POTENTIATIVE EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITOR ON CARRAGEENAN EDEMA IN RATS AND THE ROLE OF TISSUE KININOGEN

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Roles of kininogens in the development of potentiation of carrageenan edema in rats by angiotensin converting enzyme (ACE, =kininase II) inhibitor were studied. Carrageenan-induced edema was potentiated by oral administration of YS980, an ACE inhibitor, at a dose of 1 mg/kg 0.5 h before carrageenan injection. Intravenous injection of bromelain, a kininogen (KGN) depletor, at 3 mg/kg produced reduction of plasma KGN (total and high molecular weight KGN), which resulted in suppression of carrageenan edema and suppression of edema potentiation induced by YS980. Even after the plasma KGN level and inflammatory response to carrageenan returned to normal 24 h after the administration of bromelain, the potentiative effect of YS980 on the carrageenan edema remained suppressed. Thus, some factor other than plasma KGN is thought to be involved in the potentiation mechanisms on the carrageenan edema by YS980. Partially purified KGN from rat plasma (0.1 mg/site: liberated 4.4×10^{-8} g bradykinin eq by trypsin digestion) completely restored the suppressed potentiation to normal by local preinjection to the inflamed site. In addition, such restoration was not observed in the animal in which plasma KGN was reduced 3 h after administration of bromelain. These results suggest that KGN, not only in plasma but also in tissue, play an active role in the development of potentiation of carrageenan edema by ACE inhibitor.

Keywords — carrageenan; edema potentiation; angiotensin converting enzyme (ACE); inhibitor (ACE); bromelain; plasma kininogen; tissue kininogen

INTRODUCTION

Plasma kininogen (KGN) appears to play an important role in the development of acute inflammations,¹⁾ since the plasma KGN in rats bearing acute inflammation is increased²⁾ and the carrageenan-induced inflammation can be reduced by lowering plasma KGN level.^{3–5)} Recently, we have reported that carrageenan-induced edema in rats was potentiated by orally active kininase II (identical with angiotensin converting enzyme; ACE) inhibitors, (4*R*)-3-[(2*S*)-3-mercapto-2-methylpropanoyl]-4-thiazolidine carboxylic acid (YS980), (2*S*)-1-[(2*S*)-3-mercapto-2-methylpropanoyl]-proline (captopril), *etc.*, and the potentiation of edema by them was due to their inhibitory action on

kininase II (ACE) in the inflamed site.^{6,7)} We suggested that plasma KGN also plays an important role in the development of the potentiative effect of ACE inhibitors on carrageenan-induced edema in rats.^{8,9)}

On the other hand, there are found only a few reports concerning the role of KGN in tissues in the inflammatory state. Although KGN and kallikrein was found not only in plasma but also in various tissues including dermal tissue of rats,^{10,11)} it is suggested that KGN in the tissue may originate from plasma KGN.^{12,13)} In this paper, the role of KGN in tissues (temporarily calls as “tissue KGN”) in the potentiative effect of ACE inhibitor on carrageenan-induced edema was studied.

MATERIALS AND METHODS

Compounds used were as follows : stem bromelain (Jintan Dof, 888 U/mg); YS980 (synthesized at Santen Pharm. Co., Ltd.); lambda carrageenan (Pasco); trypsin (Difco); glass powder (0.09–0.14 mm diameter, Nippon Rikagaku Kikai); synthetic bradykinin (Protein Research Foundation, Japan); atropine sulfate (Nakarai); mepyramine maleate (Sigma); methysergide hydrogenmaleinate (Sandoz); aprotinin (Bayer). Stem bromelain, a Plasma KGN depletor,¹⁴⁾ dissolved in saline was administered intravenously from dorsal penal vein at a dose of 3 mg/kg. YS980 suspended in 0.5% tragacanth was administered orally at a dose of 1 mg/kg, which gave a maximum potentiation of carrageenan edema.⁷⁾ Partially purified KGN dissolved in 0.1 ml saline was injected to the subplantar region of the right hind paw.

Paw Edema — Paw edema was produced in male Wistar rats, 170–210 g, by injecting 1 mg of lambda carrageenan dissolved in 0.1 ml of saline at the subplantar region of the right hind paw. The paw volume was measured by water displacement, and the increase in the volume caused by carrageenan was expressed as percent edema relative to the normal paw volume.

