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Toxicodynamic Analysis of Inflammatory Reactions by an Angiotensin Converting Enzyme Inhibitor (Lisinopril) in Guinea-pig Skin

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Abstract

There have been reports of rash and angioedema in the treatment of hypertension with angiotensin converting enzyme (ACE) inhibitors. To evaluate the inflammatory reaction, we continuously infused lisinopril for three days into the peritoneal cavity of ovalbumin-sensitized guinea-pigs and tested intradermal inflammation with ovalbumin. Inflammatory responses were measured in two perpendicular directions serially, and the areas of rash were used as an index of inflammatory reaction induced by lisinopril.

Inflammatory responses were dose-dependently enhanced by treatment with lisinopril. Plasma concentration of lisinopril required to produce 50% of the maximum potentiation of the inflammatory reaction in guinea-pig skin was 40 times plasma unbound concentration after the clinical treatment of lisinopril in patients.

Angiotensin converting enzyme (ACE) inhibitors are the first choice drugs for the treatment of hypertension. Their use results in a lowering of blood pressure without adverse effects on central nervous system, cardiac function, and metabolism of lipids, glucose or uric acid (Joint National Committee 1988; Hiwada 1989). However, it has been reported that in some patients, persistent chronic cough is induced by all ACE inhibitors currently available (Sesoko & Kaneko 1985). Although the mechanism for ACE inhibitor-induced chronic cough remains unknown, some investigators have suggested bronchial hyperactivity and increased inflammatory substances (substance P, bradykinin) in the membranous wall of trachea, which stimulate the cough receptor (Kaufman et al 1980; Sesoko & Kaneko 1985; Kohrogi et al 1988). The inflammatory response is very similar to the IgE-mediated (Type 1) hypersensitivity reaction, such as in bronchial asthma and pollinosis, in that it is induced by the release of chemical mediators. Indeed, it is reported that eczema erythematosum, wheal and flare are induced by treatment with ACE inhibitors in some hypertensive patients (Lindgren et al 1989). Moreover, Lindgren et al (1987) reported that the inflammatory skin responses in guinea-pigs induced by injection of ovalbumin are increased by treatment with an ACE inhibitor.

The aim of the present study was to investigate the effect of an ACE inhibitor (lisinopril) on the inflammatory skin reactions in sensitized guinea-pigs, and to clarify the relationship between the potentiation of inflammatory reactions and the plasma concentration of lisinopril.

Materials and Methods

Drugs and other agents

Lisinopril was the gift from Shionogi & Co. Ltd (Osaka, Japan), ovalbumin was purchased from Sigma (St Louis, MO, USA), and $Al(OH)_3$ was from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals used were of analytical grade.

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Animals

Female Dunkin Hartley guinea-pigs, 250 g, were sensitized 14 days before use, and their body weight was 260–340 g when the inflammatory response was measured.

Sensitization and testing

We followed the method of Lindgren et al (1987) with slight modification, as follows.

Sensitization. One millilitre of sensitizing suspension (ovalbumin 10 μg together with $Al(OH)_3$ 100 mg mL^{-1} in saline) was injected intraperitoneally 14 days before testing.

Drug administration. An osmotic pump (Model 2ML1; 10 $\mu L\,h^{-1}$ at 37°C, Alzet) was filled with lisinopril solution or saline, and was inserted into the peritoneal cavity of the guinea-pig three days before testing. The osmotic pump delivers the solution continuously, and the dose of lisinopril was controlled by changing the concentration of the drug solution.

Measurement of the inflammatory reaction. The flank was shaved with an electric razor, and the hair was removed completely with hair remover (Hair Remover for medical use, Kanebo) on the day before testing (it has been confirmed that there is no allergic reaction to the hair remover). At the time of testing, 10 μL ovalbumin solution (0·02 mg mL^{-1}) was injected intradermally using a microsyringe. The diameters of the roughly circular wheal-and-flare reactions were measured in two perpendicular directions 1, 3, 6, 9 and 24 h after injection, and the area (A) was

estimated using the following equation:

A = (a radius length of the inflammatory response)
× (a radius width of the inflammatory response)
× 3·14 (1)

Blood sampling. After the last measurement, the blood was obtained by the immediate decapitation of the animal.

