Effects on Blood Pressure Decrease in Response to PAF of *Impatiens textori* MIQ.

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A 35% EtOH extract of flowers of *Impatiens textori* MIQ. showed an inhibitory effect on blood pressure decrease in response to platelet activating factor (PAF) measured with a blood pressure monitoring system. Bioassay-guided fractionation of the 35% EtOH extract (IT) led to isolation of the flavones apigenin (1) and luteolin (3), which significantly inhibited blood pressure decrease in response to PAF. Their compounds and apigenin 7-glucoside (2), chrysoeriol (4), quercetin (5), quercetin 3-glucoside (6), kaempferol (7), kaempferol 3-glucoside (8) and kaempferol 3-rhamnosyldiglucoside (9) were also isolated from the flowers of *I. textori* for the first time. This study revealed that the flowers of *I. textori* might be a possible anti-allergy agent.

Key words   *Impatiens textori*; anti-platelet activating factor effect; apigenin; luteolin; blood pressure monitoring; anti-allergy agent

Whole plants of *Impatiens textori* MIQ. of the same family as *I. balsamina* L. have been used for detoxication and treatment of carbuncle and contusion in Chinese medicine.[1] Several flavonoid derivatives have also been isolated from the whole plants,[2—4] however, there has been no report on the anti-allergic effects of the flowers of *I. textori*.

Previously, we developed *in vivo* anti-allergic screening methods to examine blood pressure (BP) decrease in response to exogenous platelet activating factor (PAF) using BP monitoring.[5] Using these methods, we found that the 35% EtOH extract of the white petals of *I. balsamina* L. and isolated compounds significantly inhibited BP decrease in response to PAF.[5] The anti-anaphylactic mechanism for *I. balsamina* has also been identified.[5—7]

Here we report the effect of the 35% EtOH extract (IT) of the flowers of *I. textori* to counter BP decrease in response to PAF and the active principal compounds from IT.
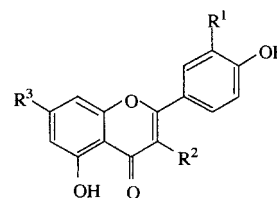
MATERIALS AND METHODS

General Experimental Procedures   Melting points were determined with a Yanagimoto micro melting point apparatus (MP A-3, Yanagimoto Seisakusyo, Japan). IR spectra were recorded on a Shimadzu 435 spectrometer and UV absorption spectra with a Hitachi 323 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-GSX 500 spectrometer (with tetramethylsilane (TMS) as internal reference). MS were measured in the positive-ion mode on a JEOL JNM-DX 303 double-focusing spectrometer using xenon atoms with kinetic energy equivalent to 6 kV at the ion-accelerating voltage of 3 kV. The mass marker was calibrated with perfluoroalkylphosphazine (ultra marker), and 3-nitrobenzyl alcohol (NBA) was used as the matrix.

Plant Material   The flowers of *I. textori* were collected in Gifu (Japan) in September, 1998. The voucher samples are kept in our university medicinal plant garden.

Extraction and Isolation Procedure   Fresh flowers (2.0 kg) of *I. textori* were extracted twice with 35% EtOH for 1 week at room temperature and filtered. The alcohol solution was evaporated *in vacuo* to remove EtOH, giving a residue (designated IT) of 15.5 g. IT was suspended in distilled water