Kininogen Assay — The total KGN level and high molecular weight (HMW) KGN level in rat plasma were determined by the method of Diniz *et al.*¹⁵⁾ (trypsin incubation) and Uchida *et al.*¹⁶⁾ (glass powder incubation), respectively. The liberated kinin was assayed against synthetic bradykinin on the contractile response of isolated guinea pig ileum suspended in Tyrode solution at 30°C aerated with a mixture of 5% CO₂ in oxygen and containing atropine sulfate, mepyramine maleate and methysergide hydrogenmaleinate (0.1 µg/ml each). The concentration of plasma KGN was expressed as bradykinin eq/ml plasma.

Partial Purification of Kininogen — Partial purification of rat plasma KGN was performed by a modification of the method of Yano *et al.*¹⁷⁾ Citrated rat plasma (64 ml : liberated 3.2×10^{-8} g bradykinin eq/mg protein), containing 10000

KIE aprotinin to avoid kallikrein activation, was precipitated between 30 and 50% saturation of ammonium sulfate, and the precipitates were dialyzed against water for 48 h at 4°C. To the dialyzed fraction, zinc acetate solution was added to make a final concentration of 20 mM. The precipitates were centrifuged and the supernatant was dialyzed against water for 72 h at 4°C. The dialyzate was heated at 60°C for 3 h in order to inactivate enzymes such as prekallikrein and other heat labile substances related to inflammations, and the supernatant was lyophilized to the 110 mg of white powder. Protein content was 0.93 mg/mg powder by Lowry's method.¹⁸⁾

Bradykinin releasing ability was estimated using trypsin incubation¹⁵⁾ of the pH 7.6 solution of partially purified KGN on the isolated guinea pig ileum. One milligram of partially purified KGN liberated 4.4×10^{-7} g bradykinin eq ($=4.7 \times 10^{-7}$ g bradykinin eq/mg protein).

RESULTS

Effect of Bromelain on Edema Potentiation and Plasma KGN Level

Carrageenan edema was potentiated by YS980 (1 mg/kg) orally administered 0.5 h before carrageenan injection (Fig. 1A). Carrageenan edema was produced 3, 24 and 48 h after the intravenous administration of bromelain. Intravenous administration of bromelain 3 h before carrageenan suppressed the carrageenan edema and the potentiative effect of YS980 (Fig. 1B). Bromelain reduced also total KGN and HMW KGN level in plasma (Fig. 2). Although the plasma KGN level and carrageenan edema formation recovered to normal within 24 h after administration of bromelain, the potentiation by YS980 remained suppressed (Fig. 1C, 2) and it required 48 h for recovery (Fig. 1D).

Restoration of Potentiation by Partially Purified KGN

Partially purified KGN from rat plasma did not produce any edema by subplantar injection at a dose of 0.01 to 1 mg. As shown in Fig. 3A, 0.1 mg of partially purified KGN gave no influence either on the normal carrageenan edema

or the edema potentiated by YS980. Carrageenan edema was produced 3, 24 and 48 h after intravenous administration of bromelain in the presence of partially purified KGN by subplantar injection of 0.1 mg 2 h before carrageenan injection. At 24 h after the administration of bromelain, partially purified KGN restored the suppressed potentiation of edema to normal (Fig. 3C). On the other hand, no restoration was observed 3 h after bromelain administration (Fig. 3B). At 48 h after bromelain, partially purified KGN did not affect the recovered potentiation (Fig. 3D).

DISCUSSION

Di Rosa *et al.*³⁾ and Katori *et al.*⁵⁾ showed that cellulose sulfate and bromelain produced depletion of plasma KGN, which resulted in suppression of carrageenan inflammations. Present study revealed that intravenous administration of bromelain at 3 mg/kg produced both suppres-

sion of edema formation and reduction of plasma KGN (total and HMW KGN) by 3 h pre-treatment. The potentiation by YS980 of the carrageenan edema was also suppressed by pre-treatment with bromelain. Oh-ishi *et al.*¹⁴⁾ suggested that intravenously administered bromelain depleted the plasma HMW KGN by activating plasma kallikrein through Hageman factor activation. Bradykinin may play an important role in the development of carrageenan edema³⁾ and the edema potentiation by YS980,⁸⁾ and accordingly it is suggested that disformation of bradykinin based on the depletion of a kinin precursor leads to the suppression of carrageenan edema and the suppression of the potentiative effect of YS980. However, in the present study, a phenomenon which could not be explained on the basis of the depletion of plasma KGN alone was observed. Even after the plasma total and HMW KGN level and inflammatory response to carrageenan returned to normal 24 h after ad-

