AT-4526, A NEW QUINOLONE DERIVATIVE: ITS CLINICAL EVALUATION ON BACTERIAL RESPIRATORY DISEASE AND DIARRHEA IN CALVES

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INTRODUCTION

Bacterial respiratory diseases and diarrhea in the calves are causing great economic losses. So, antibacterial agents are often used to prevent and medicate to the diseases. Recently, bacteria resistant to antibiotics are increasing and causing severe problem in clinical field (1, 2). 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, has broad and potent antibacterial activity, and is not cross-resistanct with antibiotics (3). It is well absorbed and distributed to most tissues of calves by intramuscular (i.m.) administration (4). Therefore, it is suggested that AT-4526 is useful for bacterial diseases in various animals. This report describes the efficacies of AT-4526 against spontaneous bacterial respiratory diseases and diarrhea in calves.

MATERIALS AND METHODS

I. Drug and administration

AT-4526 (5% injectable solution) was supplied by Dainippon Pharmaceutical Co., Ltd., Osaka Japan. It was intramuscularly administered to calves once a day for 2-5 consecutive days at doses of 1.25 mg/kg, 2.5 mg/kg and 5.0 mg/kg.

II. Animals

The 144 calves (1-15 month-old) showing acute clinical signs of respiratory diseases including pyrexia, cough and/or nasal discharge and 81 calves (4-120 day-old) showing clinical signs of bacterial diarrhea including depression and/or dehydrated symptoms, were entered into the study. The field trial was performed in the period from June, 1990 to January, 1992.

III. Observation

1. Clinical observation

(1) Respiratory disease. Observation was carried out for clinical signs of respiratory diseases such as respiration rate, cough, nasal discharge, appetite and rectal temperature every day from the initial day of drug administration for 10 days (Day 0 to Day 9) and scores were recorded as defined in Table 1. These scores were summed up indivisually for clinical evaluation.

Table 1. Clinical signs and scores used for clinical evaluation

Clinical signs	Score		
	0	1	2
Rectal temperature	Normal (≦39.9°C)	Pyrexia (40.0-40.4°C)	Pyrexia (≧40.5°C)
Respiration rate	Normal (≦60)*	Slightly rapid (61-80)	Rapid (≧81)
Cough	No	Occasionally visible	Frequently visible
Nasal discharge	No	Occasionally visible	Frequently visible
Appetite	Good	Slightly poor	Poor

* : Respiration rate per minute

(2) Bacterial diarrhea. Observation was performed for clinical signs of diarrhea such as fecal abnormality, depression and dehydrated symptoms every day from Day 0 to Day 9.

2. Bacteriological examination

(1) Respiratory disease. Isolation and identification for Pasteurella multocida, P. haemolytica and Haemophilus somnus were performed with nasal discharge before drug administration and on the next day of last drug administration. Media used are DE JONG-BORST medium (BBL), COLOMBIA medium (BBL) and Chocolate agar. Incubation was carried out at 37°C in a incubator or a candle jar.

(2) Bacterial diarrhea. Isolation and identification for E. coli, Clostridium perfringens and Salmonella spp. were performed with feces before drug administration and on the next day of last administration. Media used are DHL medium (Eiken) and CW medium (added with kanamycin, Nissui). Incubation was performed at 37°C aerobically or anaerobicaly. As to E. coli, heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST) were assayed by a latex agglutination test and an enzyme linked immunoassay.

3. Viral and parasitological examination

For differential diagnosis of bacterial respiratory diseases, the titer of serum antibody against parainfluenzavirus 3, bovine adenovirus, bovine respiratory syncytial virus and bovine herpesvirus 1 were assayed. In bacterial diarrhea, the EPG (egg per gram of feces) of Strongyloides papillosus and OPG (oocyst per gram) of Eimeria spp. were calculated.

