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In Vitro and *In Vivo* Antibacterial Activities of the Tricyclic Ketolide TE-802 and Its Analogs

TAKEO ONO, MASATO KASHIMURA*, KEIKO SUZUKI, RIKA OYAUCHI, JUNKO MIYACHI, HIROSHI IKUTA, HIROYUKI KAWAUCHI, TOSHI AKASHI, TOSHIFUMI ASAKA and SHIGEO MORIMOTO

Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd.,
1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama, 331-9530, Japan

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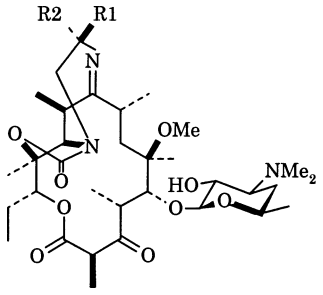
The *in vitro* and *in vivo* antibacterial activities of tricyclic ketolides (TKs: TE-802, TE-806, TE-935, TE-943) have been compared with those of clarithromycin (CAM), azithromycin (AZM) and rokitamycin (RKM). TKs were active against not only erythromycin (EM)-susceptible organisms; aerobic Gram-positive bacteria, some Gram-negative bacteria, anaerobic bacteria and *Mycoplasma pneumoniae*, but also EM-resistant *Staphylococcus aureus* (inducible macrolide-resistant strains) as well as EM-resistant *Streptococcus pneumoniae* (efflux-resistant strains). The therapeutic efficacies of TKs against systemic infections and respiratory tract infection (RTI) caused by Gram-positive bacteria in mice are superior to those of CAM and AZM. The peak plasma levels (Cmax, po) of TE-802 in mice were equal to that of CAM, but the plasma area under the concentration-time curve ($AUC_{24\,hours}$) was 4.7 times that for CAM. The plasma Cmax (po) value for TE-802 in monkey was equal to that of CAM, whereas the $AUC_{8\,hours}$ value was three-fourths that of CAM. The pharmacokinetics of TE-802 are similar to those of AZM in mice and monkeys, suggesting the potential for once-daily administration in humans.

The appearance of CAM[1] and AZM[2], so-called second-generation macrolides, greatly expanded the macrolide antibiotic market worldwide. The next target for development of new agents[3] was already set as EM-resistant pathogens, including penicillin/EM-resistant *S. pneumoniae*. We previously reported[4] the synthesis and initial results of evaluation of new ketolide compounds having a unique aglycon structure. Due to their unique skeleton and excellent antibacterial activity, the tricyclic ketolides have attracted much attention[5].

In this paper we describe the antibacterial activities of TE-802 and its analogs tricyclic ketolides, which were compared with those of CAM, AZM and RKM[6] *in vitro* and *in vivo*. In addition, the pharmacokinetic profiles of these compounds in several species of animals are presented.

Fig. 1. Structures of TE-802, TE-806, TE-935 and TE-943.



TE-802 : R1=R2=H
TE-806 : R1=R2=Me
TE-935 : R1=Me, R2=H
TE-943 : R1=H, R2=Me

Materials and Methods

Bacterial Strains

The clinically isolated strains used in this study were obtained from several Japanese hospitals. The other strains were from the Taisho Culture Collection or were ATCC strains.

* Corresponding author: m.kashimura@po.rd.taisho.co.jp

Antibacterial Agents

TE-802, TE-806, TE-935, TE-943 and CAM were prepared at Taisho Pharmaceutical Co., Ltd. RKM and AZM were obtained from Toyo Jozo (Japan) and U.S. Pharmacopeia, respectively. Stock solutions of the drugs were prepared for *in vitro* tests by dissolving 10 mg of drug in 5 ml of methanol. The drugs were suspended in physiological saline containing 5% gum arabic for oral administration in *in vivo* tests. For iv injection of drugs, the test drugs were suspended in distilled water and then dissolved by addition of the required amount of dilute hydrochloric acid, keeping the pH above 7.

