

## Full Paper

**Goshuyuto, a Traditional Japanese Medicine for Migraine, Inhibits Platelet Aggregation in Guinea-Pig Whole Blood**

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**Abstract.** The effects of *goshuyuto* and *chotosan*, traditional Japanese medicines, on collagen-induced platelet aggregation were examined using guinea-pig blood. *Goshuyuto* at the concentration of 1,000  $\mu\text{g/mL}$  inhibited collagen-induced platelet hyper-aggregation to the same degree as aspirin at the concentration of 100  $\mu\text{mol/L}$ , but *chotosan* did not. *Goshuyuto* is composed of four medicinal herbs. Of them, aqueous extracts of *Evodiae Fructus* and *Zingiberis Rhizoma* inhibited platelet aggregation, but aqueous extracts of *Zizyphi Fructus* and *Ginseng Radix* did not. Two components of *Zingiberis Rhizoma*, 6-shogaol and 6-gingerol, also inhibited platelet aggregation. These results suggest that *Evodiae Fructus* and *Zingiberis Rhizoma* may play important roles in the anti-aggregation effects of *goshuyuto* and that 6-shogaol and 6-gingerol are among the active ingredients. Therefore, *goshuyuto* may ameliorate migraine by preventing the hyper-aggregation of platelets in migraine with aura.

**Keywords:** *goshuyuto*, *Evodiae Fructus*, *Zingiberis Rhizoma*, 6-shogaol, 6-gingerol

**Introduction**

*Goshuyuto* (*Wu-Zhu-Yu-Tang* in Chinese) and *chotosan* (*Diao-Tang-San* in Chinese), traditional Japanese herbal medicines, are prescribed for treatment of headaches in Japan. In general, *goshuyuto* is prescribed for migraine-type headaches, and *chotosan* is prescribed for tension-type headaches. *Goshuyuto* is known to be clinically effective for the treatment of migraine headache, nausea, beriberi, and heart failure in Japan. Odaguchi et al. reported that *goshuyuto* was effective in reducing the frequency of headache episodes among chronic headache patients in a responder-limited, randomized controlled study (1). On the other hand, *chotosan* has been used for the treatment of chronic headache and hypertension (2). A recent clinical study reported that *chotosan* had an ameliorative effect on cognitive dysfunctions in stroke patients (3). However, there have been no reports about the mechanisms of the

preventive effects on headaches of *goshuyuto* and *chotosan*.

The pathogenesis of migraine is thought to be platelet hyper-aggregation caused by one of several stimulating factors [e.g., collagen, thrombin, adenosine diphosphate (ADP), serotonin (5-HT), thromboxane A<sub>2</sub> (TXA<sub>2</sub>)]. 5-HT is excessively released from platelets, and the blood level of 5-HT is elevated. The elevation of cerebral blood levels of 5-HT is thought to cause the aura of the first stage of migraine by contracting the cerebral blood vessels. Furthermore, it is thought that because the excessive 5-HT released is immediately metabolized, the second stage of migraine is due to relaxation of the cerebral blood vessels. While the underlying pathogenesis of 5-HT release has not been elucidated, it is generally thought to be related to the occurrence of migraine. In fact, it was reported that urinary excretion of 5-hydroxyindoleacetic acid, the main metabolite of 5-HT, is elevated in migraine patients (4, 5). It is also reported that the injection of reserpine, which is the releaser of 5-HT, induces a typical headache in migrainous subjects and that intravenous injection of 5-HT relieves migraine headache (6). Recently, a 5-HT<sub>1BD</sub>-like

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agonist was reported to be effective for treatment of migraine patients (7). These results indicate that 5-HT may play an important role in the pathogenesis of migraine.

Moreover, it is known that 86% of patients with migraine have platelet hyper-aggregability, and almost 96% of migraine headaches can be improved by treatment with antithrombotic drugs (8). Therefore, it is thought that platelet aggregation plays an important role in the pathogenesis of migraine.

Although *goshuyuto* is used for treatment of migraine, its effect on platelet aggregation has not been clarified. In this study, we investigated the effects of *goshuyuto* and *chotosan*, both of which are used for headache therapy, and several of the constituents of *goshuyuto* on collagen-induced whole-blood platelet aggregation in guinea pigs.

