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Effect of Terfenadine on Substance P and Vasoactive Intestinal Polypeptide Concentrations in Nasal Secretions from Patients with Nasal Allergy

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Abstract—Before terfenadine treatment, the mean substance P and vasoactive intestinal polypeptide (VIP) concentrations in nasal secretions from nasal allergy patients tended to be higher than the values of healthy subjects. During terfenadine treatment, the mean substance P concentrations in nasal secretions from patients allergic to house dust or pollen were significantly decreased to 62 and 39% of the initial values, respectively. The mean VIP concentrations in nasal secretions from the house dust allergy patients and the pollen allergy patients were significantly decreased to 52 and 18% of the initial values, respectively. Plasma substance P and VIP concentrations were not affected by nasal allergic symptom and terfenadine treatment.

Allergic reaction in nasal mucosa is considered to be modulated by histamine, and neuropeptides, such as substance P and vasoactive intestinal polypeptide (VIP). Substance P and VIP are generated and contained in neural cell bodies, and released from the nerve endings. However, their precise roles in the allergic reaction are still unclear.

In nasal mucosa, substance P is present in nociceptive trigeminal sensory nerves, found in epithelium, around blood vessels and surrounding submucosal glands. Substance P has been reported to induce vascular permeability, vasodilation, mucus secretion and other physiological phenomena (Lundblad et al 1983; Lundberg et al 1984; Mcdonald 1987; Baraniuk et al 1991). In particular, its mast cell-degranulating effect via axon reflex is considered to contribute to the development of neurogenic inflammation (Barnes 1991).

VIP is localized with acetylcholine in post-ganglionic para-sympathetic nerves, which originate in sphenopalatine ganglia. VIP-immunoreactive nerves are found around blood vessels and surrounding submucosal glands (Lundberg et al 1981, 1987; Barnes 1987; Baraniuk et al 1990). VIP is considered to be an inhibitory factor of the allergic reaction (Said 1990, 1991).

Substance P and VIP may be involved in sensory and autonomic neuromodulation in nasal mucosa, and may play important roles as chemical mediators. However, there are few reports concerned with the effects of anti-allergic agents on the substance P and VIP levels.

Terfenadine is frequently used for the treatment of nasal allergy. Terfenadine was originally regarded as an antihistamine (Cheng & Woodward 1982). Subsequently, the anti-allergic effect of terfenadine became considered to be closely related to its mast cell-stabilizing action as well as its antihistamine action (Tasaka et al 1986; Akagi et al 1987; Tanizaki et al 1987). Its effect on substance P and VIP levels in nasal mucosa is a matter of increasing interest.

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We have previously reported that the raised plasma substance P concentration of subjects with the common cold was decreased by terfenadine administration (Mori et al 1992). We also reported the abundance of substance P and VIP in nasal secretions from nasal allergy patients (Chaen et al 1993). In the present study, to examine the effect of terfenadine on substance P and VIP levels in upper airway more precisely, substance P and VIP concentrations in nasal secretions from nasal allergy patients were measured before and during terfenadine treatment.

Materials and Methods

Subjects

Sixteen patients with nasal allergy to house dust (HD allergy), 10 patients with nasal allergy to Japanese cedar pollen (pollinosis), and 16 healthy volunteers participated in this study (Table 1). The healthy volunteers were classified into a younger control group and an older control group. The mean ages of the HD allergy group, pollinosis group, younger control group and older control group were 15·6, 33·5, 17·5 and 31·8 years, respectively. Informed consent was obtained from all the subjects, and this study received approval from our Ethical Committees in Oita Medical University.

Medication

Patients received treatment with the usual daily dose (120 mg) of terfenadine (Triludan tablet 60 mg, Shionogi Co. Ltd, Osaka, Japan) for at least two weeks.

