PHARMACOLOGICAL STUDIES ON NEWLY SYNTHESIZED ANTI-ALLERGIC AGENTS, 2-METHYL-3-PIPERIDINO-β-PROPIONAPHTONE HYDROCHLORIDE (KZ-111) AND 3-ISOBUTYRYL-2-ISOPROPYLPYRAZOLE-[1, 5-a] PYRIDINE (KC-404)

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Abstract—Effects of 2-methyl-3-piperidino-β-propionaphtone hydrochloride (KZ-111), 3-isobutyryl-2-isopropylpyrazolo-[1, 5-a]pyridine (KC-404) and FPL-55712 on experimental allergic reactions were investigated. Homologous passive cutaneous anaphylaxis (PCA) in rats was clearly inhibited by oral and intravenous administrations of KC-404 and KZ-111. FPL-55712 inhibited the PCA reaction only by intravenous injection, but not by oral administration. Maximum inhibition of the PCA reaction by KC-404 and KZ-111 was obtained by administration of these agents 2 hr prior to challenge. The immunological release of histamine from sensitized rat peritoneal mast cells was inhibited by KZ-111 at a concentration of 10^-4 g/ml. KC-404 and FPL-55712 did not inhibit the immunological release of histamine. All three compounds had no effect on the release of histamine from rat peritoneal mast cells and on the generation of SRS from rat polymorphonuclear leucocytes by calcium ionophore A23187. KC-404 and KZ-111 produced a downward displacement of the maximum without a parallel shift in LTD4 induced concentration-response curves of guinea pig ileal and tracheal smooth muscle at concentrations between 10^-6 and 10^-5 g/ml. FPL-55712 at a concentration of 10^-6 g/ml produced a parallel shift of LTD4 induced concentration-response curves to the right in both smooth muscle preparations. The 50% inhibitory concentration to the contraction by LTD4 of each of the three compounds is lower than those of other agonists, histamine, PGF2α and BaCl2. KC-404 and KZ-111 inhibited CaCl2-induced contraction in K^+ depolarized ileal smooth muscle, but FPL-55712 had no effect on the CaCl2-induced contraction. All three compounds inhibited the Schultz-Dale reaction in guinea pig trachea. Respiratory disorder in guinea pig experimental asthma was inhibited by oral administration of KC-404 and KZ-111, but not by FPL-55712.

There are evidences to indicate that slow reacting substance of anaphylaxis (SRS-A) does play a role in inducing the bronchospasm of human allergic asthma (1-3). The discovery of a specific antagonist of SRS-A would be of considerable value in the therapy of asthma. From this point of view, we investigated the effect of some compounds on SRS-A activity and reported the inhibitory effect of propionaphtone derivatives on the SRS-A induced contraction of guinea pig ileum (4). A comparison of the activities of 16 derivatives led to the conclusion that 2-methyl-3-piperidino-β-propionaphtone hydrochloride (KZ-111) was to be preferred. KZ-111 showed either a potent antagonistic
action to SRS-A or an inhibitory effect to histamine release mediated by IgE antibody. Similarly, Nishino et al. reported an antagonistic effect of 3-isobutyryl-2-isopropylpyrazolo-[1,5-a]pyridine (KC-404) against SRS-A on guinea pig ileal and tracheal muscles (5). In addition, they reported the inhibitory effect of KC-404 on the antigen induced release of SRS-A from sensitized guinea pig lung. Because of these evidences, it interested us to compare the anti-SRS-A activity and the anti-allergic action of these two compounds. In the present study, we investigated above activities of the two compounds by using synthetic leucotriene D₄ (LTD₄) and compared them to the activities of FPL-55712, which was the first compound reported to be a potential antagonist of SRS-A (6, 7).