In general, a  $\text{Ca}^{2+}$ -channel blocker (e.g., lomerizine hydrochloride) is used as therapy for the aura of migraine (the first stage of migraine) (9), and 5-HT<sub>1BD</sub>-like agonists (e.g., sumatriptan) are used after the attack has begun (the second stage of migraine) (7). Lomerizine is reported to have an anti-aggregatory effect on platelets and an inhibitory effect on the release of 5-HT (10). Therefore, we also determined the effects of lomerizine and sumatriptan on collagen-induced whole blood aggregation in guinea pigs.

## Materials and Methods

### Reagents and drugs

The powdered extract of *goshuyuto* was manufactured at our Shizuoka factory (Tsumura & Co., Tokyo). *Goshuyuto* is a mixture of four medicinal herbs: 4 parts Fructus of *Zizyphus jujube* (*Zizyphi Fructus*), 3 parts Fructus of *Evodia rutaecarpa* (*Evodiae Fructus*), 2 parts Radix of *Panax ginseng* (*Ginseng Radix*), and 1.5 parts Rhizome of *Zingiber officinale* (*Zingiberis Rhizoma*). The qualities of these raw materials were tested according to the Japanese Pharmacopoeia and our company's standards. A mixture of the four medicinal herbs was extracted with purified water at 100°C for 1 h. Then, the extracted solution was concentrated by removing water via reduced pressure and spray dried. The yield of the extract was ca. 20%. The extract was analyzed by high-performance liquid chromatography (HPLC). The powdered extract of *goshuyuto* (No. 2030031010) is stored in our laboratory at constant temperature and humidity.

Similarly, the powdered extract of *chotosan* (No. 2010047010) is manufactured from a mixture of eleven medicinal herbs: 5 parts Gypsum fibrosum, 3 parts hook and branch of *Uncaria rhynchophylla*

(*Uncariae Uncis cum Ramulus*), 3 parts peel of *Citrus unshiu* (*Aurantii Nobilis Pericarpium*), 3 parts tuber of *Pinellia ternate* (*Pinelliae Tuber*), 3 parts root of *Ophiopogon japonicus* (*Ophiopogonis Tuber*), 3 parts sclerotinum of *Poria cocos* (*Hoelen*), 2 parts root of *Panax ginseng* (*Ginseng Radix*), 2 parts root and rhizome of *Saposhnikovia divaricata* (*Saposhnikovia Radix*), 2 parts flower of *Chrysanthemum morifolium* (*Chrysanthemi Flos*), 1 parts root of *Glycyrrhiza uralensis* (*Glycyrrhizae Radix*), and 1 parts rhizome of *Zingiber officinale* (*Zingiberis Rhizoma*). The powdered extract of *chotosan* (No. 2010047010) is stored in our laboratory at constant temperature and humidity.

Synephrine, limonin, and 5-HT were purchased from Sigma-Aldrich (St. Louis, MO, USA). Evodiamine, 6-shogaol, 6-gingerol, and aspirin (acetylsalicylic acid) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka). Rutaecarpine and lomerizine hydrochloride were purchased from LKT Laboratories Inc. (St. Paul, MN, USA). Sumatriptan succinate was purchased from GlaxoSmithKline (Tokyo). Collagen solution was purchased from MC Medical Inc. (Tokyo). The other reagents used for analysis were purchased from commercial sources.

### Animals

Seven- and twelve-week-old male Hartley guinea pigs weighing 400–700 g were obtained from SLC Co., Ltd. (Hamamatsu). The animals were allowed free access to water and a standard laboratory food, CG7 (Clea, Tokyo). They were kept in a facility at a temperature of  $24 \pm 1^\circ\text{C}$ , relative humidity of  $55 \pm 5\%$ , and with lights on from 07:00 to 19:00 daily. All experimental procedures were performed according to the "Guidelines for the Care and Use of Laboratory Animals" approved by the Laboratory Animal Committee of Tsumura & Co.

### Preparation of *goshuyuto*, its components, and *chotosan*

*Goshuyuto*, *chotosan*, and the four constituent medicinal herbs of *goshuyuto* were dissolved separately in methanol and sonicated at 25°C for 30 min. The solution was centrifuged at  $900 \times g$  for 3 min, and the supernatant was filtered through a 0.45- $\mu\text{m}$  filter.

