MECHANISM OF ACTION OF A NEW ANTI-INFLAMMATORY AGENT, NAPROXEN (II)
EFFECTS OF NAPROXEN ON ACTIVITIES OF MUCOPOLYSACCHARASE, ACID PROTEASE AND COLLAGENOLYTIC ENZYMES IN INFLAMED TISSUES

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Abstract—In order to elucidate the biochemical anti-inflammatory properties of naproxen, the effects of this compound on activities of mucopolysaccharase (β-glucuronidase (β-Gase) and lysozyme (LZ)), acid protease (APase) and collagenolytic enzyme (CL) in inflamed tissues were investigated by means of a proliferative inflammatory model in filter-paper-implanted rats. In the preventive test, naproxen strongly inhibited granuloma formation and exudate accumulation as did indomethacin and prednisolone. Although the inhibitory effects of naproxen on all these enzymes were quite evident, indomethacin failed to inhibit APase activity. Prednisolone did not significantly inhibit LZ and APase activities in granuloma. In the curative test, prednisolone caused a marked decrease in the weight of the granuloma already formed and in the volume of the exudate, but with naproxen and indomethacin there was only a slight decrease. Naproxen and indomethacin induced slight but significant inhibition of LZ and CL activities, while prednisolone showing a weak inhibition of CL activity only. From these results, it may be concluded that anti-inflammatory and anti-rheumatic effects of naproxen are partly attributable to its inhibitory actions on these lysosomal enzymes.

Naproxen is a new type of non-steroidal anti-inflammatory agent (1) developed by Syntex Research. Its effectiveness for rheumatoid arthritis has been confirmed in animal experiments (2, 3) and clinical studies (4–8).

In order to adequately ascertain the therapeutic effects of naproxen, the biochemical mechanism of the anti-inflammatory actions of this agent was investigated. In previous papers (9, 10), it is reported that by using the proliferative granulation model in filter-paper-implanted rats, investigation was conducted as to how naproxen would act on quantitative changes of the main connective tissue constituents (total mucopolysaccharide, acid mucopolysaccharide, glycoprotein, non-collagenic protein and collagen) in inflamed granulation tissue. It was found that the drug increased the 0.15 M NaCl-insoluble fraction of constituents of granulation tissue.

It is known that the degradation of the constituents of connective tissue in inflammatory regions due to lysosomal enzymes vary according to quantitative changes of the constituents of the same tissue, and also that there is an elevation of activities of several different hydrolytic enzymes originating from lysosomes present in synovial fluid and synovium of patients with rheumatoid arthritis and in inflammatory tissues (11–16). We also found that in rats with adjuvant-induced arthritis, there were apparent correlations between the
intensity of the inflammation and activities of enzymes and further between depression of inflammation by anti-inflammatory drugs and their inhibitory actions on enzymatic activities (17). In order to clarify the biochemical mechanism of the anti-inflammatory actions of naproxen, we made an attempt to determine whether or not the drug can indeed inhibit activities of hydrolytic enzymes (mucopolysaccharase, acid protease and collagenolytic enzyme) in granuloma and skin covering the granuloma pouch by employing the same inflammatory model used in a previous study (10). The effects of naproxen on these enzymatic activities were then compared with its effects on the weight of the granuloma and the volume of exudate.

MATERIALS AND METHODS

Drugs

The chemical structure of naproxen (NAP) is shown in Fig. 1. Indomethacin (IDM) [Merck-Banyu], benzydamine hydrochloride (BEN) [Daiichi Seiyaku Co., Ltd.] and prednisolone (PD) [Toyo Jozo Co., Ltd.] were employed for comparison.

Induction of inflammatory lesion

Female Wistar rats, weighing 180–200 g were placed in a prone position on a board, lightly anesthetized with ether and the skin of the dorsum was shaved with an electric clipper. A horizontal incision approx. 25 mm in width was made on dorsal skin near the base of the tails. A piece of rectangular sterile filter paper of 25 × 35 sq. mm (Toyo Filter Paper No. 26, 0.7 mm in thickness) was inserted s.c. into the dorsal region, the skin of which had been loosened by a stainless steel spatula through the incision. Procaine penicillin G (3 million units) was then applied to the incision and the wound was carefully sutured. This day was considered day 0 of implantation.