and successively extracted with EtOAc and *n*-BuOH. The EtOAc extract (ext.) and *n*-BuOH ext. were evaporated *in vacuo* to dryness. The EtOAc ext. (340 mg) was fractionated by column chromatography on silica gel eluting with a CHCl$_3$–MeOH gradient system to give seven fractions (fr.) I—VII. Fraction II (134 mg), eluted with CHCl$_3$–MeOH (20 : 1), was fractionated by column chromatography on silica gel eluting with a CHCl$_3$–MeOH gradient system, followed by Sephadex LH-20 with MeOH, and successively recrystallized from MeOH–H$_2$O to give compound 1 (32.6 mg), 3 (14 mg), 4 (0.52 mg), 5 (13 mg) and 7 (1.4 mg). Fraction IV (46 mg), eluted with CHCl$_3$–MeOH (16 : 1), was repeatedly fractionated by column chromatography on silica gel eluting with a CHCl$_3$–MeOH gradient system followed by Sephadex LH-20 with MeOH to give compound 2 (21.6 mg), 6 (1.4 mg) and 8 (0.42 mg). The *n*-BuOH ext. (3.2 g) was fractionated by flash chromatography on silica gel eluting with a CHCl$_3$–MeOH gradient system to give six fr., I—VI. Fraction IV (1.47 g) was repeatedly fractionated by flash chromatography on silica gel eluting with CHCl$_3$–MeOH–H$_2$O (7 : 3 : 0.5), followed by Sephadex LH-20 with MeOH and successively recrystallized from MeOH–H$_2$O to give compound 9 (7.6 mg).

Samples and Material Preparation   IT, EtOAc ext., *n*-BuOH ext., H$_2$O ext. and compounds 1, 2 and 3 were sus-

	R^1	R^2	R^3		R^1	R^2	R^3
1	H	H	OH	6	OH	O—glc	OH
2	H	H	O—glc	7	H	OH	OH
3	OH	H	OH	8	H	O—glc	OH
4	O—CH$_3$	H	OH	9	H	O—glc^2—rha	OH
5	OH	OH	OH			^{3}glc	

Flavonoid compounds from flowers of *Impatiens textori* Miq.

pended in $100 \mu l$ water per 10 g body weight before use in biological experiments. Doses were expressed as dried extracts and compounds (mg/kg body weight) in 100 mg/kg and 20 mg/kg.

Platelet activating factor (PAF) and *Rac*-3-(*N*-*n*-octadecylcarbamoyloxy)-2-methoxypropyl 2-thiazoliethyl phosphate (CV-3988), a PAF antagonistic agent, were purchased from Funakoshi Co., Ltd. and Cascade Biochem, Ltd. PAF and CV-3988 were dissolved in $10 \mu l$ saline per 10 g body weight before use in biological experiments. Doses were expressed as PAF (μg/kg body weight) in 1μg/kg and CV-3988 (mg/kg body weight) in 10 mg/kg.

Animals Male ddY mice (SPF grade), 6 weeks old, were obtained from Japan SLC (Shizuoka, Japan) and housed at $24 \pm 2 °C$. Food and water were available *ad libitum*.

Effects of Extracts and Isolated Compounds on BP Decrease in Response to Exogenous PAF The effects of extracts and compounds **1**, **2** and **3** on exogenous PAF-induced BP decrease were investigated as previously reported[5] using BP monitoring. The BP of unanesthetized mice was measured by the tail cuff method by a physiograph (MK-1030, Muromachi Kikai, Japan). The normal BP (90—110 mmHg) of each mouse hardly changed for one week (data not shown). Therefore, the normal BP was measured 1 d before injection with PAF in each mouse. PAF, dissolved in saline, was administered intravenously (i.v.) at 1μg/kg to the tail vein of normal mice. The BP of the mice was monitored every 2 min immediately after injection with PAF. The period of BP recording was the 24 min in which the BP significantly decreased. Test extracts and compounds were dissolved in water. One hundred mg/kg each of IT, EtOAc ext., *n*-BuOH ext. and H_2O ext. and 20 mg/kg each of compounds **1**—**3** were individually administered orally 1 h before PAF injection. CV-3988,[8] a PAF-antagonist used as a positive control, was dissolved in saline and administered i.v. at 10 mg/kg to the tail vein of normal mice 1 h before PAF injection. The results were expressed as the percentage of normal BP at given times after this injection, because there were individual differences in the BP of normal mice. If the BP decreased below the range (30 mmHg) of the monitor level, we used the value equivalent to 30% of the normal BP (90—110 mmHg).

Statistical Analysis The results are presented as the mean±S.E.M. Statistical analysis was performed using Student's *t*-test.

