

Effect of Anti-inflammatory Agents on Accelerated Granuloma Formation in Rats¹⁾

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Effects of steroidal and non-steroidal anti-inflammatory agents on the accelerated granuloma formation in rats, which was induced by the dual implantation of felt-pellets, were compared with effects on the usual granuloma formation (one time implantation of felt-pellet). The $ID_{50}\%$ value of anti-granuloma activity of each agent in the usual method was as follows: dexamethasone 0.03 mg/kg/day, indomethacin 1.4 mg/kg/day, prednisolone 2.5 mg/kg/day, flufenamic acid 18.5 mg/kg/day, phenylbutazone 20.0 mg/kg/day and Aspirin 700 mg/kg/day, *p.o.* However, indomethacin (2 and 3 mg/kg/day), phenylbutazone (30 and 100 mg/kg/day) and Aspirin (600 mg/kg/day) did not show any significant anti-granuloma activity on the accelerated granuloma formation, and these inhibitory activities were less than 20%. Flufenamic acid showed the activity at 60 mg/kg/day, but did not at 30 mg/kg/day. On the other hand, prednisolone inhibited by the same degree both usual and accelerated granuloma formations at 3 and 10 mg/kg/day, and dexamethasone showed more potent inhibitory activity on the accelerated granuloma formation than that on the usual granuloma formation. These results suggest that the mode of anti-granuloma action of steroidal anti-inflammatory agents may be different from those of non-steroidal anti-inflammatory agents.

In the previous paper,³⁾ it was reported by us that the acceleration of granuloma formation was induced by dual implantation of felt-pellets in rats. The acceleration was observed when the 2nd felt-pellets were implanted 3 or 6 days following implantation of the first pellets in the same rat. To investigate whether or not the accelerated granuloma formation is qualitatively different from the usual granuloma formation, the present experiments were performed in rats.

This paper presents the experimental results on the effects of steroidal and non-steroidal anti-inflammatory agents both on accelerated and usual granuloma formations caused by dual or one time implantation of felt-pellets in rats, respectively.

Methods

Animals and Preparation of Pellets—Male rats of Wistar strain, weighing 150 to 180 g, were maintained in an air-conditioned room at a temperature of $23 \pm 1^\circ$, and given free access to water and laboratory food (CE-2, Nippon CLEA, Tokyo). Felt-pellets, weighing 35 ± 1 mg each, were prepared according to the method of Nakamura and Shimizu.³⁾

One Time Implantation of Felt-pellets (Usual Granuloma Formation)—Two felt-pellets were subcutaneously implanted at two symmetrical sites of the back of each rat lightly anaesthetized with intraperitoneal hexobarbital sodium. On the 5th day after the implantation, the pellets along with the surrounding inflammatory tissue were carefully dissected from the animals. The removed granulomas were dried for 30 hr at 60° and weighed. The net weight increase was determined by subtracting 35 mg (original weight) from each dry granuloma weight. Each drug was dissolved or suspended in 0.5% gum tragacanth aqueous solution, and orally administered to rats in a constant volume of 5 ml/kg once a day for 5 days. Six to 12 rats were used for each dose. Statistical analysis was done according to t-test.

Dual Implantation of Felt-pellets (Accelerated Granuloma Formation)—The dual implantation of felt-

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2) Location: 33-94 Enoki-cho, Suita-shi, Osaka. 565, Japan.

3) H. Nakamura and M. Shimizu, *European J. Pharmacol.*, **27**, 198 (1974).

pellets was performed according to the procedure of Nakamura and Shimizu.³⁾ The 2nd pellets were implanted 3 days after implantation of the 1st pellets, and 5 days later the animals were killed. Each drug was orally administered once a day for 5 days from the same day as the implantation of the 2nd pellets as shown in Fig. 1. Nine to 12 rats were used for each dose.

Materials—The used drugs were as follows: phenylbutazone (Fujisawa Pharm. Co., Ltd.), flufenamic acid (Dainippon Pharm. Co., Ltd.), indomethacin (Nippon Merck-Banyu Co., Ltd.), Aspirin (Yoshitomi Pharm. Co., Ltd.), prednisolone (Takeda Chem. Ind. Ltd.) and dexamethasone (Nippon Merck-Banyu Co., Ltd.).