Materials and Methods

Drugs: FPL-55712 was kindly provided by Fisons Pharmaceutical Co. Ltd., Leicestershire, U.K. KC-404 and KZ-111 were gifts from Kyorin Pharmaceutical Co. Ltd., Tokyo, Japan and Nippon Zoki Pharmaceutical Co. Ltd., Osaka, Japan, respectively. These compounds were dissolved or if not, suspended in a physiologic saline. LTD₄ was kindly given by Dr. S. Terao (Takeda Pharmaceutical Co. Ltd., Osaka, Japan).

Animals: Male Hartley guinea pigs weighing 350-400 g or male Wistar rats weighing 150 g were purchased from Shizuoka Jikken Dobutsu Kyokai, Shizuoka, Japan.

Homologous passive cutaneous anaphylaxis (PCA) in rats: The procedure was reported previously by us (8). Antiserum containing homocytotropic antibody was obtained from rats that were immunized with 2,4-dinitrophenyl-coupled ascaris extract (DNP-As) mixed with killed Bordetella pertussis according to Tada and Okumura (9). The antiserum diluted 17-fold with a physiologic saline was injected intradermally at a 0.1 ml dose in 4 sites on the shaved backs of normal rats. After 48 hr, 1 ml of 0.25% Evans blue solution containing 2.0 mg of antigen was injected into rats. Thirty minutes later, the animals were exaguinated, and the skins were removed to measure the amount of dye which had leaked into the skin. The amount of dye was then estimated colorimetrically after extraction with 1.0 N KOH and a mixture of acetone and phosphoric acid by the method of Katayama et al. (10).

Histamine release from isolated rat peritoneal mast cells: Anesthetized rats were bled by cardiac puncture. In the experiments on immunological histamine release, the rats were sensitized by the injection of antiserum containing homocytotropic antibody against DNP-As 24 hr prior to exanguination of the animals. For the isolation of mast cells, 10 ml of Tyrode solution containing 50 µg heparin was injected into the peritoneal cavity. After gentle massage of the abdomen, the peritoneal cell suspension was harvested. The cells were washed twice with Tyrode solution. The mast cell suspension, 2.7 ml, was incubated at 37°C for 20 min with 10⁻⁴ g/ml antigen or 10⁻⁶ g/ml calcium ionophore A23187 in a volume of 0.3 ml. The reaction was stopped by immersing the test tubes into an ice bath. The cells were sedimented by centrifugation at 200 g at 4°C for 10 min, and then histamine in the supernatant was quantified by the fluorometric method of May et al. (11).

SRS release from rat polymorphonuclear (PMN) leucocytes: PMN leukocytes were obtained from the peritoneal cavity of rats by the technique described by Boyden (12). Twenty-five mg of oyster glycogen were injected intraperitoneally, and the exudate was collected 18 hr later. The exudates contained over 95% of PMN leucocytes. The cells were centrifuged lightly and re-suspended in Tyrode solution at a concen-
tration of $10^7$/ml. SRS release was caused by addition of $10^{-6}$ g/ml calcium ionophore A23187 into the PMN cell suspension. The incubation was done at 37°C for 20 min. SRS activity in the supernatant was assayed using guinea pig ileum as described previously (4).

**Contractile studies:** Guinea pig tracheal spiral strip or ileal smooth muscle strip was placed in a 5 ml Magnus bath and suspended in Tyrode solution. After a 1 hr equilibration period, responses were recorded isotonically on a smoked drum using a frontal writing lever with a magnification of 3.5 and a load of 1.0 g. Cumulative concentration-response curves for the LTD$_4$ were generated for each tissue by successive increases in the bath concentration of LTD$_4$. The antagonistic effect of drugs was tested by measuring cumulative concentration-response curves for LTD$_4$ 10 min after the addition of drugs into the organ bath. In order to minimize inter-tissue variability, contractile responses were normalized by expressing them as a percentage of the maximum response obtained by reference standards: histamine ($10^{-6}$ g/ml) on the trachea and histamine ($10^{-7}$ g/ml) on the ileum. The contraction elicited by the reference standards was measured upon completion of the concentration-response curve. The 50% inhibitory concentrations of drugs to the contraction by LTD$_4$, histamine, PGF$_{2\alpha}$ and BaCl$_2$ were measured as described below. The guinea pig ileal contraction caused by LTD$_4$ ($5\times10^{-9}$ g/ml), histamine ($10^{-8}$ g/ml), PGF$_{2\alpha}$ ($5\times10^{-8}$ g/ml) and BaCl$_2$ ($2\times10^{-4}$ g/ml) was assayed; thereafter, the contraction by each agonist in the presence of several concentrations of antagonist was determined. The concentration required to reduce the contraction by half was calculated.

