Tulathromycin: An Overview of a New Triamilide Antimicrobial for Livestock Respiratory Disease*

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Tulathromycin is a novel triamilide antimicrobial that has been approved for use in the treatment and prevention of bovine respiratory disease and the treatment of swine respiratory disease in the European Union and the United States. The agent penetrates gram-negative bacteria well, and it exhibits mixed bacteriostatic and bactericidal activity. Tulathromycin is formulated as a ready-to-use, sterile aqueous solution, and the packaged concentration of 100 mg tulathromycin/ml allows low-volume dosing. This agent is characterized by rapid absorption from the injection site, extensive distribution to tissue, and slow elimination, thereby providing high, prolonged drug concentration in the lungs. Studies show that a single dose of tulathromycin is effective in treating cattle and swine with respiratory disease and in preventing high-risk cattle from developing respiratory disease.

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and for the prevention of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis* in herds showing signs of disease presence. In the European Union, Draxxin is also indicated for treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *P. multocida*, and *Mycoplasma hyopneumoniae* sensitive to tulathromycin. In the United States, Draxxin is approved for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, and *H. somni* and for the control of respiratory disease in cattle at high risk of developing BRD associated with *M. haemolytica*, *P. multocida*, and *H. somni*. US approval has also been granted for treatment of SRD associated with *A. pleuropneumoniae*, *P. multocida*, *Bordetella bronchiseptica*, and *Haemophilus parasuis*.

This article describes the development of tulathromycin and explains the features that underpin its clinical application and potential in veterinary medicine.

**TRIAMILIDE SCREENING STRATEGY**

In the search for a novel antibiotic offering high efficacy against BRD and SRD from a single administration, scientists at Pfizer screened hundreds of novel analogs based on literature macrolide templates for desired characteristics of spectrum, potency, and an indication of pharmacokinetic behavior that would support fast onset and extended duration of activity in vivo. A laboratory mouse-*P. multocida* challenge model that incorporated lung pharmacokinetics and efficacy assessments following therapeutic, metaphylactic, and prophylactic dosing of test compounds was found to be highly predictive of clinical potential in livestock.1,2

During the course of the research program, a novel class of macrolide, subsequently termed triamilides;3 was discovered with strong activity against gram-negative respiratory pathogens and desirable pharmacokinetic behavior, characterized by high and extended tissue levels in host animals.

**CHEMISTRY**

Tulathromycin is a 15-membered ring macrolide antimicrobial (Figure 1), which has a unique chemical structure characterized by the presence of three amine groups (tribasic). Tulathromycin is structurally related to erythromycin, which is monobasic, and to azithromycin (Zithromax, Pfizer), which is dibasic. The molecular formula for tulathromycin is C₄₁H₇₉N₃O₁₂; its molecular weight is 806.23, and the chemical name is (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-dideoxy-3-C-methyl-3-O-methyl-4-C-[(propylamino)methyl]-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xyl-hexopyranosyl]oxy]-1-oxa-6-azacyclo-pentadecan-15-one.

In its raw form, tulathromycin is a white to off-white crystalline powder that is readily soluable in water at pH 8.0 or below. In solution,
tulathromycin consists of a 9:1 stable mixture of two isomers that remain in equilibrium. Because of the dominance of the major isomer, all synthetic and clinical attention has focused on that molecule. The ratio of isomers in biologic fluids and tissue also remains in equilibrium.

The three basic nitrogen/amine groups in tulathromycin exist in separate regions of the molecule. The first is within the macrolide core (this is the so-called azalide nitrogen that characterizes all azalides). The other two nitrogen atoms exist on the two sugar moieties attached to the macrolide core. Each of the nitrogen groups can become positively charged, so molecules of tulathromycin in solution may carry up to three positive charges with a range of zero (neutral) to three (3+). The relative proportions of each form depend on environmental conditions, particularly pH, and at any given pH the proportions will be in dynamic equilibrium.

**MODE OF ACTION**

The tribasic structure of tulathromycin confers attributes for penetrating gram-negative pathogens, the most common cause of BRD and SRD. The highly charged (3+) molecular forms of tulathromycin are thought to possess physicochemical properties that enhance the displacement of magnesium ions that stabilize the outer lipopolysaccharide layer, thereby disrupting its structural integrity. This disruption allows all molecular forms of tulathromycin to pass through the outer layer and subsequently the peptidoglycan cell wall. To complete cell penetration, neutral molecular forms are favored to pass across the inner, cytoplasmic membrane. Thus the mixed populations of charged tulathromycin molecules, including the triple-charged forms, collectively appear to be well equipped to penetrate the complex cell membrane and wall layers of gram-negative bacteria.

