THERAPEUTIC EFFICACY OF A NEW CEPHAMYCIN, MT-141, IN COMPROMISED MICE

KATSUMI KAWAHARAJO, KEIKO SHITOH, MASAHIRO NIIZEKI, SHINJIRO MURATA and YASUHARU SEKIZAWA†

Pharmacology & Toxicology Laboratories, Meiji Seika Kaisha, Ltd., Morooka-cho, Kohoku-ku, Yokohama 222, Japan
†Faculty of Agriculture, Tamagawa University, 6-1-1 Tamagawa-Gakuen, Machida, Tokyo 194, Japan

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The antibacterial activity of MT-141 against *Escherichia coli* and *Proteus morganii* in compromised mice was investigated and compared with that of latamoxef, cefmetazole and cefoxitin. The bactericidal activity of MT-141 in short-term contact with *E. coli* and *P. morganii* was markedly enhanced when combined with mouse serum, and the activity of MT-141 was greater than the activities of the three reference drugs. The antibacterial activities of MT-141 in the liver, spleen and kidney of neutropenic and Sarcoma 180 tumor-bearing mice infected with *E. coli* and *P. morganii* were superior to the activities of the reference drugs. MT-141 was more potent than cefmetazole and cefoxitin, and similar to latamoxef in potency against systemic *P. morganii* infection in Sarcoma 180 tumor-bearing mice.

MT-141 is a new cephamycin antibiotic, the first semisynthetic cephalosporin containing a D-amino acid moiety at the C-7 substituent[3]. MT-141 is active against a wide variety of aerobes and anaerobes, and shows minimum bactericidal concentration (MBC) values which are superior to those of cefmetazole, cefoxitin and latamoxef. It also exhibits marked *in vivo* activity, more than that would be expected from its *in vitro* minimal inhibitory concentration (MIC) data[17].

Infections of Gram-negative bacilli in compromised patients have become an important problem because there is no satisfactory treatment. Cyclophosphamide (CY), which is a widely used antitumor drug, and X-ray irradiation, are well known to deplete the total population of white blood cells[2,7,15]. BEAMAN *et al.*[1], PIERSON *et al.*[13] and KAWASAKI *et al.*[7] reported that CY treatment markedly increased the susceptibility of mice to infection due to *Escherichia coli*, *Nocardia asteroides* and *Pseudomonas aeruginosa*. TSURU *et al.*[16] reported that the bacterial growth of *E. coli* and lethality of infected mice were enhanced by X-ray irradiation. The present authors[5,6] reported that Sarcoma 180 (S-180) tumor-bearing mice were highly susceptible to *Proteus morganii*, and demonstrated marked impairment of the production of IgM antibody for opsonization of the same bacteria. The purpose of this paper is to compare the activity of MT-141 with the activities of latamoxef, cefmetazole and cefoxitin against *E. coli* and *P. morganii* infections in neutropenic and tumor-bearing mice.

Materials and Methods

Antibiotics

MT-141 was prepared by Meiji Seika Kaisha, Ltd., Tokyo. Cefoxitin (Daiichi Seiyaku Co., Ltd., Tokyo), cefmetazole (Sankyo Co., Ltd., Tokyo) and latamoxef (Shionogi & Co., Ltd., Osaka) were purchased.

Test Organisms

E. coli strain No. 29 and *P. morganii* strain 1510 were kindly supplied by Dr. T. NISHINO, Department of Microbiology, Kyoto College of Pharmacy, and the late Dr. S. YAMAGISHI, Faculty of Pharmaceutical Sciences, Chiba University, respectively. These two strains were isolated from clinical sources in Japan. The MICs of MT-141, latamoxef, cefmetazole and cefoxitin were 1.56 μg/ml, 0.2 μg/ml, 1.56 μg/ml and 1.56 μg/ml respectively, for *E. coli* No. 29 and 1.56 μg/ml, 0.39 μg/ml, 3.13 μg/ml and 3.13 μg/ml, for *P. morganii* strain 1510.