IV. Judgement for the clinical and bacteriological efficacy

1. Clinical evaluation

In the respiratory diseases, clinical improvement was evaluated by the following formula:

The clinical improvement score = (Initial clinical score before drug administration - Clinical score after the last drug administration)/ Initial clinical score

The clinical efficacy was evaluated with clinical improvement score by the criterion described as follows:

Remarkably effective ; 1.0 ∿ 0.9 of clinical improvement score
Effective ; 0.8 ∿ 0.5 of clinical improvement score
Ineffective ; 0.4 ∿ 0.0 of clinical improvement score

In the bacterial diarrhea, the clinical efficacy was evaluated by the criterion as follows:

Remarkably effective ; Normal feces within 3 days after the initiation of drug administration

Effective ; Normal feces within 5 days after the initiation of drug administration

Ineffective ; No improvement on fecal appearance until 5 days after the initiation of drug administration

2. Bacteriological evaluation

Bacteria possibly causing bacterial respiratory diseases or diarrhea were counted before and after drug administration. The bacteriological efficacy was evaluated by the criterion as follows:

Eradicated ; No bacteria detected after drug administration

Reduced; The number of bacteria after drug administration is less than one-tenths of the number before drug administration

Persistent ; The number of bacteria after drug administration is more than one-tenths of the number before drug administration

V. Determination of minimum inhibitory concentration (MIC)

AT-4526 was synthesized by Dainippon Pharmaceutical Co., Ltd. Oxytetracycline (OTC), thiamphenicol (TP), kanamycin (KM), ampicillin (ABPC) and bicozamycin (BCM) were obtained from commercial suppliers. The organisms used were isolated from animals showing clinical signs. P. multocida and P. haemolytica were isolated from nasal discharge. E. coli was isolated from feces before drug administration. MICs were determined by the two fold agar dilution method (5).

RESULTS

I. Results of field trial

1. Bacterial respiratory diseases

(1) Clinical evaluation. The clinical efficacy evaluated by the above criterion is shown in Table 2. The efficacies at doses of 1.25, 2.5 and 5.0 mg/kg were 45.5, 91.4 and 88.0%, respectly. The rate at a dose of 1.25 mg/kg was significantly lower than that at the other doses. No side effect was observed at all the doses.

Table 2. Result of clinical evaluation of AT-4526 in the field trial of bacterial respiratory diseases in calves

Dose[a] (mg/kgxdays)	Clinical evaluation			Efficacy[c] (%)
	Remarkably effective	Effective	Ineffective	
1.25 x 3~5 (11)[b]	5	0	6	45.5[d]
2.5 x 2~5 (58)	51	2	5	91.4
5.0 x 2~5 (75)	65	1	9	88.0

a : Once a day, i.m. administration, b : Number of animals

c : (Number of remarkable effect cases + Number of effect cases)/ The number of total cases

d : Differences from doses of 2.5 and 5.0 mg/kg are statistically significant. ($P<0.01$)

(2) Bacteriological evaluation. The efficacy evaluated by the above criterion is shown in Table 3. The total efficacy at doses of 1.25, 2.5 and 5.0 mg/kg were 90.0, 84.4 and 92.4%, respectively. There is no significant difference in efficacy between the isolated bacteria and between the doses employed.

Table 3. Result of bacteriological evaluation of AT-4526 in the field trial of bacterial respiratory disease in calves

Bacterium	Dose[a] (mg/kgxday)		Bacteriological evaluation			Efficacy[c] (%)
			Eradicated	Reduced	Persistent	
P. multocida	1.25 x 3∿5	(8)[b]	7	0	1	87.5
	2.5 x 3∿5	(30)	23	1	6	80.0
	5.0 x 3∿5	(40)	32	4	4	90.0
P. haemolytica	1.25 x 3∿5	(2)	2	0	0	100
	2.5 x 3∿4	(7)	7	0	0	100
	5.0 x 3∿5	(9)	9	0	0	100
P. multocida + *P. haemolytica*	2.5 x 3∿4	(8)	7	0	1	87.5
	5.0 x 2∿4	(17)	15	1	1	94.1
Total	1.25 x 3∿5	(10)	9	0	1	90.0
	2.5 x 3∿5	(45)	37	1	7	84.4
	5.0 x 2∿5	(66)	56	5	5	92.4

a : Once a day, i.m. administration, b : Number of animals
c : (Number of eradicated cases + Number of reduced cases)/Number of total cases

2. Bacterial diarrhea

(1) Clinical evaluation. The result is shown in Table 4. The efficacy at doses of 2.5 and 5.0 mg/kg were 92.3 and 83.8%, respectively. The total efficacy rate was 85.2%. No side effect was observed.

