AT-4526, A NEW QUINOLONE DERIVATIVE: ITS IN VITRO AND IN VIVO ANTIBACTERIAL ACTIVITY AS A VETERINARY MEDICINE

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Introduction

Various antibiotics have been used in the treatment of bacterial infections in domestic animals. In recent years, however, outbreaks of infections due to microorganisms resistant to antibiotics are often observed (1, 2, 3). The antibacterial quinolone derivatives does not show cross-resistance with antibiotics (4). AT-4526, 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(cis-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid (Fig. 1), is a new quinolone derivative which was synthesized in 1986 (5). This compound, as well as the structurally related enrofloxacin (6), has a high order of activity against a broad spectrum of gram-positive, gram-negative bacteria, and Mycoplasma spp..

This paper describes the in vitro and in vivo antibacterial activities of AT-4526 against field isolates in domestic animals and the comparison with those of enrofloxacin (ERFX), oxytetracycline (OTC), tylosin (TS), thiamphenicol (TP), kanamycin (KM) and ampicillin (ABPC).

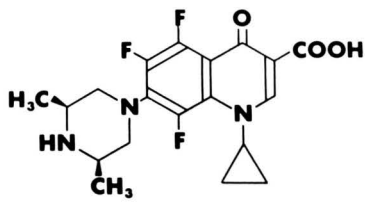

FIG. 1. Chemical structure of AT-4526

MATERIALS AND METHODS

Drugs. AT-4526 and ERFX were synthesized in Dainippon Pharmaceutical Co., Ltd., Research Laboratories (5). Other antimicrobial agents were purchased commercially. Doses and concentrations of the drugs are expressed in terms of the free bases.

Organisms. The organisms used were stock strains in our Research Laboratories and recent field isolates from infected animals in Japan.

MICs determinations. MICs were determined by a twofold agar dilution method with inocula of about 10^3 colony forming units per spot (4).

Inhibition of DNA gyrase activity. An effect on DNA gyrase activity was investigated by measuring the inhibition of supercoiling activity of DNA gyrase derived from Escherichia coli KL-16, using plasmid pBR322 as a substrate (7).

Assessment of in vivo activities. The in vivo antibacterial activity of the drugs was determined by measuring their protective effect against death caused by bacterial infections in mice. Systemic infections were induced by inoculating male mice intraperitoneally with 50 to 500 times the 50% lethal doses of the organisms. The drugs were given as a single oral (po) or intramuscular (im) dose to the mice immediately after the infection. Survivors were recorded for a week postinfection. The 50% effective doses (ED_{50}s) were calculated by the probit method, and the 95% confidence limits were calculated by the Litchfield and Wilcoxon method.

Plasma levels in mice. The AT-4526 and ERFX were given once orally or intramuscularly to mice at a dose of 5 mg/kg. The drug

TABLE 1. In vitro antibacterial activities of AT-4526 and reference compounds against field isolates

Organisms (no. of isolates)	Antimicrobial agents	MIC (μg/ml)[a] Range	50%	90%
Staphylococcus spp. (12)	AT-4526	0.39-0.78	0.39	0.78
	ERFX	0.05-0.2	0.1	0.2
	OTC	0.39->100	0.78	100
	TS	0.39->100	0.78	>100
	TP	6.25->100	12.5	50
	KM	0.2->100	0.78	3.13
	ABPC	0.1->100	0.1	0.2
Streptococcus spp. (16)	AT-4526	0.78-1.56	1.56	1.56
	ERFX	0.2-0.78	0.39	0.78
	OTC	0.2->100	0.39	100
	TS	0.05->100	0.39	>100
	TP	1.56-6.25	6.25	6.25
	KM	6.25-100	12.5	50
	ABPC	0.0125-0.1	0.0125	0.1
Escherichia coli (55)	AT-4526	0.05-1.56	0.1	1.56
	ERFX	0.025-0.78	0.05	0.78
	OTC	3.13->100	>100	100
	KM	0.78->100	100	>100
	ABPC	1.56->100	3.13	>100
Salmonella spp. (80)	AT-4526	0.05-6.25	0.1	1.56
	ERFX	0.05-1.56	0.05	0.78
	OTC	1.56->100	>100	100
	KM	0.78->100	100	>100
	ABPC	0.78->100	>100	>100