Measurement of Bactericidal Activity

E. coli strain No. 29 and *P. morganii* strain 1510 were cultivated at 37°C for 20 hours on nutrient agar (Difco) plates and suspended in Hanks' solution to a density of 10^6 colony forming units (cfu)/ml. The suspensions were mixed with serum (final 10%) from normal mice or taken from tumor-bearing mice 8 days after the transplantation of S-180, and these mixtures were incubated at 37°C for 30 minutes. Then each antibiotic was added to the culture suspensions to give final antibiotic concentrations of 3.13 μg, 6.25 μg and 12.5 μg/ml, and the suspensions were incubated for 2 hours. After incubation, 10-fold serial dilutions of the culture were prepared with nutrient broth (Difco), and 0.1 ml of each dilution was spread on the surface of a nutrient agar plate. After incubation at 37°C for 18 hours, the colonies were counted.

Measurement of Complement Activation

The content of complement was measured by a standard hemolytic assay with sheep erythrocytes sensitized with immune rabbit serum[4]. *P. morganii* strain 1510 was incubated on nutrient agar plates at 37°C for 20 hours, and suspended in Davis medium containing 7 g of K_2HPO_4, 0.1 g of $MgSO_4$, 0.5 g of $Na_3C_6H_5O_7 \cdot 2H_2O$, 2 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$ and 2 g of glucose to an optical density in the range of 0.14~0.16 at 560 nm. After shaking at 37°C for 2.5 hours, each antibiotic was added to culture to give various final concentrations (0.39 μg, 1.56 μg, 6.25 μg, 12.5 μg and 25 μg/ml of MT-141, 6.25 μg, 12.5 μg and 25 μg/ml of cefmetazole, and 0.05 μg, 0.2 μg, 0.78 μg, 12.5 μg and 25 μg/ml of latamoxef). Then, after incubation at 37°C for 20 hours, the cultures were centrifuged at 3,500 rpm for 15 minutes. Each bacterial cell pellet was suspended in 0.7 ml gelatin - glucose - Veronal buffer containing magnesium and calcium (GVB). A solution of 0.5 ml GVB containing 4 units of guinea pig complement (Research Institute for Microbial Diseases, University of Osaka) was added to 0.5 ml of each bacterial suspension. After incubation at 37°C for 30 minutes, 5.5 ml of GVB and 1 ml (5×10^8 cells) of the sensitized erythrocytes were added to each mixture. After incubation at 37°C for 60 minutes, the tubes were centrifuged. The optical density of the supernatants was read at 541 nm.

Animals

Four-week-old male ICR mice were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals.

Compromised Mice

Two kinds of compromised mice, *i.e.*, tumor-bearing mice and neutropenic mice, were prepared by the following method.

Tumor-bearing Mice: S-180 cells were suspended in saline, so that 0.1 ml contained 1×10^7 cells. Then 0.1 ml of the suspension was injected intraperitoneally to mice. The mice were used 8 days after this transplantation of the tumor cells.

Neutropenic Mice: Mice were placed in individual plastic cages. The animals were subjected to whole body irradiation with the telecasesium unit (900 rads). On the same day as the ^{137}Cs-irradiation took place, the mice were given 200 mg/kg of cyclophosphamide (CY, Shionogi & Co., Ltd., Osaka) by intraperitoneal injection. These mice were used 4 days after the treatment with ^{137}Cs plus CY.

Protection Test

Antibacterial Effects in the Main Organs: Normal mice, tumor-bearing mice and neutropenic mice were injected with *E. coli* strain No. 29 or *P. morganii* strain 1510. The two organisms had been

cultivated at 37°C for 20 hours on heart infusion agar (HI agar; Difco) and suspended in nutrient broth at $10^7 \sim 10^8$ cfu per 0.2 ml. Normal mice (6 mice/group) were inoculated intraperitoneally with 0.2 ml of the suspension mixed with an equal volume of 5% mucin. Tumor-bearing mice (6 mice/group) and neutropenic mice (5 mice/group) were inoculated intravenously with 0.2 ml of the bacterial suspensions. The challenged mice were treated by subcutaneous administration of an antibiotic at 30 minutes or at 30 minutes and 6 hours post-infection. The mice were sacrificed 24 hours after the challenge injection of the test strains. The livers, spleens and kidneys were pooled for each group and homogenized in nutrient broth. In normal mice, the organs were washed well with saline before the homogenization. Ten-fold serial dilutions of the liver, spleen and kidney homogenates were prepared with the same nutrient broth, and 0.1 ml of each dilution was spread on the surface of a nutrient agar plate. After incubation at 37°C for 20 hours, the cfu for each organ was determined. Analysis of variance was carried out on the antibacterial effects shown by MT-141 and the other cephamycins.