**Schultz-Dale reaction in guinea pig trachea:** Isolated tracheal strips were obtained from guinea pigs which had been passively sensitized with anti-benzylpenicilloyl bovine $r$-globulin (BPO-BGG) homocytotropic guinea pig serum. The animals were sensitized by injection of antiserum 48 hr prior to killing. The antiserum containing homocytotropic antibody was prepared according to the method of Levine et al. (13). A pair of tissue strips was prepared from one smooth muscle. One was used for the control, and the other one was used for testing the effect of drug. The antigen, BPO-bovine serum albumin (BSA), induced contraction was expressed as the percentage of the histamine maximal response. Response was measured for 15 min after the addition of antigen.

**Experimental asthma:** The procedure was described previously (14). In brief, experimental asthma was caused by the intravenous injection of BPO-BSA into a guinea pig which was passively sensitized with anti-BPO-BGG homocytotropic antibody previously, and the asthmatic respiration was measured by counting the changes in the rate and the volume of respiration and the ratio of expiration time to inspiration time.

**Statistics:** Results were statistically evaluated by using Student's $t$-test.

**Results**

**PCA reaction:** When each of the three agents was administered orally 1 hr prior to antigen challenge, KC-404 and KZ-111 at a dose of 100 or 200 mg/kg inhibited 48 hr homologous PCA in rats (Table 1). However, FPL-55712 did not inhibit the reaction. By the intravenous administration carried out simultaneously with antigen, each drug at a dose of 5 mg/kg showed a significant inhibition. Moreover, KC-404 and KZ-111 inhibited the reaction at a dose of 1 mg/kg. Figure 1 indicates the time courses of the inhibitory effect of the three agents on the PCA reaction (Fig. 1). KC-404 and KZ-111 inhibited the reaction by the administration at 1 or 2 hr prior to antigen challenge.

The release of histamine from isolated rat
Table 1. Effect of FPL-55712, KC-404 and KZ-111 on 48 hr homologous PCA in rats

| Treatment   | Oral Dose (mg/kg) | Oral Amount of dye (µg/site) | Intravenous Dose (mg/kg) | Intravenous Amount of dye (µg/site) |
|-------------|-------------------|------------------------------|--------------------------|-----------------------------------|
| Saline      | 8.3±0.45 (9)      |                              | 10.4±0.79 (7)            |                                   |
| FPL-55712   | 50                | 8.7±0.15 (9)                 | 0.5                      | 10.0±1.54 (7)                     |
| 100         | 8.8±0.16 (8)      | 1                            | 11.0±0.87 (6)            |                                   |
| 200         | 8.7±0.35 (8)      | 5                            | 5.5±0.84 (7)             |                                   |
| Saline      | 6.3±0.45 (6)      |                              | 6.3±0.45 (6)             |                                   |
| KC-404      | 50                | 4.9±0.88 (6)                 | 0.5                      | 6.5±0.45 (6)                      |
| 100         | 4.5±0.39 (7)      | 1                            | 4.4±0.23 (6)             |                                   |
| 200         | 4.0±0.49 (6)      | 5                            | 4.2±0.72 (6)             |                                   |
| Saline      | 8.2±1.67 (7)      |                              | 8.2±0.45 (6)             |                                   |
| KZ-111      | 50                | 8.1±0.03 (7)                 | 0.5                      | 4.9±0.88 (5)                      |
| 100         | 3.3±0.98 (7)*     | 1                            | 4.5±0.39 (6)             |                                   |
| 200         | 2.4±0.42 (7)†     | 5                            | 4.0±0.48 (6)†            |                                   |

The values represent the mean ± S.E. The number in parenthesis indicates the number of animals. Each compound was administered p.o. 1 hr prior to antigen challenge or i.v. at the same time as antigen challenge. *: P<0.05, †: P<0.01.

