# Bactericidal activity of parenteral fluoroquinolone T-3762 against *Pseudomonas aeruginosa* and *Escherichia coli*

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A high concentration of parenteral fluoroquinolone, T-3762 was used to study its antimicrobial activity against *Pseudomonas aeruginosa* and *Escherichia coli* because of the high peak plasma concentration in human healthy volunteers. Once 5-minute exposure of T-3762 with bacteria resulted in a rapid decrease in the number of viable cells and suppression of growth during 5−120 min incubation in drug-free broth, while β-lactams did not. It should be noted that parenteral fluoroquinolones may be more active at high concentrations and in a short time.

**Key words:** Bactericidal activity, Parenteral fluoroquinolone, T-3762

For effective and safe use of chemotherapeutic agents, the antimicrobial activity against causative bacteria and the pharmacokinetic properties in the human body should be fully investigated. A number of fluoroquinolones have been recognized as effective broad-spectrum antimicrobial agents[1~6]. T-3761 [S-(−)-10-(1-aminocyclopropyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido(1,2,3,-d,e)(1,4)benzoxazine-carboxylic acid], a new oral fluoroquinolone[7] and its parenteral form T-3762[8] were evaluated in antimicrobial and clinical studies. The pharmacokinetic properties of T-3762 were found to be more characteristic than those of several existing fluoroquinolones[8]. High peak plasma concentration ($C_{max}$) of T-3762 were 11μg/ml at the dose of 500 mg, while those of ofloxacin and ciprofloxacin are approximately 1.5μg/ml at the ordinary doses, respectively.

This paper evaluates the bactericidal activity of T-3762 in short time exposure at a high concentration in consideration of its pharmacokinetic properties.

*Pseudomonas aeruginosa* GN 11189, GN 17328 and *Escherichia coli* GN 16261 originating from a patient was maintained in this laboratory and used as a reference strain. Antimicrobial agents were as follows: T-3762 (Toyama Chemical Co., Ltd.), ofloxacin (Dai-ichi Seiyaku Co., Ltd.), ciprofloxacin (Bayer Yakuhin, Ltd.), ceftazidime (Nippon Glaxo) and imipenem (Banyu Pharmaceutical).

MICs were determined by the two-fold serial agar dilution method. The media used for preculture and MIC determinations were Sensitivity Test Broth and Sensitivity Disk Agar (Nissui Pharmaceutical), respectively. Bactericidal activities of the drugs were assessed by measurement of the reduction in the number of viable cells during incubation with the drugs. An overnight culture was diluted with fresh Mueller-Hinton (MH) broth (Difco Laboratories). After 10 ml of the dilution was incubated for 4 h at 37 ℃ with shaking, 0.1 ml of drug solution at various concentrations was added. A 0.1-ml portion of each culture was taken at selected intervals and diluted with buffered saline containing 0.01 % gelatin. The undiluted or the diluted culture was mixed with 10 ml of melted SDA kept at 50 ℃. Viability of bacteria after short-time exposure with drugs were determined. Mid-logarithmic phase bacteria (approximately 106 cfu/ml) were mixed for 5 min with T-3762 at a concentration of 1, 2, 4, 8 or 12 times the MIC (0.78 μg/ml). After 5-min of incubation at 37 ℃, the cultures were spun down at 4 ℃. The precipitated cells were suspended again in an original volume of drug-free MH broth and incubated at 37 ℃ with shaking. At the indicated time, a portion of each culture was withdrawn,

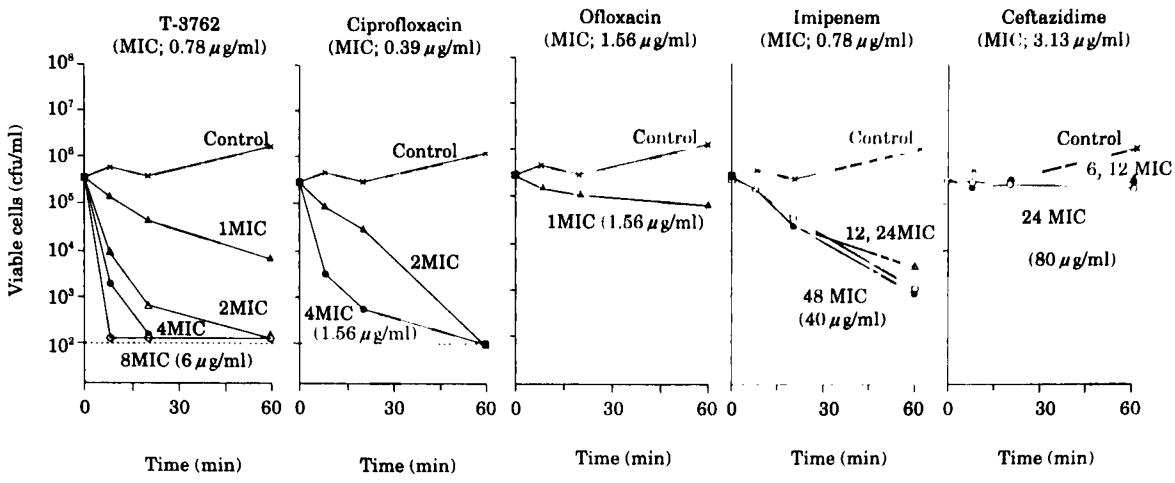


Fig. 1. Bactericidal activities of T-3762, ciprofloxacin, ofloxacin, ceftazidime and imipenem against *Pseudomonas aeruginosa* GN 11189.
·····, limit of assay.

diluted appropriately and mixed with 10 ml of melted SDA kept at 50 ℃. Three plates were used for counting the number of colonies of each dilution. After incubation at 37 ℃ for 18 h, the number of colonies was counted.