### Preparation of other compounds

Synephrine, evodiamine, rutaecarpine, limonin, 6-shogaol, 6-gingerol, and aspirin were dissolved in methanol at the final concentration of 100  $\mu\text{mol/L}$ .

### Preparation of medicines

Lomerizine hydrochloride is soluble in 100% methanol but insoluble in distilled water. Conversely, sumatriptan is soluble in water but insoluble in metha-

nol. Therefore, lomerizine hydrochloride was dissolved in 100% methanol and then diluted with water. Sumatriptan succinate was dissolved in water, centrifuged at  $900 \times g$  for 3 min, and the supernatant was filtered through a  $0.45\text{-}\mu\text{m}$  filter. The supernatant was diluted with methanol. *Goshuyuto* was prepared by the same method as lomerizine hydrochloride. All medicines were prepared as 50% methanol solutions.

#### Preparation of blood samples

Whole blood was collected from the inferior vena cava of guinea pigs under ether anesthesia. Immediately, 3.18% trisodium citrate was added to collected blood in the ratio of 1:9 (v/v).

#### Measurement of platelet aggregation by screen filtration pressure

Measurement of platelet aggregation was carried out using a screen filtration pressure method (11) with a whole blood aggregometer, WBA (MC Medical Co., Ltd.). Blood collected from guinea pigs was stored at room temperature for 1 h before assay. Four reaction tubes each containing  $195\text{ }\mu\text{L}$  of whole blood were placed in the incubation chamber at  $37^\circ\text{C}$  with a stirring bar in each tube, and  $5\text{ }\mu\text{L}$  of each component, or vehicle as a control, was added to each tube. After 5-min incubation,  $22.2\text{ }\mu\text{L}$  of collagen at 1, 2, 4, or  $8\text{ }\mu\text{g/mL}$  final concentration was added to each tube. After incubation for 5 min, filter-unit syringes with screen microsieves made of nickel and containing three hundred  $30\text{-}\mu\text{m}$  square openings per 1 mm diameter area (MC Medical, code No. SSR4421) were connected to a pressure sensor, and the blood samples were automatically aspirated into the syringes. A negative pressure of  $-130\text{ mmHg}$  was established as 100%, and  $-6\text{ mmHg}$ , rather than  $0\text{ mmHg}$ , was used as the 0% pressure rate to compensate for the viscosity of whole blood. The platelet aggregation pressure of each reaction tube was determined as the pressure rate (%). The collagen concentration ( $\mu\text{g/mL}$ ) at 50% pressure was expressed as the platelet aggregatory threshold index (PATI). Because the upper limit of determination of PATI was  $8\text{ }\mu\text{g/mL}$ , PATI values above  $8\text{ }\mu\text{g/mL}$  were expressed as  $8\text{ }\mu\text{g/mL}$ .

#### Statistics

Each value was expressed as the mean  $\pm$  S.E.M. Results were statistically evaluated using two-factor factorial ANOVA to compare the pressure rates, and a one-way analysis of variance coupled with Dunnett's test was performed to compare PATI values. Differences were accepted as significant at  $P < 0.05$ .

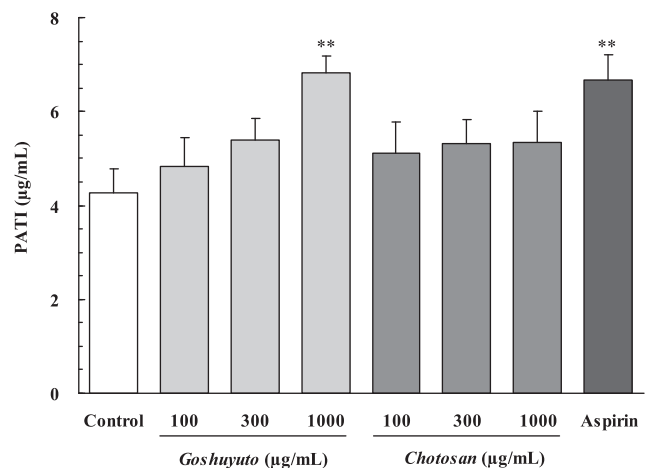
## Results

We made reference to the method of Sudo et al. as a screen filtration pressure method (11). We investigated both collagen and ADP as stimulators of platelets. Finally, we used collagen as stimulator because this agonist induced more stable platelet aggregation than ADP. In addition, the platelet-collagen interaction triggers the secretion of ADP and 5-HT from platelets and induces arachidonic acid metabolism through cyclooxygenase-1 (COX-1) in platelets.