Measurement of plasma concentration of lisinopril by RIA (Hichens et al 1981)
The immunogen was prepared by coupling *N*-(1-carboxy-3-phenylpropyl)-L-lysyl-L-proline to albumin with difluorodinitrobenzene, and antisera were raised in rabbits. The radiolabelled component was obtained by iodination of the amidine derived from the reaction of the same compound and methyl *p*-hydroxybenzimidate. The resulting assay was sensitive to 2 pg of either drug, but 5 pg was the practical limit for accurate measurement in up to 100 μL serum.

Pharmacokinetic analysis of plasma concentration
Osmotic pumps filled with lisinopril solution (10 and 50 mg mL^{-1}) were inserted into the peritoneal cavities of guinea-pigs. After 1, 3, or 4 days, the guinea-pigs were decapitated and exsanguinated. Plasma was obtained by centrifugation and lisinopril levels in the plasma were determined by RIA. We fitted the two kinds of data concurrently to the linear one-compartment model; one comprised the plasma concentrations of lisinopril after 1, 3, and 4 days, and the other comprised those after 4 days, for various concentrations of lisinopril in the osmotic pump.

Measurement of in-vivo and in-vitro plasma protein binding
The plasma protein-binding of lisinopril was measured by ultrafiltration.

Lisinopril was added to 2 mL blank plasma obtained from ovalbumin-sensitized guinea-pigs to final concentrations of 10, 100, and 1000 ng mL^{-1}. About 1 mL solution was filtered using the Amicon Centrifree Micropartition system at 37°C. The plasma unbound fraction of lisinopril was calculated as the concentration ratio of the filtered solution (C_u) and the upper non-filtered solution ($C_u + C_b$).

In the same way, in-vivo unbound fraction was obtained using the plasma sampled after measuring the inflammatory skin reaction. These data were fitted to equation 2 and the binding parameters were obtained by nonlinear least squares method (Yamaoka & Tanigawara 1983).

$$C_b = \frac{n_1 P \cdot C_u}{K_{d1} + C_u} + \frac{n_2 P \cdot C_u}{K_{d2} + C_u} \quad (2)$$

where C_b is plasma bound concentration, C_u is plasma unbound concentration, n_1 and n_2 are the number of binding sites, respectively, P is the protein concentration, and K_{d1} and K_{d2} are dissociation constants.

Toxicodynamic analysis
The relationship between plasma unbound concentration of lisinopril (C_u) and the intensity of inflammatory skin responses (E) was fitted to equation 3, and each parameter was calculated:

$$C_b = \frac{E_{max} \cdot C_u}{EC50 + C_u} + E_0 \quad (3)$$

where E_{max} is the maximum response, E_0 is the baseline response, and EC50 is the plasma unbound concentration which induced 50% of the maximum potentiation of the inflammatory response.

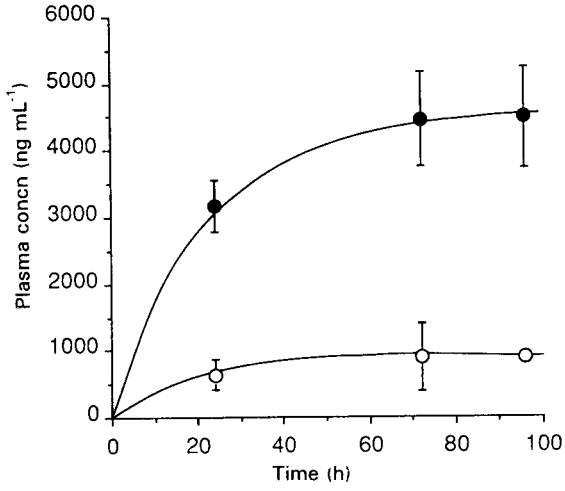

FIG. 1. Time course of plasma concentration of lisinopril. Plasma concentration was measured 1, 3 and 4 days after the osmotic pumps (filled with 10 or 50 mg mL^{-1} lisinopril solution) were inserted into the peritoneal cavity of sensitized guinea-pigs. ○ 10, ● 50 mg mL^{-1} (mean ± s.e., n = 3 for each data point).

Results

Fig. 1 shows the time course of plasma concentration of lisinopril after the osmotic pumps (filled with 10 or 50 mg mL^{-1} lisinopril solution) were inserted into the peritoneal cavity of guinea-pigs. Plasma concentration reached steady state within 70 h.