Sampling

Nasal secretion samples (50–200 μL) were quickly taken using Juhn tap tubes, and placed into chilled test-tubes containing 0·3 mL aprotinin-EDTA mixture (500 kallikrein inhibitor units mL^{-1} aprotinin and 1.2 mg mL^{-1} EDTA). At the same time, 10 mL venous blood was taken, and placed into chilled test-tubes containing 0·5 mL aprotinin-EDTA mixture.

The subjects underwent sampling before and 2–5 (median

Table 1. Subject data.

Group	n	Mean age	Condition	
HD allergy	16	15·6	Before terfenadine	Symptomatic
			During terfenadine	Respondent to medication
Pollinosis	10	33·5	Before terfenadine	Symptomatic
			During terfenadine	Respondent to medication
Control (younger)	6	17·5		Healthy
Control (older)	10	31·8		Healthy

4) weeks after the start of terfenadine treatment. Before treatment, all the patients displayed relevant symptoms. During treatment, the patients were respondent to the medication.

Samples from the healthy volunteers were collected when nasal mucosa received ordinary stimulations (not IgE-mediated), such as the fluctuation of temperature.

Preparation of nasal secretion extract

The nasal secretion samples were diluted 20-fold with 4% acetic acid, pH 4·0, and mixed for 10 min. After 20 min centrifugation (40000 *g*, 4°C), supernatants were loaded onto reversed-phase C18 cartridges (Sep-Pak C18, Waters Co. Inc., Milford, MA, USA). After washing with 4% acetic acid, substance P and VIP in nasal secretions were eluted with 70% acetonitrile in 0·5% acetic acid, pH 4·0. Eluates were concentrated by spin vacuum evaporation, lyophilized and kept at −40°C until use.

Preparation of plasma extract

After 20 min centrifugation (1670 *g*, 4°C), the plasma samples were diluted 5-fold with 4% acetic acid, pH 4·0, and treated as for the preparation of the nasal secretion extract.

Enzyme immunoassay (EIA) for substance P and VIP

EIA for substance P and VIP was performed as previously reported (Takeyama et al 1990a,b). For substance P assay, antiserum RA-08-095 (Cambridge Research Biochemicals Ltd, UK) and β-D-galactosidase-labelled Tyr^8-substance P were used. For VIP assay, antiserum 604/001 (UCB-Bioproducts SA, Belgium) and β-D-galactosidase-labelled VIP (11–28) were used. EIA was performed by the delayed addition method. Separation of bound and free material was performed by the double antibody solid phase method. The detectable minimum amounts of substance P and VIP were 0·4 and 0·1 fmol/well, respectively.

Measurement of total protein concentration in nasal secretion

Ten microlitres of nasal secretions were collected at the same time. The total protein concentration in nasal secretions was measured by the method of Lowry et al (1951).

HPLC of nasal secretion and plasma extracts

For the biochemical characterization of substance P-immunoreactive substance (SP-IS) and VIP-immunoreactive substance (VIP-IS), nasal secretion and plasma extracts were applied onto a reversed-phase C18 column (Cosmosil 5C18-AR, 4·6 × 150 mm, Nacarai Tesque, Inc., Kyoto, Japan). The column was equilibrated with 0·1% trifluoroacetic acid. The nasal secretion or plasma extracts were applied to the column. Substance P-related compounds were eluted with a linear gradient of acetonitrile (15% in 6 min and 15–50% in 35 min) in 0·1% trifluoroacetic acid. Synthetic substance P and its sulphoxide were applied to the column under the same conditions. VIP-related compounds were eluted with a linear gradient of acetonitrile (10% in 6 min and 10–45% in 35 min) in 0·1% trifluoroacetic acid. Synthetic VIP was applied to the column under the same conditions. The flow rate was 1 mL min^{-1} and the fraction size was 1 mL. Each fraction was concentrated by spin-vacuum evaporation, and lyophilized. The residue was submitted to EIA.

Statistical analysis

All results were expressed as mean ± s.d. Comparison of mean values was made by the one-way analysis of variance. A value of $P < 0·05$ was regarded as significant.

