Pharmacological Studies on 6-Amidino-2-Naphthyl[4-(4,5-
dihydro-1H-imidazol-2-yl)amino] Benzoate Dimethane
Sulfonate (FUT-187). I: Inhibitory Activities on Various Kinds
of Enzymes In Vitro and Anticomplement Activity In Vivo

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Abstract—FUT-187, a newly synthesized compound, was studied on its inhibitory
activities mainly on proteolytic enzymes, in comparison with those of FUT-175 and
FOY-305, known serine protease inhibitors. FUT-187, as well as FUT-175 and
FOY-305, had selective inhibitory activities on serine proteases including Clr, Cls,
kallikrein, trypsin, plasmin and thrombin; its activities on these enzymes except Clr
and pancreatic kallikrein were relatively lower than those of FUT-175 and FOY-305.
Further studies were conducted focusing on complement-mediated reactions. In
spite of its lower activities against Clr and Cls, inhibitions by FUT-187 on the com-
plement-mediated hemolysis in vitro and in vivo were only a little weaker than or
equivalent to that of FUT-175. FOY-305 was ineffective in these tests. Forssman
shock in guinea pigs is known to be initiated by the activation of the complement
system. The protective effect of intravenous or oral FUT-187 against this shock
was definitely superior to that of FUT-175. Furthermore, FUT-187 inhibited
changes accompanied with Forssman shock, such as increase in lung weight, the
decrease in platelet counts and CH50, and histopathological changes. These
results suggested that FUT-187 should be a more potent oral therapeutic agent
than FUT-175 for various inflammatory diseases attributed to the excessive ac-
tivation of the complement system followed by platelet aggregation.

The endopeptidases are classified into ser-
ine protease, thiol protease, metallo protease
and acid protease by the differences in their
active sites. Furthermore, serine proteases
having a serine residue in its active site are
classified into so-called trypsin-like serine
proteases, chymotrypsin-like serine proteases
and elastase depending on its substrate selec-
tivity. Trypsin-like serine protease which has
selectivity to arginyl and lysyl residue-con-
taining substrate includes enzymes in the
complement system, trypsin, plasmin, kalli-
krein and thrombin. Chymotrypsin-like serine
protease has selectivities towards phenylalanyl,
tyrosyl and tryptophan residue-containing
substrates; and elastase is selective mainly to
alanyl residue-containing substrates.

Among these enzymes, the complement
system is considered to be intimately involved
not only in host defense against infectious
agents, but also in the pathogenesis of im-
mune-complex diseases such as acute
glomerulonephritis (1), rheumatoid arthritis
and lupus erythematosus (2). In these
diseases, agents that inhibit the activation of
the complement system would be expected to
ameliorate the acute pathological changes
induced by immune complexes.

FUT-175, FOY-007 and FOY-305 have
been reported to be serine protease inhibitors
(3–5). These inhibitors, however, do not have
enough inhibitory activities on the comple-
ment system in vivo especially when administered orally.

6-Amino-2-naphthyl[4-(4,5-dihydro-1H-imidazol-2-yl)amino] benzoate dimethane sulfonate (FUT-187), a newly synthesized serine protease inhibitor, having similar structure to FUT-175 as shown in Fig. 1, has relatively higher inhibitory activity on the enzymes in the complement system including Clr and C19.

This paper reports the inhibitory effects of FUT-187 on the serine proteases, especially on the complement-mediated in vitro and in vivo reactions in comparison with those of the known serine protease inhibitors FUT-175 and FOY-305.