Table 4. Result of clinical evaluation of AT-4526 in the field trial of bacterial diarrhea in calves

Dose[a] (mg/kg x day)		Clinical evaluation			Efficacy[c] (%)
		Remarkably effective	Effective	Ineffective	
2.5 x 3	(13)[b]	6	6	1	92.3
5.0 x 2∿4	(68)	38	19	11	83.8
Total	(81)	44	25	12	85.2

a : Once a day, i.m. administration, b : Number of animals
c : (Number of remarkable effect cases + Number of effect cases)/ Number of total cases

(2) Bacteriological evaluation. The result is shown in Table 5. The efficacies at doses of 2.5 and 5.0 mg/kg were 92.3 and 83.8%, respectively. The total efficacy was 85.2%.

Table 5. Result of bacteriological evaluation of AT-4526 in the field trial of bacterial diarrhea in calves

Dose[a] (mg/kg x day)		Bacteriological evaluation			Efficacy[c] (%)
		Eradicated	Reduced	Persistent	
2.5 x 3	(13)[b]	3	9	1	92.3
5.0 x 2∿4	(68)	21	36	11	83.8
Total	(81)	24	45	12	85.2

a : Once a day, i.m. administration, b : Number of animals
c : (Number of eradicated cases + Number of reduced cases)/ Number of total cases

II. The MICs of field isolates

1. Respiratory disease

The result is shown in Figure 1. The MICs of AT-4526 for P. multocida and P. haemolytica ranged from 0.006 to 0.1 μg/ml. The antibacterial activity of AT-4526 was more potent than the reference antibacterial agents. As to resistant strains, the rate of P. multociada resistant to OTC and TP were 17.8 and 16.7%, respectively. The rate of P. haemolytica resistant to OTC and TP were 21.4 and 3.6%, respectively. Almost all strains were resistant to KM.

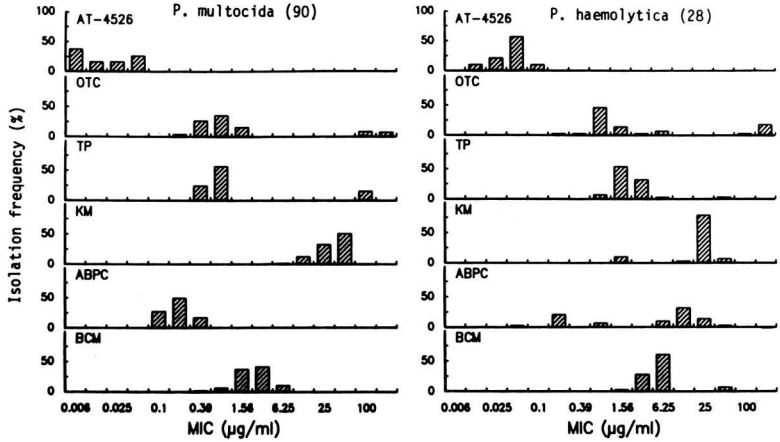

Fig. 1. The MICs of AT-4526 and reference agents for field isolates

2. Bacterial diarrhea

The MICs of AT-4526 for E. coli (280 strains) ranged from 0.025 to 0.2 μg/ml. The antibacterial activity of AT-4526 was more potent than that of the reference antibacterial agents compared. Isolation rates of strains of E. coli resistant to OTC, KM, ABPC and BCM were 75.2, 24.8, 31.2 and 1.1%, respectively.

