J. Pharm. Pharmacol. 1982, 34: 60–61
Communicated April 10, 1981

0022–3573/82/010060–02 $02.50/0


# Potentiative effects of a kininase II inhibitor (YS980) on carrageenan-induced oedema in rat hind paw and roles of plasma kininogen

H. SUDA*, H. YAMAUCHI, T. ISO, *Research Laboratory, Santen Pharmaceutical Co., Ltd., Shimoshinjo, Higashiyodogawaku, Osaka 533, Japan*

Bradykinin is formed from high molecular weight kininogen in plasma through activation of a kinin-forming enzyme, kallikrein, and is inactivated by kinin-degradating enzymes, kininase I and II. The peptide is believed to be one of important chemical mediators in carrageenan-induced inflammation. The inflammation is suppressed by cellulose sulphate and bromelain which deplete plasma kininogen (Di Rosa et al 1971; Katori et al 1978). Recently, we reported carrageenan oedema to be potentiated by an orally active kininase II (EC 3.4.15.1) inhibitor, (4*R*)-3-[(2*S*)-3-mercapto-2-methyl-propanoyl]-4-thiazolidinecarboxylic acid (YS980), and suggested that its potentiation of carrageenan oedema is due to its inhibitory action on kininase II in the inflamed site (Iso et al 1978).

We now report on the role of plasma kininogen in the development of potentiative effects of YS980 on carrageenan-induced oedema in rat hind paw.

## *Materials and methods*

Materials used were: YS980 (synthetized at Santen Pharm, Co., Ltd.); stem bromelain (Jintan Dolf, 888 U $mg^{-1}$); trypsin (Difco); synthetic bradykinin (Protein Research Foundation, Japan); atropine sulphate (Nakarai); mepyramine maleate (Sigma); methysergide hydrogenmaleinate (Sandoz); λ-carrageenan (Pasco). YS980 suspended in 0·5% tragacanth was administered orally at a dose of 1 mg $kg^{-1}$. Stem bromelain dissolved in 0·9% NaCl (saline) was administered intravenously at a dose of 10 mg $kg^{-1}$.

* Correspondence.

*Foot paw oedema.* Foot paw oedema was produced by injecting 1 mg of carrageenan dissolved in 0·1 ml saline into the subplantar region of the hind paw of male Wistar rats, 190–220 g. The paw volume was measured by water displacement, and the increase in the volume caused by carrageenan was expressed as per cent oedema relative to the normal paw volume.

*Kininogen assay.* The kininogen level of rat plasma was determined by the method of Diniz & Carvalho (1963). The liberated kinin was assayed against bradykinin on the guinea-pig isolated ileum suspended in Tyrode solution at 30 °C aerated with a mixture of 5% $CO_2$ in oxygen and containing atropine sulphate, mepyramine maleate and methysergide hydrogenmaleinate (0·1 μg per ml each). The concentration of plasma kininogen

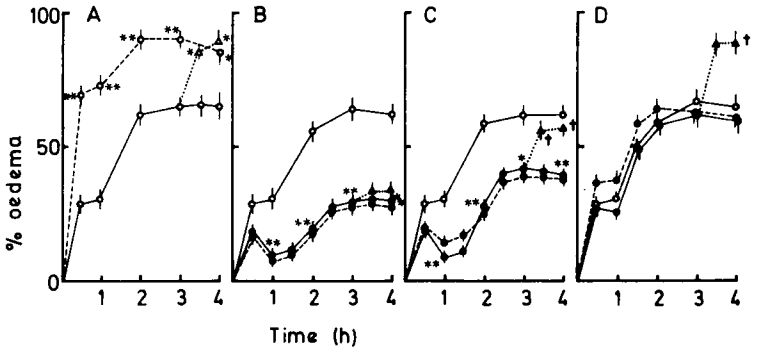


Fig. 1A. Effects of YS980 on carrageenan oedema in rats. YS980 (1 mg $kg^{-1}$) was administered orally 30 min before (- - - ○ - - -) and 3 h after (. . . . △ . . .) carrageenan injection. (—○—) control group. Each point represents the mean ± s.e.m. of 5 experiments. * Significantly different from control ($P < 0.01$). ** $P < 0.001$.

B, C and D. Suppression of oedema by bromelain and its effects on YS980-induced potentiation. Bromelain (10 mg $kg^{-1}$) was injected intravenously just before (B), 4 h before (C) and 24 h before (D) carrageenan injection. (—○—) control, (—●—) bromelain-treated group. YS980 (1 mg $kg^{-1}$) was administered orally to the bromelain-treated group 30 min before (- - - ● - - -) and 3 h after (. . . ▲ . . .) carrageenan injection. Each point represents the mean ± s.e.m. of 5 experiments. * Significantly different from control ($P < 0.01$). ** $P < 0.001$ † Significantly different from bromelain-treated group ($P < 0.01$)

was expressed as $\mu$g bradykinin equivalent $ml^{-1}$ plasma.