![Figure 2. Comparison of minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) for European Union isolates of M. haemolytica and P. multocida.](image-url)
An additional feature that may affect intrinsic antibacterial potency relates to the observed poor affinity of tulathromycin for efflux pumps in the membrane of characterized mutant strains of *Escherichia coli*. These efflux pump mechanisms are widespread in gram-negative bacteria, functioning to expel foreign substances. Thus, low binding affinity of tulathromycin for such pumps may lead to accumulation of tulathromycin within bacterial cells.

Tulathromycin, like other macrolides, binds to the 50S subunit of bacterial ribosomes and thereby inhibits protein synthesis, leading to inhibition of cell division and cell death. Although macrolides are generally regarded as bacteriostatic, tulathromycin actually exhibits mixed bacteriostatic and bactericidal activity. The minimum bactericidal concentration (MBC) was found to be the same as the minimum inhibitory concentration (MIC) for 70% (21 of 30) of a selection of *M. haemolytica* and *P. multocida* isolates from cattle and swine (Figure 2). To obtain the data in Figure 2, 10 field isolates each of *M. haemolytica* and *P. multocida* from cattle and *P. multocida* from swine were selected at random from a large collection of isolates acquired between 1998 and 2001 during the Draxxin clinical trial program in Europe. Standard broth microdilution techniques were used to assess tulathromycin MIC and MBC values for each isolate. MIC values were recorded as the lowest tulathromycin concentration that completely inhibited bacterial growth, whereas MBC was reported as the lowest concentration producing a 99.9% (1,000-fold) reduction in bacterial density within 24 hours.

**FORMULATION**

Draxxin is formulated as a ready-to-use, sterile aqueous solution containing 100 mg of tulathromycin/ml, thus permitting low-volume dosing of livestock at only 2.5 ml/100 kg body weight to deliver the commercial dose of 2.5 mg/kg via the SC route in cattle and by the IM route in swine. Tulathromycin has good chemical stability, which enables Draxxin to be stored at room temperature (≤25°C) for 36 months. The injectable solution is photostable and is packaged in clear vials.

Tulathromycin has excellent syringeability
characteristics compared with other antibiotics, such as tilmicosin (Micotil, Elanco Animal Health) and florfenicol (Nuflor, Schering-Plough Animal Health), based on low viscosity over an extreme range of temperatures (Figure 3). Viscosity was measured using a viscometer (Model LVTDV-II; Brookfield Engineering Laboratories, Middleboro, MA) that had been calibrated with viscosity standards. Data in Figure 3 are expressed in standard centipoise units (water has a viscosity of 1.0 centipoise at 20°C).

A further study was conducted to evaluate the actual force required to expel tulathromycin, tilmicosin, and florfenicol through an 18-gauge, 1.5-inch needle at room and refrigerator temperatures using an automated texture analyzer (Stable Micro Systems; Godalming, Surrey, United Kingdom). Syringes and products were stored overnight at the appropriate temperature before testing. Each product was evaluated a minimum of three times at each temperature level. The results of this syringeability study

### TABLE 2. MIC<sub>90</sub> Values for Tulathromycin against Isolates from US Cattle

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td><em>M. haemolytica</em> (n = 642)</td>
<td>2</td>
</tr>
<tr>
<td><em>P. multocida</em> (n = 221)</td>
<td>1</td>
</tr>
<tr>
<td><em>H. somni</em> (n = 36)</td>
<td>4</td>
</tr>
<tr>
<td><em>M. bovis</em> (n = 35)</td>
<td>1</td>
</tr>
</tbody>
</table>

### TABLE 3. MIC<sub>90</sub> Values for Tulathromycin against Isolates from North American Swine

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. multocida</em> (n = 55)</td>
<td>2</td>
</tr>
<tr>
<td><em>A. pleuropneumoniae</em> (n = 135)</td>
<td>32</td>
</tr>
<tr>
<td><em>H. parasuis</em> (n = 31)</td>
<td>2</td>
</tr>
<tr>
<td><em>B. bronchiseptica</em> (n = 42)</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 4. Effects of serum and pH on MIC determination for tulathromycin versus *H. somni*. The pH at the start of the incubation period was 7.2 in A and 7.4 in B; carbon dioxide was 5% for both. According to CLSI guidelines, the accepted pH range is 7.2–7.4. A slight variation in the pH of the medium significantly affected MIC values, and the addition of heat-inactivated serum to the incubation medium enhanced tulathromycin activity against all *H. somni* strains.
Table 1) show that less force was required to expel tulathromycin than tilmicosin or florfenicol ($P < .05$) at each temperature tested (approximately 3°C and 20°C).