Fig. 2 shows the plasma concentration of lisinopril on the

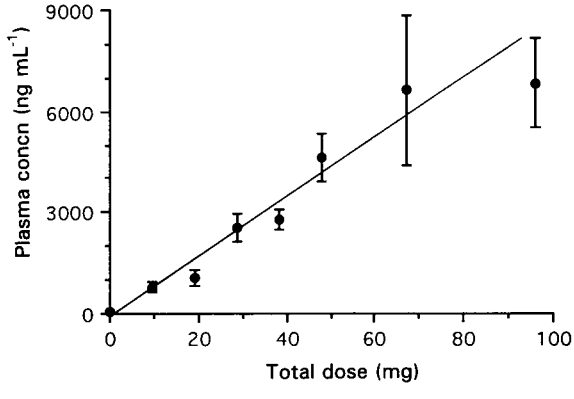

FIG. 2. Relationship between the total dose of lisinopril and the plasma concentration. The plasma concentration of lisinopril was measured on the 4th day after implantation of osmotic pumps filled with various concentrations of lisinopril (mean ± s.e., n = 4–10 for each data point).

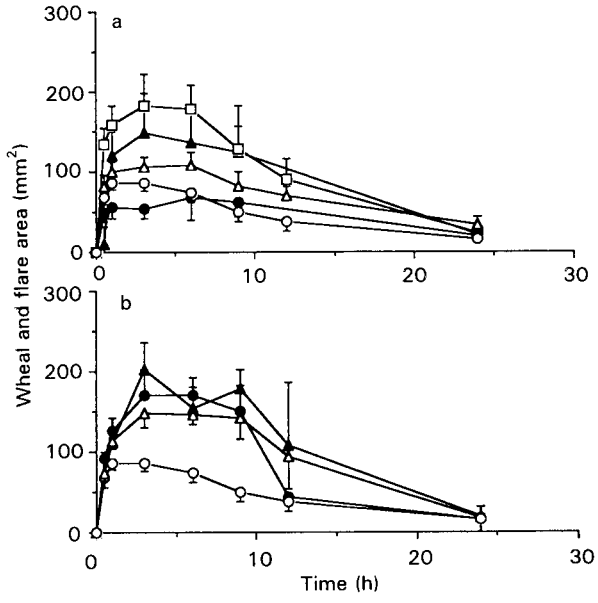

FIG. 3. Time course of wheal-and-flare area induced by lisinopril. The wheal and flare area was measured after implantation of the osmotic pumps filled with the lisinopril solutions. a. □ 40, ▲ 30, △ 20, ● 10 mg mL^{-1}; b. ▲ 100, △ 70, ● 50 mg mL^{-1}, ○ Control (mean ± s.e., n = 4–10).

4th day after implantation of the osmotic pumps filled with various concentrations of lisinopril. The total dose of lisinopril (abscissa) was calculated from the drug concentration (mg mL^{-1}) filled in the osmotic pumps and the delivering rate (mL h^{-1}). Plasma concentration of lisinopril was in proportion to the total dose at least up to 70 mg. The curve in Fig. 1 and the line in Fig. 2 were obtained by the simultaneous fitting to the linear one-compartment model with zero-order absorption.

Fig. 3 shows the time courses of wheal-and-flare area in guinea-pigs administered various doses of lisinopril. For all doses of lisinopril, including control, inflammatory area reached a maximum at 3–6 h after injection of ovalbumin, and almost completely disappeared after 24 h. Moreover, the inflammatory skin response increased with the increase in the dose of lisinopril.

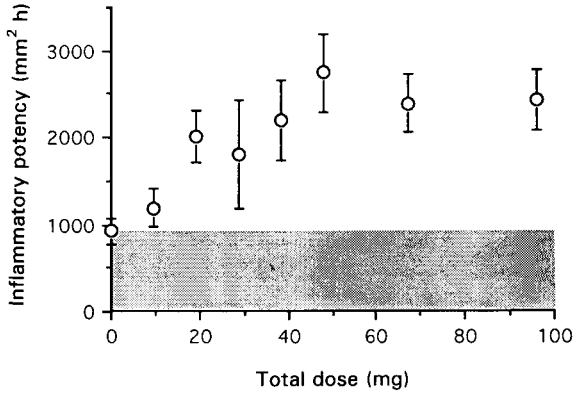

FIG. 4. Relationship between the total dose of lisinopril and the inflammatory potency. The area under the curve of inflammatory response in Fig. 3 was regarded as the inflammatory potency and plotted against the dose (mean ± s.e.). The shaded area in this figure indicates the control response.

