Effect of (±)-2-[p-(2-Thenoyl) Phenyl] Propionic Acid (Suprofen) on Experimental Allergic Reactions

Fusayuki YOKOYA, Tamaki NAKAYAMA, Kenji SAKAMOTO, Kazuo OHHIRA, Yasuhiro OHSHIKA, Yukio MORI*, Kazumi TOYOSHI*, Hiroichi NAGAI* and Akihide KODA*

Pharmaceutical Research Laboratory, Taiyo Pharmaceutical Co. Ltd., Nishinoisshiki-cho, 2-181, Takayama, Gifu 506, Japan            *Gifu College of Pharmacy, 6-1, Mitahora-higashi 5-chome, Gifu 502, Japan

Accepted August 8, 1983

Abstract—The effects of (±)-2-[p-(2-thenoyl)phenyl] propionic acid (suprofen), a new anti-inflammatory agent, on experimental allergic reaction and antibody formation were examined. The action was compared with those of ketoprofen, ibuprofen, indomethacin, tranilast, chlorpheniramine, prednisolone and/or cyclophosphamide. Suprofen inhibited homologous PCA in rats, immunological histamine release from rat peritoneal mast cells and guinea pig lung tissues, Forssman cutaneous vasculitis (FCV) and the Arthus reaction in guinea pigs. The potency for inhibition of the PCA reaction was similar to that of ketoprofen and more potent than ibuprofen and tranilast. As for the release of anaphylactic mediators, suprofen was less potent than tranilast in terms of histamine release, but not the release of the slow reacting substance of anaphylaxis (SRS-A). Suprofen inhibited FCA more potently than other nonsteroidal anti-inflammatory drugs (NSAID). The inhibition of the Arthus reaction by suprofen was similar to those of other NSAID and prednisolone. Suprofen hardly affected delayed hypersensitivity in guinea pigs and antibody (IgM or IgE) formation in mice or rats.

Suprofen ((±)-2-[p-(2-thenoyl)phenyl] propionic acid) has been reported to have marked anti-inflammatory analgesic and antipyretic activities in experimental animals (1). In addition, Fujimura et al. (2) have reported that suprofen inhibits biosynthesis of prostaglandins in vivo. The basic structure of suprofen is phenylpropionic acid, so that the agent is classified as an acidic nonsteroidal anti-inflammatory drug (NSAID). Though there have been a few reports which indicate an anti-allergic action for certain kinds of NSAID (3, 4), most of the NSAIDs, especially acidic NSAIDs, are ineffective in the therapy of allergic disease. Therefore, it is considered of interest to study the effect of suprofen on allergic reactions in experimental animals.

Materials and Methods

Drugs: Suprofen was synthesized in the Taiyo Pharmaceutical Laboratories. As comparative drugs, ketoprofen (Hokuriku Pharmaceutical Co. Ltd, Tokyo), ibuprofen (Kakenyaku Pharmaceutical Co. Ltd, Tokyo), indomethacin (Sumitomo Kagaku Co. Ltd, Osaka), tranilast (Kissei Pharmaceutical Co. Ltd, Matsumoto), chlorpheniramine maleate (CPM) (Kowa Pharmaceutical Co. Ltd, Tokyo), prednisolone acetate (Takeda Pharmaceutical Co. Ltd, Osaka) and cyclophosphamide (Shionogi Pharmaceutical Co. Ltd, Osaka) were used. Drugs were dissolved in saline. If the drug was water insoluble, it was suspended in 0.5% tragacanth gum saline solution in an appropriate concentration.

Animals: Male Wistar rats weighing 150 to 200 g, male Hartley guinea pigs weighing 250 to 300 g and male ddY mice weighing 18 to 20 g were used. All animals were purchased from the animal center of Shizuoka Experimental Laboratories. Food and water were provided ad libitum.
Homologous passive cutaneous anaphylaxis (PCA) in rats: The experimental procedure was described elsewhere (5). Briefly, rat homocytotropic antibody against dinitro-phenylated ascaris extract (DNP-As) was prepared according to the method of Tada and Okumura (6). The antiserum diluted 20-fold with physiologic saline was injected intradermally in a volume of 0.1 ml into 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 intravenously into the rats. Thirty minutes later, the animals were sacrificed by exanguination, and the skins were removed to measure the PCA blueing lesion. 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. (7).

