Inhibitory Effect of a Novel Anticomplementary Agent, K-76COONa, on the Release of Histamine Induced by Zymosan and Compound 48/80

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Abstract—A novel sesquiterpene anticomplementary agent, K-76COONa, if locally applied, was revealed to have an activity to prevent histamine release from rat connective tissue mast cells not only in a zymosan-induced inflammation of the air pouch type (zymosan-air-pouch inflammation) but also in compound 48/80-induced air-pouch inflammation. Moreover, K-76COONa inhibited in vitro histamine release from rat peritoneal mast cells induced by compound 48/80.

K-76COONa inhibits activation of the complement system through interference with the step for C5 (1), but it does not inhibit proteases such as trypsin and plasmin (2). We reported in a previous paper that K-76000Na dose-dependently inhibited leukocyte migration in a zymosan-induced experimental model of inflammation of the air pouch type, the zymosan-air-pouch inflammation (3). The present experiments were undertaken to collect further information on the pharmacological properties of K-76COONa by the use of the zymosan-air-pouch inflammation model as well as another experimental model of inflammation of the air pouch type induced by a histamine liberator, compound 48/80.

Zymosan-air-pouch inflammation was induced using male Sprague-Dawley rats, 180–210 g in body wt. and specific-pathogen-free, by injecting 4.0 ml of 1.6% (w/v) zymosan (Sigma Chemical Co., St. Louis, MO, U.S.A.) suspended in a solution of 0.8% (w/v) sodium carboxymethyl cellulose (CMC, Cellogen F-3H, Dai-ichi Kogyo Seiyaku Co., Kyoto, Japan) into the preformed air-pouch on the back of rats according to the procedure described already (3). Compound 48/80-induced air-pouch inflammation was induced similarly by injecting 4.0 ml of 0.8% CMC solution containing 1 μg/ml of compound 48/80 (Sigma Chemical Co., St. Louis, MO, U.S.A.). K-76COONa (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) was locally administered by dissolving it in 0.8% CMC solution containing zymosan or compound 48/80.

Plasma exudation was measured with the aid of fluorescein-labeled bovine serum albumin (F-BSA) as a tracer (4). The amounts of exuded F-BSA into the pouch fluid during the first thirty minute period after the intravenous injection of F-BSA were measured by reading its fluorescence intensity at 521 nm under excitation at 490 nm. The plasma exudation was expressed by the amounts of exuded F-BSA in terms of percent of the injected F-BSA.

Histamine was assayed by the method of von Redlich and Glick (5) with the modification of using the reagents and samples at 10 times the volume used in the original method. In a preliminary study, it was confirmed that K-76COONa at a concentration up to 4.0 mg/ml did not interfere with the histamine assay.

In vitro experiments for the release of histamine from rat peritoneal mast cells were done by the method of Atkinson et al. (6). Cells were collected from the peritoneal cavity of adult male Sprague-Dawley rats sacrificed immediately after intraperitoneal
injection of the Tyrode solution containing heparin (5 units/ml). The Tyrode solution consisting of (mM) NaCl, 137; NaHCO₃, 12; glucose, 5.6; KCl, 2.7; NaH₂PO₄, 0.4; MgCl₂, 1.0; and CaCl₂, 1.8 was adjusted to pH 7.4 before use. The cells collected were washed and suspended in the Tyrode solution from which calcium and magnesium had been omitted. The number of cells collected from one rat was sufficient for 12 samples, each consisting of 6 x 10⁵ cells and containing approximately 6 percent mast cells and 0.2 to 1.5 μg histamine. The cell suspension (0.5 ml) was then added to centrifuge tubes containing modified Tyrode solution (0.5 ml) which had two times higher concentrations of calcium and magnesium than normal Tyrode solution. K-76COONa was dissolved in the modified Tyrode solution. After equilibration (37°C, 5 min), various concentrations of compound 48/80 were added to the suspension, and then histamine secretion from the cells was allowed to proceed for a further 10 min. The histamine release was calculated according to the formula:

