Microbiology and Pharmacology of Enrofloxacin in Cattle

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Summary

Enrofloxacin is the first third-generation fluoroquinolone specifically developed for use in veterinary medicine. It is approved worldwide for use in dogs and cats and, for food animals outside the U.S. and Canada. Enrofloxacin has a wide spectrum of antimicrobial activity, is rapidly bactericidal, is active at very low concentrations, and shows no plasmid-mediated resistance. In cattle enrofloxacin's major pharmacokinetic characteristics are a high bioavailability after parenteral administration, a large volume of distribution and relatively high concentrations in body tissues. Antibacterial activities in serum and tissues, achieved after subcutaneous administration of a dose of 2.5 mg/kg, are substantially above the MIC_{90} for most important pathogens of the bovine respiratory and intestinal tracts.

Introduction

Enrofloxacin is the first third-generation fluoroquinolone specifically developed for use in veterinary medicine. It is used worldwide to treat a wide range of bacterial infections in many different species.

The very low minimum inhibitory concentrations (MICs) and favorable pharmacokinetic properties of enrofloxacin suggest that it would be an excellent antimicrobial for the treatment of bacterial infections in cattle. Enrofloxacin has been successfully used outside the U.S. to treat cattle affected with bacterial diseases of the respiratory, gastrointestinal and reproductive systems. In Europe, enrofloxacin is approved for parenteral treatment of bacterial infections in cattle caused by Pasteurella spp., Haemophilus spp., *Mycoplasma bovis*, *E. coli*, Salmonella spp., and Staphylococcus spp. Its high degree of efficacy is associated with its unique mechanism of action and profound bactericidal activity. Enrofloxacin is not currently approved for use in cattle in the U.S. or Canada. This paper presents the microbiological and pharmacological properties of enrofloxacin in cattle.

Structure-activity relationship

The first-generation quinolones, nalidixic and oxolinic acids, are antimicrobials characterized by good activity against most gram-negative bacteria and a limited volume of distribution. A 4-quinolone ring serves as the template for their chemical structure. Subsequent research led to the development of 6-fluoro-7-substituted-4-quinolones, the fluoroquinolones. Enrofloxacin represents the first fluoroquinolone exclusively developed for veterinary medicine. Substitution of a fluorine atom at position 6 enhances the inhibition of DNA gyrase activity and extends its spectrum to include some gram-positive bacteria. The piperazine ring at position 7 increases activity against staphylococci and adds activity against Pseudomonas spp. The cyclopropyl group at N-1 fortifies the general antimicrobial properties and expands the activity spectra to include Mycoplasma spp.

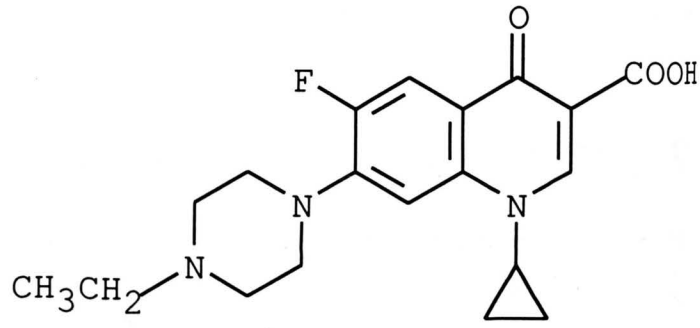

Figure 1. Molecular Structure of Enrofloxacin

Mechanism of action

Enrofloxacin's mechanism of action is mainly characterized by a rapid bactericidal effect associated with destruction of bacterial DNA. The specific action of enrofloxacin, and other quinolones, is unique among antimicrobials because it targets the bacterial enzymes required for DNA replication. The fluoroquinolones primarily inhibit the bacterial DNA gyrase (a bacterial topoisomerase II enzyme)[1,2] responsible for supercoiling of DNA within the bacterial cell; other less important targets and mechanisms are also discussed in the literature. DNA gyrase is composed of two subunits (subunits A and B) with distinct activities. Subunit A of the enzyme cuts the double-stranded DNA , subunit

B introduces negative supercoils, and subunit A reseals the DNA. Fluoroquinolones inhibit the resealing of the DNA by the A monomer, resulting in degradation of bacterial DNA by exonucleases,[1,2] and rapid cell death. After entry into the bacterial cell via porins, fluroquinolones accumulate very rapidly.[3] Morphologic alterations are decreased cell division, filamentation and cellular lysis[4]. Enrofloxacin does not disrupt the outer bacterial cell therefore endotoxin release is much lower compared to cephalosporines or aminoglycosides.[5] Endotoxemia causing septic shock is therefore less likely with enrofloxacin treatment.