Administration and evaluation of drugs

Animals were divided into groups of 6. The experiments consisted of two procedures. Firstly, the preventive test started on day 0. Here each drug dissolved or suspended in 1% Gum Arabic was given orally by gavage once a day for 12 consecutive days, and preventive effects of each drug were evaluated on day 12.

The second test was commenced on day 12 and continued until day 24. Here each drug was administered in the same way as in the first test, and curative effects were assessed on day 25. Animals serving as control were given physiological saline solution in the same amount as the drug solutions. Effects of the individual drugs were expressed by percentage ratio of inhibition of drug-treated groups to the control group.

Determination of exudate volume and weight of granuloma

Within the first 8 days, granuloma pouch was formed around the implanted filter paper and fluid accumulated in the pouch. As it was impossible to separate clearly the granuloma...
pouch from the surrounding tissue until day 5, all assays were performed after day 8. After sacrifice, the granuloma pouch was excised together with the dorsal skin covering the pouch. Separating the pouch from the skin, volume of exudate in the granuloma pouch and wet weight of pouch wall were determined, respectively. The skin covering the granuloma pouch and the pouch wall were later used for determination of enzyme activities.

**Preparation of enzyme solution**

Inflamed tissues were divided into granuloma and skin covering the granuloma pouch. Both inflamed tissues were cut finely with scissors and homogenized in a physiological saline solution. The homogenate was centrifuged at 12,000 rpm for 20 min while cooling at 0°C. The supernatant was used as enzyme solution. This solution was kept at 0–3°C and enzymatic activities were determined within 24 hr.

**Determination of enzyme activities**

As mucopolysaccharases, activities of β-glucuronidase (β-Gase) and lysozyme (LZ) were determined in accordance with Hasebe’s method (12). For the determination of β-Gase activity, p-nitrophenyl glucuronide (Chugai Pharmaceutical Co., Ltd.) was used as substrate, while for the determination of LZ activity, lyophilized Micrococcus lysodeikticus (Biochemical Industries, Ltd.) was used as substrate.

Acid protease (APase) activity [substrate: denatured hemoglobin (Sigma)] was also determined by the method of Bertelli et al. (18), a partial modification of the method of Anson.

The unit of these enzyme activities was expressed as the specific activity per 1 g of wet tissue.

Collagenolytic (CL) activity was determined in granuloma by a modification of the method of Nakagawa and Tsurufuji (19). Granulation tissue itself was used as the substrate for determination of CL activity. A piece of granuloma weighing 500 mg was cut finely into sections of 1–2 mm in thickness, which were then transferred into a 20 ml Erlenmeyer flask. Five ml of modified Krebs-Ringer solution (0.154 M NaCl 95 vol, 0.154 M KCl 4 vol, 0.11 M CaCl₂ 3 vol, 0.154 M KH₂PO₄ 1 vol, 0.154 M MgSO₄ 0.5 vol, 0.154 MgCl₂ 0.5 vol, 1.3% NaHCO₃ 3 vol, 0.16 M Na pyruvate 4 vol, 0.1 M Na fumarate 7 vol, 0.16 M Na-L-glutamate 4 vol, 5.4% glucose 5 vol and 0.1 M Na phosphate buffer 3 vol) containing 0.2 mM proline and 0.5 mg each of potassium penicillin G and dihydrostreptomycin sulfate were added. Incubation was carried out at 34°C for 40 hr under an atmosphere of 95% O₂-CO₂ gas. L-cysteine was added to the incubation medium to make a concentration of 10 mM, then the mixture was kept frozen until determination of CL activity. It was then thawed, homogenized and centrifuged. The supernatant and the liquid re-extracted from the residue were combined. A cold trichloroacetic acid (TCA) solution was then added to the combined solution to make a concentration of 2.5%. TCA soluble substances were concentrated and hydrolyzed by heating in 6 N HCl at 105°C for 16 hr. Hydroxyproline in the hyrolysate was analyzed quantitatively by the method of Kivirikko et al. (20). CL activity was calculated from the amount of hydroxyproline.
RESULTS

Changes in exudate volume and granuloma weight

Exudate volume and granuloma weight were recorded at various intervals from day 8 to day 100 of implantation. As indicated in Fig. 2, the volume of exudate increased rapidly after implantation, i.e., the exudate volume was 3.5±0.6 ml on day 8 and reached a peak (8.8±2.1 ml) on day 25. The exudate volume sharply decreased thereafter, and was hardly detected by day 100.