RESULTS

Isolation and Identification of Compounds Compounds **1**—**9** were isolated from the fresh flowers of *I. textori* by successive chromatography and recrystallization. Apigenin (**1**),[2] apigenin 7-glucoside (**2**),[3] luteolin (**3**),[2] chrysoeriol (**4**),[2] quercetin (**5**),[4] quercetin 3-glucoside (**6**), kaempferol (**7**),[4] kaempferol 3-glucoside (**8**) and kaempferol 3-rhamnosyldiglucoside (**9**)[9] were identified by direct comparison of spectral data (MS, UV, IR, ^{1}H- and ^{13}C-NMR).[10,11] These compounds were isolated from the flowers of *I. textori* for the first time. Compounds **1**, **2** and **3** which had been obtained in sufficient amounts were tested for the bioassay.

Effects of Extracts, Compounds and CV-3988 on BP Decrease in Response to PAF The control mice were injected i.v. with PAF (1μg/kg) to normal mice without pre-
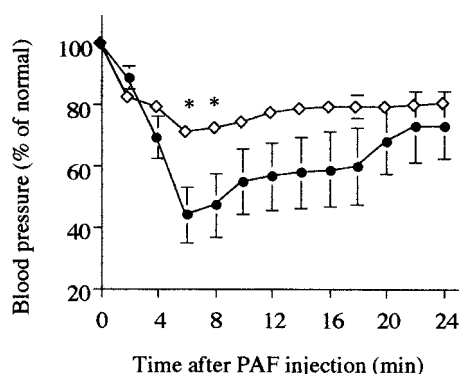

Fig. 1. Effect of the 35% EtOH Ext. (IT) from *I. textori* on BP Decrease in Response to PAF

Mice were injected i.v. with PAF (1μg/kg) without pretreatment (●: control) or following pretreatment *p.o.* with IT (◇) of 100 mg/kg 1 h before injection of PAF. The BP of each mouse was measured for 24 min after PAF injection. All values are mean±S.E.M. of 5 mice. ∗$p<0.05$ (compared with control by Student's *t*-test).
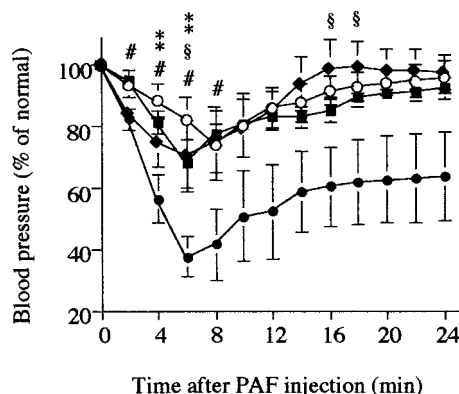

Fig. 2. Effects of EtOAc Ext., *n*-BuOH Ext. and H_2O Ext. on BP Decrease in Response to PAF

Mice were injected i.v. with PAF (1μg/kg) without pretreatment (●: control) or following pretreatment *p.o.* with EtOAc ext. (○), *n*-BuOH ext. (◆) and H_2O ext. (■) of 100 mg/kg 1 h before injection of PAF. The BP of each mouse was measured for 24 min after PAF injection. All values are mean±S.E.M. of 5 mice. Statistical significance of difference of EtOAc ext. compared with PAF challenge only (a) ∗∗$p<0.01$. Statistical significance of difference of *n*-BuOH ext. compared with (a) §$p<0.05$. Statistical significance of difference of H_2O ext. compared with (a) #$p<0.05$ (Student's *t*-test).

treatment.

Figure 1 shows the change in BP of control mice and the IT injected group monitored every 2 min for 24 min after i.v. injection with 1μg/kg of PAF. IT at 100 mg/kg significantly inhibited BP decrease.

Figure 2 shows the change in BP of control mice and those given an injection of EtOAc ext., *n*-BuOH ext. and H_2O ext. monitored every 2 min for 24 min after i.v. injection with 1μg/kg of PAF. All extracts at 100 mg/kg significantly inhibited BP decrease.