Survival Study: *P. morganii* strain 1510 was suspended in nutrient broth at 10^7 cfu per 0.2 ml. Then 0.2 ml of the bacterial suspension was inoculated intravenously to mice 4 days after tumor transplantation. The challenged mice were then treated with subcutaneous injections of an antibiotic at 30 minutes, 20, 25, 44, 49, 68, 73, 93 and 97 hours post-infection. The survival rate of the mice was calculated 14 days after the challenge. From the survival rates at 9 days after the challenge, it was observed that the transplanted and non-challenged mice began to die due to the tumor growth, and the median effective dose (ED_{50}) of each antibiotic was determined according to the formula of LITCHFIELD-WILCOXON[10].

Results

Bactericidal Activity in Presence of Serum

The bactericidal activities of MT-141, latamoxef, cefmetazole and cefoxitin in short-term contact in the presence of serum are shown in Figs. 1 and 2. MT-141 and the reference drugs alone did not reduce the number of cfu of *E. coli* strain No. 29 and *P. morganii* strain 1510. In contrast, in the presence of normal mouse serum and in Hanks' solution, all the antibiotics caused a marked reduction in the counts of the organisms. MT-141 was more active than cefmetazole, cefoxitin and latamoxef against *E. coli* (Fig. 1). At lower concentrations MT-141 (3.13 μg/ml and 6.25 μg/ml) tended to be more active than the three reference drugs against *P. morganii* (Fig. 2). The bactericidal activities of MT-141, latamoxef, cefmetazole and cefoxitin in the presence of serum from S-180 tumor-bearing mice were similar to the activities of the drugs in the presence of normal mouse serum (Figs. 1 and 2).

Complement Activation

Complement activation in guinea pig complement solution incubated with *P. morganii* strain 1510 was examined. No difference was found in the capacity for complement activation of the untreated strain 1510 *versus* the same strain treated with MT-141, latamoxef or cefmetazole (Table 1).