The weight of granuloma on day 8 was 2.1±0.2 g and the peak (2.7±0.1 g) which appeared on day 12 was maintained at nearly a constant level until day 35. The weight decreased gradually thereafter, but was still 2.0±0.4 g on day 100.

Variation of hydrolytic enzyme activities

Fig. 3 shows variations of hydrolytic enzyme activities in the granuloma and skin covering the granuloma pouch from day 8 to day 100 of implantation. The activities of β-Gase, LZ and APase in granuloma were significantly higher than those in the normal skin throughout the experimental period, viz., they were 4.3, 31.8 and 4.0 times on day 8 and 11.2, 158.2 and 5.5 times as high on day 25 as those in the normal skin tissue, respectively. The activities of these three enzymes in the skin covering the granuloma pouch were slightly higher than those in the normal skin from day 8 to day 16.

On the other hand, there was a high activity of CL in the granuloma, although such was hardly detectable in the normal skin.

β-Gase and LZ activities in granuloma reached the peak on days 12 and 25, respectively, while both APase and CL exhibited the highest activities on day 35. The activities of all enzymes thereafter showed a gradual decrease.

Anti-inflammatory effects of NAP given for 12 consecutive days after implantation of the filter paper (Preventive test)

Effects on granuloma weight and exudate volume: As seen in Table 1, at the dose levels
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of 2.5 mg/kg, 10 mg/kg and 25 mg/kg, NAP exerted dose-dependent actions which inhibited weight of granuloma 29%, 46% and 71%, respectively, and the exudate volume 17%, 60% and about 100%, respectively. The inhibitory actions of 10 mg/kg of NAP on granulation and exudation were nearly equivalent those of 2.5 mg/kg of IDM and 5 mg/kg of PD. BEN in the dose of 50 mg/kg had no significant inhibitory action on either granuloma weight or exudate volume.

**TABLE 1.** Effects of naproxen and other drugs on granuloma weight and exudate volume of filter-paper-implanted rats following oral, daily ×12 administrations beginning day 0 after implantation

| Drugs       | Dose mg/kg/day p.o. | Wet granuloma wt. (g) (mean±S.E.) | Inhibitory ratio (%) | Exudate vol. (ml) (mean±S.E.) | Inhibitory ratio (%) |
|-------------|---------------------|------------------------------------|----------------------|-------------------------------|----------------------|
| Control     |                     | 2.68±0.14                          |                      | 5.60±0.29                     |                      |
| Naproxen    | 2.5                 | 1.90±0.29c                         | 29.1                 | 4.63±0.20                     | 17.3                |
|             | 10                  | 1.45±0.40c                         | 45.9                 | 2.22±0.96c                    | 60.4                |
|             | 25                  | 0.79±0.14a                         | 70.5                 | Negligible a                  |                      |
| Indomethacin| 2.5                 | 1.25±0.07a                         | 53.4                 | 1.46±0.48a                    | 73.9                |
| Benzydamine HCl | 50       | 2.13±0.15                          | 20.5                 | 3.32±1.02                     | 40.7                |
| Prednisolone| 5                   | 1.55±0.27b                         | 42.2                 | 1.06±0.21a                    | 81.1                |

Statistical difference from the control group
(a, p<0.001; b, p<0.01; c, p<0.05)
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Effects on $\beta$-Gase activity in granuloma and skin covering granuloma pouch: As given in Table 2, $\beta$-Gase activity in granuloma was inhibited about 30% after the oral administration of NAP in doses of 10 mg/kg and 25 mg/kg. This inhibitory action tended to be stronger than that produced by 2.5 mg/kg of IDM. The enzyme activity was inhibited 53% after the administration of PD (5 mg/kg), which was the most potent inhibitory action of the drugs tested in this experiment. BEN showed no significant inhibitory action at the dose level of 50 mg/kg.