Results

Effect of Anti-inflammatory Agents on Usual Granuloma Formation

As shown in Fig. 2, 3, 4 and 5, the used drugs showed inhibitory activity (solid line) on the usual granuloma formation induced by one time implantation of felt-pellets. The oral doses of drugs to cause the decrease by 20% in dry granuloma weight compared with vehicle control group were as follows: dexamethasone 0.03 mg/kg/day, indomethacin 1.4 mg/kg/day, prednisolone 2.5 mg/kg/day, flufenamic acid 18.5 mg/kg/day, phenylbutazone 20.0 mg/kg/day and Aspirin 700 mg/kg/day.

Effect of Anti-inflammatory Agents on Accelerated Granuloma Formation

Inhibitory activities of anti-inflammatory agents on the accelerated granuloma formation induced by the 2nd implanted pellets are represented by the dotted lines in Fig. 2, 3, 4 and 5. Indomethacin, phenylbutazone and Aspirin did not show any significant anti-granuloma activity at 2 and 3 mg/kg/day, 30 and 100 mg/kg/day and 600 mg/kg/day, *p.o.*, respectively, although these agents showed significant inhibitory activities on the usual granuloma formation (Fig. 2 and 3, solid line). Flufenamic acid showed a significant anti-granuloma activity at a high dose of 60 mg/kg/day (Fig. 4, dotted line), but did not at 30 mg/kg/day. These results demonstrate that non-steroidal anti-inflammatory agents possess generally weaker inhibitory activities on the accelerated granuloma formation than those on the usual granuloma formation.

On the other hand, prednisolone inhibited by the same degree both usual and accelerated granuloma formations at 3 and 10 mg/kg/day, *p.o.* (Fig. 5b), and dexamethasone showed more potent inhibitory activity on the accelerated granuloma formation than on the usual granuloma formation (Fig. 5a).

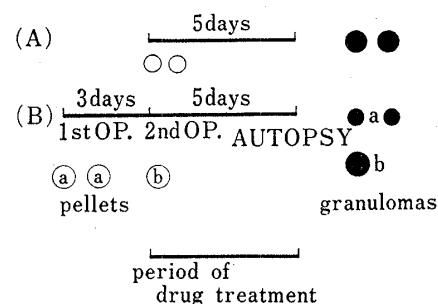


Fig. 1. Time Schedule of Experiments

- (A): usual granuloma formation-one time implantation
 (B): accelerated granuloma formation-dual implantation
 (a): the 1st implanted pellet,
 (b): the 2nd implanted pellet

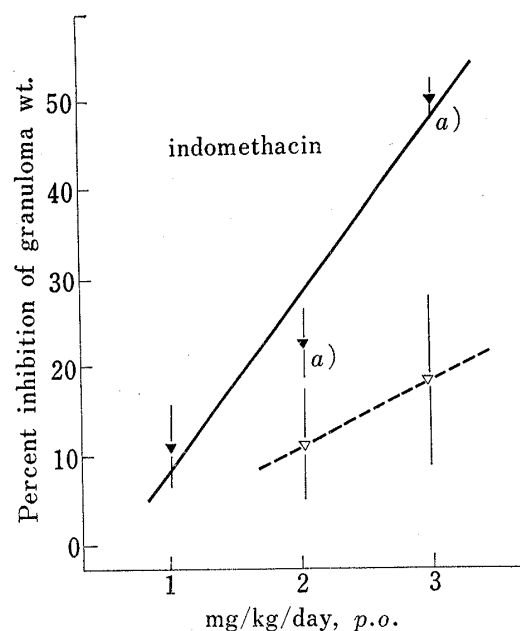


Fig. 2. Effect of Indomethacin on Usual and Accelerated Granuloma Formations

Solid and dotted lines represent inhibitory effects on usual and accelerated granuloma formations, respectively. The vertical shows the percent inhibition of drug treated group to vehicle control group in dry granuloma weights. Each point represents the mean \pm S.E. of 9 to 12 rats.