Susceptibility Studies

MICs were determined by an agar dilution method in accordance with the standard method specified by the Japan Society of Chemotherapy[7]. GAM Agar (Nissui) was used for anaerobic bacteria. PPLO Broth (Eiken) was supplemented with 1% agar, 0.2% malt extract and 20% horse serum for *Mycoplasma pneumoniae*. Inoculum sizes were 10^6 CFU/ml for both aerobic and anaerobic bacteria and 10^5 CFU/plate for *M. pneumoniae*. Each serial dilution of test drugs was mixed with the medium. The overnight culture broths of bacteria or their dilutions were seeded onto agar plates with a spot replicating device. MIC was defined as the lowest antibiotic concentration yielding less than four colonies per spot, and was read after 18 hours incubation for aerobic bacteria, 48 hours incubation for anaerobes and 7 days incubation for *M. pneumoniae*. All incubations were at 37°C.

Effects of pH and Inoculum Size on Antibacterial Activity

The effects of medium and inoculum size on antibacterial activities were assayed by determining the MICs and MBCs of test drugs against *H. influenzae*. MICs were determined by a microdilution method in accordance with the standard method specified by the Japan Society of Chemotherapy[7]. The pH of the medium was adjusted to 8, 7, or 6. Inoculum sizes tested were 10^6, 10^5 and 10^4 CFU/ml.

Acute Systemic Infection

S. aureus Smith4, *S. pyogenes* ATCC8668, *S. pneumoniae* IID553 and *S. pneumoniae* 224 were used as challenge organisms. *S. aureus* Smith4, *S. pyogenes* ATCC8668 and *S. pneumoniae* IID553 are standard strains. The MICs of CAM against these strains are 0.20, 0.05 and 0.05 μg/ml, respectively. *S. pneumoniae* 224 is clinical isolate and efflux-resistant strain encoded by *mefA*. Male ICR mice, 4 weeks of age, were infected intraperitoneally with 0.5 ml of a bacterial suspension containing 100% or more of the minimal lethal dose of bacteria. Hog gastric mucin (5%, w/v) was added to the suspensions of organisms before injection. The test drugs in 5% gum arabic were administered orally in a volume of 0.2 ml to groups of 8 mice 1 hour after inoculation. Mortality was recorded daily over 7 days and ED_{50} values were calculated by the probit method[8].

Respiratory Tract Infection

Male ICR mice, 3 weeks of age, placed in an exposure chamber were infected with *S. pneumoniae* J-4 or *H. influenzae* J-48 by the aerosol method. *S. pneumoniae* J-4 and *H. influenzae* J-48 were both clinical isolates and the MICs of test drugs were summarized in Table 7. At 24 hours after infection, a single oral dose of each drug was administered, and at 48 hours after infection the potencies of TKs were compared with those of CAM and AZM by measuring the number of viable organisms in the lungs. Detection limit is <1.48 log/lung and the reproducibility of test has been confirmed by plural experiments. Mice infected with *S. pneumoniae* J-4 develop pneumonia. An inflammatory symptom was observable to the naked eye. In mice infected with *H. influenzae* J-48, a typical pneumonic symptom was not observed, but the number of organisms in the lung was confirmed in control mice.

Determination of Plasma Levels

The antibiotics were administered orally or intravenously to animals. Plasma samples were collected at regular intervals after administration and the concentrations of antibiotic were measured by the bioassay method using *Micrococcus luteus* ATCC 9341 as a test organism. Animals used and the doses (route) of drug administration were as follows: Male ICR mice, 5 mg/kg (po); male cynomolgus monkeys, 5 mg/kg (po and iv).

Results

In Vitro Susceptibility Studies

Antibacterial Spectrum

The *in vitro* activities (MICs) of TKs were compared with those of CAM, AZM and RKM against standard strains of bacteria. The results are shown in Table 1. The potency of TKs were generally equal to that of CAM and 2- to 8-fold greater than those of AZM and RKM against aerobic Gram-positive bacteria, including *S. aureus*, *S. pneumoniae* and *S. pyogenes*. The MICs of TKs

Table 1. Antibacterial activities of TKs, CAM, AZM and RKM against standard strains.