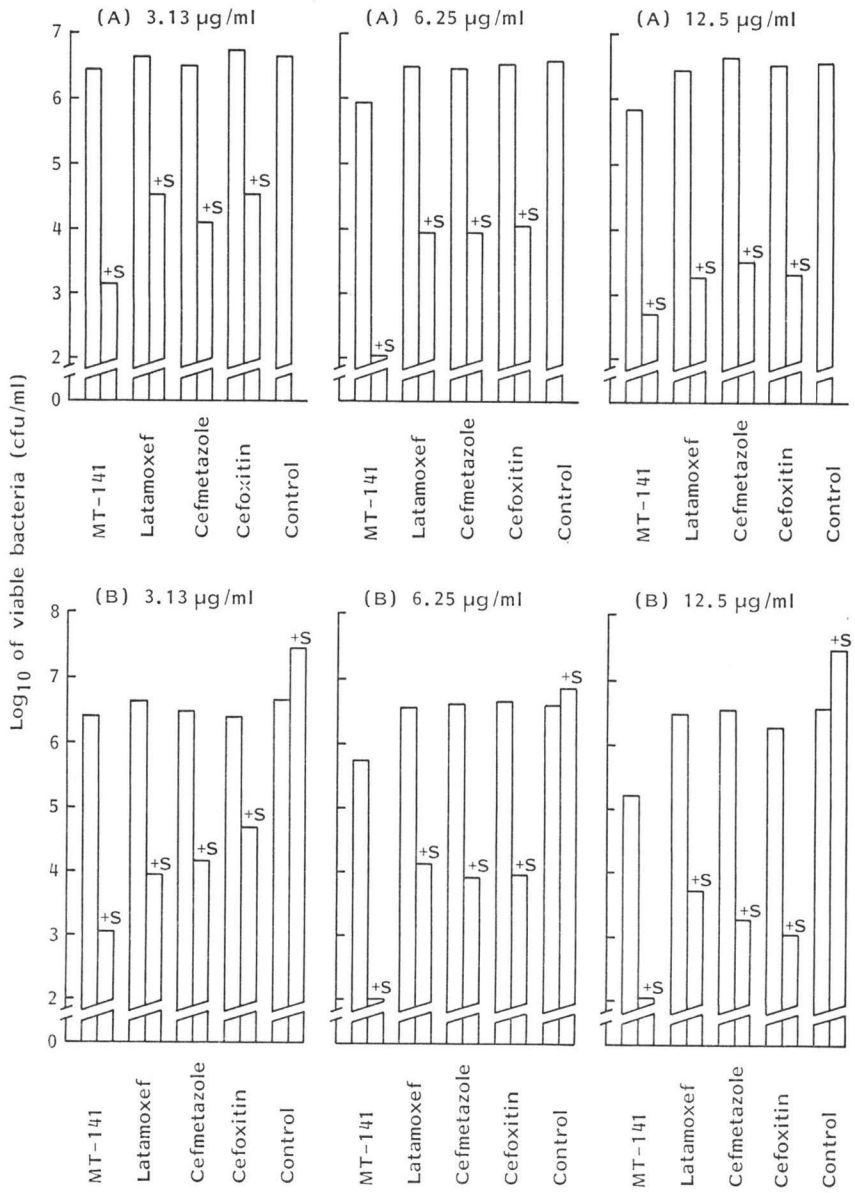
Antibacterial Activity in Mice

The antibacterial effects of MT-141, latamoxef, cefmetazole and cefoxitin against infection due to *E. coli* strain No. 29 in normal, neutropenic and tumor-bearing mice were investigated by determining the number of viable organisms on nutrient agar 24 hours after treatment with the antibiotics. In normal mice, the decrease in the viable bacterial counts in the liver, spleen and kidney observed after treatment with 1 mg of MT-141 was about 3 to 4 logarithms greater than in the control mice. In neutropenic mice, a marked reduction in the viable count was observed in the main organs when 8 mg of MT-141 was administered at 30 minutes and 6 hours post-infection. In tumor-bearing mice, a

Fig. 1. Bactericidal effect of MT-141 and other cephamycins in the presence or absence of sera of normal (A) and tumor-bearing mice (B) on *E. coli* strain No. 29.

The suspensions of strain No. 29 were mixed with serum at 37°C for 30 minutes and then were incubated with 3.13, 6.25 and 12.5 μg/ml of each agent. After incubation for 2 hours, the colonies were counted. The MICs for this strain were 1.56 μg/ml for MT-141, cefmetazole and cefoxitin, and 0.2 μg/ml for latamoxef.

+S: Presence of serum.



