ANTI-INFLAMMATORY TESTING METHODS: COMPARATIVE EVALUATION OF MICE AND RATS

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The possibility of using mice in place of rats on the anti-inflammatory screening tests was investigated.

The diversities of the responses between mice and rats on screening tests, that is, carrageenin, formalin-induced edema in the hind paw, adjuvant arthritis and cotton pellet granuloma were observed with p.o. administration of prednisolone, 0.5 mg/kg and 5.0 mg/kg, and indomethacin, 0.5 mg/kg and 5.0 mg/kg. On the effects of these drugs on screening tests, a similar type of phenomena between rats and mice was found.

Then the dose-response relationships of aspirin, flufenamic acid, phenylbutazone, cyproheptadine, prednisolone and indomethacin were examined in four kinds of screening tests mentioned above. Prednisolone, indomethacin, phenylbutazone and aspirin inhibited the carrageenin, formalin-induced edema, cotton pellet granuloma and adjuvant arthritis apparently, but flufenamic acid showed no inhibition on the carrageenin and formalin-induced edema. Cyproheptadine inhibited the formalin and serotonin-induced edema.

We have found that mice, instead of rats, can be used for the anti-inflammatory screening methods.

Keywords—anti-inflammatory testing method; comparative evaluation of mice and rats; dose-response relationship; aspirin; flufenamic acid; phenylbutazone; cyproheptadine; prednisolone; indomethacin

Inflammation seems to be a relatively non-specific response, which may be initiated by a variety of physical, mechanical, chemical or immunological means. These responses have much in common, no matter what the original stimulus and animals are used for testing. In the screening tests of anti-inflammatory agents, the usage of rats are very popular nowadays.\(^1\)\(^-\)\(^{14}\) But, on account of it's high cost, the number of rats used in screening experiments is limited. This paper reports the possibility of utilizing mice to screen the anti-inflammatory agents, instead of rats.

In the first step, the diversity of mice and rats as testing animals was investigated utilizing carrageenin,\(^15\)\(^-\)\(^{16}\) formalin\(^15\)\(^-\)\(^{18}\)-induced edema in mouse and rat hind paw, adjuvant arthritis\(^19\)\(^-\)\(^{21}\) and cotton pellet granuloma\(^22\) in both animals by the administration of prednisolone as a steroidal drug and indomethacin as a non-steroidal drug.

In the second step, the anti-inflammatory activities of aspirin, phenylbutazone, flufenamic acid and the anti-allergenic activity of cyproheptadine were investigated utilizing same experimental systems mentioned above on mice.

EXPERIMENTAL

Agents Used—Prednisolone, indomethacin (Tokyo Kasei Co.), aspirin, phenylbutazone, flufenamic acid and cyproheptadine (Maruko Chem. Co.) were suspended in a 5.0% Tween-80 (Nakarai Chem. Co.) solution. \(\lambda\)-Carrageenin

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(Sigma Chem. Co.), formalin (Nakarai Chem. Co.), serotonin (Merck) and Mycobacterium butyricum (Difco) were used as an irritant.

**Measuring Method of Edema in Mouse and Rat Hind Paw** — The volume of the mouse hind paw was measured as follows (Fig. 1). The foot above the topmost callus pad was immersed in a pool of water. A slight rise in water level (A → B) in a pool was transmitted to a drastic rise in water level (A → B') in a pipette with a capacity of 1 ml. By this method, a small increase in the volume was recognised easily. The foot volume of the rat hind paw was measured as mentioned above. But in this case, the capacity of the pipette used was 5 ml.

**Carrageenin-induced Edema in Mouse and Rat**<sup>15</sup> **Hind Paw** — Female ddY mice, 5 weeks old weighing 18—22 g, and female Wistar rats, 5 weeks old weighing 160—200 g, were used in this experiment. The animals were maintained on laboratory food (CE-2, Kureha Co.) and water in well controlled environment. The subplantar injection of 0.05 ml of 1.0% λ-Carrageenin in water for injection was carried out.