TABLE 1- Continued

Organisms (no. of isolates)	Antimicrobial agents	MIC (μg/ml)[a] Range	50%	90%
Pasteurella multocida (30)	AT-4526	0.0125-0.05	0.025	0.025
	ERFX	0.0125-0.05	0.0125	0.025
	OTC	0.39-3.13	0.78	1.56
	TS	3.13-50	12.5	25
	TP	0.39->100	0.78	0.78
	KM	3.13-50	6.25	12.5
	ABPC	0.05-0.2	0.1	0.1
Pasteurella haemolytica (24)	AT-4526	0.0125-0.1	0.025	0.1
	ERFX	0.0125-0.1	0.025	0.05
	OTC	0.39-100	0.78	100
	TS	6.25-25	12.5	25
	TP	0.39->100	1.56	3.13
	KM	0.78-12.5	6.25	6.25
	ABPC	0.025-25	0.78	25
Haemophilus parasuis (16)	AT-4526	0.0125-0.025	0.0125	0.025
	ERFX	0.0125-0.025	0.0125	0.025
	OTC	0.39-6.25	0.39	3.13
	TS	3.13-25	12.5	25
	TP	0.78-1.56	0.78	0.78
	KM	3.13-25	6.25	25
	ABPC	0.025-0.2	0.025	0.025
Haemophilus somnus (22)	AT-4526	0.0125-0.05	0.025	0.05
	ERFX	0.0125-0.05	0.025	0.05
	OTC	0.05-1.56	0.2	0.78
	TS	0.78-12.5	1.56	6.25
	TP	0.2-0.39	0.2	0.39
	KM	3.13-50	6.25	25
	ABPC	0.0063-0.78	0.025	0.39
Actinobacillus pleuropneumoniae (42)	AT-4526	0.0125-0.05	0.025	0.05
	ERFX	0.0125-0.05	0.025	0.05
	OTC	0.2-12.5	0.78	6.25
	TS	25-50	50	50
	TP	0.39->100	0.78	100
	KM	1.56->100	6.25	12.5
	ABPC	0.1-12.5	0.2	0.39
Mycoplasma hyopneumoniae (24)	AT-4526	0.1-0.2	0.1	0.2
	ERFX	0.05-0.2	0.05	0.1
	OTC	0.0125-3.13	0.05	1.56
	TS	0.0125-0.2	0.1	0.1
	TP	0.78-3.13	0.78	1.56
	KM	3.13-12.5	6.25	12.5

[a] 50% and 90%: MICs inhibiting more than 50 and 90 % of isolates, respectively. ERFX, enrofloxacin; OTC, oxytetracycline; TS, tylosin; TP, thiamphenicol; KM, kanamycin; ABPC, ampicillin.

concentration were determined by the agar well diffusion method using E. coli Kp as the assay organism seeded into heart infusion agar (Difco).

RESULTS

Susceptibility of field isolates. The MICs of AT-4526 ranged from 0.05 to 1.56 μg/ml for gram-positive organisms such as staphylococci and streptococci, from 0.0125 to 6.25 μg/ml for gram-negative organisms such as E. coli, Salmonella spp., Pasteurella spp., Haemophilus spp. and Actinobacillus pleuropneumoniae and from 0.1 to 0.2 μg/ml for Mycoplasma hyopneumoniae (TABLE 1). Its in vitro antibacterial activities were comparable to or slightly less potent than those of ERFX and generally more potent than those of antibiotics such as OTC, TS, TP, KM and ABPC with some exceptions. AT-4526 showed no cross resistant with reference drugs mentioned above except ERFX.

The MIC values of AT-4526 were scarcely affected by the pH of culture media, the inoculum size or the addition to culture media of horse serum or sodium deoxycholate.