Antagonistic action against the increase of vascular permeability induced by histamine: Rats were given intradermally 5 μg histamine in a volume of 0.5 ml. Immediately after the injection, 1 ml of saline containing 2.5 mg Evans blue was injected i.v. into the tail vein. The dermal leakage of the dye was assayed 30 min later. The amount of dye was measured by the same method as described above.

Antigen induced mediator release: The experiments in rats were done according to the method of Orange et al. (8). In brief, the rats were passively sensitized by i.p. injection of anti-DNP-As homocytotropic antibody containing serum. After 24 hr, antigen in a volume of 10 ml of Tyrode solution was injected into the peritoneal cavity. Exactly 5 min later, the animals were killed by cutting the carotid arteries, and the peritoneal fluid was collected. Following gentle centrifugation at 400 g for 5 min at 4°C, the amount of histamine in the supernatant was assayed by isolated guinea pig ileum. The experiments concerning the release of histamine and the slow reacting substance of anaphylaxis (SRS-A) from sensitized guinea pig lung was carried out essentially according to the method previously described (9). Guinea pig homocytotropic antibody was made by the method of Levine et al. (10) using benzylpenicilloyl bovine γ-globulin (BPO-BGG) mixed with alum as antigen and adjuvant. respectively. Five hundred milligram of chopped lung tissue obtained from passively sensitized guinea pig was suspended in 4.0 ml Tyrode solution and warmed for 5 min at 37°C. The drug to be tested in a volume of 0.5 ml was added to the reaction mixture and incubated for a subsequent 5 min. Antigen in a volume of 0.5 ml of 0.1% BPO-BGG was added and incubated for 20 min. The amounts of histamine and SRS-A were measured by biological assay with isolated guinea pig ileum. The procedure of SRS-A assay was described elsewhere (11).

Forssman cutaneous vasculitis (FCV): FCV was carried out according to the method described previously (12). Guinea pigs were injected with 0.1 ml of rabbit anti-sheep red blood cell (SRBC) serum diluted 8-fold with physiologic saline, i.d., into their shaved backs followed by an i.v. injection of 1.0 ml of 1% Evans blue. After 1 hr, the animals were sacrificed by exanguination, and the skin was removed. The blueing spot caused by FCV was measured by the same method as used for PCA.

Arthus reaction in guinea pigs: Guinea pigs were immunized by i.m. injection of 8 mg/kg of egg albumin (Sigma). Three and 6 days after the immunization, animals received two booster injections of antigen in a dose of 40 mg/kg. Four weeks after the last injection, 0.1 ml of 0.1% antigen solution was injected i.d. into 3 sites on the shaved backs of the animals. At 1, 3, 5, 8 and 24 hr after antigen injection, the dimensions of inflamed areas were measured macroscopically.

Delayed type hypersensitivity reaction in guinea pigs: Guinea pigs were immunized with Mycobacterium butilicum (Sigma) by three injection of complete Freund's adjuvant at 3 day intervals. Five weeks after the last injection, 0.1 ml of antigen solution containing 0.2 μg/ml purified protein derivative was injected i.d. into 3 sites on the shaved backs of the animals. At 24 hr after injection, the dimensions of the inflamed areas were measured.

Antibody formation: The production of hemolytic plaque forming cell (HPFC) in mice spleen was tested by the method of Cunningham and Szenberg (13). Mice were
immunized by i.v. injection with $2 \times 10^8$ SRBC. Five days later, the animals were killed, and the spleen was expanded for preparing a single cell suspension with 150 mesh stainless sieves. One hundred $\mu$l of the single cell suspension, 450 $\mu$l of $7.5 \times 10^8$ cells/ml SRBC suspension, and 450 $\mu$l of 10% guinea pig serum were mixed, and a Cunningham chamber was filled with the mixture. After 1 hr incubation at 37°C, the number of HPFC was counted macroscopically. The production of IgE antibody in rats was tested by the method of Tada and Okumura (6) as described previously. The IgE antibody titer was measured by the 48 hr homologous PCA reaction in rats, and IgM and IgG antibody titer was measured by the passive hemagglutination test (PHA) according to the method of Avrameas et al. (14).

Statistics: Results were statistically evaluated using Student’s $t$-test.

Results

Forty-eight hr homologous PCA in rats: The effects of suprofen and other NSAID on homologous PCA in rats were compared with that of tranilast. The dye leakage caused by PCA was inhibited dose-dependently by suprofen at doses between 5 to 25 mg/kg (Fig. 1). Ketoprofen showed similar potency in the inhibition of PCA. The inhibition of PCA by these two agents is more potent than those by ibuprofen and tranilast. Indomethacin showed no significant effect on the PCA reaction.