The volume of the foot was measured prior to the treatment with carrageenin and at 0.5, 1, 2, 3, 5 and 7 h after the injection. The increase in the volume was taken as the volume of edema. The agents were administered p.o. 1 h before the carrageenin treatment. The percent inhibition of edema induced by each agent was calculated for each animal group with respect to its vehicle-treated control group.

**Adjuvant Arthritis in Mice and Rats** — According to the method described by Pearson et al.,<sup>19</sup> adjuvant arthritis was induced in female ddY mice, 5 weeks old weighing 18—22 g, and female Wistar rats, 5 weeks old weighing 160—200 g, at the start of the experiment. The subplantar injection of 0.05 ml of 0.3% or 0.6% suspension of heat-killed Mycobacterium butyricum in liquid paraffin into the right hind paw of the mice and rats was carried out and then volume of the injected hind paw was measured prior to the injection and on 11, 13, 15, 17, 19 and 21 days after the injection. The animals which had the same degree of edema volume on day 11 were selected and used. They were administered p.o. the drugs on day 11 after the injection. And the difference between day 11 and each succeeding day — 13, 15, 17, 19 and 21 days — was called

![Diagram](image_url) **FIG. 1. Diagram of Apparatus for Measuring Foot Volume of Unanaesthetized Rats and Mice, see text for explanation.**
the edema volume. The agents were administered p.o. to the mice and rats once a day for 11 days. The percent inhibition of edema produced by each agent was calculated for each animal group with respect to its vehicle-treated control group.

**Cotton Pellet Granuloma in Mice and Rats**

The cotton pellet granuloma inhibition test was performed in our laboratory by a modification of the method of Meier et al.\(^{22}\) Cotton dental rolls (Taketora Co. LTD.) were cut into small segments so that each piece weighs 10–12 mg; in any experiment, the pellets were the same weight \(\pm 1\) mg. After sterilizing in an autoclave, cotton pellets were bilaterally implanted subcutaneously into the dorsal area — four cotton pellets were inserted in rats and two cotton pellets in mice. The agents were administered p.o. once a day for 7 days after this treatment. After 8 days, the animals were killed, and the pellets were carefully removed from surrounding tissues and weighed after being dried overnight at 65°C. The increment in dry weight was taken as the amount of granuloma. The rate of granuloma formation was calculated as follows:

\[
\frac{\text{(Dry wt of granuloma)} - \text{(Initial wt of cotton pellet)}}{\text{(Initial wt of cotton pellet)}} \times 100
\]

**Serotonin-induced Edema in Mice**\(^{16}\) — Female ddY mice, 5 weeks old weighing 18–22 g, were used. The subplantar injection of 0.05 ml of 1.0% serotonin in physiological saline solution was carried out in mice. The volume of the foot was measured prior to the treatment with serotonin and at 0.5, 1, 2, 3 and 4 h after injection. The increase in the volume was taken as the volume of

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**FIG. 2. Inhibitory Effects of Prednisolone on the Swelling of Rat and Mouse Hind Paw induced by Carrageenin (1.0%, 0.05 ml)**

Average of 8 female rats and 12 female mice.

---: control, \(\bigcirc\) : 0.5 mg/kg p.o., \(\triangle\) : 5.0 mg/kg p.o., \(\times\) : 50.0 mg/kg p.o. Small numbers mean the inhibitory rate (%).
RESULTS

Carrageenin-induced Edema in Mice and Rats

The anti-inflammatory effects of prednisolone, 0.5, 5.0 and 50 mg/kg p.o., and indomethacin, 0.5, 5.0 and 10 mg/kg p.o., on carrageenin-induced edema in the mouse hind paw were investigated and the results were compared with those in the rat hind paw. The results are shown in Fig. 2 and 3.

Prednisolone, 50 mg/kg p.o., in mice and prednisolone, 5.0 mg/kg p.o., in rats caused significant inhibitions of about 50% on an average of 7 h after the treatment of carrageenin (p < 0.001).

In contrast, indomethacin, 0.5 mg/kg p.o., tended to inhibit the development of edema in mice and rats. Indomethacin, 5.0 mg/kg p.o., significantly inhibited the edema by 33% on an average of 7 h after the treatment of carrageenin in mice and rats, respectively (p < 0.001). The inhibitory effect was also observed with indomethacin, 10 mg/kg p.o.