Activity of the enzyme present in skin covering the granuloma pouch was inhibited about 30% by NAP at the dose levels of 10 mg/kg and 25 mg/kg, and the inhibition by PD (5 mg/kg) was 37%. Neither IDM nor BEN showed any significant inhibitory actions.

Effects on LZ activity in granuloma and skin covering granuloma pouch: As illustrated in Table 3, LZ activity in the granuloma was inhibited 12%, 17% and 28% by

| Drugs              | Dose mg/kg/day p.o. | Granuloma | Skin      |
|--------------------|---------------------|-----------|-----------|
|                    | $\mu g$ $\beta$-nitrophenol/ghhr (mean±S.E.) | Inhibitory ratio (%) | $\mu g$ $\beta$-nitrophenol/ghhr (mean±S.E.) | Inhibitory ratio (%) |
| Control            |                     |           |           |
| Naproxen           | 2.5                 | 10820±743 | 2884±131  |
|                    | 10                  | 9309±731  | 2283±192  | 30.8 |
|                    | 25                  | 7701±473\textsuperscript{b} | 2073±131\textsuperscript{b} | 28.1 |
| Indomethacin       | 2.5                 | 8623±362\textsuperscript{a} | 2382±126  | 17.4 |
| Benzydamine HCl    | 50                  | 8822±560  | 2524±218  | 12.5 |
| Prednisolone       | 5                   | 5038±345\textsuperscript{a} | 1826±188\textsuperscript{b} | 36.7 |

Statistical difference from the control group
(a, $p<0.001$; b, $p<0.01$; c, $p<0.05$)

Table 2. Effects of naproxen and other drugs on $\beta$-glucuronidase activity in granuloma and skin covering the granuloma pouch of filter-paper-implanted rats following oral, daily $\times 12$ administrations beginning day 0 after implantation

| Drugs              | Dose mg/kg/day p.o. | Granuloma | Skin      |
|--------------------|---------------------|-----------|-----------|
|                    | $\mu g$ egg-white lysozyme/g (mean±S.E.) | Inhibitory ratio (%) | $\mu g$ egg-white lysozyme/g (mean±S.E.) | Inhibitory ratio (%) |
| Control            |                     |           |           |
| Naproxen           | 2.5                 | 242.5±3.7 | 12.6±1.2  |
|                    | 10                  | 212.9±11.6\textsuperscript{a} | 9.6±3.7   | 23.8 |
|                    | 25                  | 201.5±13.9\textsuperscript{a} | 5.8±1.5\textsuperscript{b} | 54.0 |
| Indomethacin       | 2.5                 | 173.8±10.9\textsuperscript{a} | 3.9±1.2\textsuperscript{a} | 69.0 |
| Benzydamine HCl    | 50                  | 193.2±9.5\textsuperscript{a} | 6.4±1.4\textsuperscript{b} | 49.2 |
| Prednisolone       | 5                   | 220.5±11.2 | 6.9±2.1\textsuperscript{c} | 45.2 |

Statistical difference from the control group
(a, $p<0.001$; b, $p<0.01$; c, $p<0.05$)

Table 3. Effects of naproxen and other drugs on lysozyme activity in granuloma and skin covering the granuloma pouch of filter-paper-implanted rats following oral, daily $\times 12$ days administrations beginning day 0 after implantation
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NAP at dose levels of 2.5 mg/kg, 10 mg/kg and 25 mg/kg, respectively, in proportion to the doses given. The activity was inhibited 20% by IDM (2.5 mg/kg). None of BEN (50 mg/kg) and PD (5 mg/kg) induced a significant inhibition of LZ activity, though PD elicited a most remarkable inhibition on β-Gase.

The activity of LZ in the skin covering the granuloma pouch was inhibited markedly by each of the drugs tested.

**Effects on APase activity in granuloma and skin covering the granuloma pouch:** Findings are illustrated in Table 4. APase activity in granuloma was inhibited 20% or more by NAP at dose levels of both 10 and 25 mg/kg, while the enzyme activity was not significantly inhibited by any other drug used.