DISCUSSION

In this clinical trial for bacterial respiratory diseases in calves, P. multocida and P. haemolytica were isolated in high frequency. When AT-4526 was administered intramuscularly to the calves for 2-5 consecutive days at doses of 1.25, 2.5 and 5.0 mg/kg, high clinical efficacy and the reduction of bacteria isolated from nasal discharge were observed at doses of 2.5 and 5.0 mg/kg. At a dose of 1.25 mg/kg, clinical efficacy was lower than those at doses of 2.5 and 5.0 mg/kg, though the reduction of bacteria isolated from nasal discharge was observed also at a dose of 1.25 mg/kg. MICs of AT-4526 against P. multocida and P. haemolytica are very low, 0.006 - 0.1 μg/ml, and AT-4526 well distributes to tissues of calves (4). This suggests that AT-4526 distributed to nasal discharge at high concentration and decreased bacteria even at a dose of 1.25 mg/kg.

In bacterial diarrhea, AT-4526 was administered intramuscularly for 2-4 days at doses of 2.5 and 5.0 mg/kg. At doses of 2.5 and 5.0 mg/kg, high clinical efficacy and the reduction of bacteria isolated from feces were observed. These result suggests that the optimum doses of AT-4526 for bacterial respiratory diseases and diarrhea in calves may be 2.5 - 5.0 mg/kg.

SUMMARY

Clinical trials of AT-4526 in bacterial respiratory diseases and diarrhea in calves were performed to assess its efficacy by intramuscular administration in Japan. The clinical efficacy rates in bacterial respiratory diseases were 45.5, 91.4 and 88.0% at doses of 1.25, 2.5 and 5.0 mg/kg, respectively. The clinical efficacy rate in bacterial diarrhea were 92.3 and 83.8% at doses of 2.5 and 5.0 mg/kg, respectively. No side effect was observed at all. The MICs of AT-4526 for P. multocida, P. haemolytica and E. coli isolated from tested calves suggested that AT-4526 was more potent than the reference compounds in antibacterial activity. Some strains were resistant to OTC, TP, KM and ABPC, while no strains were resistant to AT-4526. Present studies demonstrate that AT-4526 is effective on bacterial respiratory diseases and diarrhea in calves.

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RESUME

Des essais cliniques de l'AT-4526 vis-à-vis de la maladie respiratoire et de la diarrhée bactériennes chez les veaux furent effectués au Japon par administration intramusculaire pour déterminer son efficacité. Les taux d'efficacité clinique vis-à-vis de la maladie bactérienne de l'appareil respiratoire à une dose de 1,25, 2,5 ou 5,0 mg/kg/jour furent respectivement de 45,5, 91,4 ou 88,0%.

Les taux d'efficacité clinique vis-à-vis de la diarrhée bactérienne à une dose de 2,5 ou 5,0 mg/kg/jour furent respectivement de 92,3 ou 83.8%. Les plages MIC de l'AT-4526 vis-à-vis de la Pasteurella multocida, la Pasteurella haemolytica et l'Escherichia coli isolées des veaux testés furent plus importantes comparées aux agents antimicrobiens. Les taux d'isolation de l'effort de résistance vis-à-vis de l'oxytétracycline, le thiamphénicol, la kanamycine et l'ampicilline furent comparativement élevés.

ZUSAMMENFASSUNG

Ein klinischer Versuch von AT-4526 gegen bakteriell bedingter Erkrankungen von Atemwegen und Diarrhöe in Kälbern wurde in Japan ausgeführt, um dessen Wirksamkeit bei intramuskulärer Verabreichung zu bewerten. Die klinischen

Wirksamskeitsraten bei bakteriell bedingten Erkrankungen von Atemwegen bei einer Dosis von 1.25, 2.5 oder 5.0 mg/kg/tgl. waren 45.5, 91.4 oder 88%, respektive.

Die klinischen Wirksamskeitsraten bei bakteriell bedingter Diarrhöe bei einer Dosis von 2.5 oder 5.0 mg/kg/tgl. waren 92.3 oder 83.8%, respektive. Die MIC - Werte von AT-4526 gegen P. multocida, P. haemolytica und E. coli, welche aus getesteten Kälbern isoliert wurden, waren stärker als die verglichenen antimikrobialen Mittel. Die Isolationsraten der 'resistent strain' gegen OTC, TP, KM und ABCP waren vergleichsweise hoch.