Formalin-induced Edema in Mice and Rats

The inhibitory effects of prednisolone, 0.5, 5.0

---: control, ○: 0.5 mg/kg p.o., △: 5.0 mg/kg p.o., ▲: 10.0 mg/kg p.o. Small numbers mean the inhibitory rate (%).
and 50 mg/kg p.o., and indomethacin, 0.5, 5.0 and 10 mg/kg p.o. on formalin-induced edema were investigated in mice and rats. The results are shown in Fig. 4 and 5. The inhibition of about 50% on an average of 24 h after the treatment of formalin was observed with prednisolone, 5.0 mg/kg p.o., in mice and rats (p < 0.001). Indomethacin, 5.0 mg/kg p.o., caused significant inhibition of about 60% on an average of 24 h after the injection in mice (p < 0.001) and about 50% in rats (p < 0.001).

**Adjuvant Arthritis in Mice and Rats**

The inhibitory effects of 11-day (from 11 to 21 days after treatment) administrations of prednisolone, 0.5, 5.0 and 50 mg/kg p.o., and indomethacin, 0.5, 5.0 and 10 mg/kg p.o., on the edema formation in the mouse and rat hind paw were determined.

The results are illustrated in Fig. 6 and 7. Prednisolone and indomethacin in every dose produced dose-dependent inhibition on the edema by more than 50% on an average on the 21st day in mice and rats (p < 0.001).

**Cotton Pellet Granuloma in Mice and Rats**

The inhibitory effects of prednisolone, 0.5 and 5.0 mg/kg p.o., and indomethacin, 0.5 and 10 mg/kg p.o., per day for 7 days, on the granuloma formation in mice and rats by cotton pellet method were investigated.

The results are shown in Table I and II.

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**FIG. 4. Inhibitory Effects of Prednisolone on the Swelling of Rat and Mouse Hind Paw induced by Formalin**

(Rat: 0.75%, 0.1 ml; Mouse: 0.73%, 0.05 ml)

Average of 4 female rats and 10 female mice.

---: control, ○: 0.5 mg/kg p.o., △: 5.0 mg/kg p.o., ×: 50.0 mg/kg p.o. Small numbers mean the inhibitory rate (%).
Inhibitory effects of about 30 and 40% on an average with prednisolone, 5.0 mg/kg p.o. ($p < 0.001$) and more than 40% on an average with indomethacin, 10 mg/kg ($p < 0.001$) were observed in mice and rats, respectively.

**Dose-response Relationships of Aspirin, Flufenamic Acid, Phenylbutazone, Cyproheptadine, Indomethacin and Prednisolone in Mice**

In order to study whether other agents have any effect on the inflammatory model using mice, time course and dose-response relationships in paw edema and granuloma formation with the following agents were studied; aspirin, 11.1, 33.3, 100 and 300 mg/kg p.o.; flufenamic acid, 0.3, 1.0, 3.0 and 9.0 mg/kg p.o.; phenylbutazone, 3.3, 10, 30 and 90 mg/kg p.o.; cyproheptadine, 0.3, 1.0, 3.0 and 9.0 mg/kg p.o.; indomethacin, 0.3, 1.0, 3.0 and 9.0 mg/kg p.o. and prednisolone, 0.7, 2.0, 6.0 and 18 mg/kg p.o. The results are summerised in Fig. 8, 9, 10, 11 and 12.

Fig. 8 shows the dose-response curves obtained in these experiments with carrageenin-induced edema. Cyproheptadine, regarded as antiallergic rather than anti-inflammatory drug, was inactive while 4 anti-inflammatory drugs yielded dose-response curves that were parallel within the

**FIG. 5. Inhibitory Effects of Indomethacin on the Swelling of Rat and Mouse Hind Paw induced by Formalin**

(Rat: 0.75%, 0.1 ml; Mouse: 0.75%, 0.05 ml)

Average of 4 female rats and 10 female mice.

