ANTIPYRETIC EFFECT OF INDOMETHACIN SUPPOSITORY IN RABBITS

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We have designed a thermistor rectal probe thermometer for measuring the antipyretic activity of suppositories. Using this thermistor probe, we tested the antipyretic effect of an indomethacin suppository in comparison with oral and intravenous administrations in rabbits (male, 2.5–2.9 kg). The rectal temperature of normal rabbits remained unchanged after rectal and intravenous administration of indomethacin, 25 mg/body and 10 mg/kg, respectively. The antipyretic effect was tested in febrile rabbits injected with bacterial pyrogen, lipopolysaccharide (LPS) 0.2 μg/kg (i.v.). The dose-dependent antipyretic activities were observed in febrile rabbits administered with indomethacin by rectal (6.3–23.7 mg/body), intravenous (2.5–10 mg/kg) and oral (2.5–20 mg/kg) routes. When indomethacin was administered simultaneously or 1 h after LPS, the most potent antipyretic effect was observed in the case of rectal administration and the weakest effect was observed in that of oral administration.

These data indicate that the rectal administration of drugs can produce a potent antipyretic activity, not inferior to that of the intravenous injection.

Keywords — antipyretic activity; indomethacin; suppository; bacterial pyrogen; rabbit

Recently, many reports have been published concerning the side effects of the rectal administration of the anti-inflammatory drugs. On the other hand, the rectal suppositories of those drugs were widely used to avoid gastrointestinal lesions including erosions in the gastric mucosa. However, the mode of action of these drugs administered through the rectum has not been studied in detail. Thus, it is necessary to evaluate the pharmacological activities of these drugs administered into the rectum.

Indomethacin, 1-[(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, is a well known nonsteroidal antiinflammatory agent. It has been shown by many investigators that indomethacin has a potent antipyretic activity, and it is one of the antipyretics most frequently used as a suppository. However, the antipyretic effect of indomethacin and other antipyretics following rectal administration in animals has not been reported. In addition, the methods which measure the rectal temperature after rectal drug administration have never been reported.

The antipyretic effect of some drugs was reported to be influenced by administration time in febrile rabbits injected with bacterial pyrogen (lipopolysaccharide, LPS) because early and late febrile responses were considered to be induced by different mechanisms.

In the present report, we have studied the antipyretic effect of indomethacin administered in the rectum simultaneously and 1 h after LPS injection, using a newly designed rectal thermometer.

MATERIALS AND METHODS

1) Materials — Indomethacin was supplied by Wako Pure Chemical Industries. LPS was prepared from acetone-dried Esherichia coli UKT-B extracted with 90% hot phenol as described by Westphal and Lüderitz.

2) Preparation of Indomethacin Suppository — The suppositories were prepared using intact indomethacin and polyethylene glycol 1540 as a base by a fusion method. The content of indomethacin in one suppository (1 g weight) was 6.3, 12.5, 23.7 or 25 mg.

3) Thermistor Probe for Measurement of Body Temperature — We have improved the rod-like thermistor probe (0.5 cm in diameter) of an electric thermometer (Lio Electric Co., Ltd, Tokyo) by addition of three rubber disks (1.5 mm in thick, 1.4 cm in diameter) with 4 mm space at 3 cm from the top (thermosensor) for prevention
of leakage of melted suppository from the rectum.

4) Animal Experiment — Male rabbits, weighing 2.5–2.9 kg, were retained by loosely-fitting neck stocks so that they could sit in a normal position. Rectal temperature was obtained by insertion of the improved probe into the rectum to a constant depth of 7 cm, and the temperature read to the nearest 0.1 °C on a liio electric thermometer. For the purpose of rectal administration of the suppository, the suppository was put into the rectum at 7 cm from anus. Then the probe was inserted immediately into the rectum. For oral administration, indomethacin were suspended in 5% acacia solution and 5 ml of the suspension were given per kg of body weight. For intravenous administration, indomethacin was dissolved in a sterile isotonic phosphate buffer (pH 7.4) and 5 ml of the solution per kg of body weight were given.

5) Calculation of Fever-Index (FI-5) — Fever curves of the rabbit rectal temperature were plotted on 1 mm section paper, 20 mm on the ordinate and abscissa representing 1 °C and 0.5 h, respectively. The area under the fever curves for 5 h was calculated as mm² and it was divided by 100. This value was the Fever-index (FI-5).

6) Statistical Evaluation — Data were analyzed by the use of the Student’s t-test.

RESULTS

1. Test of the Improved Thermistor Probe

To ascertain whether the thermometer with the improved thermistor probe shows the correct body temperature, this probe was compared with a standard probe for the pyrogen test. No significant differences in temperature were observed between the standard and the improved thermistor probe in normal rabbits or febrile rabbits injected with LPS (0.2 μg/kg, i.v.).

No leakage of melted suppository and no hemorrhage in the anus were observed after using the improved probe, while the standard probe did not prevent leakage.