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\text{Histamine release} = \left( \frac{\text{Histamine released by the inducer}}{\text{Total histamine in the cells}} \right) \times 100
\]

Statistical analysis of the data was done by means of the F-test, and P values smaller than 0.05 were treated as being significant. K-76COONa has been shown to interfere with the complement system through blocking the activation of C5 (1), and it has been suggested in our previous paper to inhibit leukocyte migration in zymosan-air-pouch inflammation through blocking the generation of C5a, a chemotactic fragment of C5 (7-9). As G5a is well-known to possess anaphylatoxin activity (10-13), the present experiment was undertaken in an attempt to clarify whether or not the anti-C5 agent K-76COONa is capable of blocking the mast cell degranulation in zymosan-air-pouch inflammation.

Table 1. Effects of local application of K-76COONa on the vascular permeability and histamine level at the inflammatory site

| K-76COONa (mg/ml) | No. of rats | Pouch fluid histamine (ng/ml) | % inhibition | P | % of F-BSA injected (μg/ml) | % inhibition | P |
|------------------|-------------|-----------------------------|-------------|---|----------------------------|-------------|---|
| Zymosan-induced inflammation |
| 0                | 7           | 708±46                      | 45          | <0.005 | 3.02±0.32                  | 28          | <0.025 |
| 0.4              | 7           | 391±40                      | 45          | <0.005 | 2.17±0.30                  | 55          | <0.001 |
| 1.2              | 6           | 200±26                      | 72          | <0.001 | 1.36±0.15                  | 64          | <0.001 |
| 4.0              | 6           | 87±8                        | 88          | <0.001 | 1.09±0.14                  | 64          | <0.001 |
| Compound 48/80-induced inflammation |
| 0                | 6           | 476±54                      | 50          | 1.57±0.11 | 36                        | <0.001 |
| 0.4              | 6           | 391±49                      | 18          | N.S.   | 1.00±0.13                  | 36          | <0.001 |
| 1.2              | 6           | 141±21                      | 70          | <0.001 | 0.48±0.08                  | 69          | <0.001 |
| 4.0              | 6           | 36±6                        | 93          | <0.001 | 0.57±0.05                  | 64          | <0.001 |

Thirty minutes after the injection of 0.8% CMC solution containing compound 48/80 (1 μg/ml) or zymosan (16 mg/ml) into the air-pouch preformed on the back of rats, the amount of histamine in the pouch fluid and plasma exudation into the pouch fluid were determined as described in the text. K-76COONa dissolved in the CMC solution was applied locally together with the phlogistic agents, compound 48/80 or zymosan.

* Figures represent the mean±S.E.M.
Exudation and histamine release in compound 48/80-induced air-pouch inflammation to the same extent as in the case of zymosan-air-pouch inflammation (Table 1). As the above findings suggested a possibility that inhibitory effects of K-76COONa on the liberation of histamine is not exclusively specific to the stimulation by anaphylatoxin of mast cells, an in vitro experiment for the stimulation of mast cells by compound 48/80 was designed. Before doing the experiments with K76000Na, the dose-response relationship for the liberation of histamine from rat peritoneal mast cells by compound 48/80 was examined. The results summarized in Fig. 1A indicate that the 50% effective dose of compound 48/80 for histamine release is about 0.4 μg/ml. As shown in Fig. 1B, K76COONa was shown to be capable of inhibiting release into the medium of histamine from rat peritoneal mast cells stimulated by 0.4 μg/ml of compound 48/80 in a dose-dependent manner. It is unlikely, therefore, that the inhibitory effect of K76COONa on the histamine release in zymosan-air-pouch inflammation is derived from its anticomplementary activity. It is more likely that the drug inhibits the zymosan-stimulated histamine release from mast cells through the same mechanism as that operating in its inhibitory action on the compound 48/80-induced histamine release.

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