Fig. 1. Time course for the effect of FPL-55712, KC-404 and KZ-111 on homologous PCA in rats. Agents were administered p.o. at various times before challenge. Each point represents the mean ± S.E. of 5 to 8 experiments. (○) FPL-55712, (●) KC-404, (△) KZ-111.

Peritoneal mast cells: The release of histamine from sensitized rat peritoneal mast cells due to antigen was clearly inhibited by KZ-111 at a concentration of 10^-4 g/ml (Table 2). FPL-55712 and KC-404, however, did not inhibit the immunological release of histamine. In addition, all three compounds had no effect on the release of histamine caused by calcium ionophore A23187.

SRS release: The release of SRS from rat peritoneal PMN leukocytes caused by calcium ionophore A23187 was not influenced significantly by any of the three compounds tested. (Table 3).

Contractile studies: Incubation of tracheal or ileal strips with 10^-6 g/ml FPL-55712 resulted in significant displacement of LTD4 concentration-response curves to the right (Fig. 2). KC-404 and KZ-111 produced a downward displacement of the maximum without a parallel shift in both tissue strips at a concentration of 10^-6 or 10^-5 g/ml. The specificity of antagonism to LTD4 was examined in comparison to the histamine, PGF2α and BaCl2 in guinea pig ileum. (Table 4). Each of the 50% inhibitory concentrations of these three compounds against the contraction of LTD4 was lower than that of other agonists. The specificities of antagonistic
effects of KC-404 and KZ-111 to LTD₄ were relatively lower than that of FPL-55712. Figure 3 shows the effect of these compounds on CaCl₂-induced contraction of the ileum. FPL-55712 had no effect on the CaCl₂-induced contraction in K⁺-depolarized ileal

Table 2. Effect of FPL-55712, KC-404 and KZ-111 on the histamine release from rat peritoneal mast cells caused by antigen or Ca²⁺ ionophore A23187

| Treatment | Antigen (% to control) | Ca²⁺ Ionophore A23187 (% to control) |
|-----------|------------------------|-------------------------------------|
| Saline    | 100                    | 100                                 |
| FPL-55712 | 10⁻⁵ 85.6±5.83          | 108.2±5.63                          |
|           | 10⁻⁴ 90.6±6.17          | 109.3±10.90                         |
| KC-404    | 10⁻⁶ 112.1±7.99         | 111.1±11.80                         |
|           | 10⁻⁵ 96.3±7.89          | 110.6±7.83                          |
|           | 10⁻⁴ 88.9±1.68          | 93.2±3.47                           |
| KZ-111    | 10⁻⁵ 72.7±8.50          | 108.2±4.74                          |
|           | 10⁻⁴ 86.6±6.52          | 102.4±3.50                          |
|           | 10⁻³ 62.8±3.47          | 115.3±7.89                          |

The value represent the mean±S.E. of 4 to 6 experiments. Each compound was added to the reaction mixture 10 min prior to antigen challenge or the addition of calcium ionophore. Net histamine release from mast cells by antigen was 18.5% of the total histamine and that by calcium ionophore was 40.0%.