Results

Total protein concentration in nasal secretions

The mean total protein concentrations in nasal secretions from the HD allergy group before terfenadine treatment, the HD allergy group during treatment, the pollinosis group

Table 2. Substance P in nasal secretions and plasma (pM).

	Nasal secretion		Plasma	
Group	Without terfenadine	With terfenadine	Without terfenadine	With terfenadine
Younger control	224·4 ± 97·6 (27·2 ± 10·8)	—	2·7 ± 1·2	—
Older control	248·8 ± 86·2 (31·7 ± 13·3)	—	2·8 ± 1·0	—
HD allergy	293·8 ± 183·4 (62·6 ± 35·6)	238·5 ± 135·1 (38·8 ± 18·5)	3·0 ± 0·9	3·0 ± 1·0
Pollinosis	394·4 ± 204·1 (89·5 ± 39·9)	201·1 ± 90·8 (34·6 ± 23·3)	2·8 ± 1·1	3·1 ± 1·0

Values in parentheses are concentrations expressed as fmol (mg protein)$^{-1}$.

Table 3. VIP in nasal secretions and plasma (pM).

	Nasal secretion		Plasma	
Group	Without terfenadine	With terfenadine	Without terfenadine	With terfenadine
Younger control	35·3±29·9 (3·7±2·6)	—	0·9±0·4	—
Older control	28·1±16·0 (3·4±2·2)	—	1·0±0·3	—
HD allergy	26·3±9·1 (6·3±3·2)	19·9±7·4 (3·3±1·2)	0·8±0·4	1·0±0·5
Pollinosis	61·4±41·6 (13·7±7·5)	14·6±10·3 (2·4±1·8)	0·9±0·4	1·1±0·4

Values in parentheses are concentrations expressed as fmol (mg protein)$^{-1}$.