The bactericidal activities of T-3762 at high concentrations were studied. By contact with the drug, viability of *P. aeruginosa* GN 11189 and *E. coli* GN 16261 fell down rapidly but in any strains restored on the next day even in the broth containing less than 4 times the MICs. On the other hand, drug exposure at high concentration above 8 times MIC suppressed the regrowth of bacteria. The manners of ciprofloxacin and ofloxacin were similar to that.

Bactericidal activities of T-3762, ofloxacin, ciprofloxacin, ceftazidime and imipenem against *P. aeruginosa* GN 11189 are shown in Fig. 1. By only 5-minutes exposure of T-3762, the number of viable cells rapidly decreased and were especially undetectable at the concentration above 8 times the MIC (6 μg/ml). Other fluoroquinolones also exhibited so strong activites as T-3762 at high concentration. But they were not so effective at less than 1.5 μg/ml corresponding to $C_{max}$ of them. After 5-min incubation with T-3762, viability of *P. aeruginosa* GN 11189, GN 17328 and *E. coli* GN 16261 fell to $< 4.8 \times 10^{-3}$, $< 1.6 \times 10^{-4}$ and $2.2 \times 10^{-2}$, respectively. Ceftazidime and imipenem were not effective even at high concentrations. On the other hand, fluoroquinolones showed concentration-dependent effects in a short time.

The bactericidal activities of T-3762 in a short time were studied (Fig. 2). *P. aeruginosa* GN 11189 ($3.3 \times 10^6$) was mixed with T-3762 at the concentration above 4 times the MIC (6.25 μg/ml) for five minutes and resuspended in drug-free MH broth. After 2 h of incubation, an one milliliter aliquot was inoculated on the MH-agar plate and bacterial colonies were not seen on the next day. That is to say, once bacteria was damaged by very short-time exposure to the drug, suppression of bacteria continued even in the absence of the drug.

The pharmacokinetic properties of fluoroquinolones differ more or less from agents to agents

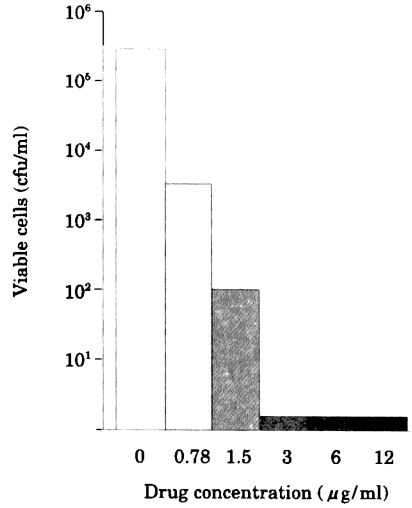


Fig. 2. Supression of bacterial growth after 5-min treatment with T-3762 and 2-h incubation in drug-free broth.

depending on the difference in chemical structures[9]. Such differences are essentially responsible for effectiveness. The advantage of parenteral use of agents are considered to act against bacteria in a short time. Patients who are likely to require parenteral fluoroquinolone therapy may be at an increased risk of side effects because of their poor underlying condition[10]. The parenteral administration of fluoroquinolones has not been yet accepted in Japan because of their side effects. However, the phase I and early phase II studies for the parenteral use of T-3762 demonstrated clinical effectiveness without severe side effects. In addition, administration of T-3762 resulted in getting high $C_{max}$ values as that is characteristic of parenteral use. In this study, it is concluded that fluoroquinolones could show strong bactericidal activity at high concentrations of the drug in a short time as comparison with $\beta$-lactam antibiotics. It should be noted also that the high $C_{max}$ value of T-3762 achieved a rapid bactericidal activity even in a short time.

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## *Pseudomonas aeruginosa* および *Escherichia coli* に対する注射用フルオロキノロン薬，T-3762の高濃度における殺菌作用


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T-3762は日本国内では初めての注射用フルオロキノロン薬として，現在臨床試験が行われている。注射剤の特徴としてT-3762は既存の経口剤と比べて高い血中濃度（$C_{max}$; 11 $\mu$g/ml）が得られることからキノロン薬としての強い殺菌作用が効果的に現れることが期待される。本研究では *Pseudomonas aeruginosa* および *Escherichia coli* を用いてフルオロキノロン薬の高濃度領域における殺菌作用についてceftazidimeやimipenemなどと比較検討した。特にT-3762は$C_{max}$に相当するような高濃度，すなわち8MIC以上で作用させた場合は5分間で強い殺菌作用を示し，薬剤をのぞいた後もbroth中で細菌の増殖が押さえられた状態で推移した。このような短い時間での殺菌作用は$\beta$-ラクタム剤では認めることができなかった。このようにフルオロキノロン薬の高濃度領域における使用は従来の薬剤では得られなかったような作用をもたらす可能性が示唆される。



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