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Inhibition of Rat Embryo Histidine Decarboxylase by Epoxyquinomicins

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Epoxyquinomicins A, B, C and D were first isolated from *Amycolatopsis* sp. MK299-95F4 as weak antibiotics (Fig. 1)[1]. Subsequent studies on their biological activities revealed that these compounds inhibited type II collagen-induced arthritis *in vivo*[2]. Epoxyquinomicins showed low acute toxicity in mice by intraperitoneal administration with the LD_{50} values of 25, 50, 100, and 100 mg/kg for epoxyquinomicins A, B, C, and D, respectively. Therefore, these epoxyquinomicins are unique candidates for anti-inflammatory agents. The mode of action appears to be different from that of common NSAIDs (non-steroidal anti-inflammatory drugs)[3], since epoxyquinomicins C and D did not inhibit cyclooxygenase-1 at the concentration of 300 μM. The mechanism of the antiarthritic effect has not been elucidated for epoxyquinomicins.

Histidine decarboxylase is considered to be involved in the mechanism of inflammation by producing histamine. Lecanoric acid was isolated from a fungus as a histidine decarboxylase inhibitor[4]. A synthetic analogue, SD-170 (Fig. 2), with a peptide bond in place of the ester bond of lecanoric acid, was 150 times stronger than lecanoric acid in the inhibition of histidine decarboxylase[5]. SD-170 inhibited delayed-type hypersensitivity in mice[5] and phorbol ester-induced tumor promotion in mice[6]. In view of the structural similarity between epoxyquinomicins and peptide-type lecanoric acid analogues, we studied the inhibition of histidine decarboxylase[7~9] by epoxyquinomicins.

As shown in Fig. 3, all of epoxyquinomicins tested concentration-dependently inhibited rat embryo histidine decarboxylase. Among them, epoxyquinomicins B and C showed potent inhibitions with IC_{50}s of 0.003 and 0.37 μM, respectively. Especially, epoxyquinomicin B was stronger than the positive control SD-170 (IC_{50}, 0.069 μM). On the other hand, epoxyquinomicins A and D, which have a chlorine atom in the salicyl group, showed relatively weak inhibitions with IC_{50}s of 6.9 and 25 μM, respectively, suggesting a chlorine atom decreases the inhibitory activity.

Nakamura *et al.* demonstrated that elevated histidine decarboxylase activity exacerbated the arthritis by increasing the number of granulocytes, macrophages and osteoclasts[10]. A histidine decarboxylase inhibitor, hypostamine had a therapeutic effect on adjuvant-induced

Fig. 2. Structure of SD-170.

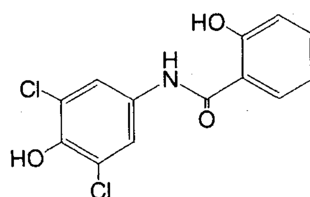


Fig. 1. Structures of epoxyquinomicins.

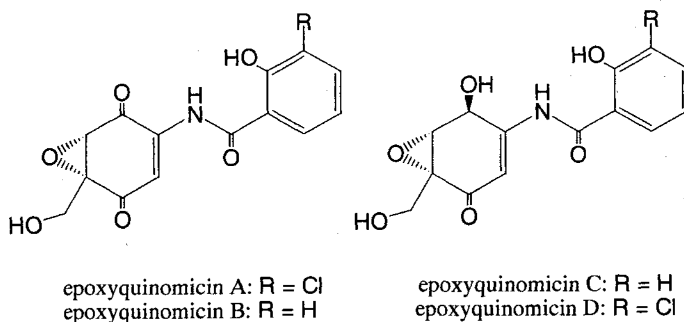
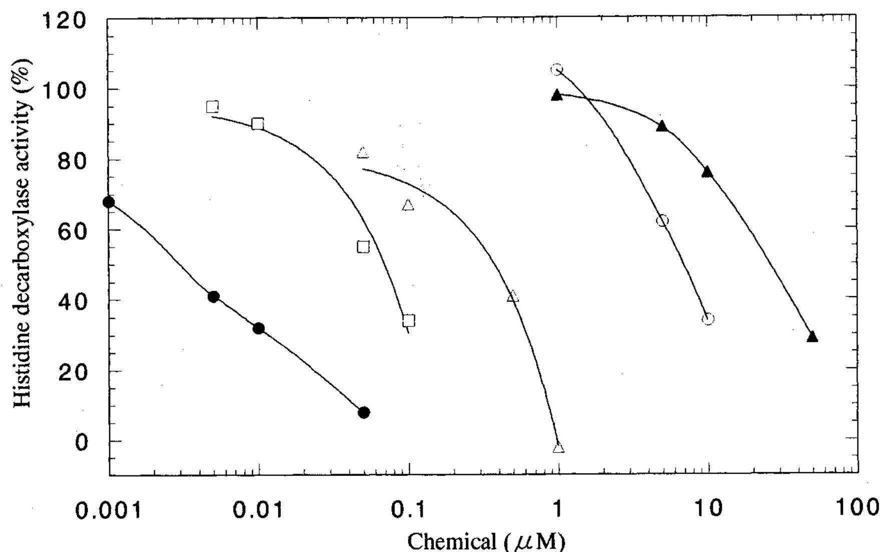


Fig. 3. Inhibition of rat embryo histidine decarboxylase by epoxyquinomicins.

○: epoxyquinomicin A, ●: epoxyquinomicin B, △: epoxyquinomicin C, ▲: epoxyquinomicin D, □: SD-170



arthritis[11]. Therefore, the antiarthritic effect of epoxyquinomicins *in vivo* may be partly due to the inhibition of histidine decarboxylase.