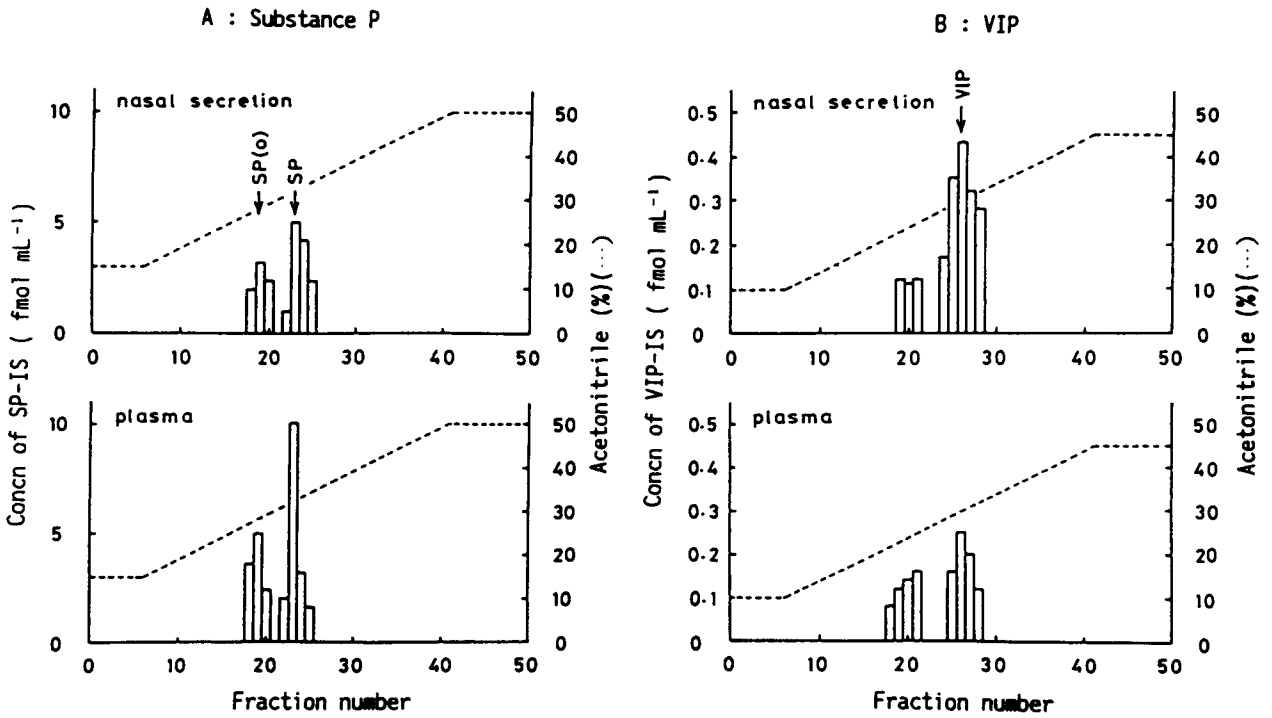

FIG. 1. HPLC profiles of nasal secretion and plasma extracts. A, substance P. Extracts from nasal secretion (100 μL) and plasma (5 mL) were applied. The arrows indicate the elution regions of synthetic substance P and substance P sulphoxide (SP(O)). SP-IS: substance P-immunoreactive substance. B, VIP. Extracts from nasal secretion (100 μL) and plasma (5 mL) were applied. The arrows indicate the elution region of synthetic VIP. VIP-IS: VIP-immunoreactive substance.

before treatment, the pollinosis group during treatment, the younger control group and the older control group were 5·3±2·6, 6·4±2·5, 4·5±1·5, 6·9±2·8, 8·6±2·1 and 9·1±2·9 mg mL^{-1}, respectively. The substance P and VIP concentrations in nasal secretions were expressed as fmol (mg protein)$^{-1}$.

Substance P concentrations in nasal secretions and plasma

Substance P concentrations in nasal secretions and plasma are shown in Table 2.

For the HD allergy group, the mean substance P concentration in nasal secretions before treatment was significantly higher than the value of the young control group ($P<0·05$). The value during treatment was significantly lower than the value before treatment ($P<0·05$).

For the pollinosis group, the mean substance P concentration in nasal secretions before treatment was significantly higher than the value of the older control group ($P<0·01$). The value during treatment was significantly lower than the value before treatment ($P<0·01$).

VIP concentrations in nasal secretions and plasma

The VIP concentrations in nasal secretions and plasma are shown in Table 3.

For the HD allergy group, the mean VIP concentration in nasal secretions during terfenadine treatment was significantly lower than the value before treatment ($P<0·01$).

For the pollinosis group, the mean VIP concentration in nasal secretions before treatment was significantly higher than the value of the older control group ($P<0·01$). The value during treatment was significantly lower than the value before treatment ($P<0·01$).

HPLC of nasal secretion and plasma extracts

Typical HPLC profiles of the nasal secretion and plasma extracts are shown in Fig. 1.

The main SP-IS in nasal secretion and plasma extracts were eluted at the same elution volume as the synthetic substance P in the region of 32% acetonitrile with a minor peak at the same elution volume of substance P sulphoxide in the region of 28% acetonitrile. The main VIP-IS in nasal secretion and plasma extracts were eluted at the same elution volume as the synthetic VIP in the region of 30% acetonitrile with an unidentified minor peak in the region of 25% acetonitrile. The assays of the nasal secretion and plasma extracts were considered to be specific.

Discussion

Substance P and VIP concentrations in nasal secretions from nasal allergy patients and healthy subjects were significantly higher than the values in plasma. Therefore, substance P and VIP were considered to exist in nasal secretions as the result of the peripheral releases from the endings of substance P- and VIP-immunoreactive nerves.

Before terfenadine treatment, the mean substance P and VIP concentrations in nasal secretions from the nasal allergy patients tended to be higher than the values of the healthy subjects. In nasal mucosa of the nasal allergy patients, substance P- and VIP-immunoreactive nerves were considered to be continuously over-excited.