Materials and Methods

1. Drugs

FUT-187 was synthesized at the Research Laboratories of Torii & Co., Ltd. Clr and C19 were purified from preserved human sera as described by Okamura et al. (6, 7). Other enzymes used were pancreatic kallikrein (Bayer), plasma kallikrein (PAESER), trypsin (Sigma), plasmin (Green Cross Corp.), thrombin (Mochida Pharm.), chymotrypsin (Biochemicals Inc.), papain (Sigma), pepsin (Sigma), cathepsin D (Sigma), lipase (Sigma), phospholipase A2 (PLA2; Boehringer Mannheim Yamanouchi, Inc.), amylase (Wako Pure Chemical) and hyaluronidase (Sigma). Substrates used were acetyl-L-arginine methyl ester (AAME), acetyl-L-glucyl-L-lysine methyl ester (AGLME), tosyl-L-arginine methyl ester (TAME), Z-Phe-Arg-MCA (ZPA), acetyl-L-tyrosine ethylester (ATEE), Suc-Ala-Pro-Ala-MCA (SAPA), Z-Gly-Pro-Leu-Gly-Pro H2O AcOEt (ZGPLGP), 2,3 dimercaptopropan-1-ol tributyratoate (BALB) and benzoyl-L-arginine ethylester (BAEE) from the Protein Research Foundation and casein, fibrinogen, hemoglobin, phosphatidyl choline, starch and hyaluronic acid from Sigma. Other chemicals and drugs used were aspirin (ASP, Yoshida Pharmaceutical Inc.), FOY-305 (Ono Pharmaceutical Inc.), indomethacin (IDM, Banyu Pharmaceutical Inc.), FUT-175 (Torii & Co., Ltd.), cobra venom factor (CVF, Sigma), sheep erythrocyte (SRBC, Pharm), rabbit anti-SRBC serum (hemolysin, Kyokuto Chemicals Inc.) and FITC-conjugated goat anti-guinea pig C3 (Cappel).

2. Animals

Male Hartley strain guinea pigs weighing 250-400 g (Takasugi Experimental Animal and Matsumoto Experimental Animal) were used.

3. Inhibitory activities of FUT-187 on various enzymes in vitro

1) Proteases

a) Clr, C19, pancreatic kallikrein, plasma kallikrein, trypsin, plasmin, thrombin and chymotrypsin (synthetic substrates): Assays were done according to the method of Hestrin as modified by Roberts (8) except in the case of plasma kallikrein. The substrates used were AAME for Clr; AGLME for C19; TAME for pancreatic kallikrein trypsin, plasmin and thrombin; and ATEE for chymotrypsin, at a substrate concentration of 10 mM. Hydrolytic activity of each protease on the corresponding substrate, either in the presence or absence of FUT-187, was determined by incubation of the reaction mixture in 100 mM phosphate buffer (PB, pH 7.4) for Clr at 37°C for 60 min; in 20 mM PB (pH 7.4) for C19, thrombin and chymotrypsin; and 100 mM tris HCl (pH 8.5) for pancreatic kallikrein trypsin, and plasmin, at 37°C for 30 min. For plasma kallikrein, 0.1 mM of ZPA was used as the substrate. Hydrolytic activity of the protease on ZPA was determined by incubation of the reaction mixture in 100 mM tris HCl (pH 8.5) at 37°C for 30 min. The fluorescence of aminomethylcoumarin released from the substrate was measured at Ex 380 nm and Em 460 nm on a fluorophotometer (Shimadzu, M. Oda et al.)
b) Trypsin, plasmin and chymotrypsin (natural substrates): The method described by Muramatsu et al. (9) was followed. The substrates used were casein for trypsin and chymotrypsin and fibrinogen for plasmin.

c) Elastase: The substrate used was SAPA, and the assay was done according to the method described by Morita et al. (10).

d) Collagenase and papain: The substrate used were ZGPLGP for collagenase and BAEE for papain. The assays were done according to the method described by Aoyama et al. (3).

e) Pepsin and cathepsin D: The substrate used was hemoglobin, and these assays were done according to the method described by Cunningham and Tang (11).

2) Other enzymes

a) Lipase: The substrate used was BALB, and the assay was done using a Lipase kit®, according to the method of Kurooka and Kitamura (12).

b) PLA2: The substrate used was phosphatidylcholine, and the assay was done according to the method described by De Haas et al. (13).

c) Amylase: The assay was done by using an Amylase Test Wako®, according to the method of Caraway (14).

d) Hyaluronidase: The substrate used was hyaluronic acid, and the assay was done according to the method described by Kakegawa et al. (15).

4. Inhibitory effects on complement-mediated hemolysis via classical pathway

1) In vitro

a) Experiments with 500-fold diluted serum: The method of Mayer (16) was followed. The mixtures consisting of SRBC sensitized beforehand with 500-fold diluted hemolysin (EA) at a final concentration of 2.5×10⁶ cells/ml, 1/2.5 volume of 200-fold diluted guinea pig serum and FUT-187 at varying concentrations in GVB++ buffer were incubated at 37°C for 10 min. After incubation, the reaction was stopped by adding 0.01 M EDTA-containing saline, the mixture was centrifuged at 3,000 r.p.m. for 10 min, and the absorbance of the supernatant was read at 540 nm.

b) Experiments with 2-fold diluted serum: The method of Plescia et al. (17) was modified. A 0.3-ml aliquot of undiluted guinea pig serum, 0.06 ml of drug solution or GVB++ buffer and 0.3 ml of 5×10⁶ cells/ml EA was mixed, and the hemolysis in the reaction mixture was determined by an aggregometer (Rikadenki, platelet aggregometer).