The bactericidal concentrations were twice higher than the

TABLE 2. In vivo antibacterial activity of AT-4526 against systemic infection in mice

Infecting organism	Drug	MIC (μg/ml)	ED_{50} (mg/kg/dose) po	im
E. coli SZ-51	AT-4526	0.1	0.892	0.656
	ERFX	0.025	1.79	1.10
	OTC	3.13	168	7.28
	KM	1.56	130	4.06
	ABPC	25	>400	>400
S. typhimurium SL-28	AT-4526	0.1	0.928	1.10
	ERFX	0.05	1.70	1.86
	OTC	3.13	172	14.3
	KM	1.56	367	8.27
	ABPC	1.56	19.6	32.5
S. typhimurium S-5	AT-4526	0.1	0.568	1.00
	ERFX	0.05	1.73	1.88
	OTC	>100	>400	>400
	KM	>100	>400	>400
	ABPC	>100	>400	>400
P. multocida NG-1	AT-4526	0.025	0.433	0.465
	ERFX	0.0125	1.10	1.31
	OTC	0.78	>400	29.8
	KM	12.5	>400	13.2
	ABPC	0.05	>400	6.46

ERFX, enrofloxacin; OTC, oxytetracycline; KM, kanamycin; ABPC, ampicillin.

MIC for selected strains of Staphylococcus aureus 209P JC-1, E. coli NIHJ JC-2, and Pseudomonas aeruginosa Tsuchijima.

Inhibition of DNA gyrase activity. The 50% inhibitory concentration (IC_{50}) of DNA gyrase activity of AT-4526 was 0.52 μg/ml, which is ten times higher than the MIC (0.05 μg/ml). The discrepancy between IC_{50} and MIC is a common phenomenon observed for other quinolone antibacterials (7). Therefore, it is considered that AT-4526 is a potent DNA gyrase inhibitor.

In vivo antibacterial activity. The ED_{50}s of AT-4526 ranged from 0.433 to 1.10 mg/kg (TABLE 2). In these experiments the in vivo efficacies of AT-4526 were consistently more potent than the those of the reference drugs including ERFX regardless of MIC value of the each compound by both administration routes.

TABLE 3. Pharmacokinetic parameters of AT-4526 and enrofloxacin on levels in plasma in mice given a single oral or intramuscular dose of 5 mg/kg

Drug	Route	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-24} (μg.h/ml)
AT-4526	po (n=5)	0.785	0.5	0.756	1.16
	im (n=5)	2.01	0.5	0.613	2.52
Enrofloxacin	po (n=5)	0.170	0.5	1.18	0.332
	im (n=5)	0.631	0.5	0.492	0.590

Plasma levels in mice. In order to give the possible explanation to the more potent in vivo efficacies of AT-4526 than ERFX in spite of the lower in vitro activities pharmacokinetic properties were examined in mice. The plasma maximal concentrations (C_{max}s) of AT-4526 and ERFX after an po administration were 0.785 and 0.170 μg/ml, respectively, and half lives ($t_{1/2}$) were 0.756 and 1.18 h, respectively (TABLE 3). The C_{max} of AT-4526 after po dosing was higher than that of ERFX, and the area under the concentration-time curve (AUC) of AT-4526 was higher than that of ERFX. After a single im dosing, C_{max}, $t_{1/2}$, and AUC values of AT-4526 were also higher than those of ERFX. AT-4526 showed the more favorable pharmacokinetic profile in mice in comparison with ERFX by both administration routes.

DISCUSSION

Some quinolone antibacterials such as oxolinic acid and nalidixic acid, have been used as a veterinary medicine for long time. Their antibacterial activities, however, were limited to gram-negative bacilli in the actual use. Furthermore, old type antibiotics as OTC, TP, KM, ABPC, have met with the problem of emergence of antibiotics-resistant bacteria. Recently, ERFX was put on the market in some countries, whose antibacterial activities and spectrum are superior to those of the existing antibacterials in the veterinary field.

We have accumulated experiences of research and development on quinolone antibacterials for human and animal uses, and then, tried to find out a newer quinolone for veterinary use.

As described above, the anti-infectious activity of AT-4526 in experimental mouse models was more potent than to that of ERFX in spite of its lower in vitro antibacterial activities. The potency could be entirely depend upon its pharmacokinetic characteristics in mice, higher and long sustained plasma level of active principle following im or po administration.