Table 3. Effect of FPL-55712, KC-404 and KZ-111 on the release of SRS from rat PMN leucocytes caused by calcium ionophore A23187

| Treatment | Concentration (g/ml) | N  | SRS (U/10⁶ PMN) |
|-----------|----------------------|----|-----------------|
| Control   |                      | 8  | 65.0±25.2       |
| FPL-55712 | 10⁻⁵ 85.2±8.0        | 7  | 63.6±13.2       |
|           | 10⁻⁴ 85.2±8.0        | 7  | 63.2±12.8       |
| KC-404    | 10⁻⁵ 85.2±8.0        | 7  | 62.9±29.0       |
|           | 10⁻⁴ 85.2±8.0        | 4  | 62.9±29.0       |
| KZ-111    | 10⁻⁵ 85.2±8.0        | 4  | 50.4±30.1       |
|           | 10⁻⁴ 85.2±8.0        | 4  | 37.8±6.4        |

The values represent the mean±S.E. Each compound was added to the reaction mixture 10 min prior to the addition of calcium ionophore.

Table 4. The 50% inhibitory concentrations of FPL-55712, KC-404 and KZ-111 against the contraction due to LTD₄, histamine, PGF₂α and Ba²⁺ in guinea pig ileum

| Treatment | LTD₄ | Histamine | PGF₂α | Ba²⁺ |
|-----------|------|-----------|-------|------|
| FPL-55712 | 2.0×10⁻⁹ (11) | 10⁻⁵ < (10) | 10⁻⁵ < (10) | 10⁻⁵ < (10) |
| KC-404    | 1.8×10⁻⁹ (8)   | 1.2×10⁻⁷ (6) | 7.3×10⁻⁸ (6) | 10⁻⁶ < (6) |
| KZ-111    | 7.6×10⁻⁹ (10)  | 6.6×10⁻⁷ (4) | 7.2×10⁻⁷ (6) | 1.5×10⁻⁶ (6) |

Each value is expressed by g/ml as the mean of 4 to 11 experiments. The number in parenthesis is the number of experiments. The concentrations of LTD₄, histamine, PGF₂α and Ba²⁺ are 5×10⁻⁹, 10⁻⁸, 5×10⁻⁶ and 2×10⁻⁴ g/ml, respectively.
Fig. 2. Effect of FPL-55712, KC-404 and KZ-111 on LTD4-induced dose-response curves in guinea pig trachea (upper position) and ileum (lower position). ○: Control, ●: 10⁻⁷ g/ml, △: 10⁻⁶ g/ml, ▲: 10⁻⁵ g/ml.

Fig. 3. Effect of FPL-55712, KC-404 and KZ-111 on CaCl₂ dose-response curves. The preparations were bathed for 60 min in Ca²⁺-free Tyrode’s solution and then immersed for 6 min in Ca²⁺-free K-Tyrode’s solution. CaCl₂ was accumulatively added to the Ca²⁺-free K-Tyrode’s solution. Each drug was added to the medium 10 min before the addition of CaCl₂. Each point represents the mean of 4 to 16 experiments. ○: Control, ●: 10⁻⁶ g/ml, △: 10⁻⁵ g/ml.

Fig. 4. Effect of FPL-55712, KC-404 and KZ-111 on the Schultz-Dale reaction in guinea pig trachea. Each drug was added to the medium 10 min before the addition of antigen (5 x 10⁻⁵ g/ml). Each point represents the mean±S.E. of 5 to 20 experiments. ○: Control, ●: 10⁻⁶ g/ml, △: 10⁻⁵ g/ml, ▲: 10⁻⁴ g/ml.

smooth muscle (Fig. 3). In contrast to FPL-55712, both KC-404 and KZ-111 inhibited the CaCl₂-induced contraction at concentrations between 10⁻⁶ to 10⁻⁵ g/ml.