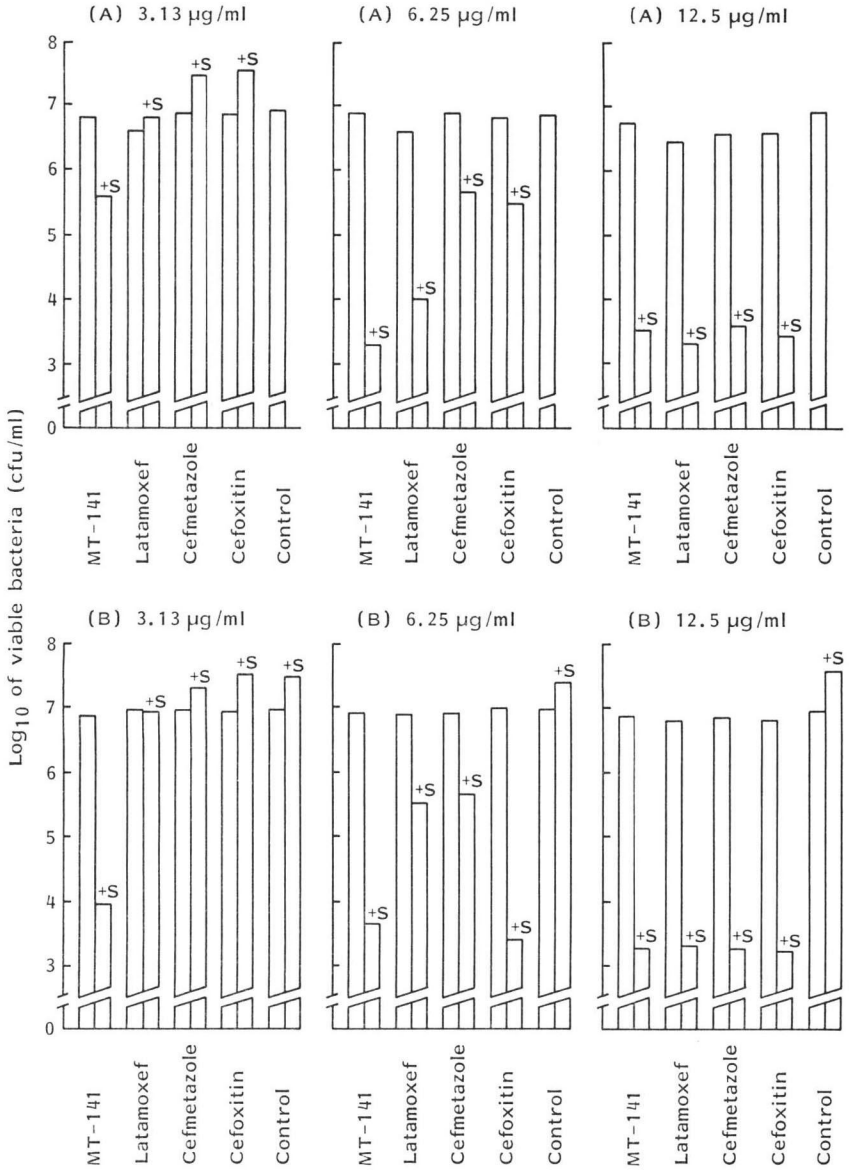
dose of 8 mg/mouse (4 mg/mouse×2 times) of MT-141 also showed a marked reduction in the viable count. These antibacterial effects of MT-141 were greater than those of latamoxef, cefmetazole and cefoxitin (Fig. 3).

The therapeutic effect of MT-141 against infection due to *P. morganii* strain 1510 in normal, neu-

Fig. 2. Bactericidal effect of MT-141 and other cephamycins in the presence or absence of sera of normal (A) and tumor-bearing mice (B) on *P. morganii* strain 1510.

The suspensions of strain 1510 were mixed with serum at 37°C for 30 minutes and then were incubated with 3.13, 6.25 and 12.5 μg/ml of each agent. After incubation for 2 hours, the colonies were counted. The MICs for this strain were 1.56 μg/ml for MT-141, 0.39 μg/ml for latamoxef and 3.13 μg/ml for cefmetazole and cefoxitin.

+S: Presence of serum.



tropenic and tumor-bearing mice was compared with that of the three reference drugs in the same manner as described for *E. coli*. In normal mice, the reductions in the viable cell counts in the liver, spleen and kidney at a single dose of 0.25 mg/mouse of MT-141 were significantly larger than the reductions brought about by latamoxef, while cefmetazole and cefoxitin were inactive. In neutropenic mice, when 0.25 mg/mouse of each antibiotic was administered at 30 minutes and at 6 hours post-infection,

Table 1. Complement activity in guinea pig complement solution incubated with *P. morganii* strain 1510 treated with MT-141, latamoxef and cefmetazole.