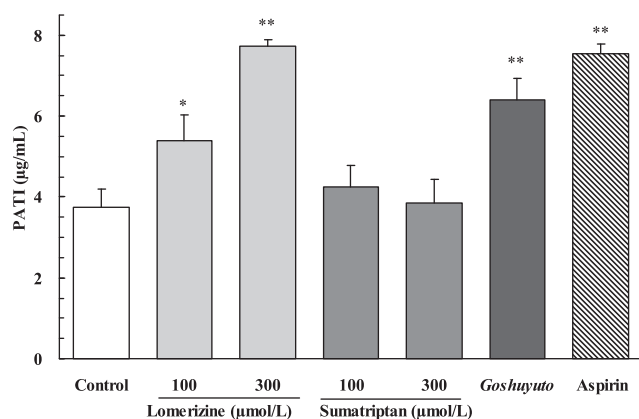
Also, we measured platelet aggregation in response to collagen at 1, 2, 4, or  $8\text{ }\mu\text{g/mL}$  final concentrations. First, we investigated 5-HT release from the platelets in response to collagen using the HPLC method under the same condition as used for the determination of platelet aggregation. We confirmed that 5-HT was dose-dependently released in response to the collagen (1, 2, 4, or  $8\text{ }\mu\text{g/mL}$ ) (data not shown).

We examined the effects of *goshuyuto* and *chotosan* on induction of platelet aggregation by collagen. Figure 1 shows that *goshuyuto* at the concentration of  $1,000\text{ }\mu\text{g/mL}$  significantly elevated the PATI values, but *chotosan* did not affect them. Aspirin ( $100\text{ }\mu\text{mol/L}$ ), which is known to inhibit platelet aggregation, significantly elevated the PATI values compared to that of the control.

On the other hand, lomerizine significantly elevated the PATI value at doses of 100 and  $300\text{ }\mu\text{mol/L}$ , although sumatriptan (100 and  $300\text{ }\mu\text{mol/L}$ ) did not affect it (Fig. 2).



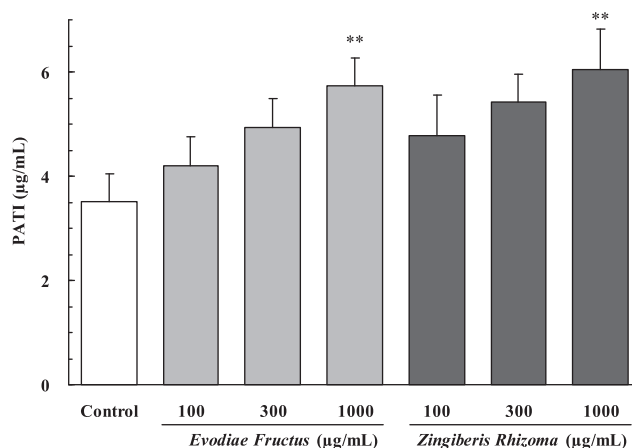
**Fig. 1.** Effects of *goshuyuto* and *chotosan* on collagen-induced platelet aggregation of guinea-pig whole blood. *Goshuyuto* and *chotosan* were examined at 100, 300, and  $1,000\text{ }\mu\text{g/mL}$ . Aspirin was used at  $100\text{ }\mu\text{mol/L}$ . The effects of each dose of *goshuyuto* or *chotosan* on the PATI were compared with that of the vehicle (methanol) by a one-way analysis of variance coupled with Dunnett's test (\*\* $P < 0.01$ ). Data are each expressed as the mean  $\pm$  S.E.M. of 11 determinations.



**Fig. 2.** Effects of lomerizine, sumatriptan, and *goshuyuto* on collagen-induced platelet aggregation of guinea-pig whole blood. Lomerizine and sumatriptan were examined at 100 and 300  $\mu\text{mol/L}$ . *Goshuyuto* was used at 1,000  $\mu\text{g/mL}$ . Aspirin was used at 100  $\mu\text{mol/L}$ . All medicines were prepared as 50% methanol solutions. The effects of each medicine on the PATI were compared with that of vehicle (50% methanol) by a one-way analysis of variance coupled with Dunnett's test (\* $P<0.05$ , \*\* $P<0.01$ ). Data are each expressed as the mean  $\pm$  S.E.M. of 11 determinations.