*Results*

Carrageenan-induced oedema in rat hind paw was potentiated by oral administration of YS980 at a dose of 1 mg $kg^{-1}$ 30 min before and 3 h after carrageenan injection (Fig. 1A). The effect was dose-dependent with a maximum at 1 mg $kg^{-1}$. Local administration of YS980 at doses of 0·3 to 10 $\mu$g into subplantar region of hind paw produced a similar response.

Intravenous administration of bromelain at 10 mg $kg^{-1}$ rapidly produced both suppression of oedema formation and reduction of plasma kininogen in rats, both effects disappearing within 24 h (Figs 1, 2). The potentiation by YS980 of the early and latter phases of carrageenan oedema was also suppressed by pretreatment with bromelain; the suppression at the early phase appeared rapidly after administration of bromelain (Fig. 1B) and lasted for more than 24 h (Fig. 1D), while the suppression of the latter phase disappeared within 24 h (Fig. 1C, 1D).

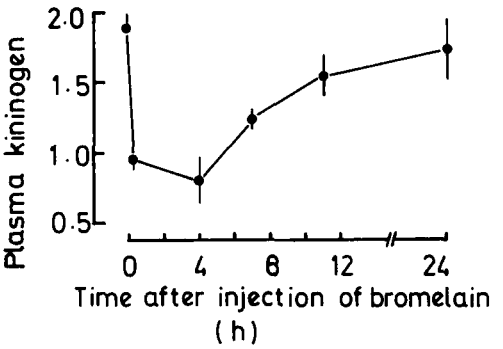


FIG. 2. Time-course of plasma kininogen after intravenous injection of bromelain (10 mg $kg^{-1}$) to rats. Blood was collected after decapitation, before and 0·25, 4, 7, 11 and 24 h after bromelain injection. Kininogen was estimated using trypsin incubation (Dinitz & Carvalho 1963) of the plasma from individual rats. Results are expressed as $\mu$g bradykinin equivalent $ml^{-1}$ plasma (ordinate). Each point represents the mean $\pm$ s.e.m. of 5 experiments. * Significantly different from 0 time value ($P < 0{\cdot}001$)

*Discussion*

Di Rosa et al (1971) have divided a process of carrageenan-induced rat paw oedema to three phases: an early phase (0 to 1·5 h), a second phase (1·5 to 2·5 h) and latter phase (after 2·5 h) and have suggested that bradykinin is released transiently only in the second phase. On the other hand, it has been reported that bradykinin is formed during all of the three phases of the carrageenan inflammation (Ferreira et al 1974; Bonta et al 1976). The present study revealed that YS980, a potent kininase II inhibitor, potentiates the inflammation at all three phases, and the effects are suppressed by intravenous administration of bromelain just before carrageenan injection. From these findings, it is suggested that bradykinin may play an active role in the development of potentiative effects of YS980 on the carrageenan inflammation at all the phases.

However, there was some difference in quality between two potentiative phenomena observed at the early and latter phases. At 24 h after administration of bromelain, the plasma kininogen level and inflammatory response to carrageenan had returned to normal, and the potentiative effect of YS980 on the latter phase of the inflammation had completely recovered. However, the potentiation at the early phase remained suppressed. Thus, plasma kininogen is thought to play a role in the development of the potentiative effect of YS980 at the latter phase of the carrageenan inflammation, but not at the early phase. Since it has been reported that both kininogen and kallikrein exist not only in plasma but also in various tissues including dermal tissue of rats (Sardesai 1968; Werle & Zach 1970), tissue kininogen might participate in the development of potentiative effect of YS980 at the early phase of carrageenan-induced oedema in rat hind paw. There remains the possibility that some pharmacological action of YS980 other than its inhibitory effect on kininase II may be involved in the potentiative mechanism on carrageenan-induced inflammation.

## REFERENCES

Bonta, I. L., Bult, H., Ven, L. L. M., Noordhoek, J. (1976) Agents Actions 6: 154–158

Diniz, C. R., Carvalho, I. F. (1963) Ann. N.Y. Acad. Sci. 104: 77–89

Di Rosa, M., Giroud, J.P., Willoughby, D. A. (1971) J. Pathol. 104: 15–29

Ferreira, S. H., Moncada, S., Parsons, M., Vane, J. R. (1974) Br. J. Pharmacol. 52: 108p–109p

Iso, T., Yamauchi, H., Suda, H., Nakajima, N., Nishimura, K., Iwao, J. (1978) Experientia 34: 1202–1203

Katori, M., Ikeda, K., Harada, Y., Uchida, Y., Tanaka, K., Oh-ishi, S. (1978) Agents Actions 8: 108–112

Sardesai, V. M. (1968) Can. J. Physiol. Pharmacol. 46: 77–79

Werle, E., Zach, P. (1970) Z. Klin. Chem. u. Klin. Biochem. 8: 186–189