Mammalian DNA replication enzymes are inhibited by drug concentrations 1,000 to 2,000 fold higher than needed to inhibit bacterial enzymes. For this reason fluoroquinolones have a favorable margin of safety.

Spectrum

Enrofloxacin is a broad-spectrum, bactericidal chemotherapeutic that is effective against aerobic bacteria. It has an excellent intrinsic activity against bacteria of the family enterobacteriaceae and fastidious gram-negative bacteria like Pasteurella spp. and Haemophilus spp. Enrofloxacin also has good activity against Mycoplasma spp., Staphylococcus spp., Pseudomonas spp., and Chlamydia spp. However, enrofloxacin possesses little activity against anaerobic bacteria. In 1993 Greene and Budsberg[6] reviewed MIC values for enrofloxacin from several original publications:

Table 1. MICs for Enrofloxacin as Reviewed by Greene and Budsberg[6]

	MIC range (µg/ml) [no. of strains]
Mycoplasma spp.	0.01 - 1.0 [108]
Gram-negative bacteria	
Haemophilus somnus	0.015 [10]
Haemophilus parasuis	<0.001 [10]
Actinobacillus pleuropneumoniae	0.01 - 0.06 [10]
Actinomyces pyogenes	1.0 [10]
Actinomyces suis	0.001 - 0.015 [7]
Pasteurella multocida and *P. haemolytica*	0.007 - 0.12 [88]
Pseudomonas aeruginosa	0.25 - 8.0 [93]
Escherichia coli	0.01 - 2.0 [330]
Proteus mirabilis	0.03 - 0.5 [91]
Klebsiella pneumoniae	0.03 - 0.5 [144]
Gram-positive bacteria	
Staphylococcus intermedius	0.01 - 1.0 [147]
Corynebacterium pseudotuberculosis	0.125 - 1.0 [10]
Rhodococcus equi	0.5 - 1.0 [10]
Streptococcus equi	1.0 [10]
Streptococcus suis	0.5 - 1.0 [10]
Streptococcus zooepidemicus	1.0 [10]
Anaerobic bacteria	
Bacteroides fragilis group	0.8 - 12.5 [27]

More recently we determined the susceptibility of Pasteurella spp., Mycoplasma spp., Salmonella spp. and *E. coli* isolates collected in Europe against enrofloxacin.[7] Bacteria were routinely collected by veterinary diagnostic laboratories during postmortem examinations associated with enteric or respiratory disease in cattle. The susceptibility of the pathogens to various antimicrobials was determined by means of an agar dilution technique according to the procedure described by the NCCLS.[8]

Table 2. MICs (µg/ml) Values[7] of Antimicrobial Compounds Against Pasteurella spp., *Mycoplasma bovis*, Salmonella spp. and *E. coli*, Isolated From Cattle.

	MIC			
	Minimum (n)	Maximum (n)	MIC_{50}	MIC_{90}
Pasteurella spp. (63)				
Enrofloxacin	0.015 (48)	0.12 (4)	0.015	0.06
Amoxicillin	0.015 (4)	16 (2)	0.12	0.5
Ampicillin	0.03 (2)	32 (2)	0.12	0.25
Chloramphenicol	0.015 (4)	>128 (2)	0.5	1.0
Gentamicin	0.12 (2)	16 (2)	1	2
Nalidixic Acid	0.5 (16)	16 (4)	1	8
Oxytetracycline	1 (8)	>128 (4)	2	2
Sulfathiazol/ Trimethoprim	0.03 (10)	>128 (2)	0.12	1
Mycoplasma spp. (18)				
Enrofloxacin	0.05 (3)	0.25 (4)	0.1	0.25
Flumequin	0.5 (7)	50 (2)	25	50
Oxytetracycline	0.25 (3)	10 (3)	1	10
Tylosin	0.5 (5)	100 (2)	1	100
Salmonella spp. (234)				
Enrofloxacin	0.015 (63)	1 (3)	0.03	0.06
Amoxicillin	0.25 (1)	128 (40)	1	128
Ampicillin	0.5 (34)	>128 (19)	1	>128
Chloramphenicol	2.0 (1)	128 (25)	4	128
Gentamicin	0.25 (17)	2.0 (8)	1	1
Oxytetracycline	0.5 (1)	128 (71)	4	128
Sulfathiazol/ Trimethoprim	0.06 (1)	>128 (19)	0.5	1
E. coli (109)				
Enrofloxacin	≤0.025 (68)	0.8 (1)	≤0.025	0.05
Gentamicin	0.05 (1)	100 (2)	0.8	1.56
Nalidixic Acid	0.4 (1)	> 100 (4)	3.13	12.5
Oxytetracycline	0.8 (4)	>100 (73)	>100	>100
Thiamphenicol	1.56 (2)	>100 (28)	100	>100