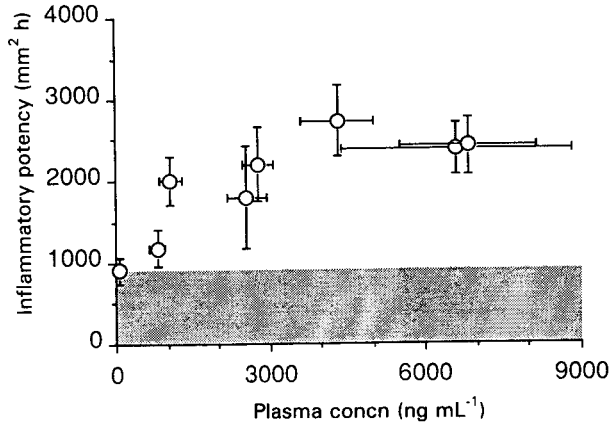

FIG. 5. Relationship between the plasma concentration of lisinopril and the inflammatory potency. The abscissa in Fig. 4 was converted from dose of lisinopril to plasma concentration of lisinopril (mean ± s.e.). The shaded area in this figure indicates the control response.

To investigate the relationship between the intensity of inflammatory response and the dose of lisinopril, the area under the curve of inflammatory response in Fig. 3 was regarded as the inflammatory potency and plotted against the dose (Fig. 4). This shows the saturable relationship between the dose of lisinopril and the inflammatory potency.

The abscissa in Fig. 4 was converted from dose of lisinopril to plasma concentration of lisinopril (Fig. 5). A saturable relationship was obtained (Fig. 4), because the plasma concentration is proportional to the dose as shown in Fig. 2. Fig. 6 shows the relationship between plasma unbound concentration of lisinopril and the unbound fraction. Binding parameters (± s.d.) were obtained by fitting according to equation 2; $n_1P = 100 \pm 21$ ng mL^{-1}, $n_2P = 76\,000 \pm 29\,000$ ng mL^{-1}, $K_{d1} = 2{\cdot}0 \pm 5{\cdot}5$ ng mL^{-1}, $K_{d2} = 390\,000 \pm 76\,000$ ng mL^{-1}.

Fig. 7 was obtained by converting plasma concentration of lisinopril (the abscissa in Fig. 5) to plasma unbound concentration using the unbound fraction of lisinopril in Fig. 6. Each

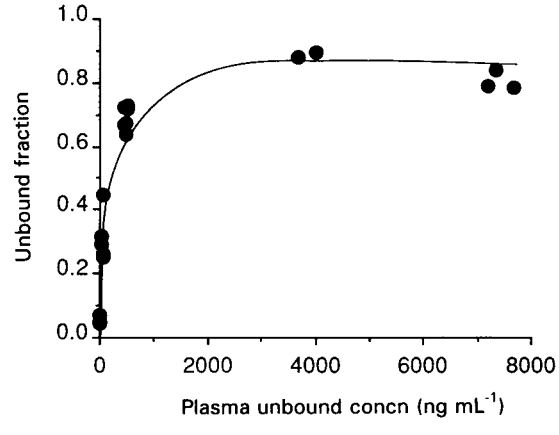

FIG. 6. Relationship between the plasma unbound concentration of lisinopril and the unbound fraction. Protein binding was measured by ultrafiltration.

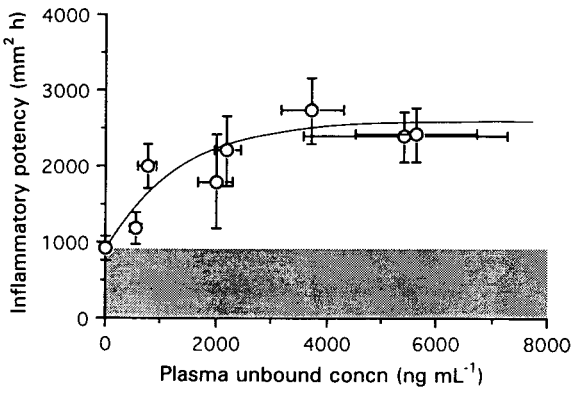

FIG. 7. Relationship between the plasma unbound concentration of lisinopril and the inflammatory potency. The abscissa in Fig. 5 was converted from plasma concentration of lisinopril to plasma unbound concentration using the unbound fraction of lisinopril in Fig. 6 (means ± s.e.).

parameter was calculated according to equation 3; $E_{max} = 2200 \pm 640\,mm^2\,h$, $E_0 = 890 \pm 140\,mm^2\,h$, $EC50 = 1900 \pm 1500\,ng\,mL^{-1}$. The apparent maximum value of inflammatory response ($E_{max} + E_0$) was $3090\,mm^2\,h$.

