AT-4526, A NEW QUINOLONE DERIVATIVE: ITS PHARMACOKINETICS IN CATS, DOGS, PIGS AND CALVES

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INTRODUCTION

AT-4526, 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(cis-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, a new quinolone derivative, is under development as veterinary medicine. It has broad and potent antibacterial activities against gram-positive and gram-negative bacteria, anaerobes and Mycoplasma spp. (1). Its in vivo activity was generally higher than those of structurally related enrofloxacin and certain antibiotics such as oxytetracycline, kanamycin, and ampicillin in experimental infections in mice even in the case of less potent in vitro activity (1).

This paper describes the pharmacokinetics of AT-4526 in cats, dogs, pigs and calves.

MATERIALS AND METHODS

Drug. AT-4526 was synthesized in Dainippon Pharmaceutical Co., Ltd., Research laboratories as reported previously (2). Doses and concentrations of the drugs are expressed in term of the free bases.

Animals. Male and female mongrel cats weighing 2.6 to 4.5 kg, male beagle dogs weighing 11 to 12 kg, male pigs (Landrace x Large Yorkshire) 15 to 30 kg, male and female calves (Japanese cattle and Holstein) weighing 79 to 143 kg were employed.

Drug Administration. For intramuscular (im) and subcutaneous (sc) administration, 5% solution of AT-4526 was prepared in sterile water containing lactic acid equimolar to the active ingredient. For oral (po) administration, the drug was suspended in 0.2% carboxymethyl cellulose solution. The drug was administered once at a dose of 5 mg/kg to animals, unless otherwise specified.

Preparation of assay samples. Blood samples were withdrawn by venipuncture at 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h postadministration, and centrifuged to separate the plasma. Organs and tissues were harvested from exsanguinated pigs 1, 3, and 6 h postadministration and exsanguinated calves 1 h postadministration. These samples were weighed, homogenized with three times the weight of 0.067M phosphate buffer solution (pH 7.0) with Polytron homogenizer, heated at 80°C for 15 min., cooled, and centrifuged to separate the supernatant. Urine and feces were collected from animals individually housed in metabolic cages except calves. All samples were stored at -20°C until assayed.

Assay. The AT-4526 concentrations were determined by the agar

well diffusion method using Escherichia coli Kp (3) as an assay organism and heart infusion agar (Difco), or high-performance liquid chromatography (HPLC) with fluorometric detection. The sensitivities of the bioassay and HPLC assay were about 0.1 and 0.02 μg/ml or μg/g, respectively.

Pharmacokinetic analysis. Plasma level parameters were estimated as to following items: C_{max}, maximal concentration observed (μg/ml) ; T_{max}, time of C_{max} (h); $t_{1/2}$, elimination half-life (h) calculated by linear regression analysis on the elimination phase; and AUC, area under the concentration-time (μg.h/ml) calculated by the trapezoidal rule.

RESULTS

Plasma levels. The AT-4526 levels in plasma in animals after a single im, sc, or po administration are shown in Fig. 1. The mean plasma levels of AT-4526 in cats, dogs, pigs and calves reached peaks of 2.03 to 2.84 μg/ml at 0.5 to 1 h after an im or sc administration. Even at 8 h after postadministration, plasma levels were between 0.281 (calves) and 1.14 μg/ml (dogs), and no drug was detected in the plasma at 24 h, except for dogs (0.191 μg/ml at 24 h).

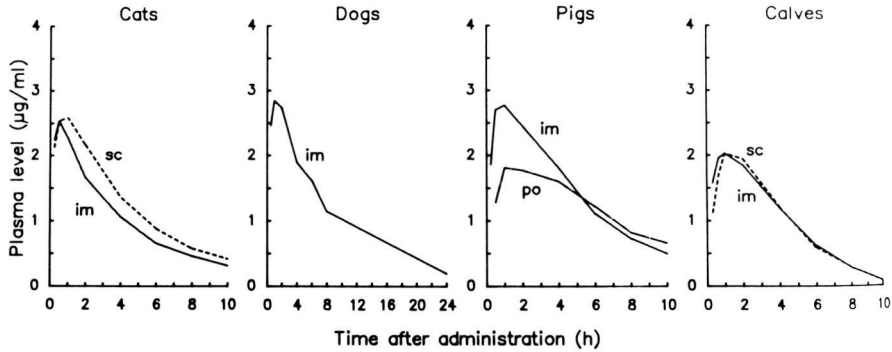

Fig. 1. Mean plasma concentrations of AT-4526 in cats, dogs, pigs, and calves given a single dose of 5 mg/kg (bioassay).

