Inhibitory Effects of Copiamycin A, a Macrocyclic Lactone Antibiotic, on Gastric H⁺,K⁺-ATPase, Acid Secretion and Ulcer Formation

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ABSTRACT—When 17 macrocyclic lactone antibiotics were examined for their abilities to inhibit gastric H⁺,K⁺-ATPase, copiamycin A was found to have the strongest and relatively specific activity with IC₅₀s of 15.7 µg/ml and > 100 µg/ml against the hog gastric H⁺,K⁺-ATPase and the dog kidney Na⁺,K⁺-ATPase, respectively. Furthermore, this antibiotic inhibited the histamine-induced gastric acid secretion in the isolated gastric mucosal membrane of guinea pigs and the gastric ulcer formation in pylorus-ligated rats.

It has been demonstrated that recently developed peptic ulcer therapeutics such as substituted benzimidazoles can effectively reduce the gastric acidity by selective inhibition of gastric H⁺,K⁺-ATPase, which plays a major role in gastric acid secretion (1).

In our search for inhibitory activity of the gastric H⁺,K⁺-ATPase among microbial metabolites, copiamycin A, well-known as an antifungal antibiotic (2), has been isolated as an active one from the culture broth of Streptomycetes hygroscopicus sp. M931-1, which was isolated from a soil sample collected in Kanagawa Prefecture, Japan. Therefore, we comparatively tested copiamycin A and 16 macrocyclic lactone antibiotics against H⁺,K⁺-ATPase. In this experiment, the gastric microsomal membrane was used as an H⁺,K⁺-ATPase enzyme fraction, which was prepared from the hog stomach according to the method of Saccomani et al. (3) with a minor modification. Briefly, the fundic mucosa scraped from the muscular layer of the hog stomach with a spatula was suspended in about 10 volumes of a cold solution (I) of 250 mM sucrose, 20 mM Tris-HCl buffer (pH 7.4), 0.2 mM phenylmethylsulfonylfluoride (Sigma) and 0.1% mercaptoethanol (Sigma) and then homogenized with a Teflon-glass homogenizer. The resulting homogenate was centrifuged at 12,000 × g for 15 min. The supernatant was collected and recentrifuged at 100,000 × g for 60 min at 5°C. The pellets were re-suspended in a cold solution (I), homogenized gently with a Teflon-glass homogenizer, layered carefully on a 37% (W/V) sucrose cushion in solution (I) and then centrifuged overnight at 100,000 × g at 5°C. The broad interface, containing the membranes with the ATPase, was carefully collected and then directly used in this experiment.

ATPase inhibition was assayed as follows: Because the hog gastric H⁺,K⁺-ATPase prepared above and the dog kidney Na⁺,K⁺-ATPase purchased from Sigma Chemical Co. are able to hydrolyze p-nitrophenylphosphate (pNPP, Sigma) as well as ATP, pNPP was used as the enzyme substrate. Reaction mixture (250 µl) containing appropriate amounts of enzymes, test compound and tris(hydrox-
(R)-2-(4-methyl)aminomethane (Wako) of 9.35 μmole (pH 7.4) was pre-incubated for 3 hr at 37°C. The enzyme reaction was started by adding 10 μl of 100 mM pNPP, carried out for 30 min at 37°C and then stopped by adding 20 μl of 1 M NaH₂PO₄ - 200 mM EDTA. The amount of p-nitrophenol released by the enzyme reaction was determined calorimetrically at 405 nm.