We also investigated the effects of the four components of *goshuyuto* on collagen-induced platelet aggregation. *Evodiae Fructus* and *Zingiberis Rhizoma* significantly inhibited the pressure rates and elevated the PATI value to the same level that *goshuyuto* did, while *Zizyphi Fructus* and *Ginseng Radix* did not (Table 1). The dose-dependent (100 to 1,000  $\mu\text{g/mL}$ ) effects of *Evodiae Fructus* and *Zingiberis Rhizoma* on platelet aggregation were also examined separately. Both *Evodiae Fructus* and *Zingiberis Rhizoma* showed dose-dependent anti-aggregatory effects (Fig. 3).

In addition, the effects of components of *Zingiberis Rhizoma* and *Evodiae Fructus* on platelet aggregation



**Fig. 3.** Effects of *Evodiae Fructus* and *Zingiberis Rhizoma* on collagen-induced platelet aggregation of guinea-pig whole blood. The effects were determined at 100, 300, and 1,000  $\mu\text{g/mL}$ . The effects of the two components of *goshuyuto* were compared with that of vehicle (methanol) by a one-way analysis of variance coupled with Dunnett's test (\*\* $P<0.01$ ). Data are each expressed as the mean  $\pm$  S.E.M. of 10 determinations.

were examined separately (Table 2). Both 6-gingerol and 6-shogaol, ingredients of *Zingiberis Rhizoma*, significantly inhibited the pressure rates and elevated the PATI values. Evodiamine, rutaecarpine, synephrine, and limonin, components of *Evodiae Fructus*, did not elevate the PATI values.

## Discussion

We investigated the anti-aggregatory effects of *goshuyuto* and *chotosan*, both of which are used in Japan for headache therapy, on platelet aggregation. *Goshuyuto* had an anti-aggregatory effect on collagen-

**Table 1.** Effects of *goshuyuto* and components on collagen-induced platelet aggregation of guinea-pig whole blood

	Pressure rate (%)				PATI ( $\mu\text{g/mL}$ )
	Concentration of collagen ( $\mu\text{g/mL}$ )				
	1	2	4	8	
Control (vehicle)	13.35 $\pm$ 3.47	19.18 $\pm$ 2.90	43.78 $\pm$ 8.43	83.75 $\pm$ 3.39	4.78 $\pm$ 0.46
<i>Zizyphi Fructus</i>	7.36 $\pm$ 2.24	11.85 $\pm$ 2.83	40.70 $\pm$ 8.72	83.14 $\pm$ 2.60	4.84 $\pm$ 0.44
<i>Evodiae Fructus</i>	9.77 $\pm$ 2.92	12.84 $\pm$ 3.18	18.83 $\pm$ 3.22	57.18 $\pm$ 6.44**	7.14 $\pm$ 0.32**
<i>Ginseng Radix</i>	10.51 $\pm$ 2.51	15.04 $\pm$ 3.68	32.76 $\pm$ 8.63	74.01 $\pm$ 6.73	5.69 $\pm$ 0.54
<i>Zingiberis Rhizoma</i>	14.71 $\pm$ 3.32	17.30 $\pm$ 5.03	23.23 $\pm$ 3.45	59.17 $\pm$ 8.24**	6.35 $\pm$ 0.58*
<i>Goshuyuto</i>	10.75 $\pm$ 3.90	8.16 $\pm$ 2.39	22.01 $\pm$ 6.34	66.58 $\pm$ 6.54**	6.29 $\pm$ 0.50*

Anti-aggregatory effects of *Zizyphi Fructus*, *Evodiae Fructus*, *Ginseng Radix*, *Zingiberis Rhizoma*, and *goshuyuto* were determined at 1,000  $\mu\text{g/mL}$ . Effects of *goshuyuto* and components of *goshuyuto* on the pressure rate (%) and PATI ( $\mu\text{g/mL}$ ) are indicated. Statistical analysis was performed by two-factor factorial ANOVA to compare the pressure rates, and a one-way analysis of variance coupled with Dunnett's test was used to compare PATI values. The effects were compared with that of the control (vehicle) (\*\* $P<0.01$ , \* $P<0.05$ ). Data are each expressed as the mean  $\pm$  S.E.M. of 11 determinations.