The listed MICs for enrofloxacin are in agreement with the results of previous published investigations.[9,10] In 1995, the results of an investigation were published which compared MIC values of several antimicrobials against pathogens isolated from swine in the U.S.. MIC results for antimicrobials of contemporary interest are listed in the following table:

Table 3. MIC Values[11] of Antimicrobial Compounds Against *Actinobacillus pleuropneumo-niae, Pasteurella multocida, Streptococcus suis, Salmonella cholerae-suis* and *Escherichia coli (n)*

	MIC (µg/ml)			
	Range	50%	90%	mode
A. pleuropn. (50)				
Enrofloxacin	≤ 0.03 - 0.13	≤ 0.03	0.13	≤ 0.03
Ceftiofur		≤ 0.03	≤ 0.03	≤ 0.03
Tetracycline	0.25 - >32	8	32	8
Tilmicosin	1 - >64	2	4	2
P. multocida (50)				
Enrofloxacin	NR	≤ 0.03	≤ 0.03	≤ 0.03
Ceftiofur	≤ 0.03 - 0.06	≤ 0.03	≤ 0.03	≤ 0.03
Tetracycline	0.25 - >32	1	16	1
Tilmicosin	0.5 - 8	4	8	4
S. suis (50)				
Enrofloxacin	0.13 - 1	0.13	0.13	0.13
Ceftiofur	1 - 2	1	1	1
Tetracycline	1 - >32	>32	>32	>32
Tilmicosin		>64	>64	>64
S. cholerae-suis (50)				
Enrofloxacin	≤ 0.03 - 0.5	0.25	0.5	0.25
Ceftiofur	≤ 0.03 - 1	≤ 0.03	0.13	≤ 0.03
Tetracycline	0.5 - >32	32	>32	32
Tilmicosin	0.06 - >64	>64	>64	>64
E. coli (50)				
Enrofloxacin	≤ 0.06 - 16	0.06	0.06	0.06
Ceftiofur	0.25 - 4	0.5	1	0.5
Tetracycline	1 - >32	>32	>32	>32
Tilmicosin		>64	>64	>64

It is likely that bovine pathogens show an MIC pattern against recently developed antimicrobials similar to that of porcine isolates.

Microbiological studies show that both the MIC and MBC (minimum bactericidal concentration) values for enrofloxacin are low and often times similar. The bactericidal activity of enrofloxacin is very pronounced and is illustrated by the low MBC values. In an investigation of 338 bacterial isolates, 10% of the MBC values equalled the MIC value, in 40%, MBCs were one dilution greater and in 30%, the MBCs were two dilutions higher than the corresponding MICs.[12]

Until recently, third-generation quinolones were not thought to be active against aerobic bacteria exposed to strict anaerobic conditions.[13] New studies with enrofloxacin have shown it to be highly bactericidal against *E. coli* under both aerobic and anaerobic conditions.[14]

Aerobic flora were almost entirely wiped out after oral fluoroquinolone treatment in humans (and returned to normal a week after the last administration) whereas anaerobic bacteria were only mildly affected.[15] A recent study[16] showed that methanogenic bacteria of the normal rumen microflora were not affected by in-vitro concentrations up to 44 µg/ml, which might explain why, clinically, no adverse effects are observed.