Anaphylactic mediator release and antagonistic action: The release of histamine by the injection of antigen into sensitized rat peritoneal cavity and the increase of vascular permeability due to histamine in rat back skin were inhibited by suprofen at a dose of 25 mg/kg (Table 1). The effects of suprofen and some other drugs on the release of histamine and SRS-A from sensitized guinea pig lung are shown in Fig. 2 and Table 2. Suprofen ($10^{-4}$ g/ml), ketoprofen ($10^{-4}$ g/ml), indomethacin ($10^{-4}$ g/ml) and tranilast ($10^{-5}$ and $10^{-4}$ g/ml) inhibited the release of histamine. The inhibition by all NSAID tested were shown not to be dose-dependent. In contrast to the NSAID, tranilast inhibited the reaction dose-dependently. The generation of SRS-A was inhibited by suprofen.
(10^{-4} \text{ g/ml}) \text{ and indomethacin (10^{-4} \text{ g/ml}). However, indomethacin at a dose of 10^{-6} \text{ g/ml} enhanced the generation of SRS-A. Other NSAID and tranilast showed no significant effect on the generation of SRS-A.}

**FCV:** Suprofen at a dose of 25 mg/kg and CPM at a dose of 5 mg/kg inhibited the dye leakage caused by Forssman antibody in guinea pig skin (Fig. 3). Ketoprofen, ibuprofen and indomethacin did not affect the reaction.

**Arthus reaction in guinea pigs:** Suprofen (5 and 25 mg/kg), ketoprofen (5 mg/kg), ibuprofen (10 mg/kg), indomethacin (5 mg/kg) and prednisolone (5 mg/kg) inhibited the increase of the inflammed area due to the Arthus reaction at the period between 3 to 8 hr after the injection of antigen. Ketoprofen and prednisolone inhibited the reaction at 24 hr after the challenge (Fig. 4).

**Delayed type hypersensitivity cutaneous reaction in guinea pigs:** Suprofen, ibuprofen and indomethacin hardly affected the delayed type cutaneous reaction in guinea pigs when the inflammed area was measured at 24 hr after the injection of antigen. Ketoprofen and

---

**Table 2. Effect of suprofen, ketoprofen, ibuprofen, indomethacin and tranilast on SRS-A release from sensitized guinea pig lung**

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (g/ml)</th>
<th>SRS-A (u)</th>
<th>% to control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>10^{-5}</td>
<td>40.2±4.28</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>37.2±6.21</td>
<td>92.5</td>
</tr>
<tr>
<td><strong>Suprofen</strong></td>
<td>10^{-5}</td>
<td>36.0±3.47</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>30.2±3.30</td>
<td>83.9</td>
</tr>
<tr>
<td><strong>Ketoprofen</strong></td>
<td>10^{-5}</td>
<td>36.0±3.43</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>33.8±3.45</td>
<td>93.9</td>
</tr>
<tr>
<td><strong>Ibuprofen</strong></td>
<td>10^{-5}</td>
<td>33.8±3.45</td>
<td>93.9</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>28.5±5.81</td>
<td>84.3</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>10^{-6}</td>
<td>36.0±3.47</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10^{-5}</td>
<td>57.8±6.47</td>
<td>160.6</td>
</tr>
<tr>
<td><strong>Indomethacin</strong></td>
<td>10^{-6}</td>
<td>18.6±7.39</td>
<td>51.7</td>
</tr>
<tr>
<td></td>
<td>10^{-5}</td>
<td>40.0±5.58</td>
<td>111.1</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>40.2±4.28</td>
<td>100</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>10^{-5}</td>
<td>38.8±6.26</td>
<td>91.5</td>
</tr>
<tr>
<td></td>
<td>10^{-4}</td>
<td>39.0±1.79</td>
<td>97.0</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. of 5 experiments. *P<0.05.
prednisolone inhibited the reaction (Fig. 5).

**Antibody formation:** The productions of HPFC in mice and IgE antibody in rats were not affected by the administration of 5 to 50 mg/kg suprofen for 5 days after the immunization (Fig. 6 and Table 3). Cyclophosphamide at doses of 5 to 10 mg/kg inhibited the production of both antibodies.

