J. Pharm. Pharmacol. 1992, 44: 856–858
Communicated December 9, 1991

COMMUNICATIONS

Effect of terfenadine on the plasma concentrations of substance P and vasoactive intestinal polypeptide in volunteers

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Abstract—The effect of terfenadine on the plasma concentrations of substance P and vasoactive intestinal polypeptide (VIP) was studied in 7 healthy subjects and 8 subjects with the common cold. Before terfenadine administration, the mean plasma substance P concentration of the subjects with the common cold was significantly higher than that of the healthy subjects. The increased mean plasma substance P concentration of the subjects with the common cold was decreased after terfenadine administration. In the healthy subjects, the mean plasma substance P concentration was unchanged by terfenadine administration. The mean plasma VIP concentration of the subjects with common cold was slightly higher than that of the healthy subjects before and after terfenadine administration, with no significant difference.

The neuropeptides, substance P and vasoactive intestinal polypeptide (VIP), have a widespread distribution in the central and peripheral nervous system. These peptides exert an important regulatory influence on neuroendocrine, respiratory, cardiovascular, intestinal, and other functions.

In airways, substance P- and VIP-immunoreactive nerves are widely distributed. Substance P-immunoreactive nerves are found beneath and within airway epithelium, around blood vessels, surrounding submucosal glands, and within smooth muscle (Lundberg et al 1984). VIP-immunoreactive nerves are found in airway smooth muscle, around bronchial vessels, and surrounding submucosal glands (Dey et al 1981; Laitinen et al 1985; Barnes 1987).

Recently, the close connection of substance P and VIP to the inflammatory reaction has been suggested in human skin and lung. Substance P has been considered to mediate the axon reflex flare (Foreman et al 1983; Barnes 1986), and VIP has been considered to be an inhibitory factor of the inflammatory reaction (Said 1990, 1991). Substance P and VIP may play important roles in concert with histamine and other chemical mediators in the inflammatory reaction (Barnes 1987, 1991). However, their precise roles in the airway inflammatory reaction are still unclear, and there are few reports concerned with the plasma concentrations of substance P and VIP in human subjects with inflammatory disease.

Terfenadine is a typical basic anti-allergic agent, frequently used for the treatment of the allergic inflammation. It has been reported to have a superior mast cell-stabilizing action and an antihistamine action (Cheng & Woodward 1982; Akagi et al 1987; Tanizaki et al 1987).

Airway hyper-responsiveness caused by the common cold is well known (Jackson 1975; McDonald 1987). Therefore, the effect of terfenadine on the actions of substance P and VIP in subjects with the common cold is a matter of interest.

In this study, we examined the plasma concentrations of substance P- and VIP-like immunoreactive substances (SP-IS and VIP-IS, respectively) before and after terfenadine administration in healthy subjects and subjects with the common cold.

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Materials and methods

Subjects. Seven healthy volunteers (males) and 8 with the common cold (7 males and 1 female) from whom informed consent had been obtained, participated in the study. The mean age of the healthy volunteers was $33{\cdot}3 \pm 2{\cdot}7$ years, and the volunteers with the common cold $34{\cdot}5 \pm 6{\cdot}2$ years. The mean body weight of the healthy volunteers was $67{\cdot}6 \pm 4{\cdot}6$ kg, the volunteers with the common cold $63{\cdot}5 \pm 11{\cdot}6$ kg. None received any medication other than terfenadine during the study.

This study received approval from our Ethics Committees in Oita Medical University.

Study schedule. Terfenadine (Triludan 60 mg, Shionogi Co. Ltd, Osaka, Japan) was orally administered at a dose of 120 mg with 150 mL water.

Ten mL venous blood samples were taken from a forearm vein before and 2 h after the administration of terfenadine. The interval between the samplings approximately corresponded to the t_{max} of terfenadine (Garteiz et al 1982). The study was carried out from 1400 to 1600 h.

In order to study the background of the plasma concentration (Gamse et al 1978), the physiological fluctuations of the plasma concentrations of SP-IS and VIP-IS in four other healthy volunteers were examined from morning to evening. Blood samples were taken at 0800, 1000, 1400 and 1600 h.

Preparation of plasma extracts. The blood samples were placed in chilled tubes containing aprotinin (500 kallikrein inhibitor units mL^{-1}) and EDTA (1 mg mL^{-1}). After centrifugation (3000 *g*, 4°C, 20 min), plasma samples were diluted fivefold with 4% acetic acid pH 4·0 and 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 plasma were eluted with 70% acetonitrile in 0·5% acetic acid, pH 4·0. Eluates were concentrated by spin-vacuum evaporation, lyophilized and stored until use. The recoveries of substance P and VIP with this extracting procedure were 92 ± 10 and 94 ± 6%, respectively.