Strains	MIC (μg/ml)						
	TE-802	TE-806	TE-935	TE-943	CAM	AZM	RKM
Gram-positive bacteria							
Bacilllus subtilis ATCC6633	0.025	0.05	0.05	0.05	0.10	0.78	0.39
Staphylococcus aureus 209P-JC	0.10	0.20	0.10	0.20	0.10	0.39	0.39
S. aureus Smith4	0.20	0.20	0.20	0.20	0.20	0.78	0.78
S. epidermidis IID866	0.10	0.20	0.10	0.10	0.10	0.39	0.39
Streptococcus pneumoniae IID553	0.05	0.05	0.05	0.05	0.05	0.20	0.20
S. pneumoniae J-4	0.025	0.025	0.012	0.025	0.05	0.10	0.10
S. pyogenes IID689	0.05	0.05	0.05	0.05	0.05	0.10	0.20
S. pyogenes ATCC8668	0.10	0.10	0.20	0.10	0.05	0.20	0.39
Enterococcus faecalis ATCC29212	0.20	0.20	0.20	0.20	0.78	12.5	0.78
E. faecalis CSJ1212	0.10	0.10	0.10	0.10	0.78	6.25	0.39
E. faecium ATCC19434	0.025	0.05	0.05	0.025	0.78	3.13	0.39
Gram-negative bacteria							
Haemophilus influenzae ATCC43095	6.25	6.25	6.25	3.13	6.25	1.56	12.5
H. influenzae ATCC33533	6.25	12.5	12.5	6.25	6.25	3.13	6.25
Moraxella catarrhalis ATCC25238	0.20	0.20	0.39	0.20	0.20	0.10	0.39
Klebsiella pneumoniae IFO3317	6.25	12.5	12.5	6.25	50	3.13	>100
Pseudomonas aeruginosa NCTC10490	>100	>100	>100	>100	>100	>100	>100
Escherichia coli NIHJ JC-2	100	100	>100	50	100	12.5	>100
Anaerobic bacteria							
Peptostreptococcus asaccharolyticus 10-2	0.39	0.78	0.39	0.78	0.39	0.78	--
P. prevotii ATCC9321	0.10	--	--	--	0.10	0.78	--
P. magnus ATCC29328	1.56	--	--	--	1.56	6.25	--
Bacteroides fragilis FA-32	12.5	6.25	6.25	6.25	0.78	6.25	--
B. fragilis NCTC9343	25	6.25	6.25	6.25	0.78	6.25	--

Inoculum size: 10^6 CFU/ml.

against EM-susceptible organisms ranged from 0.012 to 0.20 μg/ml. On the other hand, the potency of TKs against Enterococci were greater than those of CAM, AZM and RKM. The MICs against *E. faecalis* and *E. faecium* of TKs ranged from 0.025 to 0.20 μg/ml. The potency of TKs against *H. influenzae* (MICs: 6.25～12.5 μg/ml) were comparable to those of CAM and RKM, but 2- to 4-fold less than AZM against these organisms. TKs were found to be inactive against *P. aeruginosa* and *E. coli*, as were CAM, AZM and RKM. The potency of TE-802 against anaerobic bacteria (MICs: 0.10～1.56 μg/ml) was equal to that of CAM, but 8- to 32-fold less than that of CAM against *B. fragilis*.

Next, *in vitro* activities (MICs) of TKs against macrolide-resistant strains were compared with those of CAM, AZM and RKM. The results are shown in Table 2. TKs had excellent potency against macrolide-resistant *S. aureus* (inducible type), against which CAM and AZM were ineffective (MICs: >100 μg/ml). The MICs of TKs against these organisms ranged from 0.20 to 1.56 μg/ml, and those of RKM ranged from 0.20 to 3.13 μg/ml. TKs were inactive against constitutively resistant *S. aureus* (MICs: >100 μg/ml), as were CAM, AZM and RKM. The potencies of TKs against efflux-mediated EM-resistant *S. pneumoniae* were superior to those of CAM, AZM and RKM. The MICs of TKs against these organisms ranged from 0.05 to 0.10 μg/ml. TKs were slightly more active against methylase-mediated or both efflux- and methylae-mediated EM-resistant *S. pneumoniae* strains (MICs: 1.56～>100 μg/ml) CAM and AZM (MICs: >100 μg/ml).

The potencies of TE-935 and TE-943 against *H. pylori* were equal to that of CAM, while that of TE-802 was inferior to that of CAM (Table 3). All TKs were more active than AZM against *H. pylori*. The potencies of TKs

Table 2. Antibacterial activities of TKs, CAM, AZM and RKM against macrolide-resistant strains.

