Inhibitory Effects of a Novel Synthetic Protease Inhibitor, FUT-175, on the Paw Edema in Rats and Zymosan-induced Complement Activation in Vitro

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Abstract—FUT-175 inhibited the zymosan-induced rat paw edema in a dose-dependent manner, while indomethacin exhibited no significant activities in this model. FUT-175 also inhibited the decrease in hemolytic complement (CH50) induced by zymosan in vitro, and indomethacin was inactive. These results suggest that FUT-175 has potent in vitro and in vivo inhibitory activity against the activation of the complement system induced by zymosan.

Activation of complement is one of the effector systems of the humoral immune response of the inflammatory process (1). The biological activities generated by activation of the complement system are manifest in increased vascular permeability, chemotaxis of leukocytes, modulation of antibody production, immune adherences and membrane damage (2). Several compounds, including protease inhibitors, inhibit the activation of the complement system (3, 4).

FUT-175, 6-amidino-2-naphthyl p-guanidinobenzoate dimethanesulfonate (nafamostat mesilate), is a novel synthetic protease-inhibiting agent that has inhibitory activity against complement (5, 6). The present study was undertaken to examine the effects of FUT-175 on the edema formation and complement activation induced by zymosan in comparison with those of indomethacin.

Prophylactic effects on various phlogogens-induced edemas: Male Sprague-Dawley rats weighing 150 to 220 g (Japan Charles River) were used. Animals were divided into groups (7 rats per group). Suspension or solutions of the phlogogens in saline solution were injected into the subplanter region of the right hind paw in a volume of 0.1 ml per rat. The test compounds were orally administered at 1 hr before the injection of phlogogens. The concentrations of phlogogens are as follows: zymosan (3%), carrageenin (1%) and kaolin (3%). The volume of the hind paw was measured by the water displacement method. The edema formation rate was calculated as the percentage increase in volume of the inflamed paw to the noninflamed paw at various times after the challenges of various phlogogens.

Zymosan-induced complement activation: The mixture consisting of 1 ml of guinea pig serum and 5 mg of zymosan was incubated at 37°C for 20 min in the presence or absence of test drugs. The mixture was centrifuged at 3,000 rpm for 10 min at 4°C, and the supernatant was dialyzed against gelatin veronal buffer (pH 7.5) at 4°C for 12 hr. The levels of complement in dialyzates were assayed according to the method of Mayer (7), and results were expressed in terms of CH50.

Statistical significance was evaluated by Student's t-test, and they were presented as the mean±S.E.

Drugs used: FUT-175 was synthesized at the Research Laboratories of Torii & Co., Ltd. The compound used for comparison was indomethacin (Indacin®, Nippon Merck Banyu). Drugs were dissolved or suspended in 5% glucose solution. Other chemicals used were carrageenin (Picnin-A®, Pasco., Inc.).
kaolin (Wako), sheep erythrocytes (SRBC, Nippon Biotest Labs., Inc.), rabbit anti-SRBC serum (hemolysin, Kyokuto chemical, Inc.) and zymosan (Sigma).

Figure 1 shows the effects of FUT-175 and indomethacin on various phlogogens-induced paw edemas in rats. The injection into the subplanter region of zymosan induced a rapid edema formation. The time course of development of edema differed from that of carrageenin- and kaolin-induced edemas. FUT-175 inhibited the edema formation induced by zymosan in a dose-dependent manner, but indomethacin inhibited only the edema at 3 hr after zymosan injection. FUT-175 had dose-dependent and significant effects on both carrageenin- and kaolin-induced paw edema, but it was inferior to indomethacin. These results show that the relative potencies of FUT-175 to indomethacin in these tests differ among the phlogogens used and indicate that FUT-175 possesses a different mechanism of action from indomethacin on inflammatory responses.

Zymosan is known to activate the complement system via the alternative pathway (8). In fact, as shown in Table 1, CH50 in zymosan treated guinea pig serum was markedly lowered compared to that in normal

![Graph](image)

**Fig. 1.** Effects of orally administered FUT-175 and indomethacin (IDM) on various phlogogens-induced edemas in rats. Values and vertical bars represent mean±S.E. of 7 rats. *P<0.05, **P<0.01: Significantly different from the control by Student’s t-test.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>CH50</th>
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<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>66.0±0.67**</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>14.4±0.78</td>
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<tr>
<td>FUT-175</td>
<td>10^-6 M</td>
<td>22.3±2.59*</td>
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<tr>
<td></td>
<td>10^-5 M</td>
<td>39.1±4.80**</td>
</tr>
<tr>
<td></td>
<td>10^-4 M</td>
<td>65.1±2.05**</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10^-3 M</td>
<td>13.4±0.71</td>
</tr>
</tbody>
</table>

Guinea pig serum and zymosan were incubated at 37°C for 20 min in the presence or absence of test drugs, and the residual complement level (CH50) was assayed. Each value represents the mean±S.E. of 4 samples. *P<0.05, **P<0.01: Significantly different from the control by Student’s t-test. Normal: normal guinea pig serum. Control: zymosan treated guinea pig serum.
guinea pig serum. FUT-175 inhibited the decrease in CH50 induced by zymosan in a concentration-dependent manner, but indomethacin was inactive.

The complement system has been speculated by Gemmell et al. (9) to play important roles in zymosan-induced paw edema (9). We reported in the preceding paper (6) that FUT-175 has potent in vitro and in vivo inhibitory activity on complement, as well as on other serine proteases such as trypsin, kallikrein, thrombin and plasmin. Therefore, it is considered that the antiinflammatory activity of FUT-175 on zymosan-induced edema is probably attributable to its anticomplementary activity in addition to inhibitory effects against kallikrein and plasmin, which are chemical mediators of the inflammatory process.

The cause of the antiedema effects of FUT-175 on carrageenin- and kaolin-induced paw edema could not be clearly elucidated in the present study, but the antiprotease activities mentioned above may partly contribute to these effects. Recently, we observed that pretreatment with FUT-175 (10 mg/kg, i.v.) markedly inhibited both kallikrein and plasmin activities in the exudate of carrageenin-induced pleurisy in rats (T. Sato et al., unpublished data). Further investigation is required to clarify this point.

References