2. Effect of Indomethacin on Body Temperature of Normal Rabbits

Some Antipyretics are known to influence normal body temperature after administration. To determine the effect of indomethacin on normal body temperature, a suppository (containing indomethacin 25 mg) was administered rectally to a normal rabbit. The body temperature was measured for 5 h. No changes in body temperature were observed.

Intravenous injections of indomethacin (10 mg/kg) also had no effect on body temperature in five normal rabbits.

3. Effect of Indomethacin Given Simultaneously with LPS

![FIG. 1. Antipyretic Effect of Indomethacin (IM) by Rectal Administration on LPS-Induced Fever in Rabbits](image)

**FIG. 1. Antipyretic Effect of Indomethacin (IM) by Rectal Administration on LPS-Induced Fever in Rabbits**

IM (rectal) and LPS (0.2 μg/kg, i.v.) were given simultaneously. Each point represents the mean with S.E. of three to six rabbits. Doses (mg/body) of IM are 0 (control, ○), 6.3 (●), 12.5 (△) and 23.7 (▲).

Significant differences (p < 0.05) from the control were observed in the following times: 6.3 mg/kg (0.25 h, 1.5–5.0 h), 12.5 mg/kg (0.75–5.0 h), 23.7 mg/kg (0.25–5.0 h).

![FIG. 2. Antipyretic Effect of IM by Intravenous Injection on LPS-Induced Fever in Rabbits](image)

**FIG. 2. Antipyretic Effect of IM by Intravenous Injection on LPS-Induced Fever in Rabbits**

IM (i.v.) and LPS (0.2 μg/kg, i.v.) were given simultaneously. Each point represents the mean with S.E. of three to six rabbits. Doses (mg/body) of IM are 0 (control, ○), 2.5 (●), 5.0 (△) and 10.0 (▲).

Significant differences (p < 0.05) from the control were observed in the following times: 2.5 mg/kg (0.25–3.75 h, 4.75 h), 5.0 mg/kg (0.25–5.0 h), 10.0 mg/kg (0.25–5.0 h).
The activity of antipyretics has been reported to be influenced by the route of administration and the interval between the administrations of LPS and antipyretics.8,9

In this study, indomethacin was administered by various routes simultaneously or 1 h after LPS injection.

As shown in Fig. 1, LPS (0.2 μg/kg i.v.) evoked a biphasic fever and produced a maximum rise in temperature of about 2.5 °C at about 3 h after the injection. The suppository containing 6.3, 12.5 or 23.7 mg of indomethacin and LPS (0.2 μg/kg, i.v.) was given simultaneously. Even the minimum dose of indomethacin (6.3 mg) significantly inhibited the increase of body temperature induced by LPS. The dose-dependent inhibition of the febrile response was observed. The dose of indomethacin in the rectal administration of 6.3, 12.5 or 23.7 mg represented about 2.3, 4.6 or 8.8 mg per kg of body weight in these studies, respectively.

When indomethacin was given intravenously, the febrile response was inhibited significantly by the minimum dose (2.5 mg/kg, i.v.), but the inhibition was observed for only a short period. The increase of body temperature after LPS injection was inhibited in the manner of dose-dependency (Fig. 2). The figure shows that the inhibition of febrile response by the intravenous administration was the same or slightly lower than by rectal administration of similar doses.

Oral administrations of indomethacin, on the other hand, showed weak antipyretic activity in comparison with the same dose by rectal or intravenous administration (Fig. 3). Twenty mg per kg in oral administration showed a weaker activity than the 5 mg per kg in the intravenous injection and the 12.5 mg (about 4.6 mg/kg) in

![Figure 3](image_url)

**Fig. 3. Antipyretic Effect of IM by Oral Administration on LPS-Induced Fever in Rabbits**

IM (p.o.) and LPS (0.2 μg/kg, i.v.) were given simultaneously. Each point represents the mean with S.E. of three to six rabbits. Doses (mg/body) of IM are 0 (control, ○), 2.5 (●), 5.0 (△), 10.0 (▲) and 20.0 (▽).

Significant differences (p < 0.05) from the control were observed in the following times; 5.0 mg/kg (3.0–4.25 h, 5.0 h), 10.0 mg/kg (2.0–4.75 h), 20.0 mg/kg (1.5–5.0 h).

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose</th>
<th>Fever index (FI-5)</th>
</tr>
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<tbody>
<tr>
<td>control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rectal</td>
<td>23.7 mg/body</td>
<td>b)</td>
</tr>
<tr>
<td>rectal</td>
<td>12.5 mg/body</td>
<td>a)</td>
</tr>
<tr>
<td>rectal</td>
<td>6.3 mg/body</td>
<td>a)</td>
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<tr>
<td>i.v.</td>
<td>10.0 mg/kg</td>
<td>b)</td>
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<tr>
<td>i.v.</td>
<td>5.0 mg/kg</td>
<td>a)</td>
</tr>
<tr>
<td>i.v.</td>
<td>2.5 mg/kg</td>
<td>b)</td>
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<tr>
<td>p.o.</td>
<td>20.0 mg/kg</td>
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<td>p.o.</td>
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<td>p.o.</td>
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<tr>
<td>p.o.</td>
<td>2.5 mg/kg</td>
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![Figure 4](image_url)

**Fig. 4. Antipyretic Effect of IM on LPS-Induced Fever in Rabbits**

IM and LPS (0.2 μg/kg, i.v.) were given simultaneously. Each column represents the mean with S.E. of three to six rabbits.

a) Significant differences from the control (p < 0.05). b) Significant differences each other.
the rectal administration.