Strains	MIC (μg/ml)						
	TE-802	TE-806	TE-935	TE-943	CAM	AZM	RKM
Inducible type							
Staphylococcus aureus B1 (*erm C*)	0.20	0.20	0.20	0.20	>100	>100	1.56
S. aureus C1	0.39	0.39	0.20	0.39	>100	>100	0.20
S. aureus 166	1.56	1.56	0.39	1.56	>100	>100	3.13
Constitutive type							
S. aureus K-2	>100	>100	>100	>100	>100	>100	>100
EM-resistant							
Streptococcus pneumoniae 210 (*mefA*)*	0.10	0.10	0.10	0.10	0.78	0.78	0.39
S. pneumoniae 217 (*mefA*)	0.10	0.05	0.10	0.10	1.56	1.56	0.20
S. pneumoniae 224 (*mefA*)	0.10	0.10	0.10	0.10	0.78	1.56	0.20
S. pneumoniae 114 (*ermB*)	25	6.25	1.56	1.56	>100	>100	3.13
S. pneumoniae 207 (*ermB*)**	50	50	50	100	>100	>100	3.13
S. pneumoniae 225 (*ermB* + *mefA*)**	6.25	3.13	1.56	3.13	>100	>100	3.13
S. pneumoniae 229 (*ermB*)	>100	>100	>100	>100	>100	>100	>100

Inoculum size: 10^6 CFU/ml.

* Penicillin-resistant strain.

** Penicillin-intermediate resistant strain.

Table 3. Antibacterial activities of TKs, CAM and AZM against other pathogens.

Strains	MIC (μg/ml)					
	TE-802	TE-806	TE-935	TE-943	CAM	AZM
Helicobacter pylori ATCC43504	0.10	0.025	0.05	0.05	0.05	0.20
H. pylori ATCC43579	0.10	0.025	0.05	0.05	0.05	0.20
H. pylori ATCC43629	0.10	0.05	0.10	0.05	0.05	0.39
Mycoplasma pneumoniae 002	0.006	0.012	0.012	0.006	0.012	≦0.0002
M. pneumoniae 003	0.006	0.012	0.012	0.006	0.012	≦0.0002
M. pneumoniae 004	0.006	0.006	0.012	0.003	0.006	≦0.0002
M. pneumoniae 005	0.003	0.006	0.006	0.003	0.006	≦0.0002
M. pneumoniae FH	0.012	0.012	0.025	0.012	0.012	≦0.0002
Legionella pneumophila ATCC33152	0.78	0.78	0.20	0.78	0.10	0.20
L. pneumophila ATCC33153	0.78	0.78	0.20	0.39	0.10	0.10
L. pneumophila ATCC33154	0.78	0.78	0.20	0.39	0.10	0.10
Mycobacterium avium N339	12.5	6.25	12.5	12.5	1.56	12.5
Chlamydia trachomatis F/UW-6/CX	0.125	0.063	--	--	0.004	0.016
C. trachomatis D/UW-3/CX	0.125	0.125	--	--	0.008	0.016

Inoculum size: 10^6 CFU/ml.

Table 4. Antibacterial activities of TKs, CAM, AZM and RKM against clinical isolates.

Strains (No. of strain)	MIC (μg/ml)						
	TE-802	TE-806	TE-935	TE-943	CAM	AZM	RKM
Staphylococcus aureus (25)*							
MIC50	0.39	0.78	0.39	0.78	0.39	1.56	1.56
MIC90	0.78	0.78	0.39	0.78	0.39	1.56	3.13
S. aureus (17)**							
MIC50	0.20	0.20	0.20	0.20	>100	>100	0.78
MIC90	0.39	0.39	0.39	0.39	>100	>100	1.56
Streptococcus pneumoniae (10)*							
MIC50	0.025	0.05	0.025	0.025	0.05	0.20	0.20
MIC90	0.05	0.05	0.05	0.05	0.05	0.20	0.20
S. pyogenes (23)							
MIC50	0.025	0.05	0.05	0.05	0.05	0.10	0.20
MIC90	0.05	0.05	0.05	0.05	0.05	0.20	0.39
Enterococcus faecalis (9)*							
MIC50	0.20	0.20	0.20	0.20	0.39	3.13	1.56
MIC90	0.20	0.20	0.20	0.20	0.39	3.13	1.56
Enterococcus faecalis (7)***							
MIC50	0.20	0.20	0.20	0.20	1.56	12.5	1.56
MIC90	0.20	0.20	0.20	0.39	3.13	25	1.56
Haemophilus influenzae (20)							
MIC50	6.25	6.25	6.25	3.13	6.25	1.56	6.25
MIC90	6.25	12.5	6.25	6.25	6.25	3.13	12.5
Moraxella catarrhalis (26)							
MIC50	0.20	0.20	0.39	0.20	0.20	0.20	0.39
MIC90	0.39	0.20	0.39	0.20	0.20	0.10	0.39
Neisseria gonorrhoeae (10)							
MIC50	0.39	0.39	0.20	--	0.20	0.013	0.78
MIC90	0.39	0.78	0.39	--	0.78	0.025	1.56