2) In vivo

Groups of 6 guinea pigs were used. Three min after intravenous injection of 1 ml of 10¹⁰ cells/ml sensitized SRBC, 0.5 ml of blood was withdrawn by cardiac puncture. After addition of 4.5 ml EDTA-saline, the supernatant was obtained by centrifugation at 2,000 r.p.m. for 10 min, and the absorbance was recorded at 541 nm. Drugs were dosed orally 1 hr prior to the intravenous injection of sensitized SRBC.

5. Protective effect on Forssman shock in guinea pigs

1) Surviving time

Groups of 3–28 guinea pigs were used. Forssman shock was provoked as described by Glovsky et al. (18). Hemolysin at a lethal dose of 0.5 ml/animal was intravenously injected. The surviving time after hemolysin injection was recorded. For the intravenous route FUT-187 was given 5 min before hemolysin injection, and for the oral route, it was given 1 hr before. CVF was injected intravenously at a dose of 100 U/kg 24 hr prior to hemolysin injection.

2) The changes in lung weight, platelet counts and CH50

The effects of FUT-187 on the changes in lung weight, platelet counts and CH50 in Forssman shock were studied in groups of 4–7 guinea pigs. Hemolysin at a sublethal dose of 0.5 ml/kg was intravenously injected. Ten minutes later, blood specimens containing one tenth volume of 3.8% sodium citrate solution were taken by cardiac puncture. Lungs were isolated and weighed. Hemolytic complement level in whole plasma was determined as CH50 by the method of Mayer (16), and the platelet counts were measured by a platelet counter (PL-100, Sysmex). The CH50 is the reciprocal of the serum dilution which contains the quantity of complement required for 50% lysis of sheep erythrocytes coated with hemolysin in a hemolytic system.

3) C3 deposition and histopathological changes in the lung

Hemolysin at a lethal dose of 0.5 ml/animal
was intravenously injected into guinea pigs, and the lungs were taken 10 min later. FUT-187, 200 mg/kg, were orally administered 1 hr prior to hemolysin injection.

a) C3 deposition: The fresh lung specimens were frozen in dry ice acetone. Each thin section of 10 μm was incubated with FITC-conjugated goat anti-guinea pig C3 at 37°C for 30 min. After washing three times with phosphate-buffered saline, the immunofluorescent staining of the section was observed microscopically.

b) Histopathological evaluation: The lung tissue specimens were fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. These sections were examined microscopically.

Results
1. Inhibitory activities of FUT-187 on various enzymes in vitro

1) Proteases

Table 1 represents the inhibitory activities of FUT-187, FUT-175 and FOY-305 in terms of IC50 values, the concentration of each agent that inhibits each protease activity by 50%. FUT-187 showed inhibitions against Clr, Cls, pancreatic kallikrein, plasma kallikrein, trypsin, plasmin and thrombin with IC50s of 0.20–23 μM. These potencies, however, were weaker than those of FUT-175 except that on pancreatic kallikrein and weaker than those of FOY-305 except for the potencies on pancreatic kallikrein and Cls.

Against collagenase, FUT-187 with an IC50 of 74 μM as well as FUT-175 and FOY-305 had moderate inhibitory activity.

Against chymotrypsin, elastase, papain, pepsin and cathepsin D, FUT-187 as well as FUT-175 and FOY-305 had practically no