In vitro studies[17] have demonstrated that enrofloxacin induces a concentration dependent post-antibiotic effect (PAE) or temporary suppression of bacterial growth after exposure to the antimicrobial drug. It is speculated that post-antibiotic effects extend the duration of antimicrobial coverage (exposed bacteria are inhibited even though antimicrobial concentrations fall below MIC levels) and therefore, less frequent dosing is required.

Table 4. Duration of PAE Following Exposure to Enrofloxacin[17]

Organism*	Duration of PAE** after exposure for 2 hours to enrofloxacin concentration of		
	2 X MIC	4 X MIC	8 X MIC
Escherichia coli (ATCC 8739)		1.3 ± 0.5 (2)	2.6 ± 0.1 (3)
*Pasteurella multocida***		2.1 ± 0.2 (3)	3.6 ± 0.2 (2)
Pseudomonas aeruginosa (ATCC 9027)		3.1 ± 0.9 (4)	n.d.
*Salmonella typhimurium****		1.2 ± 0.2 (2)	3.2 ± 0.1 (3)
Staphylococcus aureus (ATCC 6538)		2.1 ± 0.6 (2)	2.9 ± 0.6 (5)
Mycoplasma bovirhinis[18] (PG 43)	5.9 ± 1.0 (4)		

* from liquid cultures in early stationary phase
** average duration in hours ± range
( ) number of independent experiments
*** isolated from infected poultry
n.d. not determined

Resistance

Resistance to many commonly used antibiotics by respiratory and intestinal tract pathogens of bovine origin is a significant problem. This is particularly true for Pasteurella spp., Salmonella spp. and *E. coli*.

Enrofloxacin maintains efficacy against gram-negative and gram-positive strains (including ß-lactamase producing staphylococci) that possess multiple resistance to conventional antimicrobials (ß-lactam antibiotics, aminoglycosides, tetracyclines, macrolides, polypeptide antibiotics, sulfonamides, diaminopyrimides and nitrofurans). The infrequent development of resistance to fluoroquinolones usually is a multi-step process and results from bacterial chromosome mutations. The mutations either alter bacterial DNA gyrase or change the porin channels of the cell membrane that determine drug penetration.[2] Bacterial enzymes that degrade or inactivate fluoroquinolones have not been found. Importantly, no evidence of plasmid-mediated resistance to quinolones[2] has been observed. In fact, it has been shown that sub-inhibitory concentrations of quinolones eliminate plasmids from their bacterial hosts.[2] When resistance to a

fluoroquinolone occurs, there is complete cross resistance to other third-generation quinolones.

Pharmacokinetic properties

The disposition of enrofloxacin, after administration of single intravenous or subcutaneous doses of 2.5 mg enrofloxacin/kg body weight (BW) to calves (mean BW of 55kg), was investigated in a cross-over experiment with 6 animals.[19]

Enrofloxacin was administered as a proprietary formulation (commercially available outside the US) containing 50 mg of enrofloxacin per ml solution (Bayer AG, Leverkusen, Germany).

Determination of antibacterial activity in the serum was performed by a bioassay with *E. coli* DSM 10650 as the test organism. The lower detection limit was 0.01 µg/ml with a mean recovery of 91%. Serum antibacterial concentration versus time data for each animal and route of administration were incorporated into a two-compartment model for kinetic analysis (TOPFIT). The main study results appear in the following table:

Table 5. Serum Pharmacokinetics after Administration of 2.5 mg/kg BW Enrofloxacin in Calves

	Route of Administration	
	Intravenous	Subcutaneous
Cmax (mg/L)[a]		1.27 ± 0.18
Tmax (h)[b]		1.67 ± 0.26
T½β (h)[c]	6.27 ± 0.82	6.54 ± 0.84
MRT (h)[d]	8.74 ± 1.10	9.86 ± 1.25
AUC (mg h/L)[e]	14.17 ± 2.81	12.96 ± 1.59
Vss (L/kg)[f]	1.56 ± 0.18	
CL (L/h)[g]	9.19 ± 2.50	
F (%)[h]		91.2

[a]maximum concentration
[b]time to reach maximum concentration
[c]elimination half-life
[d]mean residence time
[e]area under the curve
[f]volume of distribution at steady state
[g]clearance
[h]bioavailability

Peak concentration in serum occurs 1 to 2 hours after subcutaneous administration and the serum elimination half-life for enrofloxacin is 6.5 ± 0.8 h. The bioavailability of enrofloxacin after subcutaneous administration was calculated to be 91.2%.