Inoculum size: 10^6 CFU/ml.
* Erythromycin-susceptible strains.
** Inducible resistant strains.
*** EM-intermediate resistant strains.

against *M. pneumoniae* were similar to that of CAM and less than that of AZM. The MICs of TKs against *M. pneumoniae* ranged from 0.003 to 0.025 μg/ml. TKs were also active against *L. pneumophila*, *M. avium* and *C. trachomatis*.

Susceptibility of Clinical Isolates

The *in vitro* antibacterial activities of TKs, compared with those of CAM, AZM and RKM, were tested against 147 strains of clinical isolates, including *S. aureus* (25, EM-susceptible; 17, inducible resistant type), *S. pneumoniae* (10), *S. pyogenes* (23), *E. faecalis* (9, EM-susceptible; 7, EM-intermediate resistant), *H. influenzae* (20), *M. catarrhalis* (26) and *N. gonorrhoeae* (10). As shown in table 4, The potencies of TKs were equal to or slightly greater than that of CAM and 2- to 8-fold more than that of RKM against EM-susceptible organisms. The potencies of TKs against Gram-positive bacteria were 2- to 128-fold greater than that of AZM, but 2- to 32-fold less against Gram-negative bacteria.

The most prominent characteristic of the *in vitro* antibacterial activity of TKs was excellent potency against *S. aureus* (inducible resistant type), against which CAM and AZM were ineffective (MIC >100 μg/ml, respectively). The MIC_{50} and MIC_{90} of TKs against *S. aureus* (inducible resistant type) were 0.20 and 0.39 μg/ml, respectively. In addition, the potencies of TKs against EM-intermediate resistant *E. faecalis* were 4- to 128-fold those of CAM, AZM and RKM.

Effects of Inoculum Size and Medium pH on Antibacterial Activity

As shown in Table 5, decrease of medium pH from 8 to 6 resulted in a 4- to 16-fold decrease in observed activities of TKs against *H. influenzae* ATCC43095. For AZM over the same pH range, there was a 32-fold decrease in measured activity. Like CAM and AZM, the TKs were not affected by changes in inoculum size between 10^4～10^6 CFU/ml.

In Vivo Therapeutic Activity

Acute Systemic Infections

Table 6 shows activities of TKs, CAM and AZM against experimental systemic infections caused by *S. aureus* Smith4, *S. pyogenes* ATCC8668, *S. pneumoniae* IID553 or *S. pneumoniae* 224 in mice. When administered orally, TE-802 was 1.5 to 12.3 times more potent than CAM and

Table 5. Effects of inoculum size and medium pH on antibacterial activities of TKs, CAM and AZM against *H. influenzae* ATCC43095.

Inoculum size (CFU/ml)	Medium pH	MIC/MBC (μg/ml)					
		TE-802	TE-806	TE-935	TE-943	CAM	AZM
10^4	7.0	8/8	16/16	16/16	8/8	8/16	2/4
	6.0	32/32	64/64	32/32	32/32	16/32	16/16
10^5	7.0	16/16	16/16	16/16	8/16	8/8	2/2
	8.0	4/4	4/4	4/4	2/2	4/4	0.5/0.5
10^6	7.0	16/16	16/16	16/16	8/8	8/8	2/2

Table 6. Protective effects of TKs, CAM and AZM against systemic infections in mice.