Enzyme immunoassay (EIA) for SP-IS and VIP-IS. EIA for SP-IS was performed as previously described (Takeyama et al 1990a). Antiserum (RA-08-095) purchased from Cambridge Research Biochemicals Ltd (Cambridge, UK), was specific to the carboxy-terminal portion of substance *P* as examined with synthetic substance P and its fragments. Tyr^8-substance P was labelled with β-D-galactosidase. 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 amount of substance P was 0·4 fmol/well. EIA for VIP-IS was essentially the same, except that the

antiserum for VIP (604/001, UCB-Bioproducts SA, Belgium) was used to detect the central region of human VIP. The sensitivity was 0·1 fmol/well (Takeyama et al 1990b).

HPLC of plasma extract. HPLC was performed using 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 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 (SP(O)) were applied to the column under the same conditions. VIP and 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. Comparisons between the two groups were performed using Student's unpaired *t*-test.

Results

Physiological fluctuations of SP-IS and VIP-IS in plasma. The physiological fluctuations of the plasma SP-IS and VIP-IS are shown in Fig. 1. The plasma SP-IS concentrations in the morning tended to be lower, but subsequently, the physiological fluctuation of SP-IS was small. The physiological fluctuation of VIP-IS was capricious, but the extent was small. In the afternoon, the concentrations of plasma SP-IS and VIP-IS appeared to be constant. Thus the schedule of the study described above was considered to be suitable.

Effect of terfenadine on SP-IS in plasma. In the healthy subjects, the mean plasma SP-IS concentrations before and after terfenadine administration were 2·02 ± 0·48 and 1·88 ± 0·52 pM, respectively. There was no significant difference between these values. In the subjects with the common cold, the mean plasma SP-IS concentration before terfenadine administration was 3·94 ± 1·12 pM, significantly higher than that of the healthy subjects ($P < 0·01$). The increased mean plasma SP-IS concentration of the subjects with the common cold was significantly decreased to 2·99 ± 0·81 pM by terfenadine ($P < 0·05$) (Fig. 2A).

Effect of terfenadine on VIP-IS in plasma. In the healthy subjects, the mean plasma VIP-IS concentrations before and after the terfenadine were 0·77 ± 0·25 and 0·74 ± 0·38 pM, respectively. There was no significant difference between these values. In the subjects with the common cold, the mean plasma VIP-IS concentrations before and after the terfenadine were 1·06 ± 0·26 and 0·93 ± 0·44 pM, respectively. There was no significant difference between these values (Fig. 2B).

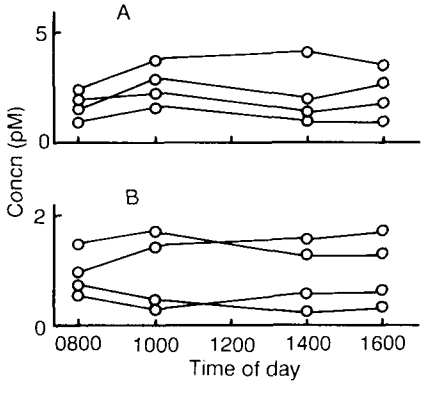

FIG. 1. Physiological fluctuations of substance P (A) and vasoactive intestinal polypeptide (B).

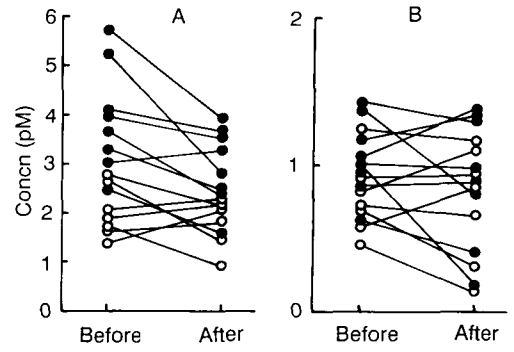

FIG. 2. Plasma concentrations of substance P (A) and vasoactive intestinal polypeptide (B) before and after terfenadine administration. ○ Healthy, ● common cold.

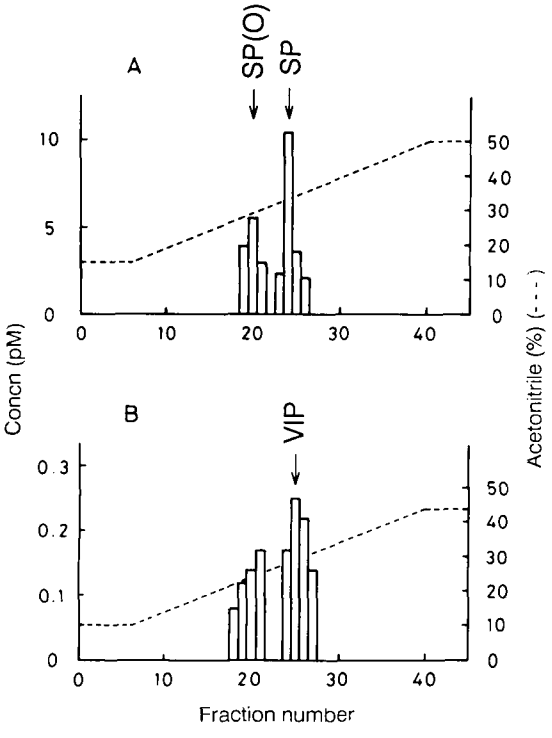

FIG. 3. HPLC profiles of plasma extracts. The arrows indicate the elution regions of synthetic substance P (SP), substance P oxide (SP (O)) and vasoactive intestinal polypeptide (VIP). A. Substance P. B. Vasoactive intestinal polypeptide.