Discussion

The plasma concentration of lisinopril reached steady state within three days of implantation of the osmotic pump. Thus, we could assume that the plasma concentration was at steady state when the inflammatory skin responses were measured. The inflammatory response was dose-dependently enhanced by treatment with lisinopril, and saturated at the total dose of 40–50 mg (Fig. 4). EC50 and E_{max} values for the relationship between plasma unbound concentration of lisinopril and the inflammatory potency were calculated based on the toxicodynamic model (Fig. 7).

Lindgren et al (1987) investigated the effect of an ACE inhibitor (enalapril) on allergen- and neurogen-induced inflammatory skin reactions in sensitized guinea-pigs. In addition to the inflammatory skin response testing, 4-mm biopsy samples were taken from the reaction area, and the dermal inflammatory cell infiltrate was counted. They found that pretreatment with the ACE inhibitor enhanced the inflammatory response, as assessed both by measurement of the wheal-and-flare area, and by counting of the dermal inflammatory cell infiltrate. They suggested that ACE inhibitors inhibit the metabolism of inflammatory substances such as bradykinin and tachykinins which stimulate afferent sensitive nerve endings, leading to further release of tachykinins, vasodilatation, extravasation, and finally, the increase in the inflammatory responses. It was also suggested that such inflammatory responses in the membranous wall of trachea may be one of the reasons for bronchial hypersensitivity (cough) induced by treatment with ACE inhibitors. However, they did not investigate the relationship between inflammatory responses and plasma concentration of ACE inhibitors.

In this study, we found that the inflammatory skin response in guinea-pig increased dose- and plasma concentration-dependently by treatment with lisinopril. Furthermore, the plasma unbound concentration of lisinopril which induced 50% potentiation of inflammatory reaction (EC50) in guinea-pig was $1900\,ng\,mL^{-1}$, which is higher than the therapeutic unbound plasma concentration (40~60 ng mL^{-1}) during clinical treatment (Nakajima et al 1990). The pharmacokinetic and toxicodynamic approach in this study might be useful in evaluating the inflammatory responses in man and guinea-pig.

References

Hichens, M., Hand, E. L., Mulcahy, W. S. (1981) Radioimmunoassay for angiotensin converting enzyme inhibitors. Ligand Quarterly 4: 43

Hiwada, K. (1989) Side effect and countermeasure of angiotensin converting enzyme inhibitor. Iyaku J. 25: 1524

Joint National Committee (1988) The 1988 Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Arch. Intern. Med. 148: 1023–1038

Kaufman, M. P., Coleridge, H. M., Coleridge, J. C., Baker, D. G. (1980) Bradykinin stimulates afferent vagal C-fibers in intrapulmonary airways of dogs. J. Appl. Physiol. 48: 511–517

Kohrogi, H., Graf, P. D., Sekisawa, K., Borson, D. B., Nodel, J. A. (1988) Neutral endopeptidase inhibitors potentiate substance P- and capsaicin-induced cough in awake guinea pigs. J. Clin. Invest. 82: 2063–2068

Lindgren, B. R., Anderson, C. D., Anderson, R. G. (1987) Potentiation of inflammatory reactions in guinea-pigs skin by an angiotensin converting enzyme inhibitor (MK-422). Eur. J. Pharmacol. 135: 383–387

Lindgren, B. R., Rosenqvist, U., Ekstrom, T., Gronneberg, R., Karlberg, B. E., Andersson, R. G. (1989) Increased bronchial reactivity and potentiated skin responses in hypertensive subjects suffering from coughs during ACE-inhibitor therapy. Chest 96: 1225–1230

Nakajima, M., Kanemaru, M., Oguma, T., Yosimoto, K. (1990) Phase I clinical study of lisinopril (MK-521), a new angiotensin-converting enzyme inhibitor. Yakuri to Chiryou 18: 525–547

Sesoko, S., Kaneko, Y. (1985) Cough associated with the use of captopril. Arch. Intern. Med. 145: 1524

Yamaoka, K., Tanigawara, T. (1983) A nonlinear least squares program based on differential equations, MULTI, for microcomputers. J. Pharmacobiodyn. 6: 595–606