The degrees of febrile response induced by LPS with or without indomethacin were calculated as Fever-index (FI-5), and on shown in Fig. 4. The figure represents the dose-dependent antipyretic activity observed in each route of administration. Activities were significantly stronger in the rectal and intravenous administrations than in the oral administration. The most effective antipyretic activity was observed in rectal administration.

FIG. 5. Antipyretic Effect of IM by Rectal Administration on LPS-Induced Fever in Rabbits
IM (rectal) was given 1 h after LPS (0.2 μg/kg, i.v.). Each point represents the mean with S.E. of five to six rabbits. Doses (mg/body) of IM are 0 (control, ○), 12.5 (△) and 23.7 (▲).

Significant differences (p < 0.05) from the control were observed in the following times; 12.5 mg/kg (2.0–5.0 h), 23.7 mg/kg (2.0–5.0 h).

It was also observed that indomethacin inhibited both the first and second fever phases which was considered to be induced by different mechanisms.4,7,8)

4. Effect of Indomethacin Given 1 h after LPS
The effect of indomethacin given 1 h after LPS (0.2 μg/kg, i.v.) by the various routes on febrile response was studied. The results are shown in Figs. 5–7.

The rapid and strong antipyretic activities of indomethacin were observed by rectal and intravenous administrations similar to that seen in the simultaneous administration experiment, but not by oral administration.

The Fever-index (FI-5) shows the dose-dependent activity observed by each route of administration and the antipyretic activities by the various routes of administration were in the same order of magnitude as with the simultaneous administration (Fig. 8).

DISCUSSION
There are few reports which have demonstrated the antipyretic effect of antipyretic suppositories. The rabbit has been known to be the most suitable animal for the evaluation of antipyretic activity of the drugs. It is important, however, to restrain the rabbit in a normal posture for measurement of body temperature9) and to prevent the leakage of melted suppository from the anus.

We have designed a thermistor probe as a

FIG. 6. Antipyretic Effect of IM by Intravenous Injection on LPS-Induced Fever in Rabbits
IM (i.v.) was given 1 h after LPS (0.2 μg/kg, i.v.). Each point represents the mean with S.E. of three to six rabbits. Doses (mg/body) of IM are 0 (control, ○), 2.5 (●), 5.0 (△) and 10.0 (▲).

Significant differences (p < 0.05) from the control were observed in the following times; 2.5 mg/kg (1.5–4.75 h), 5.0 mg/kg (1.25–5.0 h), 10.0 mg/kg (1.25–5.0 h).

FIG. 7. Antipyretic Effect of IM by Oral Administration on LPS-Induced Fever in Rabbits
IM (p.o.) was given 1 h after LPS (0.2 μg/kg, i.v.). Each point represents the mean with S.E. of three to six rabbits. Doses (mg/body) of IM are 0 (control, ○), 5.0 (△), 10.0 (▲).

Significant differences (p < 0.05) from the control were observed in the following times; 10.0 mg/kg (3.5 h, 4.25–5.0 h).
rectal thermometer, for rabbits to measure the body temperature following the rectal administration of a suppository. No differences were observed between our designed thermistor probe and a standard probe with respect to body temperature in normal and febrile rabbits. No leakage of melted suppository and no hemorrhage near the anus were observed. These results showed that the improved thermistor probe was suitable for the antipyretic test of the suppository.

When we applied the suppository containing a dye, trypan blue, to rabbits, we observed that the dye was diffused in the whole descending colon and rectum within 1 h after the application. These results suggested that the broad area of intestine was involved in the absorption of drug and the posture of the rabbit might be important for the reproducibility of both the measurement of body temperature and the absorption of drugs.

The immediate and strong antipyretic effect of indomethacin was observed by rectal and intravenous administrations, but not by oral administration. The most potent effect was observed by the rectal route among the three routes examined in this study. The results suggested that the absorption of indomethacin from the rectum might be rapid. Kuroda et al. reported that the maximum plasma concentration was achieved rapidly after rectal administration (25 mg/body), i.e., maximum plasma concentration was observed 30–40 min after the administration in normal rabbits.

The rectal administration of indomethacin produced a antipyretic effect for a longer period than the intravenous route. The results indicate that the period of blood concentration in the effective level might be long. But, it is reported that about 1 μg/ml of plasma concentration remained at 5 h after the rectal administration of 25 mg/body of indomethacin, whereas after intravenous injection the concentration was of similar level at the same time. Previously, we observed that the absorption and elimination rates of aspirin and aminopyrine were changed in febrile animals. Thus, for similar reasons, further studies are need to determine the rates of indomethacin absorption and elimination in febrile states.

REFERENCES
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