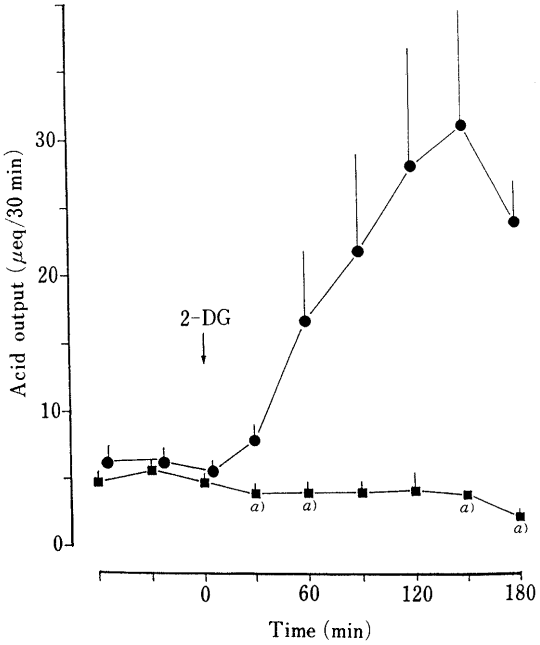

Fig. 6. Effects of Vexibinol on Gastric Acid Secretion Induced by 2-Deoxy-D-glucose in Anesthetized Rats

Acid secretion was stimulated by intravenous administration of 2-deoxy-D-glucose (2-DG, 100 mg/kg). The test drug was administered intraduodenally 15 min before 2-DG treatment. Each point represents the mean ± S.E. Significantly different from the control at *a*) $p<0.05$.
—●—, control ($n=7$); —■—, vexibinol (300 mg/kg, $n=5$).

hibitory action continued until 2.5 h after the carbachol injection (Fig. 4).

A subcutaneous injection of pentagastrin at 0.5 mg/kg rapidly increased the acid secretion, which reached the peak level after 30 min and gradually decreased thereafter. VX administered intraduodenally at 300 mg/kg did not inhibit the increase in the acid secretion. Cimetidine at 100 mg/kg significantly inhibited the increase in the acid secretion, and the inhibitory action continued for 150 min (Fig. 5).

An intravenous injection of 2-deoxy-D-glucose at 100 mg/kg gradually increased the acid secretion, which reached the peak level after 150 min. VX administered intraduodenally at 300 mg/kg significantly inhibited the increase in the acid secretion at 30, 60, 150 and 180 min after the administration of 2-deoxy-D-glucose (Fig. 6).

Discussion

The methanol extract of Sophora has been shown to inhibit markedly ulcerations caused by HCl–ethanol.[1] The active constituents in the methanol extract were further analyzed through fractionation and VX, a flavanol, was obtained as one of the active constituents. In the present study, in order to examine further the antiulcer action of VX, the effect of VX was examined in various experimental ulcer models.

An oral administration of VX had an inhibitory action on the formation of HCl–ethanol-induced ulcers but the intraperitoneal injection of VX was ineffective. In addition, in the presence of indomethacin and *N*-ethylmaleimide, VX still inhibited the formation of ulcers induced by HCl–ethanol. Further, in the animals whose gastric contents were eliminated prior to the HCl–ethanol treatment, VX still showed an inhibitory action. An intraperitoneal injection of VX, however, did not have any significant effect. These results suggest that VX directly affects the gastric mucosa and the inhibitory action is not related to endogenous PGs or internal SH compounds,[16] and also that the inhibitory action of VX is not due to physical factors such as dilution of HCl–ethanol by the gastric contents. The inhibitory action of spizofurone, a reference drug used in the present study, was not affected by pretreatment with indomethacin as in the case of VX, but either oral administration or intraperitoneal injection of spizofurone was inhibitory, which is different from the case with VX.

VX inhibited the ulcer induction by several necrosis-inducing substances such as absolute ethanol and 0.6 N HCl in addition to HCl–ethanol. VX also inhibited the ulcer formation induced by 1% NH_3, reported to be a necrosis inducing substance whose action is not inhibited by PGs,[10] suggesting the presence of gastric mucosal protecting action.

VX was also shown to inhibit the ulceration induced by stress caused by water restraint, Shay's ulcers and phenylbutazone-induced ulcers. Attack by the stored gastric solution an important factor for the development of Shay's ulcers and intraduodenally administered VX inhibited the acid secretion, suggesting that inhibition of acid secretion is involved in the antiulcer action of VX in the Shay's ulcer.

The inhibitory effect of VX on stress-induced ulcers due to water restraint and phenylbutazone induced ulcers may also be due to the inhibition of acid secretion. Since gastric motility[17] and gastric blood flow[18] are also considered to be important factors affecting the development of stress ulcers due to water restraint, VX may have effects on these factors. VX, however, did not show any significant action against indomethacin-induced ulceration, histamine-induced ulcers or serotonin-induced ulcers.

The results in the present study indicated that intraduodenal administration of VX inhibited the basal acid secretion. In addition, the gastric secretion stimulated by histamine, carbachol and pentagastrin, stimulants of acid secretion at the peripheral level, was not significantly inhibited by VX, though there appered to be a tendency of inhibition. VX, however, strongly inhibited the acid se-

cretion stimulated by 2-deoxy-D-glucose, a stimulant of acid secretion acting at the central nervous system. Acid secretion due to the pylorus ligation, which is thought to be a basal secretion, can be inhibited completely by elimination of parasympathetic nerves, and is therefore considered to be governed by parasympathetic nerves.[19] Further, the acid secretion induced by 2-deoxy-D-glucose can be inhibited completely by elimination of parasympathetic nerve influence, suggesting the activation of histamine, acetylcholine and gastrin secretion related to parasympathetic nerves.[20] Based on these reports and the results on the basal and stimulated acid secretion, it is possible that VX may affect the parasympathetic nervous system.

The results in the present study, therefore, indicated that VX, a flavanol contained in sophora, has a protective action on gastric mucosal membranes and also at a high dosage inhibited the gastric acid secretion.

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