| Enzymes        | Substrates | FUT-187 | FUT-175 | FOY-305 |
|----------------|------------|---------|---------|---------|
| Proteases      |            |         |         |         |
| Clr            | AAME       | 23      | 0.18    | 8.6     |
| Cls            | AGLME      | 0.20    | 0.030   | 110     |
| Panc. kallikrein | TAME      | 3.6     | 12      | 180     |
| Plas. kallikrein | ZPA       | 0.26    | 0.0021  | 0.068   |
| Trypsin        | TAME       | 2.7     | 0.027   | 0.062   |
|                | Casein     | 3.5     | 0.20    | 0.48    |
| Plasmin        | TAME       | 1.9     | 0.14    | 0.90    |
|                | Fibrinogen | 1.2     | 0.052   | 0.20    |
| Thrombin       | TAME       | 6.4     | 0.5     | 4.8     |
| Chymotrypsin   | ATEE       | 280     | 150     | >1000   |
|                | Casein     | >1000   | >1000   | >1000   |
| Elastase       | SAPA       | >1000   | >1000   | >1000   |
| Collagenase    | ZGPLGP     | 74      | 150     | 340     |
| Papain         | BAEE       | >1000   |         |         |
| Pepsin         | Hemoglobin | >1000   |         |         |
| Cathepsin D    | Hemoglobin | >1000   |         |         |
| Other enzymes  |            |         |         |         |
| Lipase         | BALB       | 320     | 34      | 900     |
| PLA₂           | PC         | 70      | 20      | 70      |
| Amylase        | Starch     | >1000   | >1000   | >1000   |
| Hyaluronidase  | Hyaluronic acid | >1000 | >1000 | >1000 |

Panc.: Pancreatic, Plas.: Plasma, AAME: Acetyl-L-arginine methylester, AGLME: Acetyl-L-glycyl-L-lysine methylester, TAME: Tosyl-L-arginine methylester, ZPA: Z-Phe-Arg-MCA, ATEE: Acetyl-L-tyrosine ethylester, SAPA: Suc-Ala-Pro-Ala-MCA, ZGPLGP: Z-Gly-Pro-Leu-Gly-Pro H2O AcOEt, BAEE: Benzoyl-L-arginine ethylester, BALB: 2,3 dimercaptopropan-1-ol tributylate, PC: Phosphatidylcholine. Each value represents IC50 values (μM), the concentration of drugs to inhibit 50% of each enzyme activity.
inhibitory activity (Table 1).

2) Other enzymes

FUT-187 had moderate inhibitory activity against PLA2 with an IC50 of 20 μM and practically no inhibitory activity against lipase, amylase and hyaluronidase. The inhibitory effects of FUT-187 on these enzymes were almost similar to those of FUT-175 and FOY-305 (Table 1).

2. Inhibitory effect on the complement-mediated hemolysis via the classical pathway

1) In vitro

a) Experiment with 500-fold diluted serum:

As shown in Fig. 2, FUT-187 and FUT-175 inhibited the complement-mediated hemolysis via the classical pathway with IC50s of 0.22 and 0.077 μM, respectively, while FOY-305 had only a weak inhibitory activity with an IC50 of 170 μM.

b) Experiment with 2-fold diluted serum:

As shown in Fig. 3, FUT-187 inhibited the hemolysis of SRBC in a concentration-dependent manner. The potency of FUT-187 was lower at 10^{-6} M and higher at 3×10^{-5} M than that of FUT-175 at 10^{-5} M.

2) in vivo

As shown in Table 2, oral dosing of FUT-187 and FUT-175 inhibited the complement-mediated hemolysis via the classical pathway in guinea pigs. FUT-187 showed 15.3 and 100% of inhibition at doses of 100 and 300 μg/kg, respectively.

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Table 2. Effects of FUT-187 and FUT-175 on complement-mediated in vivo hemolysis in guinea pigs

| Drugs  | Dose mg/kg p.o. | No. of animal | OD_{545} (Mean±S.E.) | Inhibition of hemolysis (%) |
|--------|-----------------|--------------|----------------------|----------------------------|
| Control |                 | 6            | 0.206±0.0263         |                            |
| FUT-187 | 30              | 6            | 0.219±0.0327         | -9.5                       |
|         | 100             | 6            | 0.185±0.0392         | 15.3                       |
|         | 300             | 6            | 0.063±0.0076**       | 100.0                      |
| FUT-175 | 30              | 5            | 0.253±0.0260         | -34.7                      |
|         | 100             | 6            | 0.188±0.0524         | 27.7                       |
|         | 300             | 6            | 0.109±0.0273**       | 70.8                       |
| NC     |                 | 6            | 0.069±0.0079**       |                            |

NC: Normal control. **: Significant difference from the control at P<0.01.
mg/kg, respectively. The potency of FUT-187 was comparable to that of FUT-175.