Strain	Challenge dose (CFU/mouse)	Drug	MIC (μg/ml)	ED_{50} (mg/mouse)*
Staphylococcus aureus Smith4	4.8×10^7	TE-802	0.20	0.22 (0.11～0.52)
		TE-806	0.20	0.38 (0.19～0.73)
		TE-935	0.20	0.14 (0.07～0.27)
		TE-943	0.20	0.40 (0.24～0.72)
		CAM	0.20	0.33 (0.18～0.59)
		AZM	0.78	0.47 (0.25～0.83)
Streptococcus pyogenes ATCC8668	1.1×10^7	TE-802	0.10	0.06 (0.04～0.11)
		TE-806	0.10	0.04 (0.02～0.09)
		TE-935	0.20	0.04 (0.02～0.07)
		TE-943	0.10	0.07 (0.04～0.13)
		CAM	0.05	0.31 (0.10～1.02)
		AZM	0.20	0.34 (0.15～0.80)
S. pneumoniae IID553	1.1×10^3	TE-802	0.05	0.047 (0.022～0.095)
		TE-806	0.05	0.047 (0.022～0.095)
		TE-935	0.05	0.062 (0.036～0.113)
		TE-943	0.05	0.066 (0.038～0.112)
		CAM	0.05	0.58 (0.30～1.09)
		AZM	0.20	0.45 (0.28～0.75)
Streptococcus pneumoniae 224 (efflux-resistant)	2.1×10^7	TE-802	0.10	0.35 (0.21～0.65)
		TE-935	0.10	0.19 (0.13～0.28)
		TE-943	0.10	0.46 (0.30～0.73)
		CAM	0.78	2.50 (1.57～7.29)
		AZM	1.56	>3.00

* 95% Confidence limits.

AZM; the ED_{50} values for TE-802 varied from 0.047 to 0.22 mg/mouse against the test organisms (EM-susceptible). TE-806, TE-935 and TE-943 were equal to or 12.3 times more potent than CAM, and 1.2 to 9 times more potent than AZM.

The ED_{50} values for TE-802, TE-935, TE-943 and CAM were 0.35, 0.19, 0.46 and 2.50 mg/mouse, respectively, against *S. pneumoniae* 224. When administered orally, TE-802, TE-935 and TE-943 were 7.1 to 13.2 times more active than CAM. AZM was shown to be ineffective against the above organism; the ED_{50} value for AZM was >3.00 mg/mouse.

Respiratory Tract Infection

Table 7 shows the activities of TKs, CAM and AZM against experimental respiratory infections caused by *S. pneumoniae* J-4 or *H. influenzae* J-48 in mice. The efficacies of TKs were consistently better than those of CAM and AZM against pulmonary infection caused by *S. pneumoniae* J-4. However, the efficacies of TKs against *H. influenzae* J-48 were slightly less than that of AZM and approximately equal to that of CAM.

Pharmacokinetic Studies in Animals

The plasma levels of TKs, compared with those of CAM and AZM, were measured after single po or iv

Table 7. Activities of CAM, AZM, TE-802, TE-806, TE-935 and TE-943 against experimental respiratory infections caused by *S. pneumoniae* and *H. influenzae* in mice.