Concentration of antibiotic (μg/ml)	Optical density at 541 nm			
	MT-141	Latamoxef	Cefmetazole	Control
0.05		0.425 (96.6%)[a)]		0.440
0.20		0.437 (99.3%)		0.440
0.39	0.451 (102.5%)			0.440
0.78		0.426 (96.8%)		0.440
1.56	0.432 (98.2%)			0.440
6.25	0.420 (95.5%)		0.428 (97.3%)	0.440
12.5	0.510 (96.8%)	0.544 (103.2%)	0.537 (101.9%)	0.527
25.0	0.541 (102.7%)	0.558 (105.9%)	0.568 (107.8%)	0.527

a) Complement activity was calculated as follows:

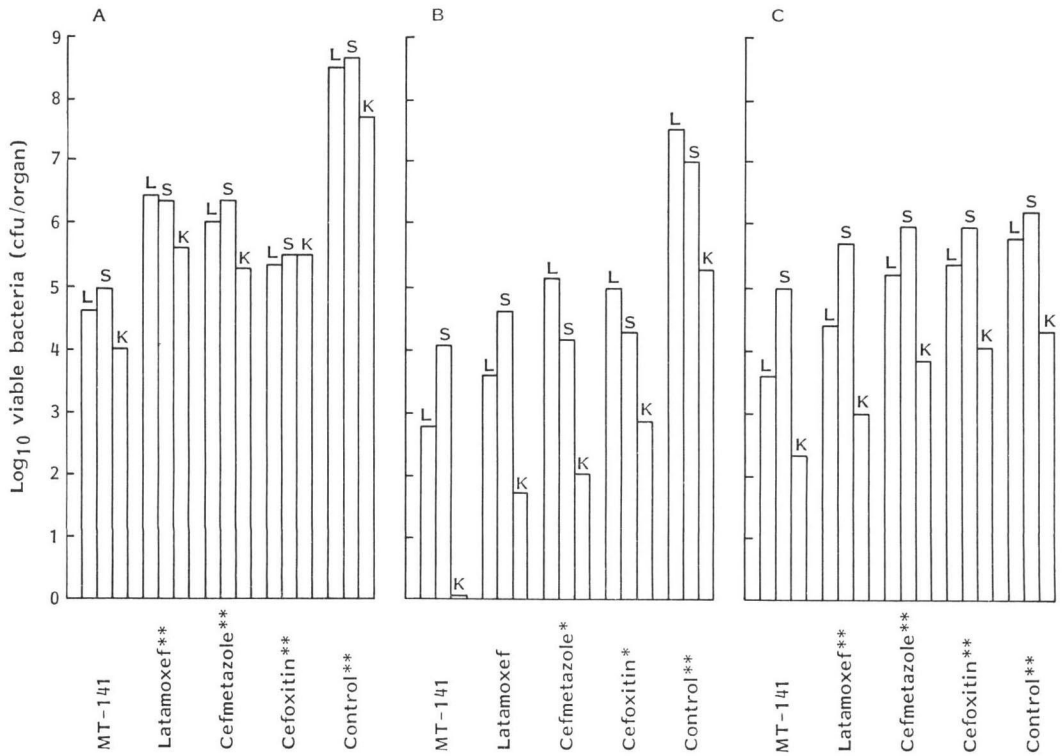
$$\text{Complement activity (\%)} = \frac{\text{O.D. of antibiotic-treated group}}{\text{O.D. of untreated group}} \times 100$$

Fig. 3. Antibacterial effect of MT-141 against infection due to *E. coli* strain No. 29 in normal (A), neutropenic (B) and tumor-bearing mice (C).

1 mg (1 mg/mouse×1 time), 32 mg (16 mg/mouse×2 times) and 8 mg (4 mg/mouse×2 times) of antibiotic were given subcutaneously to normal, neutropenic and tumor-bearing mice, respectively.

* ($P<0.05$), ** ($P<0.01$) significantly different from MT-141.

L: Liver, S: spleen and K: kidney.



the greatest reductions in the viable counts were achieved by treatment with MT-141. At a dosage of 2×0.25 mg/mouse the antibacterial activity of MT-141 in infected tumor-bearing mice was superior to the activities of cefmetazole and cefoxitin, and similar to latamoxef in potency (Fig. 4).