Barnes (1986, 1991) reported that the axon reflex of substance P-immunoreactive nerves could account for the pathophysiology of asthma. Perhaps the axon reflex mechanism may also be functioning in nasal allergic reaction. Substance P may act as a promoting factor of the neurogenic inflammation in nasal mucosa.

The mean substance P concentrations in nasal secretions from the patients during terfenadine treatment were significantly lower than the values before treatment. This significant decrease in the substance P concentrations in nasal secretions may reflect the indirect substance P-decreasing effect of terfenadine. Terfenadine has a mast cell stabilizing action and an antihistamine action. Mast cells were reported to be in close contact to the endings of substance P-immunoreactive nerves (Newson et al 1983; Skofitsch et al 1985). Substance P-immunoreactive nerves possess histamine receptors, and are directly excited by the degranulation of mast cells (Payan et al 1984). The stabilization of mast cell and the blockage of histamine receptor which were caused by terfenadine might be followed by the suppression of the axon reflex of substance P-immunoreactive nerves; the peripheral release of substance P from the endings of SP-immunoreactive nerves would then be decreased. This decreasing effect of terfenadine on the substance P level in nasal mucosa may contribute to its anti-allergic effect.

Undem et al (1983) reported that VIP inhibited antigen-induced histamine release. Said (1990, 1991) has suggested that VIP is an inhibitory modulator of inflammation. Therefore, VIP was considered to be released, not as the inflammation promoter, but as the inflammation controller. In nasal mucosa of the symptomatic patients, VIP may be actively released from the endings of parasympathetic nerves, in order to control the inflammation, with VIP being diluted into nasal secretions.

The mean VIP concentrations in nasal secretions from the nasal allergy patients during terfenadine treatment were significantly lower than the values before treatment. VIP-immunoreactive nerves are essentially efferent. Therefore, the inactivation of VIP-immunoreactive nerves might follow the suppression of substance P-immunoreactive nerves reflexively, via the central nervous system. The decrease in VIP concentration in nasal secretions may reflect the termination of the inflammation. However, the meaning of the decreasing effect of terfenadine on the VIP level in nasal mucosa is still unclear.

The alterations of the substance P and VIP concentrations in nasal secretions were observed more clearly in the pollinosis patients, rather than in the HD allergy patients. This disparity may be due to the immunochemical characteristics of HD and Japanese cedar pollen as antigens.

Plasma substance P and VIP concentrations were not affected by nasal allergic symptom and terfenadine treatment. Neuropeptides such as substance P and VIP are considered to be released from the nerve endings, and diluted into circulating blood. The inflammation as occurs with nasal allergy may be restricted within nasal mucosa. The extent of the local alterations of substance P and VIP levels in nasal mucosa might not be large enough to affect the background levels of the plasma substance P and VIP concentrations.

References

Akagi, M., Mio, M., Miyoshi, K., Tasaka, K. (1987) Antiallergic effects of terfenadine on immediate type hypersensitivity reactions. Immunopharmacol. Immunotoxicol. 9: 257–279

Baraniuk, J. N., Lundgren, J. D., Okayama, M., Mullol, J., Merida, M., Shelhamer, J. H., Kaliner, M. A. (1990) Vasoactive intestinal peptide in human nasal mucosa. J. Clin. Invest. 86: 825–831

Baraniuk, J. N., Lundgren, J. D., Okayama, M., Goff, J., Mullol, J., Merida, M., Shelhamer, J. H., Kaliner, M. A. (1991) Substance P and neurokinin A in human nasal mucosa. Am. J. Respir. Cell Mol. Biol. 4: 228–236

Barnes, P. J. (1986) Asthma as an axon reflex. Lancet i: 242–245

Barnes, P. J. (1987) Neuropeptides in human airway: function and clinical implications. Am. Rev. Respir. Dis. 136: S77–S83

Barnes, P. J. (1991) Neuropeptides and asthma. Am. Rev. Respir. Dis. 143: S28–S32