Their estimated pharmacokinetic parameters are shown in Table 1. There was little difference of C_{max}, $T_{1/2}$, and AUC values between sc and im administration in cats and calves. AT-4526 was well absorbed by a po administration. The plasma C_{max} and $t_{1/2}$ after po administration in pigs were 1.88 μg/ml and 5.09 h, respectively. These data indicates that the plasma levels of AT-4526 in animals were about twice higher than those of enrofloxacin reported by Scheer (4).

The repeated im administrations of AT-4526 at the dose of 5 mg/kg/day for 5 days to pigs and calves gave a mean plasma C_{max}s

of 2.76 and 1.10 μg/ml, respectively, and $t_{1/2}$s 2.82 and 4.93 h, respectively, after the last dose, which were comparable to those after the first dose (data not shown).

Table 1. Pharmacokinetic parameters of AT-4526 in plasma of animals given a single dose of 5 mg/kg (bioassay).

Animal	Route (n)	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-24} (μg.h/ml)
Cats	im (3)	2.63±0.15[a]	0.4±0.1	3.36±0.29	11.8±1.16
	sc (3)	2.62±0.10	0.8±0.2	3.33±0.30	14.6±1.01
Dogs	im (3)	2.85±0.15	1.7±0.3	5.84±0.42	26.3±1.27
Pigs	im (5)	2.95±0.16	0.8±0.1	3.53±0.09	17.4±0.54
	po (5)	1.88±0.02	1.0±0.3	5.09±0.31	17.1±0.59
Calves	im (11)	2.04±0.08	1.0±0.0	2.27±0.07	10.1±0.37
	sc (8)	2.08±0.08	1.2±0.2	2.11±0.07	9.95±0.49

[a] mean±standard error.

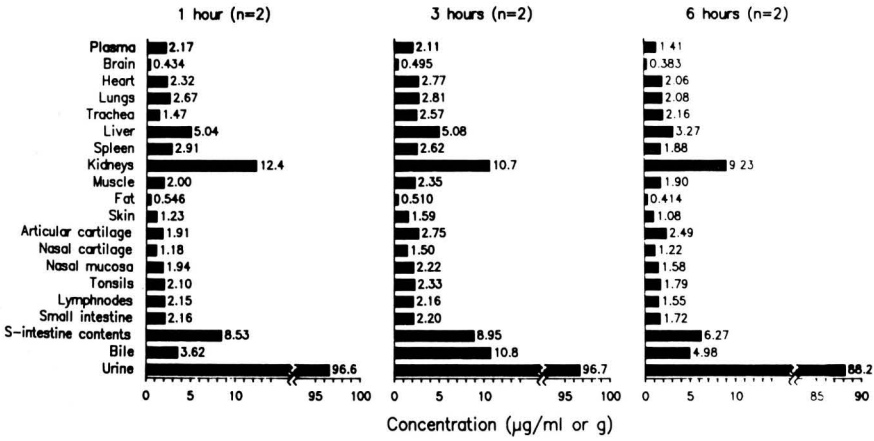

Fig. 2. Mean concentrations of AT-4526 in organs and tissues of pigs given a single im dose of 5 mg/kg (bioassay).

Tissue levels. The AT-4526 levels in organs and tissue of pigs given a single im dose of 5 mg/kg are shown in Fig. 2. The mean peak concentrations of AT-4526 in plasma was 2.17 μg/ml, and

in small intestine, nasal mucosa, muscle, heart, lung, spleen, liver, and kidney were 2.20, 2.22, 2.35, 2.77, 2.81, 2.91, 5.08, and 12.4 μg/g, respectively. The levels in main organs and tissues were comparable to or higher than that in plasma. In contrast, the peak levels in brain and fat were about one-fourth to one-fifth of the plasma levels, and those in bladder bile and bladder urine were 5 and 45 times higher than those in pigs, respectively.

Two calves received a single im dose of 5 mg/kg was sacrificed 1 h after administration, when plasma level was presumed to be highest. The distribution pattern was similar to that of pigs (data not shown). These results show that AT-4526 is well distributed in organs and tissues, except for brain and fat tissue.

The elimination from tissues was studied. AT-4526 was not detected by HPLC in any organs and tissues examined 10 or 14 days after consecutive im administration for 5 days at the dose of 10 mg/kg/day in pigs and calves (data not shown).