Fig. 4. Antibacterial effect of MT-141 against infection due to *P. morganii* strain 1510 in normal (A), neutropenic (B) and tumor-bearing mice (C).

0.25 mg (0.25 mg/mouse×1 time), 0.5 mg (0.25 mg/mouse×2 times) and 0.5 mg (0.25 mg/mouse× 2 times) of antibiotic were given subcutaneously to normal, neutropenic and tumor-bearing mice, respectively.

* ($P<0.05$), ** ($P<0.01$) significantly different from MT-141.

L: Liver, S: spleen and K: kidney.

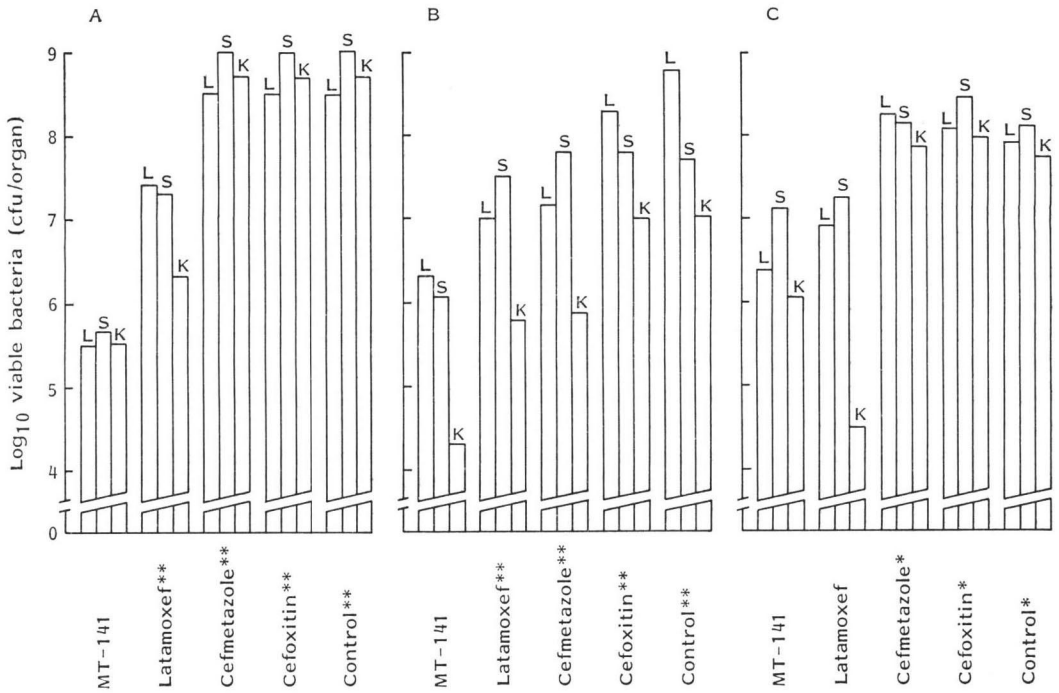
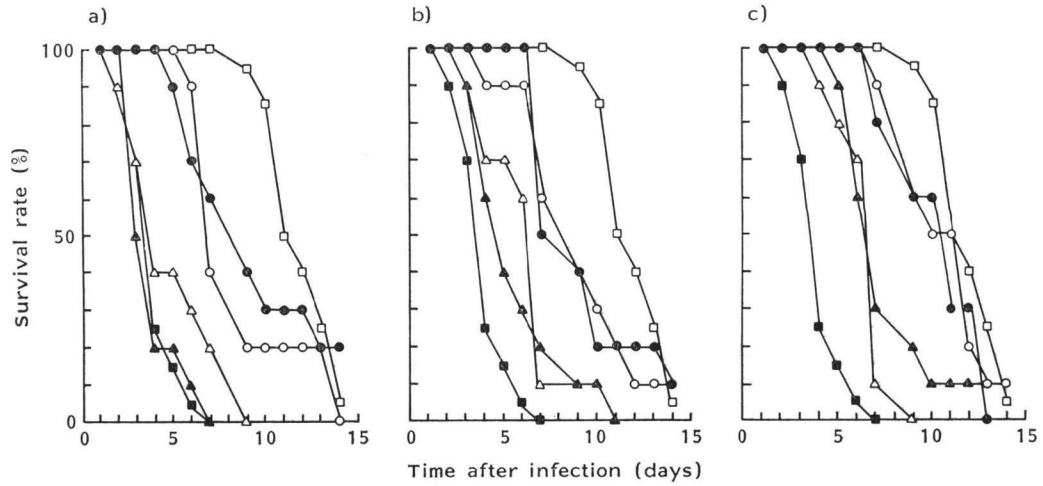