3. Protective effect on Forssman shock in guinea pigs

1) Surviving time
   a) Intravenous administration: Intravenous dosing of FUT-187 resulted in dose-dependent protection of guinea pigs from the lethal shock: 33, 100 and 100% of guinea pigs that received FUT-187 at doses of 0.3, 1.0 and 3.0 mg/kg, respectively, survived more than 24 hr. Comparing the effect of FUT-187 with that of FUT-175, 1 mg/kg of FUT-187 was equivalent to 3 mg/kg of FUT-175 (Fig. 4).

   b) Oral administration: Oral dosing of FUT-187 resulted in dose-dependent protection of guinea pigs from the lethal shock: 28.6, 41.7 and 87.5% of guinea pigs that received FUT-187 at doses of 50, 100 and 200 mg/kg, respectively, survived more than 24 hr, while all 28 animals of the control group died within 30 min after hemolysin injection (Fig. 5).

   c) Comparison with FUT-175, FOY-305, ASP, IDM and CVF: The protective effect of oral FUT-187 on Forssman shock was com-

Fig. 4. Effects of intravenously administered FUT-187 and FUT-175 on surviving time in lethal Forssman shock. Groups of 3–4 guinea pigs were used. Drugs were dosed 5 min before hemolysin injection, and surviving times were recorded. Each point represents the surviving time of an individual guinea pig. P<0.05 indicates a significant difference from the control by Wilcoxon’s rank sum test.

Fig. 5. Effect of orally administered FUT-187 on surviving time in lethal Forssman shock. Groups of 8–28 guinea pigs were used. Drugs were dosed 1 hr before hemolysin injection, and surviving times were recorded. Each point represents the surviving time of an individual guinea pig. P<0.01 indicates a significant difference from the control by Wilcoxon’s rank sum test.

Fig. 6. Effects of orally administered FUT-187, FUT-175, FOY-305, indomethacin (IDM) and aspirin (ASP) on surviving time in lethal Forssman shock. Groups of 6–14 guinea pigs were used. Drugs were dosed 1 hr before hemolysin injection, and surviving times were recorded. Each point represents the surviving time of an individual guinea pig. P<0.01 indicates a significant difference from the control by Wilcoxon’s rank sum test.
pared to those of other drugs.

Oral dosing of FUT-187 at 100 mg/kg resulted in protection of 6 out of 14 guinea pigs from the lethal shock. On the other hand, FUT-175 at 100 mg/kg had only a tendency to prolong the surviving time in 3 out of 6 guinea pigs. FOY-305 at 300 mg/kg and IDM at 10 mg/kg showed little effect. ASP at 100 mg/kg protected 1 out of 8 guinea pigs from the lethal shock. CVF used as a complement depletor was intravenously administered 24 hr prior to hemolysin injection. All guinea pigs treated with CVF at 100 U/kg were completely protected from lethal shock (Fig. 6).

FUT-187 clearly exhibited a protective effect, and its effect was definitely stronger than those of other drugs except CVF.

2) The changes in lung weight, platelet counts and CH50 in Forssman shock

In order to retain uniformly the condition of blood sampling and lung isolating time, the shock was induced by a sublethal dose of hemolysin.

Injection of hemolysin caused decrease in the platelet counts and CH50 and increase in the lung weights. FUT-187 at doses of 30, 100 and 300 mg/kg significantly inhibited these changes in a dose-dependent manner (Figs. 7 and 8).

3) Histopathological changes and C3 deposition in the lung

In some guinea pigs that were considered too moribund to survive 10 min after hemolysin injection, isolation of lung tissue was performed before the 10-min period elapsed. Guinea pigs that received a lethal dose of hemolysin had congestion in alveolar capillaries, oedema in the alveolus, expansion of the alveolus in the peripheral pulmonary lobe, thinning and destruction of the alveolar wall and aggregation of platelets in capillaries as indicated by the optical microscopic observation and definite deposition of C3 along alveolar walls as indicated by immunofluorescent microscopic examination. The aggregation of platelets were also observed electron microscopically.

These changes mentioned above were almost or completely inhibited by oral administration of FUT-187 at a dose of 200 mg/kg 1 hr prior to hemolysin injection (Table 3, Fig. 9).