Chaen, T., Watanabe, N., Mogi, G., Mori, K., Takeyama, M. (1993) Substance P (SP) and vasoactive intestinal peptide (VIP) in nasal secretions and plasma from patients with nasal allergy. Ann. Otol. Rhinol. Laryngol. In press

Cheng, H. C., Woodward, J. K. (1982) A kinetic study of the antihistaminic effect of terfenadine. Arzneim. Forsch. 32: 1160–1166

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265–275

Lundberg, J. M., Änggård, A., Emson, P., Fahrenkrug, J., Hökfelt, T. (1981) Vasoactive intestinal polypeptide and cholinergic mechanisms in cat nasal mucosa: studies on choline acetyltransferase and release of vasoactive intestinal polypeptide. Proc. Natl. Acad. Sci. USA 78: 5255–5259

Lundberg, J. M., Hökfelt, T., Martling, C.-R., Saria, A., Cuello, C. (1984) Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. Cell Tissue Res. 235: 251–261

Lundberg, J. M., Lundblad, L., Martling, C.-R., Saria, A., Stjärne, P., Änggård, A. (1987) Coexistence of multiplepeptides and classical transmitters in airway neurons: functional and pathophysiological aspects. Am. Rev. Respir. Dis. 136: S16–S23

Lundblad, L., Saria, A., Lundberg, J. M., Änggård, A. (1983) Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons. Acta Otolaryngol. 96: 479–484

Mcdonald, D. M. (1987) Neurogenic inflammation in the respiratory tract: actions of sensory nerve mediators on blood vessels and epithelium of the airway mucosa. Am. Rev. Respir. Dis. 136: S65–S72

Mori, K., Asakura, S., Morikawa, N., Takeyama, M. (1992) Effect of terfenadine on the plasma concentrations of substance P and vasoactive intestinal polypeptide in volunteers. J. Pharm. Pharmacol. 44: 856–858

Newson, B., Dahlström, A., Enerbäck, L., Ahlman, H. (1983) Suggestive evidence for a direct innervation of mucosal mast cells. Neuroscience 10: 565–570

Payan, D. G., Levin, J. D., Goetzl, E. J. (1984) Modulation of immunity and hypersensitivity by sensory neuropeptides. J. Immunol. 132: 1601–1604

Said, S. I. (1990) Neuropeptides as modulators of injury and inflammation. Life. Sci. 47: PL19–PL21

Said, S. I. (1991) VIP as a modulator of lung inflammation and airway constriction. Am. Rev. Respir. Dis. 143: S22–S24

Skofitsch, G., Savitt, J. M., Jacobowitz, D. M. (1985) Suggestive evidence for a functional unit between mast cells and substance P fibers in the rat diaphragm and mesentery. Histochemistry 82: 5–8

Takeyama, M., Mori, K., Takayama, F., Kondo, K., Kitagawa, K., Fujii, N. (1990a) Enzyme immunoassay of a substance P-like immunoreactive substance in human plasma and saliva. Chem. Pharm. Bull. 38: 3494–3496

Takeyama, M., Wakayama, K., Takayama, F., Kondo, K., Fujii, N., Yajima, H. (1990b) Micro-enzyme immunoassay of vasoactive intestinal polypeptide (VIP)-like immunoreactive substance in bovine milk. Chem. Pharm. Bull. 38: 960–962

Tanizaki, Y., Komagoe, H., Sudo, M., Kitani, H. (1987) Study on anti-allergic action of terfenadine. Institute for Environmental Medicine. Okayama University 58: 5–11 (in Japanese)

Tasaka, K., Mio, M., Okamoto, M. (1986) Intracellular calcium release induced by histamine releasers and its inhibition by some anti-allergic drugs. Ann. Allergy 56: 464–469

Undem, B. J., Dick, E. C., Buckner, C. K. (1983) Inhibition by vasoactive intestinal peptide of antigen-induced histamine release from guinea-pig minced lung. Eur. J. Pharmacol. 88: 247–250