---

**Discussion**

In the present report, we demonstrated that suprofen, a new anti-inflammatory drug, inhibited certain experimental allergic reactions, but did not affect antibody formation. Type I allergic reactions, including rat homologous PCA, histamine release from rat peritoneal mast cells and histamine and...
SRS-A release from guinea pig lung tissues were inhibited by suprofen. The Arthus reaction in guinea pigs, classified as a Type III allergic reaction, and the Type II reaction FCA were also inhibited. However, Type IV reaction, delayed hypersensitivity in guinea pig skin, was not affected by suprofen.

Little is known about the effect of NSAID on experimental Type I allergic reaction. The NSAID indicating anti-allergic action, so far reported, are tiaramide, salicylate and phenylbutazone (3, 4). These drugs except for tiaramide inhibit the IgE antibody mediated reaction only at a high dose. In the present study, suprofen, ketoprofen and ibuprofen were indicated to inhibit Type I reactions at a high dose. These findings suggest that the anti-allergic action of suprofen is no more potent than tiaramide. However, since only few NSAID inhibit the Type I reaction, suprofen is one of the useful NSAID, to investigate the more potent NSAIDs carrying anti-allergic action. Generally, NSAIDs inhibit the production of energy, the migration of

Fig. 4. Effect of suprofen, ketoprofen, ibuprofen, indomethacin and prednisolone on the Arthus reaction in guinea pigs. Each point represents the mean±S.E. of 5 to 6 animals. *P<0.05. □□□□□□□: Control. ●●●: 5 mg/kg Suprofen, △△△△△△△: 25 mg/kg Suprofen, ○○○: 5 mg/kg Ketoprofen, △△△△△△△: 10 mg/kg Ibuprofen, ●●●: 5 mg/kg Indomethacin, △△△△△△△: 5 mg/kg Prednisolone.
Fig. 5. Effect of suprofen, ketoprofen, ibuprofen, indomethacin and prednisolone on delayed type hypersensitivity cutaneous reaction in guinea pigs. Each column represents the mean±S.E. of 5 to 6 animals. Drugs were administered p.o. 1 hr prior to the injection of challenging antigen. *P<0.05.

Fig. 6. Effect of suprofen and cyclophosphamide on HPFC formation in spleen of mice immunized with SRBC. Each column represents the mean±S.E. of 5 to 6 animals. Suprofen was administered p.o. for 5 days after immunization, and cyclophosphamide was administered for 3 days. *P<0.05, †P<0.01.
leukocytes and the increase of vascular permeability. These characteristics of NSAIDs must be good for the therapy of allergy. However, many NSAIDs are insufficient for the therapy of human allergic disease. Therefore, a NSAID that possesses both anti-inflammatory and anti-allergic action will be desirable for the therapy of allergic inflammation. For the above reasons, suprofen is a good tool for investigating NSAIDs which show anti-allergic action.

Another interesting fact observed in the present study is the effect of NSAID on SRS-A production. It is generally accepted that a NSAID which inhibits cyclooxygenase activity, for example, indomethacin, enhances the production of SRS-A (15). In the present results, indomethacin at a dose of $10^{-6}$ g/ml enhanced the release of SRS-A. However, contrary to indomethacin, suprofen at doses of $10^{-6}$ and $10^{-5}$ g/ml did not enhance the release of SRS-A. These evidences suggest that suprofen may hardly affect cyclooxygenase activity. These data seem to disagree with the results reported by Fujimura et al. (2). They reported that suprofen inhibited biosynthesis of prostaglandins from the results of in vivo experiments employing arachidonic acid induced shock in rabbits (2). Further experiments will be necessary to clarify the effect of suprofen on the production of prostaglandins and leukotrienes. The experiments should be done by measuring enzyme activity participating in the arachidonic acid cascade and the amount of products.

In addition to the Type I reaction, Type II and III reactions are clearly inhibited by suprofen. Since suprofen inhibits the migration of rat neutrophils in vitro (H. Nagai et al., unpublished data), this action must be closely related to the inhibition of these reactions. Suprofen showed little effect on the Type IV reactions. The reason why suprofen inhibits Type I, II and III reactions rather than the Type IV reaction is not clear. Since this tendency is not observed in the case of ketoprofen, ibuprofen and indomethacin, suprofen may possess a particular mechanism in the anti-allergic action.

As for the antibody formation, suprofen showed no effect on the production of IgM and IgE antibodies. The effect of NSAID on humoral antibody formation is obscure. A few drugs, salicylates, benzindamine and phenylbutazone, were reported to show an immunosuppressive activity (4). Much attention must be paid in this field.

In summary, suprofen inhibits Type I, II and III allergic reactions at a high dose, but it hardly affects the Type IV reaction and antibody formation.

References