Anti-asthmatic action: Figure 4 indicates the results of the Schultz-Dale reaction in guinea pig trachea (Fig. 4). In the control group, the contraction caused by antigen reached the maximum 3 min after addition of antigen and continued until 15 min. All three compounds at concentrations of 10⁻⁵ and 10⁻⁴ g/ml produced a delay in onset and decreased the duration of response to antigen. KC-404 showed a similar inhibition even at 10⁻⁶ g/ml. In experimental asthma of guinea pigs, the respiratory disorder caused by antigen was clearly inhibited by the adminis-
Fig. 5. Effect of EPL-55712, KC-404 and KZ-111 on BPO-BSA induced respiratory disorders in guinea pigs passively sensitized with anti-BPO-BGG IgE serum. Agents were given in a dose of 5 mg/kg p.o. 1 hr prior to challenge. Each point represents the mean of 4 to 8 animals. *: P<0.05, †: P<0.01.

Discussion

The present study indicated that the potencies and the modes of the anti-allergic actions of KC-404 and KZ-111 are very similar to each other. In the experiments of 48 hr homologous PCA in rats, KC-404 and KZ-111 inhibited the reaction clearly by either oral or intravenous administration. FPL-55712 showed the efficacy only when administered intravenously. In the case of oral administration, tests of the above two
compounds showed inhibition by the administration at 1 or 2 hr prior to challenge with antigen, however, FPL-55712 did not show inhibition by the administration at any time. The most significant difference of KC-404 and KZ-111 from FPL-55712 is the effectiveness when administered orally. This may be one of the beneficial characteristics of these two compounds if they are planned to be applied as a remedy for human disease.

Concerning the mode of the anti-allergic action of KC-404 and KZ-111, it seems to be occurred by the interference of SRS activity in both cases, because both compounds showed a potent and relatively specific antagonism to LTD₄ and did not show the inhibition of histamine release, except for a high concentration of KZ-111. As for the release of SRS, Nishino et al. reported that KC-404 inhibited SRS-A release from sensitized guinea pig lung (5). In the present study, however, the release of SRS by calcium ionophore A23187 was not inhibited by KC-404 as in the case of FPL-55712. This difference might be based on the difference in experimental conditions, for example, species of animals or the kind of eliciting agents. In addition to the above evidences, the antagonistic action of the two compounds against SRS was clearly demonstrated in the experiments of the Schultz-Dale reaction in guinea pig trachea. According to Adams and Lichtenstein (15), the inhibitory patterns of the Schultz-Dale reaction by diphenhydramine and FPL-55712 were different. Whereas diphenhydramine inhibited the initial rates of contraction but not the duration of response, FPL-55712 inhibited both of them. From the present results, the inhibitory patterns of the Schultz-Dale reaction by KC-404 and KZ-111 are similar to that of FPL-55712. These findings confirm that the main mechanisms of the anti-allergic action of these two compounds are closely related to the antagonistic action against SRS.

Regarding the antagonistic action of the two compounds against LTD₄, KC-404 and KZ-111 showed inhibition of the LTD₄-induced contraction of smooth muscle in a non-competitive manner. Additionally, both of them showed a clear inhibition of the contraction induced by CaCl₂ in K⁺ depolarized guinea pig ileum. These evidences suggest the participation of calcium antagonistic action in the inhibitory mechanisms of the two compounds against LTD₄-induced smooth muscle contraction. However, the calcium antagonistic action would not play an important role in the release of histamine because all three compounds did not inhibit the histamine release induced by calcium ionophore from rat mast cells. The present evidences suggest the different role of calcium ion between the release of histamine and the contraction of smooth muscle. To elucidate the precise mechanisms of antagonistic action, further experiments are necessary.

As for the anti-asthmatic action, both KC-404 and KZ-111 caused the remission of asthmatic respiratory disorder when administered orally in guinea pigs. The present results confirmed the efficacy of these drugs on experimental asthma reported by us or Nishino et al.

FPL-55712, however, did not inhibit the respiratory obstruction in guinea pig experimental asthma; this would be result from a rapid inactivation of FPL-55712 in the blood.

In conclusion, both KC-404 and KZ-111 showed a clear anti-allergic action. This action is mainly due to their antagonistic action against SRS. These evidences and the results from experimental asthma promote the possibility for the application of these two compounds as a remedy for asthma by oral administration.
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