---: control, ○: 0.5 mg/kg p.o., △: 5.0 mg/kg p.o., ▲: 10.0 mg/kg p.o. Small numbers mean the inhibitory rate (%).
**Anti-inflammatory Testing Methods**

**FIG. 6. Inhibitory Effects of Prednisolone on the Swelling of Rat and Mouse Hind Paw induced by Adjuvant**

Average of 4 female rats and 8 female mice. 
--- : Control, ○ : 0.5 mg/kg p.o., △ : 5.0 mg/kg p.o., × : 50.0 mg/kg p.o. Small numbers mean the inhibitory rate (%).

**FIG. 7. Inhibitory Effects of Indomethacin on the Swelling of Rat and Mouse Hind Paw induced by Adjuvant**

Average of 4 female rats and 8 female mice. 
--- : control, ○ : 0.5 mg/kg p.o., △ : 5.0 mg/kg p.o., ▲ : 10.0 mg/kg p.o. Small numbers mean the inhibitory rate (%).
limits of experimental error. But flufenamic acid showed no inhibition in mice, while the strong inhibitory effect was reported in rats.\textsuperscript{14)}

Fig. 9 shows the inhibition of edema induced by formalin. All the drugs show the inhibition but cyproheptadine seems to be more potent than any of the anti-inflammatory compounds. Indomethacin and phenylbutazone show a significant inhibition in mice, while they are reported to show no activity in the rat hind paw edema induced by formalin.\textsuperscript{14) 14)}

Fig. 10 shows the inhibition of the cotton pellet granuloma formation in mice. Flufenamic acid shows no dose-response relationship but other 3 anti-inflammatory and antiallergic drugs caused similar inhibitory effects. The activity of aspirin is not so potent.

Fig. 11 shows the inhibition of the adjuvant arthritis in mice. Cyproheptadine shows no effect. Four anti-inflammatory drugs inhibited the adjuvant arthritis significantly, while aspirin caused only a slight inhibition.

Another type of edema, in which cyproheptadine is especially active, is that produced by

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### TABLE I. Effects of Prednisolone on Granuloma Formation by Cotton Pellet Method in Rats and Mice (7-day Test)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Drug</th>
<th>Dose (mg/kg p.o.)</th>
<th>Body weight (g)</th>
<th>Granuloma (mg/cotton mg)</th>
<th>Inhibitory (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Control</td>
<td>-</td>
<td>111 141</td>
<td>1.030±0.175\textsuperscript{a)}</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Prednisolone</td>
<td>0.5</td>
<td>118 130</td>
<td>0.781±0.159</td>
<td>24.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>122 121</td>
<td>0.629±0.103</td>
<td>38.9**</td>
</tr>
<tr>
<td>Mouse</td>
<td>Control</td>
<td>-</td>
<td>17.4 20.5</td>
<td>0.530±0.0540</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Prednisolone</td>
<td>0.5</td>
<td>17.9 16.2</td>
<td>0.420±0.0910</td>
<td>21.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>18.6 15.8</td>
<td>0.380±0.0270</td>
<td>28.0**</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Each value is the mean of sixteen experiments ± S.E.

\textsuperscript{*} p < 0.05, \textsuperscript{**} p < 0.001.

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### TABLE II. Effects of Indomethacin on Granuloma Formation by Cotton Pellet Method in Rats and Mice (7-day Test)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Drug</th>
<th>Dose (mg/kg p.o.)</th>
<th>Body weight (g)</th>
<th>Granuloma (mg/cotton mg)</th>
<th>Inhibitory (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Control</td>
<td>-</td>
<td>111 141</td>
<td>1.030±0.175\textsuperscript{a)}</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>0.5</td>
<td>112 132</td>
<td>0.780±0.123</td>
<td>24.3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>112 140</td>
<td>0.581±0.148</td>
<td>43.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>115 145</td>
<td>0.572±0.0970</td>
<td>44.5**</td>
</tr>
<tr>
<td>Mouse</td>
<td>Control</td>
<td>-</td>
<td>17.4 20.5</td>
<td>0.530±0.0540</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>0.5</td>
<td>18.4 19.8</td>
<td>0.450±0.0510</td>
<td>15.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>18.1 18.8</td>
<td>0.300±0.0270</td>
<td>43.0**</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Each value is the mean of sixteen experiments ± S.E.