Drug	*S. pneumoniae* J-4		*H. influenzae* J-48	
	Dose (mg/mouse)	Viable cell counts in lung Log/lung (mean)	Dose (mg/mouse)	Viable cell counts in lung Log/lung (mean)
Control		>6.75 (±0.54)*		5.22 (±0.28)*
CAM	2.7	<1.48	8	<1.81 (±0.65)
(0.05, 12.5) **	0.9	3.16 (±0.54)	4	<1.81 (±0.66)
	0.3	4.03 (±0.62)	2	3.98 (±0.90)
	0.1	5.25 (±1.94)	1	3.77 (±0.74)
AZM	0.9	<1.48	4	<1.48
(0.10, 3.13) **	0.3	3.08 (±1.04)	2	<1.48
	0.1	6.57 (±0.43)	1	3.47 (±0.37)
	0.03	5.84 (±1.55)	0.5	<3.88 (±1.65)
TE-802	0.3	<1.48	8	<1.48
(0.025, 12.5) **	0.1	3.74 (±1.66)	4	<1.48
	0.03	5.40 (±1.31)	2	<2.22 (±1.49)
	0.01	>6.36 (±1.02)	1	4.52 (±0.13)
TE-806	0.3	<1.48	8	<1.48
(0.025, 12.5) **	0.1	<1.48	4	<2.10 (±1.25)
	0.03	3.65 (±1.69)	2	<3.08 (±1.46)
	0.01	6.65 (±0.32)	1	<3.78 (±1.55)
TE-935	0.3	<1.48	8	<1.48
(0.012, 6.25) **	0.1	<1.48	4	<1.48
	0.03	4.39 (±0.45)	2	<3.41 (±1.56)
	0.01	5.55 (±1.18)	1	4.27 (±0.97)
TE-943	0.3	<1.48	8	<1.48
(0.025, 6.25) **	0.1	<1.48	4	<1.48
	0.03	4.02 (±1.09)	2	2.24 (±1.32)
	0.01	6.50 (±1.11)	1	<2.93 (±1.09)

* Numbers in parentheses are standard deviations (n=3 or 4).

** MICs (μg/ml) for challenge strains (*S. pneumoniae*, *H. influenzae*).

Table 8. Pharmacokinetics of TE-802, TE-806, TE-935, TE-943, CAM and AZM in mice and monkeys.

Compound	Animal (Number)	Route*	Plasma level**					
			Cmax (μg/ml)	AUC_{8hr}(μg·hour/ml)	T1/2(hour)	CL (ml/hour/kg)	Vdss (ml/kg)	BA (%)
TE-802	Mouse (3)	po	0.26	3.14***	--	--	--	--
TE-806	Mouse (3)	po	0.27	3.38***	--	--	--	--
TE-935	Mouse (3)	po	0.38	4.16***	--	--	--	--
TE-943	Mouse (3)	po	0.16	2.25***	--	--	--	--
CAM	Mouse (3)	po	0.32	0.67***	--	--	--	--
AZM	Mouse (3)	po	0.27	1.84***	--	--	--	--
TE-802	Monkey (3)	po	0.68±0.38	1.94±0.72	4.23±1.33	--	--	65.76
TE-935	Monkey (3)	po	0.13±0.04	0.48±0.23	3.27±0.80	--	--	20.60
TE-943	Monkey (3)	po	0.10±0.01	0.42±0.13	12.38±3.91	--	--	20.29
CAM	Monkey (2)	po	0.72	2.47	1.56	--	--	42.44
AZM	Monkey (2)	po	0.33	0.57	8.93	--	--	38.78
TE-802	Monkey (3)	iv	--	2.95±0.69	4.83±0.74	1760.86±440.67	4022.13±832.47	--
TE-935	Monkey (3)	iv	--	2.33±0.13	5.65±1.39	2154.33±122.24	5980.36±729.64	--
TE-943	Monkey (3)	iv	--	2.07±0.22	8.82±3.12	2434.02±272.98	6754.57±1020.69	--
CAM	Monkey (2)	iv	--	5.82	2.75	883.31	1907.25	--
AZM	Monkey (2)	iv	--	1.47	6.82	3416.37	6719.54	--

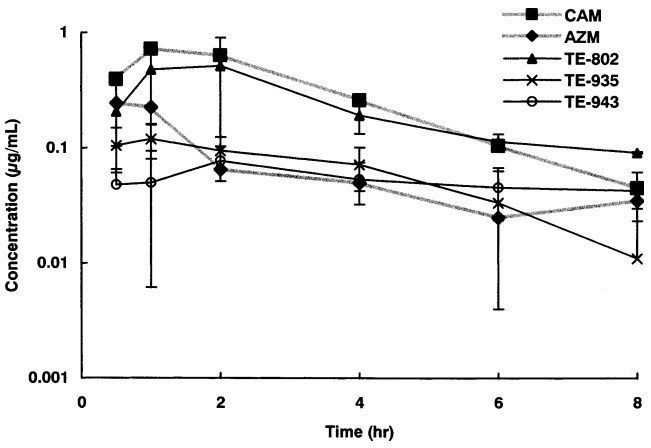
* Drugs (5mg/kg) were adoministered orally (po) or intravenously (iv).