AT-4526 as well as ERFX did not show any cross-resistance with the reference antibiotics in vitro.

These results indicate that AT-4526 will be one of useful veterinary medicines.

SUMMARY

AT-4526, 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(cis-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, is a new quinolone derivative with broad and potent antibacterial activities. It inhibited the growth of major pathogens for swine and cattle including Escherichia coli, Salmonella spp., Pasteurella multocida, Pasteurella haemolytica, Haemophilus parasuis, Haemophilus somnus and Actinobacillus pleuropneumoniae at concentrations of 0.0125-6.25 μg/ml. Its in vitro activities were comparable to or slightly less potent than those of enrofloxacin and generally more potent than those of antibiotics such as oxytetracycline, tylosin, thiamphenicol, kanamycin and ampicillin. AT-4526 did not show cross-resistance with the antibiotics. Orally or intramuscularly administered AT-4526 showed good efficacies against systemic infections due to Escherichia coli, Salmonella typhimurium and P. multocida in mice. The in vivo efficacies of AT-4526 were consistently higher than the those of the reference drugs including enrofloxacin by both administration routes regardless of MIC value of the each compound.

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RESUME

L'AT-4526, acide 1-cyclopropyle-5, 6, 8-trifluoro-1, 4-dihydro-7-(cis-3, 5-diméthyle-1-piperazinyle)-4-oxoquinoléine-3-carboxylique, est un nouveau dérivatif quinolone possédant d'importantes et vastes activités antibactériennes. Il arrête la croissance des principaux microbes pathogènes chez les porcs et le bétail comprenant l'Escherichia coli, la Salmonella spp., la Pasteurella multocida, la Pasteurella haemolytica, l'Haemophilus parasuis, l'Haemophilus somnus et l'Actinobacillus pleuropneumoniae à des concentrations de 0,0125 - 6,25 μg/ml. Ses activités in vitro furent comparables à ou d'une efficacité légèrement inférieure à celles de l'enrofloxacine et généralement plus efficaces que celles d'antibiotiques tels que l'oxytétracycline, la tyrosine, la kanamycine, le thiamphénicol et l'ampicilline.

L'AT-4526 n'a pas montré de résistance opposée avec les antibiotiques. Administré oralement ou intramusculairement, l'AT-4526 a montré une bonne efficacité vis-à-vis des infections systémiques dûes à l'Escherichia coli, la Salmonella typhimurium et la Pasteurella multocida chez la souris. L'efficacité in vivo de l'AT-4526 a été considérablement plus élevée que celle de produits pharmaceutiques de références comprenant l'enrofloxacine par les deux voies d'administration, sans tenir compte de la valeur MIC de chaque composé.

ZUSAMMENFASSUNG

AT-4526, 1-Cyclopropyl-5, 6, 8-Trifluoro-1, 4-Dihydro-7-(cis-3, 5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid ist ein 'New Quinolone' Derivat mit breiten und wirksamen Aktivitäten. Es hemmt das Wachstum von Hauptpathogenen in Schweinen und Vieh, - eingeschlossen Escherichia coli, Salmonella spp., Pasteurella multocida, Pasteurella haemolytica, Haemophilus parasuis, Haemophilus somnus und Actinobacillus pleuropneumoniae in einer Konzentration von 0.0125 - 6.25 μg/ml. Seine in vitro Aktivitäten waren vergleichbar oder leicht weniger wirksam als die Aktivitäten von Enroflaxin und generell wirksamer als die Aktivitäten der Antibiotika wie Oxytetracycline, Tylosin, Thiamphenicol, Kanamycin und Ampicillin.

AT-4526 zeigte keine Kreuz-Resistenz mit den Antibiotika. Oral oder intramuskulär verabreichtes AT-4526 wies gute Wirksamkeit gegen systemische Infektionen von Escherichia coli, Salmonella typhimurium und P. multocida in Mäusen auf. Die in vivo Wirkungen von AT-4526 waren gleichbleibend höher als die der oben erwähnten Mittel (eingeschlossen Enrofloxacin) bei beiden Verabreichungsmethoden unabhängig vom MIC-Wert eines jeden Mittels.