**Table 2.** Effects of *goshuyuto* ingredients on collagen-induced platelet aggregation of guinea-pig whole blood

	Pressure rate (%)				PATI ( $\mu\text{g/mL}$ )
	Concentration of collagen ( $\mu\text{g/mL}$ )				
	1	2	4	8	
Control (vehicle)	12.98 $\pm$ 4.49	15.68 $\pm$ 6.05	36.44 $\pm$ 8.83	89.29 $\pm$ 2.25	4.89 $\pm$ 0.47
Evodiamine	7.15 $\pm$ 1.96	12.89 $\pm$ 4.65	30.34 $\pm$ 8.63	70.16 $\pm$ 8.75	5.91 $\pm$ 0.59
Rutaecarpine	16.31 $\pm$ 4.82	17.31 $\pm$ 5.51	29.89 $\pm$ 9.47	73.38 $\pm$ 8.86	5.77 $\pm$ 0.61
Synephrine	14.56 $\pm$ 5.83	12.04 $\pm$ 3.79	38.98 $\pm$ 9.52	79.36 $\pm$ 7.57	4.82 $\pm$ 0.75
Limonin	13.65 $\pm$ 3.19	20.35 $\pm$ 5.31	38.96 $\pm$ 8.48	74.34 $\pm$ 6.38	5.42 $\pm$ 0.62
6-Gingerol	8.83 $\pm$ 3.21	5.68 $\pm$ 1.26	11.98 $\pm$ 2.88	49.44 $\pm$ 8.06**	7.37 $\pm$ 0.35**
6-Shogaol	10.54 $\pm$ 4.62	5.16 $\pm$ 0.60	14.35 $\pm$ 6.51	53.00 $\pm$ 7.47**	7.11 $\pm$ 0.51**

The effects of components of *Evodiae Fructus*, evodiamine, rutaecarpine, synephrine, and limonin, were determined at the concentration of 100  $\mu\text{mol/L}$ . The effects of components of *Zingiberis Rhizoma*, 6-shogaol and 6-gingerol, were determined at 100  $\mu\text{mol/L}$ . Effects of the components of *Evodiae Fructus* and *Zingiberis Rhizoma* on the pressure rate (%) and PATI ( $\mu\text{g/mL}$ ) are indicated. Statistical analysis was performed by two-factor factorial ANOVA to compare the pressure rates, and a one-way analysis of variance coupled with Dunnett's test was used to compare PATI values. The effects were compared with that of the control (vehicle) (\*\* $P < 0.01$ ). Data are each expressed as the mean  $\pm$  S.E.M. of 8 determinations.

induced platelet aggregation, but *chotosan* did not. Generally, *goshuyuto* is prescribed for migraine (1), and *chotosan* is prescribed for tension-type headaches (2). Therefore, it is thought that the anti-aggregatory effect of *goshuyuto* is effective in patients in whom the aura of migraine occurs.

It is reported that lomerizine hydrochloride, which is a  $\text{Ca}^{2+}$ -channel blocker, has an anti-aggregatory effect on platelets in ADP-induced platelet-rich plasma (10), but sumatriptan, which is a 5-HT<sub>1BD</sub> agonist, does not (12). We confirmed that lomerizine had an anti-aggregatory effect on the collagen-induced platelet aggregation of guinea pigs in this study, while sumatriptan did not. Fujishima et al. reported that lomerizine inhibited specific [<sup>3</sup>H]spiperone binding to 5-HT<sub>2</sub> receptors in a competitive manner, but exhibited negligible affinity for radioligand binding to other 5-HT receptor subtypes such as 5-HT<sub>1</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1C</sub>, and 5-HT<sub>3</sub> in rat cortical membrane (13). Also, as we found that ketanserin (5-HT<sub>2</sub> antagonist) significantly elevated the PATI value at the dose of 100  $\mu\text{mol/L}$  in our experiments (data not shown), it is thought the anti-aggregatory effect may involve the 5-HT<sub>2</sub> receptor. Therefore, the anti-aggregatory effect of lomerizine might be thought to be related to not only its effect as a  $\text{Ca}^{2+}$ -channel blocker but also its antagonistic effect on the 5-HT<sub>2</sub> receptor.