Discussion

In vitro studies on various enzymes activities revealed that FUT-187 as well as FUT-175 and FOY-305 had inhibitory activities...
Table 3. Effect of FUT-187 on C3 deposition and histopathological change in the Forssman shocked guinea pig lung

| Drug          | Animal No. | Immunofluorescent C3 deposition | Histopathological Aggregated platelet | Alveolar wall thinning |
|---------------|------------|---------------------------------|--------------------------------------|------------------------|
| Control       |            | +                               | ++                                   | +                      |
| 1             | ++         | +                               | ++                                   | +                      |
| 2             | +++        | ++                              | ++                                   | +                      |
| 3             | +++        | ++                              | ++                                   | +                      |
| 4             | +          | +                               | ++                                   | +                      |
| 5             | +          | +                               | ++                                   | +                      |
| 6             | +++        | +                               | ++                                   | +                      |
| FUT-187 200 mg/kg p.o. | 1 | +                               | +                                    | +                      |
| 2             | ±          | -                               | -                                    | -                      |
| 3             | ±          | -                               | -                                    | -                      |
| 4             | +          | +                               | +                                    | +                      |
| 5             | ±          | +                               | +                                    | +                      |
| 6             | ±          | -                               | -                                    | -                      |

-, negative; ±, slight; +, mild; ++, moderate; ++++, severe.

selective to trypsin-like serine proteases including Clr, Cl9, kallikrein, trypsin, plasmin and thrombin. In the above study using synthetic substrate, inhibitory activities of FUT-187 on Clr and Cl9 were 10 and 100 times lower, respectively, than those of FUT-175. In order to investigate further the inhibitory effect of FUT-187 on the complement system, some in vitro and in vivo reactions mediated by complement were chosen, and the effects of FUT-187 on these reactions were examined in comparison with those of FUT-175 and other drugs.

First, FUT-187 was examined for its effect on complement-mediated hemolysis in vitro in the presence of 500-fold diluted guinea pig serum. The inhibitory effect of FUT-187 was only about 3 times weaker than that of FUT-175. It is known that the complement system has two activation routes, i.e., the classical pathway and the alternative pathway, and the classical pathway is activated by antigen-antibody complex. Various proteases such as Clr, Cl9 and C3 convertase, etc. are involved in the activation of classical pathway. We previously have reported that FUT-175 inhibited complement-mediated hemolysis by the classical pathway, as a consequence of the inhibitory activities on Clr and Cl9 (3). FUT-187 is one of the FUT-175 derivatives, so the chemical structure of FUT-187 is similar to that of FUT-175. Hence, it was supposed that FUT-187 had the inhibitory activities on complement components, like FUT-175 does. The discrepancy between the relative potency of FUT-187 against FUT-175 in the inhibitory activities on Clr and Cl9 and that in the inhibition of complement-mediated hemolysis may be accounted for the difference in substrates used, because the synthetic substrate was used in the former experiment and the serum was used in the latter experiment.

Second, the effect of FUT-187 on complement-mediated hemolysis was examined using 2-fold diluted serum. This reaction was considered more physiological than that using 500-fold diluted serum. FUT-187 and FUT-175 both have an ester-linkage in their chemical structures. It was suspected that the ester-linkage in FUT-175 was cleaved by esterases (19). The esterase level in 2-fold diluted serum was considered to be higher than in 500-fold diluted serum. Comparing with the experiment using 500-fold diluted serum, the inhibitory potency of FUT-187 approximated that of FUT-175 in the experiment using 2-fold diluted serum. This might be due to the difference of their susceptibility to esterases.

Third, in the in vivo hemolysis mediated by the classical pathway, FUT-187 administered
Fig. 9. Effect of orally administered FUT-187 on histological changes and C3 deposition in Forssman shocked guinea pig. 1) Congestion in alveolar capillary and edema in alveolus, etc. (x100). a: Control; b: FUT-187, 200 mg/kg, p.o. 2) Aggregation of platelets (x200). c: Control; d: FUT-187, 200 mg/kg, p.o.; arrow indicates aggregation of platelets in capillary. 3) C3 deposition of alveolar wall (x100). e: Control; f: FUT-187, 200 mg/kg, p.o.
orally produced inhibition comparable to FUT-175. This might be due to the differences of blood levels of FUT-187 and FUT-175, i.e., in our preliminary experiments, after the oral administration of FUT-187 and FUT-175 at a dose of 100 mg/kg, the blood level of FUT-187 was 1.3 times higher in Cmax and 1.6 times higher in AUC_{0-4hr} than the respective values for FUT-175. From the experiments described above, FUT-187 was suggested to have anti-complementary activity comparable to FUT-175 in vivo because of their differences of susceptibility to esterase and oral absorption. Consequently, we investigated the effects of FUT-187 and FUT-175 on the experimental animal models in which the activation of the complement system was considered to be involved in its onset and development.