Other studies have reported bioavailability values between 88.2 and 96.9%[19,20] following parenteral administration.

Because enrofloxacin is lipophilic and has a low degree of ionization, its distribution in tissues is very good. The steady-state volume of distribution obtained for enrofloxacin in calves (1.56 ± 0.18 L/kg) indicates it is widely distributed. Richez *et al.*[20] determined an even higher volume of distribution (1.9 ± 0.27 L/kg) in calves (mean bw 159 ± 4 kg).

In preliminary experiments the tissue penetration of enrofloxacin was investigated[21] in 10 calves (mean body weight 166 kg) treated i.m. with 2.5 mg/kg BW of enrofloxacin. Two calves each were euthanized at 1, 2, 4, 6 and 8 hours post injection, tissue specimens were collected, homogenized and the antibacterial actitivity was measured by bioassay.

In almost all investigated tissues and fluids the maximum detected antibacterial activity exceeded the maximum activity in the serum. Enrofloxacin was found to have good penetration into lung, synovia, cerebrospinal fluid, bone and cartilage. The mean peak lung concentration was 1.75 times greater than that of serum (figure 2). The results of investigations, using canine prostatic tissue as a model, indicate that the concentration of enrofloxacin in diseased tissue is at least equivalent to its concentration in normal tissue.[22]

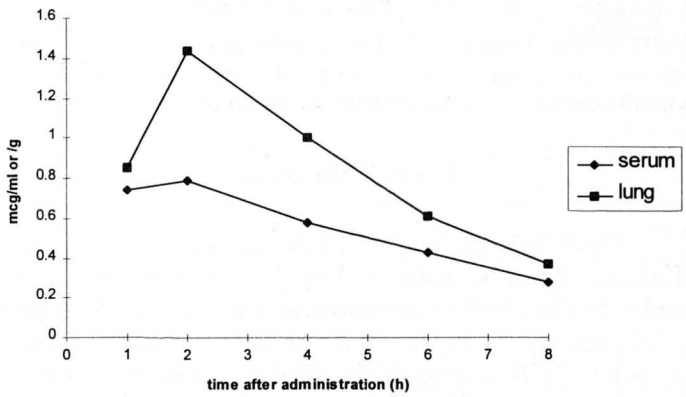

Figure 2. Mean Lung and Serum Concentrations of Antibacterial Activity after Intra-muscular Administration of Enrofloxacin to Calves (2.5mg/kg body weight)[21]

In cattle enrofloxacin is partially metabolized in the liver and excreted in bile or urine as active drug. In phase I metabolism oxoquinolones metabolize by hydroxylation and oxidation. Glucuronidation occurs at the carboxylic acid at position 3. Glucuronide conjugates are excreted predominantly through urine and bile. Therefore high concentrations are found in bile, the urinary tract, and liver. Bile concentrations are eight times greater than serum concentrations; an enterohepatic circulation of enrofloxacin has been demonstrated in laboratory animals. Urine concentrations exceed serum concentrations by 60 fold.

Concentration of Enrofloxacin in Macrophages and Neutrophils

Fluoroquinolones concentrate in macrophages and neutrophils. This fact is particularly important because some intracellular pathogens of cattle proliferate within macrophages[23] or are engulfed by neutrophils. Unfortunately, extensive data for veterinary quinolones are lacking. Research with quinolones used to treat humans has shown that the accumulation in peritoneal macrophages is 2 to 3 times greater than the extracellular concentration,[24] the concentration in neutrophils is 7 times the extracellular levels[25] and the concentration in alveolar macrophages is 14 to 18 times the serum level.[26] Ciprofloxacin was found to be freely soluble in macrophages and could fully exhibit its bactericidal potency against a variety of organisms.[27] In contrast, macrolides, in spite of enhanced accumulation, showed only bacteriostatic activity. In addition it has been demonstrated that modification of bacteria by ciprofloxacin leads to their enhanced engulfment by polymorphonuclear leukocytes.