Figure 3 shows the change in BP of control mice and the compound **1**, **2** and **3** injected group monitored every 2 min for 24 min after i.v. injection with 1μg/kg of PAF. Of these compounds, compounds **1** (Fig. 3A) and **3** (Fig. 3B) at 20 mg/kg significantly inhibited BP decrease, but compound **2** did not (data not shown).

Figure 4 shows the inhibitory effect of CV-3988, the positive control, on the BP decrease induced by 1μg/kg of PAF. CV-3988 at 10 mg/kg significantly inhibited this decrease.
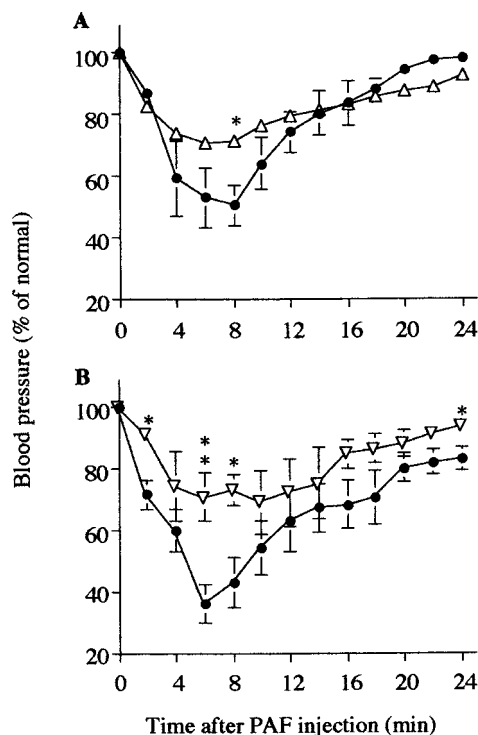
Fig. 3.   Effects of Apigenin (**1**) and Luteolin (**3**) on BP Decrease in Response to PAF

Mice were injected i.v. with PAF (1 μg/kg) without pretreatment (●: control) or following pretreatment *p.o.* with **1** (A; △) and **3** (B; ▽) of 20 mg/kg 1 h before injection of PAF. The BP of each mouse was measured for 24 min after PAF injection. All values are mean±S.E.M. of 5 mice. *$p<0.05$ and **$p<0.01$ (compared with control by Student's *t*-test).

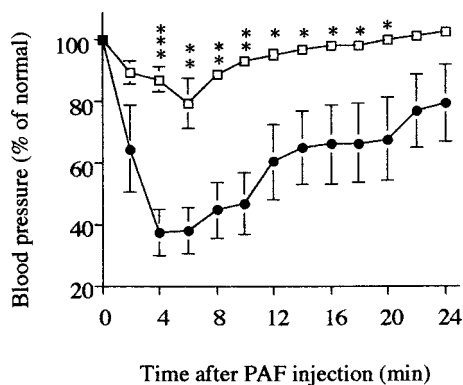Fig. 4.   Effect of CV-3988 on BP Decrease in Response to PAF

Mice were injected i.v. with PAF (1 μg/kg) without pretreatment (●: control) or following pretreatment i.v. with CV-3988 (□) of 10 mg/kg at 1 h before injection of PAF. The BP of each mouse was measured for 24 min after PAF injection. All values are mean±S.E.M. of 5 mice. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ (compared with control by Student's *t*-test).

Administration of IT, EtOAc ext., *n*-BuOH ext., H$_2$O ext., compound **1**—**3** and CV-3988 to mice did not cause a change in the BP of the normal (non-treated) mice (data not shown).

DISCUSSION

PAF shows various biological effects on different organs. However, most anti-PAF effects of natural or synthetic PAF antagonists have been tested on the inhibitory activity of blood platelet aggregation *in vitro*. Because *in vivo* and *in*

vitro activities do not necessarily parallel each other, clear evidence is needed for PAF antagonistic activity *in vivo*. Our assay method using BP decrease in response to PAF as a guide offers an approach for initial screening of PAF antagonists *in vivo*.