At dose levels ranging from 2.5 to 25 mg/kg, NAP inhibited 17 to 30% of APase activity in skin covering the granuloma pouch, thus showing a dose-dependent inhibitory action. The enzymatic activity was also inhibited 27% by PD (5 mg/kg).

**Table 4.** Effects of naproxen and other drugs on acid protease activity in granuloma and skin covering the granuloma pouch of filter-paper-implanted rats following oral, daily \( \times 12 \) administrations beginning day 0 after implantation

| Drugs          | Dose mg/kg/day p.o. | Granuloma O.D. (280 nm) of TCA soluble peptides (mean±S.E.) | Inhibitory ratio (%) | Skin O.D. (280 nm) of TCA soluble peptides/g/hr (mean±S.E.) | Inhibitory ratio (%) |
|----------------|---------------------|-------------------------------------------------------------|---------------------|------------------------------------------------------------|---------------------|
| Control        |                     | 43.22±2.41                                                  |                     | 11.29±0.62                                                 |                     |
| Naproxen       | 2.5                 | 37.18±1.82                                                  | 14.0                | 9.32±0.56                                                  | 17.4                |
|                | 10                  | 33.10±3.00                                                  | 23.4                | 8.22±0.77                                                  | 27.2                |
|                | 25                  | 33.57±1.08                                                  | 22.3                | 7.92±0.65                                                  | 29.8                |
| Indomethacin   | 2.5                 | 39.44±1.61                                                  | 8.7                 | 9.68±0.49                                                  | 14.3                |
| Benzydamine HCl| 50                  | 41.75±1.80                                                  | 3.4                 | 9.53±0.57                                                  | 15.6                |
| Prednisolone   | 5                   | 37.31±1.96                                                  | 13.7                | 8.26±0.23                                                  | 26.8                |

Statistical difference from the control group
(b, \( p<0.01 \); c, \( p<0.05 \))

**Effects on CL activity in granuloma:** Actions of drugs against CL activity were examined only in granuloma tissue and results are shown in Table 5. The activity of this enzyme was inhibited 25%, 37% and 59% by NAP at the dose levels of 2.5 mg/kg, 10 mg/kg and 25 mg/kg, respectively, viz., the action was dose-dependent. CL activity was significantly inhibited 49% and 56% by IDM (2.5 mg/kg) and PD (5 mg/kg), respectively. On the other hand, BEN (50 mg/kg) produced no significant inhibition of the enzymatic activity.

**Anti-inflammatory effects of NAP given for 13 successive days starting day 12 after implantation of filter paper (Curative test)**

**Effects on granuloma weight and exudate volume:** As shown in Table 6, the weight of wet granuloma was decreased 11–16% after the administration of NAP at the dose levels of 5 to 25 mg/kg, but the inhibitory action was not significant. Among the other drugs used,
PD was the only one having strong inhibition as in the case of the preventive test. In fact, 42% loss in weight was seen with PD (5 mg/kg). The exudate volume was inhibited slightly over 40% by NAP (10 mg and 25 mg/kg) and 36% by IDM. As in the case of the preventive test, PD induced the strongest inhibition (81%).

**Effects on $\beta$-Gase activity in granuloma and skin covering the granuloma pouch:** As given in Table 7, $\beta$-Gase activity in granuloma was inhibited slightly (11–16%) by NAP at the dose level of 2.5 to 25 mg/kg, though the inhibition was not significant. No other drug used showed a significant inhibition on this enzyme.

The enzyme activity in skin covering the granuloma pouch was inhibited slightly but significantly (13%) by NAP (25 mg/kg) only.