a) significantly different from each vehicle control group ($p < 0.01$)

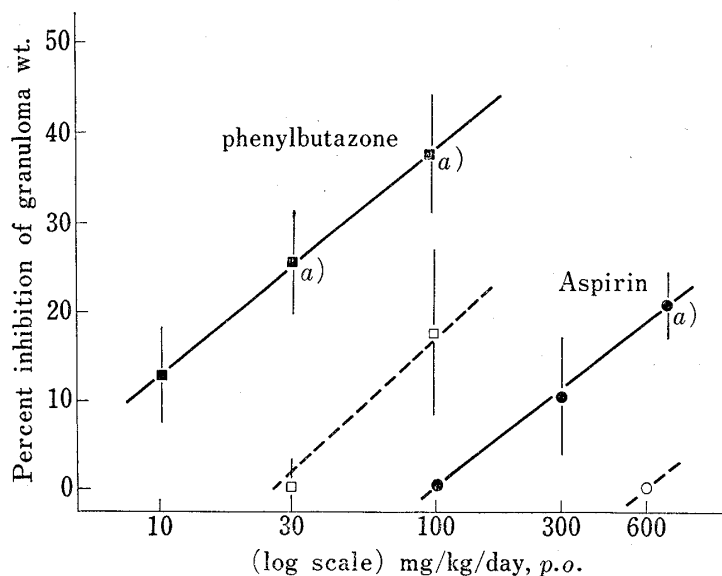


Fig. 3. Effect of Phenylbutazone and Aspirin on Usual and Accelerated Granuloma Formations

See Fig. 2 footnotes.

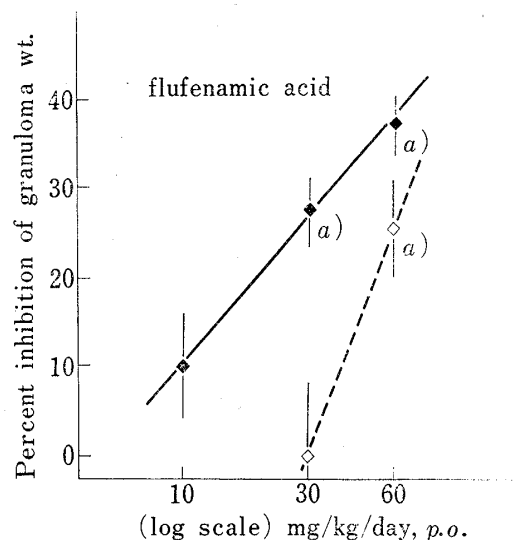


Fig. 4. Effect of Flufenamic Acid on Usual and Accelerated Granuloma Formations

See Fig. 2 footnotes.

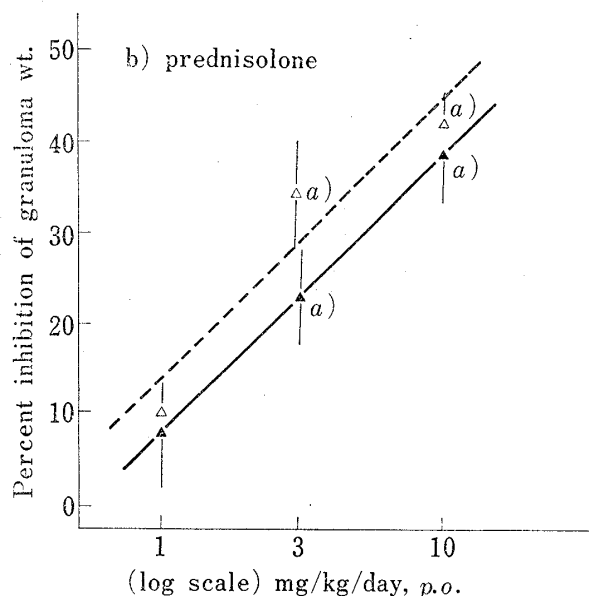
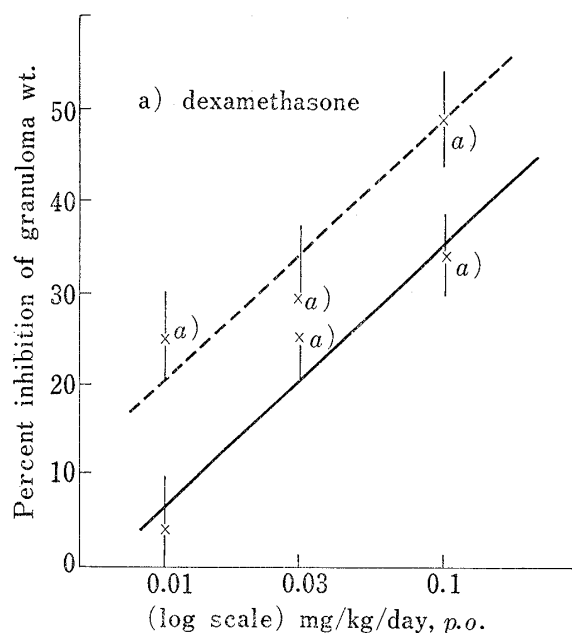