HPLC of plasma extracts. For biochemical characterization of SP-IS and VIP-IS, plasma extracts were applied to reversed phase HPLC. The main SP-IS in the plasma was eluted at the same elution volume of the synthetic substance P in the region of 32% acetonitrile with a minor peak at the same elution volume of SP(O) in the region of 28% acetonitrile (Fig. 3A).

The main VIP-IS in the plasma was eluted at the same elution

volume of the synthetic VIP in the region of 30% acetonitrile with an unidentified minor peak in the region of 25% acetonitrile (Fig. 3B). Thus the assay of the plasma extracts was considered to be specific.

Discussion

In the present study, we have demonstrated the high concentration of the plasma SP-IS of subjects with the common cold.

The cause of this high concentration of plasma SP-IS may be related to the sensory neuromodulation by neuropeptides in airways. Barnes (1986) has proposed the axon reflex mechanism of SP-immunoreactive nerves in respiratory tract. The axon reflex may be elevated in subjects with upper airway inflammation, as occurs with the common cold. Thus, the high concentration of plasma SP-IS of subjects with the common cold was considered to be caused by the continued elevation of the axon reflex in upper airway.

Substance P is a potent inducer of contractions of airway smooth muscle. Therefore, the high concentration of plasma SP-IS was considered to be related to the common cold-induced airway hyper-responsiveness (McDonald 1987).

The increased mean plasma SP-IS concentration of the subjects with the common cold was decreased by terfenadine administration. This decreasing effect of terfenadine on plasma SP-IS concentration may contribute to its anti-allergic effect.

The precise cause of the decrease of plasma SP-IS concentration of subjects with the common cold by terfenadine was unclear. Nevertheless, the direct effect of terfenadine on the conduction of substance P-immunoreactive nerves was considered to be weak, because the mean plasma SP-IS concentration of the healthy subjects was unchanged by terfenadine. Akagi et al (1987) reported that the anti-allergic effect of terfenadine was closely related to its mast cell stabilizing action. Moreover, the contact between substance P-immunoreactive nerve fibres and mast cells has been demonstrated (Skofitsch et al 1985). Therefore, the decrease of plasma SP-IS concentration by terfenadine was considered to be caused by the indirect inhibitory effect of terfenadine on the axon reflex of substance P-immunoreactive nerves via mast cells.

The changes of the plasma VIP-IS concentrations were difficult to interpret. The rate of plasma VIP-IS originating from the inflammation site might be small in comparison with the background level of plasma VIP-IS. Nevertheless, VIP may be assumed to play some role in the airway inflammatory reaction, because the mean plasma VIP-IS concentration of subjects with the common cold was slightly higher than that of healthy subjects.

In conclusion, the close connection of substance P with upper airway inflammation is suggested, and the decreasing effect of terfenadine on the raised plasma SP-IS concentration of the subjects with the common cold is revealed.

References

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

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

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

Barnes, P. J. (1991) Neuropeptides and asthma. Ibid. 143: S28–S32

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

Dey, R. D., Shannon, W. A., Said, S. I. (1981) Localization of VIP-immunoreactive nerves in airways and pulmonary vessels of dogs, cats, and human subjects. Cell Tissue Res. 220: 231–238

Foreman, J. C., Jordan, C. C., Oehme, P., Renner, H. (1983) Structure-activity relationships for some substance P-related peptides that cause wheal and flare reactions in human skin. J. Physiol. 335: 449–465

Gamse, R., Mroz, E., Leeman, S., Lembeck, F. (1978) The intestine as source of immunoreactive substance P in plasma of the cat. Naunyn Schmiedebergs Arch. Pharmacol. 305: 17–21

Garteiz, D. A., Hook, R. H., Walker, B. J., Okerholm, R. A. (1982) Pharmacokinetics and biotransformation studies of terfenadine in man. Arzneim-Forsch./Drug Res. 32: 1185–1190

Jackson, G. G. (1975) Viral (and filter-passing microbial) infections of the respiratory tract; III. The common cold. In: Beeson, P. B., McDermott, W. (eds) Textbook of Medicine. 14th edn, Saunders, Philadelphia, pp 184–187

Laitinen, A., Partanen, M., Hervonen, A., Pelto-Huikko, M., Laitinen, L. A. (1985) VIP like immunoreactive nerves in human respiratory tract. Light and electron microscopic study. Histochemistry 82: 313–319

Lundberg, J. M., Hokfelt, 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

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

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 fibres 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. Ibid. 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)