**Effects on LZ activity in granuloma:** Because LZ activity in the skin covering the granuloma pouch had already returned to the normal by day 25 (refer to Fig. 3), it was not taken up in the comparative study. As indicated in Table 8, the inhibitory actions of NAP...
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Table 7. Effects of naproxen and other drugs on $\beta$-glucuronidase activity in granuloma and skin covering the granuloma pouch of filter-paper-implanted rats following oral, daily $\times$13 administrations beginning day 12 after implantation

| Drugs           | Dose | Granuloma | Skin          |
|-----------------|------|-----------|---------------|
|                 | mg/kg/day | $\mu g$ $\beta$-nitrophenol/in/hr (mean $\pm$ S.E.) | Inhibitory ratio (%) | $\mu g$ $\beta$-nitrophenol/in/hr (mean $\pm$ S.E.) | Inhibitory ratio (%) |
| Control         |      | 8368$\pm$594 | 1119$\pm$46   |
| Naproxen        | 2.5  | 7219$\pm$145 | 13.7          | 1071$\pm$44 | 4.3 |
|                 | 10   | 7011$\pm$105 | 16.2          | 1003$\pm$57 | 10.4 |
|                 | 25   | 7481$\pm$302 | 11.0          | 972$\pm$53c | 13.1 |
| Indomethacin    | 2.5  | 7799$\pm$435 | 6.8           | 1118$\pm$83 | 0    |
| Benzydamine HCl | 50   | 9231$\pm$311 | $-9.3$        | 1125$\pm$77 | $-0.5$ |
| Prednisolone    | 5    | 6453$\pm$417 | 22.9          | 1053$\pm$26 | 5.9  |

Statistical difference from the control group
(c, p<0.05)

Table 8. Effects of naproxen and other drugs on lysozyme activity in granuloma of filter-paper-implanted rats following oral, daily $\times$13 administrations beginning day 12 after implantation

| Drugs           | Dose | Granuloma |
|-----------------|------|-----------|
|                 | mg/kg/day | $\mu g$ egg-white lysozyme/g (mean $\pm$ S.E.) | Inhibitory ratio (%) |
| Control         |      | 552$\pm$9.3 | 10.7 |
| Naproxen        | 2.5  | 466$\pm$22.8c | 9.0 |
|                 | 10   | 475$\pm$17.5c | 15.1 |
|                 | 25   | 443$\pm$21.7c | 12.1 |
| Indomethacin    | 2.5  | 459$\pm$13.2b | 2.3 |
| Benzydamine HCl | 50   | 510$\pm$20.7 | 2.1 |
| Prednisolone    | 5    | 511$\pm$6.8  | 1.1 |

Statistical difference from the control group
(b, p<0.01; c, p<0.05)

(10 mg/kg and 25 mg/kg) and IDM (2.5 mg/kg) against LZ activity in the granuloma were slight but significant, viz., the enzymatic activities were inhibited 10–15% by these drugs. Such were not inhibited significantly either by BEN (50 mg/kg) or by PD (5 mg/kg).

Effects on APase activity in granuloma and skin covering the granuloma pouch: As shown in Table 9, APase activity in the granuloma was inhibited 10% by NAP (2.5 to 25 mg/kg), but the inhibitory effect was not significant. Likewise, the other drugs used were not so effective as to demonstrate significant inhibition against the activity of APase.

APase activity in the skin covering the granuloma pouch was inhibited by none of the drugs used herein.

Effects on CL activity in granuloma: As shown in Table 10, NAP at the dose levels of 2.5 mg/kg, 10 mg/kg and 25 mg/kg inhibited CL activity 20%, 23% and 29%, respectively, in proportion to the dosage increase. The inhibitory actions of IDM (2.5 mg/kg) and PD
DISCUSSION

In order to elucidate the biochemical mechanism of anti-inflammatory action of NAP, the preventive test and the curative test were carried out. Inhibitory actions on activities of the hydrolytic enzyme (β-Gase, LZ, APase and CL) in granuloma and skin covering the granuloma pouch and inhibitory actions on weight of granuloma and volume of exudate in the proliferative inflammatory model of filter-paper-implanted rats were studied. Correlation between the two is summarized in Table 11.

The preventive test revealed that NAP at the dose level of 10 mg/kg inhibited granulation and exudation remarkably, the inhibitory action being nearly equal that of IDM (2.5 mg/kg) or PD (5 mg/kg). In the curative test, however, NAP as well as IDM, which is an acidic

(5 mg/kg) were 21% and 30%, respectively, however, no significant inhibition was seen by BEN (50 mg/kg).