Fig. 5. Effect of Steroidal Anti-inflammatory Agents on Usual and Accelerated Granuloma Formations

a) dexamethasone b) prednisolone
See Fig. 2 footnotes.

Effect of Anti-inflammatory Agents on Granuloma Formation Induced by Implantation of the 1st Pellets in Dual Implantation

Inhibitory activities of anti-inflammatory agents on the granuloma formation induced by the 1st implanted pellets is shown in Table I. Phenylbutazone (100 mg/kg/day) and flufenamic acid (30 and 60 mg/kg/day) showed a significant anti-granuloma activity, but indomethacin and Aspirin did not. With phenylbutazone (100 mg/kg/day) and flufenamic acid (60 mg/kg/day), the ratios of the inhibition of granuloma formation induced by the 1st pellets to that by the 2nd pellets were 1.06 and 1.12, respectively, and the ratios by the 1st pellets to the pellets in one time implantation study were 2.28 and 1.75, respectively (Table I.).

On the other hand, with prednisolone at the doses of 3 and 10 mg/kg/day the ratios to the 2nd pellets were 2.08 and 2.95, respectively, and the ratios to the latter were 1.38 and 2.75, respectively. With dexamethasone at a dose of 0.1 mg/kg/day the ratios were 2.67 and 1.83, respectively. Consequently, it is suggested that steroidal and non-steroidal anti-inflammatory agents are less effective against the granuloma formation if the medication is performed from the late stage of developmental process of granuloma formation.

TABLE I. Effect of Anti-inflammatory Agents on Granuloma Formation Caused by the 1st Implanted Felt-pellets in Dual Implantation Study