\textsuperscript{*} p < 0.05, \textsuperscript{**} p < 0.001.
Anti-inflammatory Testing Methods

FIG. 8. Dose-response Regression Lines for Inhibition by Single Oral Doses of Various Drugs in Carrageenin Edema in Mice

○: flufenamic acid, △: aspirin, □: prednisolone, ●: indomethacin, ▲: phenylbutazone, ■: cyproheptadine. For doses of drugs used in Fig. 8—12, see text explanation.

FIG. 9. Effects of Drugs on Edema induced by Injection of 0.75% Formalin

FIG. 10. Effects of Drugs on Granuloma Formation by Cotton Pellet Method in Mice, 7-day Test

FIG. 11. Inhibition of Adjuvant Arthritis-Induced Foot Edema in Mice

FIG. 12. Inhibition of Serotonin-Induced Foot Edema in Mice

serotonin (Fig. 12). Indomethacin and phenylbutazone also inhibited the edema.

DISCUSSION

Recently, S. Tsurufuji et al. and H. Hikino et al. reported the anti-inflammatory screening methods using mice, but the comparative evaluation of mice and rats was not reported in detail. This paper described a possible method of using mice as test animals.

Steroidal agent, prednisolone, and non-steroidal agent, indomethacin, gave dose-related response in the hind paw edema induced by carrageenin, formalin and adjuvant arthritis, and granuloma in mice as well as in rats.

A linear relationship between logarithmic dose
and the percent increase in the paw volume and granuloma was observed with aspirin, phenylbutazone, flufenamic acid, indomethacin and prednisolone in mice. But flufenamic acid in carrageenin edema showed only a slight effect in mice compared with the report of Winter in rats.\textsuperscript{14}

One of the most widely used substances for producing foot edema in rats is dilute formalin. Domenjoz\textsuperscript{25,26} and others \textsuperscript{27,28} have published a number of papers on the inhibition of formalin-induced edema by anti-inflammatory drugs. In spite of the wide use of formalin, several workers, including Lorenz,\textsuperscript{29} Warner-Jauregg, Jahn and Büch\textsuperscript{30} and Winter et al\textsuperscript{31} have pointed out that the inhibition of formalin-induced edema by drugs of the phenylbutazone and indomethacin class has been observed only after intraperitoneal doses within the grossly toxic range. But in our experiment using mice, not only cyproheptadine as an antiallergic agent but also phenylbutazone and indomethacin as an antiinflammatory agent inhibited the formalin-induced edema sufficiently. Although a difference in the sensitivity on drugs between mice and rats exists, it is not reasonable to conclude that indomethacin shows no effect on the edema induced by formalin which has a destructive effect on the tissue. The formalin-induced edema seems to be not suitable for an anti-inflammatory drug screening test, because cyproheptadine shows the stronger effect than anti-inflammatory drugs, indomethacin, flufenamic acid, prednisolone, aspirin and phenylbutazone.

There is no report concerning the occurrence of arthritis induced in mice by injection of Mycobacterial Adjuvant. In our experiment the edema on the arthral region was observed apparently in mice with the same susceptibility in rats. But it is difficult to decide as to whether this phenomena is derived from the adjuvant arthritis or not. Therefore, the histological study should be conducted in the future.

Another problem in this experiment is the method of measuring the edema of the mouse hind paw. The volume of the mouse hind paw is so small that there might be a wide range of experimental error in measuring the edema, compared with that of the rat hind paw. To confirm this point, three investigators read the scale of a pipette at the same time in each experiment. Experimental error gained proved to be negligible, after they grew familiar with the experiment.

Of the many other methods used for the evaluation of anti-inflammatory compounds, none has been more widely used than procedures involving inhibition of granuloma formation and edema induced in the hind paw of animals by subplantar injection of an irritant. These methods are important in the earliest screening stage for its rapidity and low price. From the present experiment, it seems reasonable to draw a conclusion that mice, instead of rats, can be used in the anti-inflammatory screening study.

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REFERENCES

Antinflammatory Testing Methods