Forssman systemic shock is a lethal reaction induced in normal guinea pigs by intravenous injection of anti-sheep erythrocyte rabbit serum (hemolysin). This reaction is characterized by anaphylactoid symptoms including ruffling of hair, coughing, nose scratching, convulsions, respiratory distress and death within 30 min after hemolysin injection (18). The lungs of shocked animals are markedly oedematous and lung weight increases; and in many instances, a frothy, blood-stained fluid fills the trachea and exudates through the nose, and this is accompanied by a systemic decrease in CH50, platelet counts and leucocytes (3, 20, 21). May and Frank (22) reported that C4-deficient guinea pigs were resistant to Forssman shock, but that reconstitution of the classical pathway by the administration of normal guinea pig serum restored the deficient animal's susceptibility to the Forssman shock. Newman et al. (23) reported that with the use of the purified low molecular weight factor from covra venom (CVF) to deplete C3, guinea pigs with less than 1% of intravascular C3 were protected from Forssman shock. This finding was reconfirmed in our present study. These information suggest complement activation is essential for the development of Forssman shock.

FUT-187 administered orally or intravenously had a protective effect against Forssman lethal shock in guinea pigs that was superior to the effect of FUT-175. In order to discuss the detailed effect of FUT-187 on this experimental animal model, we investigated the effects of FUT-187 on the changes accompanying Forssman shock. The increase in lung weight might be considered as an indication of increased vascular permeability. It is known that the increase in vascular permeability results from the sequential release of chemical mediators, such as histamine, serotonin, bradykinin and prostaglandins (24, 25), while Wedmore and Williams (25) suggested the essential involvement of the complement metabolite C5a in the extravasation of blood components. In this study, the increase of lung weight was also observed, and lung oedema was histopathologically determined in guinea pigs subjected to Forssman shock. Furthermore, the decrease of the IC50 level of blood and C3 deposition on alveolar wall were observed. FUT-187 inhibited not only the increase in the lung weight, but also the decrease in CH50 in Forssman shock. Furthermore, FUT-187 had a protective effect against C3 deposition along alveolar wall as observed by immunofluorescent microscopy. In the histopathological observation, FUT-187 reduced the destructive changes in guinea pig lung tissue after hemolysin injection. These results, taking the above information into account, suggested that the protective effect of FUT-187 on these parameters mainly could be attributed to its anti-complement activity.

Anaphylatoxin generated by the activation of the complement system has been reported to aggregate platelets (26). It has been reported that guinea pigs subjected to Forssman shock exhibit systemically a marked decrease in platelet counts (21, 27). In the present study, FUT-187 inhibited the decrease in platelet counts in sublethal Forssman shock and histopathologically alleviated the aggregation and deposition of platelets in the lung tissue in lethal Forssman shock. On the other hand, FUT-175 had no effect on the decrease of platelets in lethal Forssman shock (3). The aggregation and deposition of platelet counts in the pulmonary circulation might accelerate the internal pressure of the capillaries, resulting in hemorrhage and increased capillary permeability in lung tissue.
In fact, Tsai et al. (27) reported that antiplatelet serum protected animals from Forsman shock. As mentioned above, FUT-187 had an anti-complementary effect that was comparable to FUT-175 in vivo, FUT-187 and FUT-175 were expected to have an equivalent potency against Forsman shock mediated by the activation of the complement system. FUT-187, however, had a protective effect definitely superior to that of FUT-175. The superiority of FUT-187 to FUT-175 might be attributed to its inhibitory effect on platelet aggregation that accompanies Forsman shock.

Summarizing these results, it is concluded that FUT-187 is a potent and selective inhibitor of the so-called trypsin-like serine protease and, notwithstanding its relatively lower activity in vitro compared to that of FUT-175, FUT-187 shows a stronger protective effect against Forsman shock than FUT-175. This protective effect of FUT-187 by systemic administration is considered to mainly result from its inhibitory activities on serine protease especially on complement and partly by its inhibition of platelet aggregation which might contribute to the superiority of FUT-187 to FUT-175. Therefore, it is anticipated that FUT-187 should be a more potent therapeutic agent by the oral route than FUT-175 for various inflammatory diseases in which the excessive activation of the complement system followed by platelet aggregation is considered to play a major role for their onset and development.

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