It is reported that lomerizine is effective if taken during the aura of migraine, and it is useful for prevention of migraine (9). Moreover, it is reported that 86% of patients with migraine have platelet hyper-aggregation, and migraine can be improved in almost 96% of them by treatment with antithrombotic drugs (8). It is known that platelet aggregation is enhanced and that the cerebral blood level of 5-HT is increased in the early stage of

migraine. Therefore, it is thought that migraine can be prevented by inhibiting platelet aggregation. In this study, we demonstrated that *goshuyuto* showed the same anti-aggregatory effect that lomerizine did. Therefore, we hypothesized that *goshuyuto* may be a candidate for a preventive medicine if taken at the time of the aura of migraine.

All the active constituents of the components of *goshuyuto* were not identified. *Goshuyuto* consists of four medicinal herbs, *Evodiae Fructus*, *Zingiberis Rhizoma*, *Zizyphi Fructus*, and *Ginseng Radix*. We demonstrated that aqueous extracts of both *Evodiae Fructus* and *Zingiberis Rhizoma* significantly inhibited collagen-induced platelet aggregation, but extracts of *Zizyphi Fructus* and *Ginseng Radix* did not. We also investigated the active ingredients of *Zingiberis Rhizoma*. 6-Shogaol and 6-gingerol, which are components of *Zingiberis Rhizoma*, demonstrated inhibitory effects on platelet aggregation. It has been already reported that 6-shogaol and 6-gingerol had anti-aggregatory effects in arachidonic acid-induced human whole blood aggregation (14). It has been reported that 6-gingerol inhibits arachidonic acid-induced human platelet serotonin release and aggregation and that it also inhibits COX-1 enzyme activity (15). Moreover, it has been reported that 6-shogaol inhibits serotonin release during arachidonic acid-induced rabbit platelet aggregation and that 6-shogaol had an inhibitory effect on COX-1 enzyme activity (16). Therefore, 6-gingerol and 6-shogaol are thought to inhibit platelet aggregation by inhibiting COX-1 activity.

Also, it is well known that collagen caused platelet aggregation mediated through TXA<sub>2</sub>. TXA<sub>2</sub> has the ability to contract vascular smooth muscles, and it is

reported that the plasma levels of TXA<sub>2</sub> in migrainous patients was significantly higher than those in healthy controls (17). Therefore, TXA<sub>2</sub> might be involved in the case of migraine. So, we determined the TXA<sub>2</sub> level after 5-min incubation when the collagen at the concentration of 8 µg/mL was added to whole blood. We found that aspirin inhibited the TXA<sub>2</sub> release significantly, and 6-shogaol tended to inhibit it, although *goshuyuto* was not inhibitory (data not shown). In the future, we will investigate the antiaggregatory mechanism of *goshuyuto* in detail.

It has been reported that rutaecarpine, one of the components of *Evodiae Fructus*, had an inhibitory effect on occlusion time during induced thrombus formation in the mouse (18). In this study, rutaecarpine did not significantly inhibit platelet aggregation at the doses of 100 and 300 µmol/L (data not shown). It is thought that the difference between our results and theirs derives from differences in methods and species. We did not find active anti-aggregatory ingredients in *Evodiae Fructus*, but we will investigate it for other anti-aggregatory ingredients in future studies. On the other hand, *Evodiae Fructus* and *Zingiberis Rhizoma* may bring about synergistic inhibition of platelet aggregation. Taken together, it is speculated that the anti-aggregatory effect of *goshuyuto* might be due the inhibition of COX-1 activity by these active ingredients, although further studies are necessary to confirm this hypothesis. Also, in the future, we may investigate the possibility that *goshuyuto* has antagonistic effects on the 5-HT<sub>2</sub> receptor.

In conclusion, we demonstrated that *goshuyuto* had an inhibitory effect on platelet aggregation. *Evodiae Fructus* and *Zingiberis Rhizoma*, constituent medicinal herbs of *goshuyuto*, showed anti-aggregatory effects, and 6-shogaol and 6-gingerol in *Zingiberis Rhizoma* were determined to be active ingredients in the anti-aggregatory effects of *goshuyuto*. These results suggest that *goshuyuto* ameliorates the aura of migraine by inhibiting hyper-aggregation of platelets.

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