Urinary and fecal excretion. The results of Urinary excretion of AT-4526 in dogs, pigs and calves after a single im administration are shown in Table 2. The mean concentrations of AT-4526 in urine were several tens μg/ml or more for 24 h after an im administration. Total urinary recoveries of AT-4526 for 72 h (for 24 h in dogs) were 50.8, 71.1, and 37.3 % of the dose in dogs, pigs, and calves, respectively.

Table 2. Urinary excretion of AT-4526 in dogs, pigs, and calves given a single im dose of 5 mg/kg (bioassay).

Animal	Time (h)	Concentration of urine (μg/ml)	Recovery (% of dose)
Dogs (n=3)	0-24	99.6 ± 5.40[a]	50.8 ± 3.58
Pigs (n=3)	0-6	170 ± 23.7	42.1 ± 4.43
	6-24	31.8 ± 7.76	28.2 ± 2.88
	24-48	2.54 ± 0.18	2.61 ± 0.41
	48-72	0.87 ± 0.22	0.88 ± 0.27
	0-72	23.1 ± 3.14	71.1 ± 4.35
Calves (n=2)	0-6	244 ± 7.00	19.9 ± 2.38
	6-24	86.9 ± 9.05	15.4 ± 3.57
	24-48	10.9 ± 8.64	1.73 ± 0.78
	48-72	1.94 ± 1.51	0.30 ± 0.08
	0-72	53.7 ± 20.9	37.3 ± 5.09

[a] Mean±standard error.

The mean peak concentrations of AT-4526 in feces of pigs (0 to 24 h sample) and calves (24 to 48 h sample) after an im administration were 9.83 and 7.74 μg/g, respectively. Mean fecal recoveries of AT-4526 for 72 h were 9.12 and 5.46% of the dose in

pigs and calves, respectively.

Metabolites in urine. To determine the active principle in vivo, the urine samples of pigs and calves intramuscularly given AT-4526 were examined by HPLC. As shown in Fig. 3, in the urine of pigs and calves, unchanged AT-4526 was detected mainly, and its content in those samples were more than 90 %. These data indicate that AT-4526 is hardly metabolized and behaves as the unchanged form for the most part in pigs and calves.

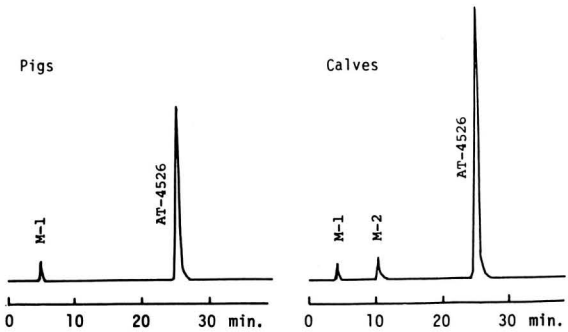

Fig. 3. HPLC chromatograms of urine in pigs and calves given AT-4526. M-1 and M-2: unknown metabolite.

DISCUSSION

It is known that the quinolone antibacterials have a unique antibacterial profile in vitro and in vivo (1). Some quinolones have already used as a antibacterial therapeutics in the veterinary field. However, their usage is relatively limited because of low antibacterial activity, metabolic instability or short persistence of an active moiety in animal bodies. AT-4526 was selected as a compound which shows very strong anti-infectious activities in experimental mouse models in comparison with its in vitro potency (1). Pharmacokinetic properties of AT-4526 were studied to predict the anti-infectious efficacy in domestic animals.

When AT-4526 was administered intramuscularly or subcutaneously to animals at a dose of 5 mg/kg, its maximum concentrations in plasma were between about 2 and 3 μg/ml and half-lives of elimination phase were between about 2 and 6 h. Even 8 h after the im administration about 0.7 μg/ml of AT-4526 in plasma were detected in pigs and about 0.3 μg/ml in calves. Furthermore, the studies on the distributions pattern after an im injection revealed that the active form of the compound existed at higher concentration for considerable long time in many tissues such as lung, trachea, heart, small intestine, than in the plasma. These concentrations are higher than MIC_{90}s against

main pathogens for pigs and calves. The total excretion rates of active principles into urine and feces in pigs and calves through 72 h following single im injection were approximately 80 % and 43 % respectively. We postulate that the relatively low excretion rate in calves does not mean that AT-4526 is extensively metabolized into inactive form(s), because by the HPLC method, only 2 extremely minor peaks could be detected in urine. It might be due to an incomplete system for collecting urine and feces in calves. No tendency of accumulation was observed after the repeated administration for 5 days at the dose of 5 mg/kg/day. Moreover, there seems to be no problem in the elimination pattern from the organs and tissues of animals given even at the higher dosage level, 10 mg/kg, than the scheduled daily dosage level, 2.5 to 5 mg/kg/day. When administered orally in pigs, AT-4526 seemed to be well absorbed through digestive tract. This result suggested the possibility of an oral formulation for domestic animals.