Thus, epoxyquinomicins B and C are new naturally occurring histidine decarboxylase inhibitors.

Experimental

Preparation of Histidine Decarboxylase

A crude fraction containing histidine decarboxylase was prepared from fetal rats as described by WATANABE *et al.*[7]. Pregnant rats (purchased from Charles River Japan, Inc.) were decapitated 16 days after mating, and their litters were collected. Whole fetal rats (85 g) were homogenized for 2 minutes at 0°C in 170 ml of 0.1 M sodium acetate buffer (pH 5.4) containing 0.01 mM pyridoxal phosphate, 0.2 mM dithiothreitol, and 1% polyethylene glycol. The homogenate was then centrifuged at 8,000 rpm for 40 minutes at 0°C. Ammonium sulfate (60 g) was added to the supernatant, which was thereafter kept at 0°C for 15 minutes. The precipitate obtained by centrifugation at 8,000 rpm for 20 minutes was dissolved in 50 ml of 0.02 M potassium phosphate buffer (pH 7.0) containing 0.01 mM pyridoxal phosphate, 0.2 mM dithiothreitol, and 1% polyethylene glycol, and dialyzed against 500 ml of the same potassium phosphate buffer overnight at 7°C. The crude histidine decarboxylase preparation thus obtained was used for enzyme reaction.

Histidine Decarboxylase Assay

Histidine decarboxylase assay was carried out, as also described by WATANABE *et al.*[7]. The reaction mixture contained 100 μl of 0.25 mM histidine, 100 μl of the above enzyme preparation, and inhibitor in 1 ml of 0.2 M potassium phosphate buffer (pH 6.8) containing 0.02 mM pyridoxal phosphate, 0.4 mM dithiothreitol, and 2% polyethylene glycol. The mixture was incubated at 37°C for 5 hours, after which 1 ml of 1 N NaOH was added. The mixture was saturated with solid NaCl, and shaken with 2 ml of *n*-butanol; and then after centrifugation, the organic phase was extracted with 1 ml of 0.1 N HCl. The extract was finally washed with 1 ml of *n*-heptane. The histamine content in the acid extract was estimated by the method of SHORE[8]. A 80 μl volume of 10 N NaOH was added to the HCl whole extract, followed by 66 μl of 1% *o*-phthalaldehyde-methanol solution (OPT reagent). After 5 minutes, the mixture was acidified with 150 μl of 3.5 N H_3PO_4. An aliquot (200 μl) was then transferred to a 96-well microplate, and the fluorescence at 460 nm resulting from excitation of 360 nm was measured with a CytoFluor 2350 multiwell fluorescence plate reader. The calibration curve showed linear correlation between the fluorescence values and 0~40 μM histamine. The extraction efficiency was 76%.

References

1) MATSUMOTO, N.; T. TSUCHIDA, M. UMEKITA, N. KINOSHITA, H. IINUMA, T. SAWA, M. HAMADA & T. TAKEUCHI: Epoxyquinomicins, new antibiotics from *Amycolatopsis*. I. Taxonomy, fermentation, isolation and antimicrobial activities. J. Antibiotics 50: 900~905, 1997
2) MATSUMOTO, N.; H. IINUMA, T. SAWA, T. TAKEUCHI, S. HIRANO, T. YOSHIOKA & M. ISHIZUKA: Epoxyquinomicins, new antibiotics from *Amycolatopsis*. II. Effect on type II collagen-induced arthritis in mice. J. Antibiotics 50: 906~911, 1997
3) WALLACE, J. L.: Mechanisms of nonsteroidal anti-inflammatory drug (NSAID) induced gastrointestinal damage—potential for development of gastrointestinal tract safe NSAIDs. Can. J. Physiol. Pharmacol. 72: 1493~1498, 1994
4) UMEZAWA, H.; N. SHIBAMOTO, H. NAGANAWA, S. AYUKAWA, M. MATSUZAKI & T. TAKEUCHI: Isolation of lecanoric acid, an inhibitor of histidine decarboxylase from a fungus. J. Antibiotics 27: 587~596, 1974
5) UMEZAWA, K.; S. MURAMATSU, M. ISHIZUKA, T. SAWA, T. TAKEUCHI & T. MATSUSHIMA: Inhibition of histidine decarboxylase and tumour promoter-induced arachidonic acid release by lecanoric acid analogues. Biochem. Biophys. Res. Commun. 110: 733~739, 1983
6) UMEZAWA, K.; T. MATSUSHIMA, T. SAWA, T. TAKEUCHI & I. HIRONO: Inhibition of tumor promotion by a lecanoric acid analogue. Experientia 40: 100~101, 1984
7) WATANEBE, T.; H. NAKAMURA, L. Y. LIANG, A. YAMATODANI & H. WADA: Partial purification and characterization of L-histidine decarboxylase from fetal rats. Biochem. Pharmacol. 28: 1149~1155, 1979
8) SHORE, P. A.; A. BURKHALTER & V. H. COHN: A method for the fluorometric assay of histamine in tissues. J. Pharmacol. Exp. Ther. 127: 182~186, 1959
9) ENDO, Y.: A method for the assay of histidine decarboxylase activity. Folia Pharmacol. Jpn. (Nippon Yakurigaku Zasshi) 112: 307~314, 1998
10) NAKAMURA, M.; T. ANDO, M. ABE, K. KUMAGAI & Y. ENDO: Contrast between effects of aminobisphosphonates and nonaminobisphosphonates on collagen-induced arthritis in mice. Br. J. Pharmacol. 119: 205~212, 1996
11) PELCZARSKA, A.: Treatment of adjuvant arthritis in rats with the histidine decarboxylase inhibitor hypostamine. J. Pharm. Pharmacol. 21: 692~693, 1969