There is good evidence that enrofloxacin has similar characteristics. Preliminary experiments have shown that enrofloxacin accumulates in alveolar macrophages (porcine). The killing activity of these macrophages against *Actinobacillus pleuropneumoniae,* in comparison to alveolar macrophages without enrofloxacin, is enhanced 20 to 40 times.[28]

Local Tolerance

Local tolerance after intramuscular administration can either be evaluated subjectively by inspection and palpation of the injection site (clinical examination) or objectively by measuring the serum creatine phosphokinase (CK) levels which increase in response to local muscle necrosis. The CK values following administration of different chemotherapeutics were compared[29] and are revealed in the following table (all chemotherapeutics were administered at recommended dose rates and volumes):

Table 6. CK-Activities After Parenteral Administration of Chemotherapeutics in Cattle[29]

Compound	Brand Name	Route of Admin.	Dosage (mg/kg)	Injection Volume (ml)	CKmax ± SD (U/L)
Oxytetracycline	Terramycin LA	i.m.	20	40	794 ± 292
Tylosin	Tylosin 20%	i.m.	10	21	561 ± 197
Trimethoprim/ Sulfadoxine	Borgal[i]	i.m.	12/2.4	22	1347 ± 630
Enrofloxacin (arginine)	Baytril® 10%	i.m.	5	20	486 ± 167
NACL (control)		i.m.		12.5	25 ± 3

[i]licensed for cattle in Germany by Hoechst

During and after intramuscular administration of enrofloxacin cattle showed no signs of pain. The few mild swellings that were observed were transient and no longer detectable after 48 hours. These findings agree with results found by Pyörälä *et al.*[30]

In most countries, subcutaneous injection is the recommended route for parenteral administration of enrofloxacin. After subcutaneous administration of an enrofloxacin 10% solution, only a slight increase in the CK values (CK_{max} 192 ± 45) was detected and no clinical signs were observed.

Pharmacokinetic predictors of efficacy

Although not completely understood, literature review suggests that primary pharmocokinetic parameters correlated with successful treatment are the area under the curve (AUC) and the peak serum concentration in relation to MIC values. In contrast to bacteriostatic drugs, time of serum concentration above MIC is a secondary parameter of efficacy for fluoroquinolones.[31,32] At a dosage of 2.5 mg/kg BW enrofloxacin in calves, antibacterial activity is above the MIC values for most significant pathogens over the entire dosing period. Peak antimicrobial activities exceed MIC values by 5 fold for Mycoplasma spp. and 20 fold for Pasteurella spp., Haemophilus spp., *E. coli* and Salmonella spp.

Although the clinical importance of these two ratios was substantiated for enrofloxacin in a murine infection model,[33] this hypothesis has to be confirmed under field conditions for food animals.

Clinical Use of Enrofloxacin

The effective dose of enrofloxacin for the treatment of bovine respiratory disease (BRD) was investigated by researchers in the U.S.[34] Results of a well controlled, blinded dose titration study (table 6) indicate that calves affected with naturally-acquired respiratory disease, and subcutaneously treated with doses of 2.5 and 5.0 mg/kg body weight once daily for 5 days, had significantly higher success rates than negative controls (p=0.000) or calves administered 1.25 mg/kg BW for 5 days (p=0.028). Further worldwide clinical studies and experience support the use of enrofloxacin at a daily treatment rate of 2.5 to 5.0 mg/kg, administered subcutaneously, for 3 to 5 days.

The primary parameters evaluated statistically were body temperature change at day 6 compared to day 1, treatment successes, relapses, mortality and lung scores. A 0.05 level of significance was used for all comparisons.

Table 7. Enrofloxacin 10% Injectable Solution, Dose Titration Study[34]

Treatment Group	Numbers of Animals	Mortalities	Successes	Relapses	Average Lung Scores (%)
Control	12	2	1	1	23.70
1.25 mg/kg	12	1	8	1	13.93
2.5 mg/kg	12	0	12	1	1.97
5.0 mg/kg	12	0	12	5	6.20

Conclusion

The microbiological and pharmacological properties of enrofloxacin make it useful for treating bacterial diseases in cattle, pigs, poultry, dogs and cats. In addition, the mode of action makes selection of resistant bacterial subpopulations uncommon. Experiential use and numerous clinical trials in Europe have confirmed the chemotherapeutic attributes of enrofloxacin for veterinary use.

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