TABLE 9. Effects of naproxen and other drugs on acid protease activity in granuloma and skin covering the granuloma pouch of filter-paper-implanted rats following oral, daily ×13 administrations beginning day 12 after implantation

| Drugs         | Dose (mg/kg/day) | Granuloma O.D. (280 nm) of TCA soluble peptides/g/hr (mean ± S.E.) | Inhibitory ratio (%) | Skin O.D. (280 nm) of TCA soluble peptides/g/hr (mean ± S.E.) | Inhibitory ratio (%) |
|---------------|-----------------|---------------------------------------------------------------|----------------------|---------------------------------------------------------------|----------------------|
| Control       | 2.5             | 56.57 ± 3.12                                                  | 10.2                 | 13.41 ± 0.57                                                  | 9.6                  |
| Naproxen      | 10              | 50.81 ± 2.94                                                  | 13.2                 | 12.12 ± 0.44                                                  | 7.6                  |
|               | 25              | 49.88 ± 2.68                                                  | 11.8                 | 12.13 ± 0.41                                                  | 9.5                  |
| Indomethacin  | 2.5             | 54.09 ± 2.53                                                  | 4.4                  | 13.19 ± 0.66                                                  | 1.6                  |
| Benzydamine HCl | 50            | 55.14 ± 2.56                                                  | 2.5                  | 12.87 ± 0.45                                                  | 4.0                  |
| Prednisolone  | 5               | 43.70 ± 2.17                                                  | 22.8                 | 12.39 ± 0.51                                                  | 7.6                  |

TABLE 10. Effects of naproxen and other drugs on collagenolytic activity in granuloma of filter-paper-implanted rats following oral, daily ×13 administrations beginning day 12 after implantation

| Drugs            | Dose (mg/kg/day) | Granuloma μg hydroxyproline degraded/0.3 g/40 hr (mean ± S.E.) | Inhibitory ratio (%) |
|------------------|-----------------|---------------------------------------------------------------|----------------------|
| Control          | 2.5             | 46.5 ± 3.1                                                    | 20.2                 |
| Naproxen         | 10              | 37.1 ± 2.3                                                   | 22.6                 |
|                  | 25              | 36.0 ± 1.4                                                   | 28.6                 |
| Indomethacin     | 2.5             | 36.8 ± 3.1                                                   | 20.9                 |
| Benzydamine HCl | 50              | 42.4 ± 2.7                                                   | 8.8                  |
| Prednisolone     | 5               | 32.6 ± 3.0                                                   | 29.9                 |

Statistical difference from the control group
(b, p<0.01; c, p<0.05)
| Drugs          | Dose (mg/kg/day p.o.) | Granuloma wt. | Exudate vol. | β-Glucuronidase | Lysozyme | Acid protease | Collagenolysis |
|---------------|-----------------------|--------------|--------------|-----------------|----------|--------------|---------------|
|               |                       |              |              | S*             | G**      | S            | G             |                |
| Preventive test|                       |              |              |                 |          |              |               |                |
| Naproxen      | 2.5                   | ++c          | +            | ++              | ++       | ++           | +c            | ++c            |
|               | 10                    | ++++c        | ++++         | +++             | +c       | ++++         | ++c           | ++c            |
|               | 25                    | +++++a       | ++++         | +++             | +b       | ++++         | ++a           | ++++           |
| Indomethacin  | 2.5                   | ++++a        | +++          | +               | ++c      | ++++         | +a            | ++++b          |
| Benzydamine HCl | 50              | +           | +++           | +               | +        | ++++         | +c            | +              |
| Prednisolone  | 5                     | ++++         | ++++         | ++              | ++       | +++a         | +             | ++++b          |
| Curative test |                       |              |              |                 |          |              |               |                |
| Naproxen      | 2.5                   | -            | +            | -               | -c       | -            | +c            | ++c            |
|               | 10                    | +           | ++++         | +               | -c       | -            | +c            | ++c            |
|               | 25                    | -           | ++++         | +               | +c       | -            | +c            | ++b            |
| Indomethacin  | 2.5                   | +           | +++           | -               | +b       | -            | +c            | ++c            |
| Benzydamine HCl | 50              | -           | +            | -               | +c       | -            | +c            | -              |
| Prednisolone  | 5                     | ++++         | ++++         | -               | +++      | -            | ++            | ++b            |