| | Dose mg/kg/day <i>p. o.</i> | <i>n</i> | Body weight mean (g) before/final | Granulation tissue (1st pellet) | | | Ratios of the inhibition of granuloma formation | |
|-----------------|-----------------------------------|----------|---|------------------------------------|-------------------|--------------------|--|------|
| | | | | dry weight mean (mg) \pm S.E. | (%) inhibition | <i>p</i> -value | | |
| Control | vehicle | 20 | 172/185 | 53.4 \pm 1.7 | | | | |
| Indomethacin | 2 | 11 | 176/186 | 51.6 \pm 1.5 | 3.4 | | | |
| Flufenamic acid | 30 | 11 | 167/183 | 46.9 \pm 1.3 | 12.2 | 0.01 ^{a)} | 2.10 ^{b)} | |
| Phenylbutazone | 30 | 11 | 166/174 | 53.2 \pm 2.5 | 0.4 | | | |
| Control | vehicle | 10 | 182/195 | 52.6 \pm 1.8 | | | | |
| Indomethacin | 3 | 6 | 176/183 | 47.5 \pm 2.6 | 9.6 | | | |
| Flufenamic acid | 60 | 5 | 179/190 | 40.5 \pm 2.3 | 22.8 | 0.01 | 1.12 ^{c)} | 1.75 |
| Phenylbutazone | 100 | 5 | 171/177 | 43.4 \pm 3.1 | 16.5 | 0.05 | 1.06 | 2.28 |
| Control | vehicle | 10 | 153/169 | 49.4 \pm 1.4 | | | | |
| Aspirin | 600 | 7 | 154/155 | 46.1 \pm 1.6 | 6.7 | | | |
| Control | vehicle | 10 | 153/169 | 49.4 \pm 1.4 | | | | |
| Prednisolone | 1 | 8 | 153/169 | 50.5 \pm 1.8 | -2.2 | | | |
| Control | vehicle | 10 | 182/195 | 52.6 \pm 1.8 | | | | |
| Prednisolone | 3 | 6 | 182/182 | 43.8 \pm 2.9 | 16.7 | 0.01 | 2.08 | 1.38 |
| Control | vehicle | 10 | 176/191 | 50.0 \pm 2.1 | | | | |
| Prednisolone | 10 | 8 | 179/188 | 42.9 \pm 1.1 | 14.2 | 0.05 | 2.95 | 2.75 |
| Control | vehicle | 11 | 191/209 | 38.5 \pm 1.6 | | | | |
| Dexamethasone | 0.01 | 12 | 189/201 | 35.5 \pm 1.1 | 8.1 | | | |
| | 0.03 | 12 | 190/200 | 35.5 \pm 1.5 | 8.1 | | | |
| Control | vehicle | 14 | 210/222 | 44.1 \pm 1.4 | | | | |
| Dexamethasone | 0.1 | 10 | 200/197 | 36.0 \pm 1.4 | 18.4 | 0.01 | 2.67 | 1.83 |

The 2nd implantation was performed 3 days after implantation of the 1st pellet in a rat. On the 8th day following the 1st implantation, the granulomas were removed. Drugs were orally administered once a day for 5 days following the 2nd implantation.

a) significantly different from each vehicle control group

b) and c) the ratios of the inhibition of granuloma formation induced by the 1st pellets to that by the 2nd pellets and the pellets in one time implantation study, respectively

Discussion

In general, non-steroidal anti-inflammatory agents showed weaker inhibitory activity on the accelerated granuloma formation induced by dual implantation (Fig. 2, 3, 4). On the other hand, prednisolone, unlike non-steroidal anti-inflammatory agents, inhibited by the equal degree both usual and accelerated granuloma formations at 3 and 10 mg/kg/day (Fig. 5b), and dexamethasone showed more potent activity on the accelerated granuloma formation at 0.01 and 0.1 mg/kg/day (Fig. 5a). If both the usual and accelerated granuloma formations are induced by the same mechanism, the anti-granuloma activities of non-steroidal agents would be almost equal in both experiments, or the net decrease in dry granuloma weights would be by the equal degree. However, the net decrease in dry weights of formed granulomas by non-steroidal agents was less in dual implantation than in one time implantation, except for flufenamic acid at a dose of 60 mg/kg/day (Table II.). Granuloma formation caused by filter paper pellets has been accelerated by soaking a carrageenin solution into the pellets in

rats,⁴⁾ as by the felt-pellets,³⁾ and both the granuloma formations caused by filter paper pellets with or without carrageenin have been inhibited by both steroidal and non-steroidal anti-inflammatory agents.^{5,6)} Furthermore, a significant acceleration of granuloma formation in the dual implantation has not been observed in adrenalectomized rats, whereas the usual granuloma formation has not been influenced by adrenalectomy.³⁾ These findings suggest that the developmental mechanism of the accelerated granuloma formation may be partly different from that of the usual granuloma formation.