In previous studies,[5] we established that the main flavonols from *I. balsamina*, quercetin (**5**), quercetin 3-glucoside (**6**), kaempferol 3-glucoside (**8**) and kaempferol 3-rhamnosyldiglucoside (**9**), significantly inhibited BP decrease in response to PAF, and that those inhibitory effects were stronger than CV-3988,[8] a PAF antagonistic agent. *I. balsamina* has been shown to prevent allergic pruritus[12] and also other allergic symptoms from becoming serious and chronic.[13]

In the present experiment, *I. textori* was confirmed to have an inhibitory effect on BP decrease in response to PAF like that of *I. balsamina*, although the active flavones, apigenin (**1**) and luteolin (**3**) were different from those of *I. balsamina*. It has been reported that **1** markedly inhibits transcriptional activation of cyclooxygenase-2 and inducible nitric oxide synthase[14] and shows inhibitory activities on interleukin-5 (IL-5)[15] and degranulation.[16] Compound **3** also shows inhibitory activities on IL-5,[15] degranulation,[16] 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (lysoPAF) acetyltransferase[17] and the immunoglobulin E-mediated biphasic cutaneous reaction.[18]

Considering these reports and our findings, *I. textori* can be used as a PAF-antagonist agent and will also prevent allergic symptoms from becoming serious and chronic by mechanisms different from those of *I. balsamina*. Details of the mode of action of *I. textori* will be reported in a future paper.

REFERENCES

1) Chang Su New Medical College, "Dictionary of Chinese Crude Drugs," Shanghai Scientific Technological Publishers, Shanghai, 1977.
2) Kim C. W., Choi K. S., *Kor. J. Pharmacogn.*, **24**, 26—31 (1993).
3) Kim C. W., Choi B. J., *Kor. J. Pharmacogn.*, **26**, 8—12 (1995).
4) Lee H. Y., Kim C. W., *Kor. J. Pharmacogn.*, **31**, 105—108 (2000).
5) Oku H., Ishiguro K., *Phytother. Res.*, **13**, 521—525 (1999).
6) Ishiguro K., Fukumoto H., Osada S., Isoi K., Senma M., *Phytother. Res.*, **8**, 301—304 (1994).
7) Ishiguro K., Fukumoto H., *Phytother. Res.*, **11**, 48—50 (1997).
8) Negro Alvarez J. M., Miralles Lopez J. C., Oritiz Martinez J. L., Abellan Aleman A., Rubio del Barrio R., *Allergol. Immunopathol.*, **25**, 249—258 (1997).
9) Fukumoto H., Ishiguro K., Murashima T., Yamaki M., Isoi K., *Phytochemistry*, **37**, 1486—1488 (1994).
10) Wollenweber E., "Flavonoids," ed. by Harborne J. B., Academic Press, New York, 1986, pp. 259—335, 448—454.
11) Agrawal P. K., Thakur R. S., Bansal M. C., "Carbon-13 NMR of Flavonoids," ed. by Agrawal P. K., Elsevier Science Publishing, New York, 1989, pp. 123—158, 292—354.
12) Ishiguro K., Oku H., *Phytother. Res.*, **11**, 343—347 (1997).
13) Oku H., Ishiguro K., *Phytother. Res.*, **15**, 506—510 (2001).
14) Liang Y. C., Huang Y. T., Tsai S. H., Lin-Shiau S. Y., Chen C. F., Lin J. K., *Carcinogenesis*, **20**, 1945—1952 (1999).
15) Park K. Y., Lee S. H., Min B. K., Lee K. S., Choi J. S., Chung S. R., Min K. R., Kim Y., *Planta Med.*, **65**, 457—459 (1999).
16) Cheong H., Ryu S. Y., Oak M. H., Cheon S. H., Yoo G. S., Kim K. M., *Arch. Pharm. Res.*, **21**, 478—480 (1998).
17) Yanoshita R., Chang H. W., Son K. H., Kudo I., Samejima Y., *Inflamm. Res.*, **45**, 546—549 (1996).
18) Kimata M., Inagak N., Nagai H., *Planta Med.*, **66**, 25—29 (2000).