In vitro anti-gastric acid secretory activity was evaluated in the isolated guinea pig gastric mucosal preparation according to the method of Barnett et al. (4) with a minor modification. Male guinea pigs weighing 250 to 350 g were fasted for 48 hr with water ad libitum, killed and then the stomachs were quickly excised. The outer muscle layer of the stomach was removed by blistering with a slow continuous stream of air through a 24-gauge needle until a section of 2 to 3 cm in diameter could be stripped. This tissue was then placed over the end of a plastic tube with the mucosal side facing inward (1.33 cm² surface area exposed). Each preparation was placed into an organ bath containing 50 ml of serosal aqueous buffer solution (II) of the following ionic composition: 143 mM Na⁺, 5.9 mM K⁺, 2.5 mM Ca²⁺, 1.2 mM Mg²⁺, 149 mM Cl⁻, 1.2 mM SO₄⁻⁻, and 16 mM glucose, which was continuously aerated with 100% O₂. Two milliliters of solution (II) with or without the test compound was added to the mucosal chamber. The mucosal solution was collected and replenished at a 30 min-interval. The acidity of 1 ml of the mucosal solution was titrated with 0.0001 N NaOH to pH 7.0 (phenolphthalein method). The titrable acid is expressed as μEq H⁺/cm²/30 min.

In vivo anti-gastric ulcer activity was evaluated in the Shay rat model (5). The abdomen of each male Wistar rat (280–350 g), which had been fasted for 24 hr, was incised; and the pylorus was ligated under ether anesthesia. Ten hours later, the animals were killed, and the forestomach was examined for ulcer formation. The test compound suspended in 0.5% carboxymethyl cellulose (Iwai Kagaku) were given i.p. in a volume of 0.5 ml/100 g body weight soon after the pylorus-ligation. One milliliter of 1% Brilliant Blue 6B (Tokyo Kasei) was given i.v. 10 min before killing the animal to stain the ulcerated area. The number of ulcers was counted and arbitrarily classified into five stages: 0 = no lesion, 1 = one to three small ulcers (3 mm or smaller), 2 = more than three small ulcers or large ulcer, 3 = one large and several small ulcers, 4 = severe large ulcers, and 5 = perforated ulcers.

Among the 17 macrocyclic lactone antibiotics listed in Table 1, copiamycin A, azalomycins B and F, and scopafungin were found to markedly inhibit the gastric H⁺,K⁺-ATPase with IC₅₀s of 15.7, 42.6, 16.4 and 35.9 μg/ml, respectively. Their inhibitory effects were more potent on the hog gastric H⁺,K⁺-ATPase than on the dog kidney Na⁺,K⁺-ATPase. The other antibiotics including 14-ring macrolides such as erythromycin and oleandomycin and polyene macrolides such as nystatin, amphotericin B and rifamycin S did not inhibit the hog gastric H⁺,K⁺-ATPase even at 100 μg/ml. Among the active compounds, the inhibitory potency of copiamycin A was found to be highest and comparable to that of omeprazole (6, 7) and SCH-28080 (8), both specific inhibitors of the gastric H⁺,K⁺-ATPase in vitro and in vivo. These results suggested that copiamycin A and other antibiotics of the azalomycin group have properties to reduce gastric acid secretion from the gastric parietal cells by inhibition of the gastric H⁺,K⁺-ATPase. To confirm the above possibility, the effect of copiamycin A on the histamine-induced gastric acid secretion was examined in the isolated guinea pig gastric mucosal preparations, which have been frequently used in physiological and pharmacological studies on the histamine-associated gastric acid secretion due to lack of complications induced by vascular, neuronal and hormonal influences. When 1 mM histamine was added, the rate of the acid output markedly increased and reached a plateau of around 2 μEq H⁺/cm²/30 min, which was approx. 2-fold elevation of the basal acid output, and then this level was maintained for at least 120 min.
Table 1. Effects of copiamycin A, 16 related macrocyclic lactone antibiotics, omeprazole, SCH-28080 and ouabain on the hog gastric H⁺,K⁺-ATPase and the dog kidney Na⁺,K⁺-ATPase