** Plasma levels were determined by bioassay method using *Micrococcus luteus* ATCC 9341.

*** AUC value of each drug from 0 to 24 hours (AUC_{24hr}).

administration to mice or monkeys. Peak concentration in plasma (Cmax) and AUCs are shown in Table 8. The Cmax values for TKs were approximately equal to those of CAM and AZM after po administration to mice, while AUC values were 3.4 to 6.2 times and 1.2 to 2.3 times greater than those of CAM and AZM. The Cmax and AUC values for TE-935 and TE-943 were lower than those of CAM and AZM in monkeys after po administration, while these values for TE-802 (Cmax: 0.68 μg/ml, AUC: 1.94 μg·hour/ml) were approximately equal to or three-fourths of those for CAM (Cmax: 0.72 μg/ml, AUC: 2.47 μg·hour/ml) and greater than those for AZM (Cmax: 0.33 μg/ml, AUC: 0.58 μg·hour/ml).

Fig. 2. Drug concentrations in plasma after 5 mg/kg oral dosing in monkeys.

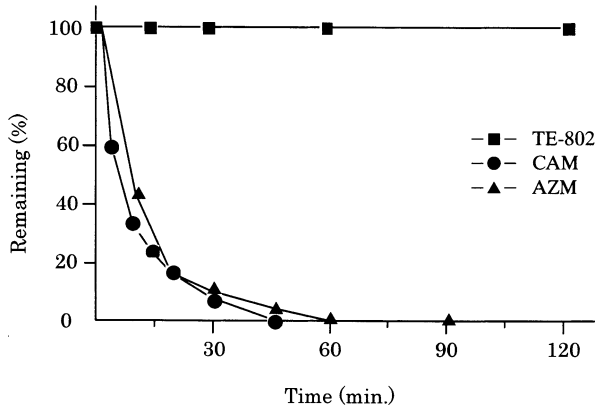


Discussion

One of the limitations of EM-antibiotics (EM, CAM and AZM) is acid instability. Tricyclic ketolides have been improved in this respect by the introduction of a keto moiety at the 3-position instead of the original cladinose sugar. As shown in Figure 3, TE-802 exhibited great stability under acidic conditions, and had not been decomposed 2 hours after dissolution of it (pH=1.2), while CAM and AZM were completely decomposed within 1 hour.

TKs, represented by TE-802, have demonstrated excellent *in vitro* activities against not only EM-susceptible organisms (standard strains and clinical isolates), but also

Fig. 3. Acid stability of TE-802, CAM and AZM. (pH 1. 2, 37°C).



EM-resistant *S. aureus* (inducible macrolide-resistant strains) as well as EM-resistant strains such as *S. pneumoniae*. In terms of genotype of EM-resistant strains, TKs exhibited excellent *in vitro* activity against efflux-mediated (*mefA*) EM-resistant *S. pneumoniae* (MICs: 0.05～0.10 μg/ml), but was less active against methylase-mediated (*ermB*) and both efflux- and methylase-mediated EM-resistant *S. pneumoniae* (MICs: 1.56～>100 μg/ml) (Table 2). Interestingly, unlike *S. pneumoniae*, TKs exhibited good activity against methylase-mediated (*ermC*) EM-resistant *S. aureus* (MIC: 0.20 μg/ml). These results suggest that the mechanism of action of TKs may differ between *S. pneumoniae* and *S. aureus*. Actually, no 'D'-shaped zone was observed around the RKM disc in the presence of TE-802 on plate of *S. aureus* B1. This result clearly differed from that for *S. pneumoniae*[9].

TKs were several times more potent *in vivo* than CAM and AZM against systemic infections in mice caused by *S. aureus*, *S. pyogenes* or *S. pneumoniae* (EM-susceptible and EM-resistant (efflux)). Moreover, TKs were more potent than CAM and AZM against respiratory tract infection caused by *S. pneumoniae*, but slightly less potent than AZM against *H. influenzae*.

The higher therapeutic efficacy of TKs in the above models may be related to the acid stability, strong *in vitro* activity and longer-lasting plasma levels. The pharmacokinetics of TKs, especially those of TE-802, are similar to those of AZM in mice and monkeys, suggesting the potential for once-daily administration in humans.

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