Fig. 5. Activities of MT-141 and other cephamycins against an experimental systemic infection in tumor-bearing mice.

Infection was induced by intravenous injection of 1.0×10^7 cfu of strain 1510 of *P. morganii*. Each antibiotic was injected subcutaneously nine times. Ten mice were used for each antibiotic dose.

●: MT-141, ○: latamoxef, ▲: cefmetazole, △: cefoxitin, ■: control (S-180 plus bacteria) and □: control (S-180).

a) 0.5 mg/mouse×9 times, b) 2.0 mg/mouse×9 times, c) 8.0 mg/mouse×9 times.



Survival Study

The therapeutic efficacy of MT-141 in an experimental systemic infection in tumor-bearing mice was compared with the efficacies of latamoxef, cefmetazole and cefoxitin. MT-141 showed activity *in vivo* against *P. morganii* strain 1510 with an ED_{50} of 3.6 mg/mouse. MT-141 was more effective than cefmetazole (ED_{50} 18 mg/mouse) and similar to latamoxef (ED_{50} 4.0 mg/mouse) in the protection afforded. Cefoxitin was only slightly active (Fig. 5).

Discussion

MT-141 has shown greater activity *in vivo* than would be expected from its activity *in vitro*[17]. Attempts were made in this study to clarify the therapeutic efficacy of MT-141 against infections in compromised hosts. TSURU *et al.*[10] reported that protection of mice from a fatal infection with *E. coli* depends mainly on polymorphonuclear leukocytes, at least in the early phase. Treatment with CY and X-ray irradiation is known to deplete the total population of white blood cells[2,7,15]. The number of white blood cells in the circulating blood of the X-ray plus CY-treated mice decreased markedly. The susceptibility of X-ray plus CY-treated mice to *E. coli* and *P. morganii* correlated well with that decrease in circulating white blood cells. In the present experiments, MT-141 and the control drugs showed potent antibacterial activity in neutropenic mice infected with *E. coli* or *P. morganii*. This finding suggests that the activity of white blood cells may not be the dominant factor in enhancing the *in vivo* activity of MT-141.

Digestive activity, chemotactic response and delayed-type hypersensitivity reaction of macrophages are depressed from an early stage in tumor-bearing animals[8,11,12,14]. S-180 tumor-bearing mice were highly susceptible to *P. morganii*, and showed markedly impaired production of the IgM antibody necessary for optimal opsonization and protection[5,6]. In the present experiments, the antibacterial activity of MT-141 was also potent against infections with *E. coli* and *P. morganii* in S-180 tumor-bearing mice. From these results, although it is suggested that bacteria treated with antibiotics are effectively opsonized by the serum complement system, activation by *P. morganii* treated with MT-141, latamoxef and cefmetazole was not observed.

LEGGETT *et al.*[9] reported that serum contains an ultrafilterable factor(s) that enhances the activity of cephalosporins against many Gram-negative bacilli. In the present experiments, MT-141's *in vitro* bactericidal activity was also enhanced by the serum of S-180 tumor-bearing mice. Furthermore, MT-141 showed good activity against acute lethal infection due to *P. morganii* in tumor-bearing mice. The *in vitro* bactericidal activities of MT-141, latamoxef, cefmetazole and cefoxitin on short-term contact with bacteria in the presence of serum correlated well with the therapeutic activity of those drugs in compromised mice infected with *E. coli* or *P. morganii*.

These findings indicate that it would be worthwhile to conduct further studies to evaluate the efficacy of MT-141 in humans with deficient defense mechanisms.

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