TABLE II. Difference in Dry Granuloma Weight between Vehicle Control Group and Drug Treated Group

| | Dose mg/kg/day <i>p.o.</i> | Difference from control group in dry granuloma weight | | | | | |
|-----------------|----------------------------------|---|-----------|--------------------|--|-----------|--------------------|
| | | Usual granuloma formation | | | Accelerated granuloma formation (2nd pellet) | | |
| | | <i>n</i> | mean (mg) | <i>p</i> -value | <i>n</i> | mean (mg) | <i>p</i> -value |
| Indomethacin | 0.3 | 6 | - 0.3 | | | | |
| | 1 | 6 | - 6.9 | | | | |
| | 2 | 6 | -14.5 | 0.01 ^{a)} | 11 | -10.5 | |
| | 3 | 5 | -32.6 | 0.01 | 6 | -19.5 | |
| Flufenamic acid | 10 | 5 | - 5.4 | | | | |
| | 30 | 6 | -16.6 | 0.01 | 11 | - 0.7 | |
| | 60 | 6 | -25.9 | 0.01 | 5 | -26.7 | 0.01 ^{a)} |
| Phenylbutazone | 10 | 6 | - 6.1 | | | | |
| | 30 | 6 | -15.3 | 0.01 | 11 | + 6.5 | |
| | 100 | 5 | -24.4 | 0.01 | 5 | -18.5 | |
| Aspirin | 100 | 6 | + 3.3 | | | | |
| | 300 | 6 | - 6.2 | | | | |
| | 600 | 12 | -12.5 | 0.01 | 7 | + 0.2 | |
| Prednisolone | 1 | 6 | - 6.0 | | 8 | - 9.7 | |
| | 3 | 6 | -17.6 | 0.01 | 6 | -36.3 | 0.01 |
| | 10 | 5 | -26.9 | 0.01 | 8 | -35.4 | 0.01 |
| Dexamethasone | 0.01 | 9 | - 1.9 | | 12 | -13.9 | 0.01 |
| | 0.03 | 9 | -11.9 | 0.01 | 12 | -16.3 | 0.01 |
| | 0.1 | 9 | -16.7 | 0.01 | 10 | -32.3 | 0.01 |

The mean dry granuloma weights of vehicle control groups are 65.9 mg (*n*=70) and 91.7 mg (*n*=60) in usual and accelerated granuloma formation, respectively.

a) significantly different from each vehicle control group

Phenylbutazone, flufenamic acid, prednisolone and dexamethasone were less effective against the granuloma formation induced by the 1st implanted pellets in dual implantation than against the usual granuloma formation. And also indomethacin and Aspirin did not show significant effects against the granuloma formation induced by the 1st pellets (Table I.). These results imply that both steroidal and non-steroidal anti-inflammatory agents possess more potent anti-granuloma activity on the early stage of granuloma formation than that on the late stage. Fukuhara, *et al.*⁷⁾ have reported that a steroidal drug, betamethasone, revealed an ability both to inhibit the formation of granuloma and to reduce the pre-existing granuloma in the carrageenin induced granuloma pouch in rats, while non-steroidal drug, indomethacin, phenylbutazone and salicylic acid, failed to reduce the pre-existing granuloma, though they had some effects on the granuloma formation. However, in the study, drugs

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have been injected locally as suspensions into the carrageenin sac, and only the wet weight of pouch wall has been determined. Hicks⁸⁾ has reported that a peak in the wet weights of granulomas induced by cotton pellets in rats was different from that in the dry weights. On the other hand, it has been reported that the carrageenin granuloma pouch reached a peak in wet weight at day 5 and then gradually decreased, while accumulation of the pouch fluid took place mainly 5 to 9 days or more.^{9,10)} However, it was found that the cotton pellet granuloma in rats reached a peak in vascular permeability at day 3 and in dry weight at day 15.¹¹⁾ The granuloma formation by felt-pellets has reached a peak at day 3 to 7 and then quickly subsided after day 8.³⁾ It has been reported that hydrocortisone acetate was less effective against the late stage of cotton pellet induced granuloma formation¹²⁾ and the effectiveness against the carrageenin induced granuloma pouch declined gradually with the age of the granuloma in rats,¹⁰⁾ and a steroidal drug, betamethasone, inhibited collagen synthesis and the degradation of collagen.¹³⁾ Subsequently, the less effectiveness of steroidal agents against the late stage of granuloma formation induced by the felt-pellets may be explained by the inhibition of the degradation of collagen with the agents.

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