*... Skin covering the granuloma pouch, **... Granuloma

Inhibition: ++++, >50%; ++++, 40~50%; +++, 30~40%; ++, 20~30%; +, 10~20%; −, <10%

Statistical difference from the control group (a, p<0.001; b, p<0.01; c, p<0.05)
non-steroidal anti-inflammatory agent exerted only slight reducing actions on pre-existing granuloma and exudate, while PD, a steroidal anti-inflammatory agent strongly acted on these targets. On the other hand, BEN (50 mg/kg), a basic non-steroidal anti-inflammatory agent was proved to be ineffective in both tests. These facts are essentially in accord with those reported in the previous paper (10) and suggest the following:

If administration commences immediately after onset of inflammation, an acidic non-steroidal anti-inflammatory agent exerts much the same action as does a steroidal one. However, such is almost ineffective when administration is started in the active stage of granulation. Consequently, in the granulation period corresponding to stage 3 of inflammation the inhibitory actions of adrenocorticoids, e.g. against the activities of fibroblasts or other cells synthesizing intercellular substances, far surpass those of acidic non-steroidal anti-inflammatory agents.

The effects of NAP on activities of β-Gase, LZ, APase and CL in granuloma and skin covering the granuloma pouch were evaluated herein. APase activity was determined at pH 3.1 as cathepsin-D. All these enzymes are said to have their source in lysosomes (21, 22) of leucocytes and various cells which infiltrate into the inflammatory tissue, and it is known that they not only take part in degradation and resorption (23) of granulation tissue during the repairing process of inflammation but also induce damage to cells and tissues (24), thus participating in the outbreak and progress of inflammation. There are a number of reports dealing with the relationship between these enzymatic activities and rheumatoid arthritis. For example, Hasebe (12) reported that activities of mucopolysaccharases (β-Gase and LZ) were high in the synovial fluid of patients with rheumatoid arthritis, as compared with their activities in the fluid of patients suffering from other diseases. It was also reported by Pruzanski and his associates (15) that patients with rheumatoid arthritis revealed LZ activity as high as 35% in the serum and 55% in the synovial fluid. Granda et al. (16) demonstrated that among lysosomal enzymes, cathepsin-D was the most closely related to the lesion of rheumatoid arthritis. Harris and his colleagues (14) demonstrated that collagenase was non-specific to rheumatoid arthritis, although this enzyme was liberated during culture of synovial tissues obtained from these patients, because it was also detected in some patients with non-rheumatic inflammatory synovitis. These reports suggest that hydrolases of the lysosome system participate in the occurrence of rheumatoid arthritic lesion and destruction of cartilage and other connective tissues.

In the preventive test of the present study, NAP at the dose level of 10 mg/kg was found to strongly inhibit β-Gase, LZ and APase activities in the granuloma and the skin covering the granuloma pouch as well as CL activity in granuloma. Findings in the tests with the other drugs were 1) though 2.5 mg/kg of IDM did not inhibit significantly the activity of APase in the granuloma and the skin covering the granuloma pouch, its inhibitory actions on activities of other enzymes were comparable to those of 10 mg/kg NAP, 2) PD in the dose of 5 mg/kg inhibited β-Gase and CL activities more strongly than NAP in the dose of 10 mg/kg, however, the former did not significantly inhibit activities of LZ and APase in granulation tissue, and 3) BEN (50 mg/kg) inhibited significantly LZ activity only in the skin covering
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the granuloma pouch.

The inhibitory actions of the respective drugs examined on all these enzymes in the curative test were considerably weaker than those in the preventive test, i.e., NAP (10 mg/kg) as well as IDM (2.2 mg/kg) slightly inhibited LZ and CL activities and PD (5 mg/kg) also slightly inhibited CL activity only, although the inhibitory actions were significant in both cases.

Thus it would appear that the mechanism of anti-inflammatory and anti-rheumatic actions of NAP may be explained in part by the inhibitory action against activities of hydrolytic enzymes of the lysosomal system.

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