| Compound                     | IC₅₀ (µg/ml) | H⁺,K⁺-ATPase (A) | Na⁺,K⁺-ATPase (B) | B/A |
|------------------------------|-------------|-----------------|--------------------|-----|
| Copiamycin A                 | 15.7        | > 100           | > 100              | > 6.8 |
| Azalomycin B                 | 42.6        | > 100           | > 100              | > 2.3 |
| Azalomycin F                 | 16.4        | > 50            | > 3.1              |
| Scopafungin                 | 35.9        | > 100           | > 2.8              |
| Oleandomycin                 | > 100       | NT              | NT                 |
| Triacetyl oleandomycin       | > 100       | NT              | NT                 |
| Erythromycin                 | > 100       | NT              | NT                 |
| Mydecamycin                 | > 100       | NT              | NT                 |
| Josamycin                   | > 100       | NT              | NT                 |
| Leucomycin                  | > 100       | NT              | NT                 |
| Tylosin                     | > 100       | NT              | NT                 |
| Cirracycmin A               | > 100       | NT              | NT                 |
| Cirracycmin B               | > 100       | NT              | NT                 |
| Geldamycin                  | > 100       | NT              | NT                 |
| Nystatin                    | > 100       | NT              | NT                 |
| Amphotericin B              | > 100       | NT              | NT                 |
| Rifamycin S                 | > 100       | NT              | NT                 |
| Omeprazole                   | 12.3        | > 100           | > 8.1              |
| SCH-28080                   | 11.5        | > 100           | > 8.7              |
| Ouabain                      | > 100       | 15.6            | < 0.6              |

NT: Not tested. *: Prepared at Bristol-Myers Research Institute. b,c,d and f: Purchased from Shionogi Co., Squibb Japan Co., Daiichi Pharmaceutical Co. and Sigma Chemical Co., respectively. #: Prepared at Bristol-Myers Squibb Co.

Table 2. Effect of copiamycin A, SCH-28080 and atropine sulfate on the gastric ulcers induced by pylorus-ligation in rats

| Compound         | Dose (mg/kg, i.p.) | No. of rats | Ulcer index (mean ± S.E.) | Inhibition (%) |
|------------------|--------------------|-------------|--------------------------|----------------|
| Control (0.5% CMC) | –                  | 12          | 4.7 ± 0.3                | –              |
| Copiamycin A     | 100                | 5           | 1.8 ± 0.9**              | 62             |
|                  | 50                 | 5           | 2.4 ± 1.1*               | 49             |
| SCH-28080        | 20                 | 5           | 0.4 ± 0.4***             | 91             |
| Atropine sulfate | 50                 | 5           | 0.2 ± 0.2***             | 96             |
|                  | 25                 | 5           | 1.4 ± 0.9***             | 70             |

*, ** and *** indicate significant differences from the 0.5% CMC control group at P < 0.02, P < 0.01 and P < 0.001, respectively.

Copiamycin A and SCH-28080 were added 30 min after reaching a plateau of acid output. Copiamycin A significantly inhibited the histamine-stimulated acid output by 48 ± 6.6 and 109 ± 10% at 10 and 100 µg/ml, respectively; and 0.1 µg/ml of SCH-28080 completely reduced the acid output to or lower than the basal level (120 ± 10%). Each value rep-
resents the mean ± S.E. of three experiments.

As shown in Table 2, copiamycin A, given at 50 and 100 mg/kg, i.p., significantly prevented the formation of gastric ulcer in pylorus ligated rats and the inhibition ratios were 49 and 62%, respectively. Arai et al. (2) reported that mice tolerated 1,000 mg/kg, i.p., of copiamycin A without any toxic signs, suggesting that this antibiotic may have a relatively wide safety margin. In the same test, SCH-28080 also showed 91% inhibition at 20 mg/kg, i.p.

These results indicated that copiamycin A was able to reduce gastric acid secretion and to inhibit the development of acute ulcers induced in the stomach of rats, although its in vivo efficacy was much weaker than that expected by the in vitro potency of H⁺,K⁺-ATPase inhibition, when compared with that of SCH-28080. This weaker in vivo efficacy of copiamycin A might be attributable largely to its pharmacological properties such as tissue distribution and pharmacokinetics. In addition, we also consider the following possibility: in the H⁺,K⁺-ATPase experiment, pNPP was used as an enzyme substrate instead of ATP to measure inhibitor activity. As a result, it is most likely that copiamycin A is equipotent to SCH-28080 in a pNPP-assay but not in an ATP-assay. Further studies will be published elsewhere.

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