These pharmacokinetic properties together with it's antibacterial activities in vitro and in vivo suggested that AT-4526 could be useful in the treatment of many kinds of infections in domestic animals.

SUMMARY

The pharmacokinetic properties of AT-4526 administered by an intramuscular, subcutaneous, and oral routes at a dose of 5 mg/kg were assessed in cats, dogs, pigs, and calves. AT-4526 was well absorbed into plasma by various dosing routes. The maximal plasma levels of AT-4526 were about 2 to 3 μg/ml, elimination half-lives , 2 to 6 h. Its distributions into various tissues in pigs and calves by intramuscular route were very rapid and in most tissues the levels were equal to or higher than the plasma level. AT-4526 was excreted mainly into urine as an unchanged form. Urinary recoveries of AT-4526 in dogs, pigs, and calves were about 51, 71, and 37 % of the dose, respectively. AT-4526 which was injected into muscle of pigs and cattle at a dose of 20 mg/kg/day once a day for 5 days, disappeared in 10 and 14 days from any tissues, respectively.

These results indicate that AT-4526 possesses a favorable pharmacokinetic profile as an anti-infectious agent in domestic animals.

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RESUME

Les propriétés pharmacocinétiques de l'AT-4526 administré par voies intramusculaires, sous-cutanées et orales à une dose de 5 mg/kg ont été établies chez des chats, des chiens, des porcs et des veaux. L'AT-4526 fut bien absorbé en plasma selon des voies de dosages variées. Les niveaux de plasma maximums de l'AT-4526 furent d'environ 2 à 3 μg/ml et l'elimination de demi-vies de 2 à 6 h. Sa distribution dans divers tissus de porcs et de veaux par voie intramusculaire fut très rapide et dans la plupart des tissus, les niveaux furent égaux ou plus élevés que le niveau du plasma. L'AT-4526 fut principalement excrété dans l'urine sans aucune modification. Les récupérations urinaires de l'AT-4526 chez les chiens, les porcs et les veaux furent respectivement d'environ 51, 71 et 37% de la dose. L'AT-4526 qui fut injecté dans le muscle de porcs et de bestiaux à une dose de 20 mg/kg/jour une fois par jour pendant 5 jours, a disparu respectivement des tissus 10 et 14 jours après.

Ces résultats indiquent que l'AT-4526 possède un profil pharmacocinétique favorable en tant qu'agent anti-infectieux pour des animaux domestiques.

ZUSAMMENFASSUNG

Die pharmakokinetischen Eigenschaften von AT-4526, verabreicht über intramuskulärem, subkutanem und oralem Wege in einer Dosis von 5 mg/kg wurden bei Katzen, Hunden, Schweinen und Kälbern bewertet. AT-4526 wurde auf verschiedenen Dosierungswegen gut vom Plasma absorbiert. Die maximalen Plasmakonzentrationen von AT-4526 lagen um 2 bis 3 μg/ml, Halbwertszeit der Elimination, 2 bis 6 Stunden. Die Verteilung des Mittels in die verschiedenen Gewebe von Schweinen und Kälbern über intramuskulärem Wege ging sehr schnell vonstatten, und in den meisten Geweben waren die Konzentrationen gleich oder höher als die Konzentration im Plasma. AT-4526 wurde hauptsächlich im Urin in unveränderter Form ausgeschieden. Die ausgeschiedenen Mengen im Urin von AT-4526 in Hunden, Schweinen und Kälbern lagen jeweils bei ungefähr 51, 71 und 37% der verabreichten Dosis. AT-4526, welches in die Muskeln von Schweinen und Vieh zu einer Dosis von 20 mg/kg/tgl. einmal pro Tag für 5 Tage injiziert wurde, hinterliess innerhalb von 10 bis 14 Tagen keine Spuren in irgendeinem Gewebe, respektive.

Diese Ergebnisse indizieren, dass AT-4526 ein günstiges pharmakokinetisches Profil als anti-infektiöser Wirkstoff in Vieh und Haustieren besitzt.