FIG. 1. Potentiative Effects of YS980 on Carrageenan Edema in Rats (A) and Suppressive Effects of Bromelain on YS980-Induced Potentiation of Edema (B, C and D)

Carrageenan (1 mg/paw) was injected (A, B, C and D) to the subplantar region of the right hind paw. Bromelain (3 mg/kg) was administered intravenously 3 h before (B), 24 h before (C) and 48 h before (D) carrageenan injection. YS980 (1 mg/kg) was administered orally 0.5 h before carrageenan injection. ○ : control, ● : YS980. Each point represents the mean $\pm$ s.e.m. of 5 experiments. a) Significantly different from control ($p < 0.01$).

ministration of bromelain, the potentiative effect of YS980 on the carrageenan edema remained suppressed. Therefore, some factor other than plasma KGN is thought to be involved in the potentiative mechanism on the carrageenan edema by YS980. Partially purified KGN completely restored the suppressed potentiation to normal by local pre-injection to the inflamed tissue. These results suggest that KGN in the tissues plays an active role in the development of potentiative effect of ACE inhibitor on the carrageenan inflammation. Partially purified KGN used in the present studies, 0.1 mg/site, did not produce any edema, gave no influence either on the normal carrageenan edema or the edema potentiated by YS980, and liberated 4.4×10^{-8} g bradykinin eq by trypsin digestion. This amount of bradykinin is considered to be sufficient to

exert various physiological effects relating to inflammation.¹⁹⁾ In addition, such restoration was not observed in the animal in which plasma KGN was reduced by the 3 h preinjection of bromelain. These findings suggest that KGN, not only in plasma but also in tissue, plays an important role in the development of potentiation of edema and support the conclusion⁸⁾ demonstrating the importance of plasma KGN in the potentiative mechanism of ACE inhibitor.

Furthermore, the present results also suggested that *i.v.* administration of bromelain presumably depletes local KGN in tissues as well as plasma KGN and that the depletion of KGN in tissues may continue for longer time than in plasma since the suppression of potentiation continued even after the recovery of plasma KGN to the normal level. Plasma KGN in rats is synthesized by liver and flows into the circulating blood.²⁾ Komiya *et al.*¹²⁾ and Horiuchi *et al.*¹³⁾ suggested from the results of purification of plasma and lung tissue KGN in bovine that plasma KGN may be stored in tissues as the tissue KGN. Therefore, there must be a time lag between the storage of tissue KGN and that of plasma KGN. Consequently, the recovery of tissue KGN to the normal level may require longer time than that of plasma KGN after the depletion of both kininogens.

There still remains the possibility that bromelain exerts certain inhibitory mechanism other than the depletion of KGN and causes the suppression of potentiative effect of ACE inhibitor, since bromelain as a thiol protease may show various actions such as degrading effect against bradykinin.²⁰⁾

In conclusion, it is suggested that local KGN in the inflamed tissues as well as plasma KGN plays an active role in the development of potentiation of carrageenan edema by ACE inhibitor.

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FIG. 2. Time-Course of Plasma Total and High Molecular Weight Kininogen after Intravenous Administration of Bromelain (3 mg/kg) to Rats

Blood was collected before and 3, 24 and 48 h after bromelain injection. Carrageenan (1 mg/paw) was injected to the subplantar region of the right hind paw 1 h before blood collection. Plasma kininogen content is expressed as μg bradykinin eq/ml plasma. ● : Total kininogen, ○ : high molecular weight kininogen. Each point represents the mean $\pm$ s.e.m. of 4 to 5 experiments. a) Significantly different from 0 time value ($p < 0.001$).

FIG. 3. Effects of Partially Purified Kininogen on Carrageenan Edema and YS980-Induced Potentiation of Edema in Rats with (B, C and D) or without (A) Bromelain

Carrageenan (1 mg/paw) was injected (A, B, C, and D) to the subplantar region of the right hind paw. Partially purified kininogen (0.1 mg/paw) was injected (A, B, C and D) to the same region 2 h before the injection of carrageenan. Bromelain (3 mg/kg) was administered intravenously 3 h before (B), 24 h before (C) and 48 h before (D) carrageenan injection. YS980 (1 mg/kg) was administered orally 0.5 h before carrageenan. ○ : Control, ● : YS980. Each point represents the mean $\pm$ s.e.m. of 5 experiments. a) Significantly different from control ($p < 0.01$).

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