**Microbiology**

The MICs of tulathromycin for cattle and swine respiratory bacterial pathogens in the United States were evaluated using microbial isolates collected during the respective clinical development programs. Bovine isolates were collected during 1999 from cattle with naturally occurring disease enrolled in clinical feedlot efficacy studies, and swine samples from North America were collected between 2000 and 2002 during a clinical SRD program conducted in California, Iowa, Nebraska, Ohio, and Canada.

Bacterial isolation, identification, and MIC determinations were conducted by GLP (Good Laboratory Practice)–accredited diagnostic laboratories using Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards [NCCLS]) methods. The MIC$_{90}$ (lowest concentration inhibiting 90% of isolates) values for tulathromycin against US isolates of *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis* from cattle were 2 µg/ml, 1 µg/ml, 4 µg/ml, and 1 µg/ml, respectively (Table 2). The corresponding values for North American swine isolates were: *P. multocida*, 2 µg/ml; *A. pleuropneumoniae*, 32 µg/ml; *H. parasuis*, 2 µg/ml; and *B. bronchiseptica*, 8 µg/ml (Table 3). MIC data for European cattle and swine respiratory pathogens are published elsewhere.$^5$

Microbiologic studies early in the development of tulathromycin pointed to several factors that might influence in vitro assessments of potency. Some variability was noted in repeat MIC valuations involving European Union isolates,$^5$ and this led to further investigations that focused on characterizing the effects of pH, carbon dioxide, and serum. Two organisms, *H. somni* and *A. pleuropneumoniae*, were chosen for special attention during these investigations.
because they are known to be “fastidious” in terms of culture requirements in vitro.

Studies were conducted to evaluate the effects of pH and serum on in vitro activity (MIC values and time-killing kinetics) of tulathromycin against *H. somni* using four US isolates (three field strains and an American Type Culture Collection [ATCC] strain). Tulathromycin MICs were determined in veterinary fastidious medium (VFM) adjusted to various starting pH values. Tulathromycin MICs for *H. somni* were highly sensitive to pH change: Even a slight variation in pH of the medium (from pH 7.4 to 7.2) significantly affected the resulting MIC values (Figure 4). Addition of heat-inactivated serum to the incubation medium enhanced tulathromycin activity against all *H. somni* strains, particularly at pH 7.4 (Figure 4).

Killing kinetics were determined for two *H. somni* field isolates, strains 2027 and 8037. The pattern of bacterial killing was similar for both strains, and only the data for strain 2027 are shown here (Figure 5). Organisms were inoculated into VFM containing various concentrations of tulathromycin with or without supplementation of 40% heat-inactivated bovine serum. Under standard CLSI conditions, the MIC for strain 2027 was 1.0 µg/ml. Killing kinetics were assessed at tulathromycin concentrations of 0.01, 0.1, 1.0, 2.0, and 4.0 µg/ml (0.01 to 4 times the MIC).

In the absence of serum, cell numbers and viability were reduced at a faster rate with increasing tulathromycin concentration, with 4.0 µg/ml producing bactericidal (>99.9% kill) activity within 24 hours (Figure 5A). Bactericidal effects were enhanced in the presence of 40% bovine serum (Figure 5B). At a concentration of 1.0 µg/ml, viable counts decreased at a rate identical to that seen at 4.0 µg/ml in the absence of serum, with no detectable viable cells remaining after 24 hours. At 4.0 and 2.0 µg/ml, in the presence of serum, virtually no viable cells were detected by 4 and 6 hours, respectively.

Using methods similar to those reported above for *H. somni*, the effects of pH, carbon dioxide, and serum were also evaluated for 12 US field isolates of the swine respiratory pathogen *A. pleuropneumoniae*. Tulathromycin MIC$_{90}$ values were lowered significantly when carbon dioxide fell and pH rose in the test medium (Figure 6). In addition, the presence of 40% heat-inactivated porcine serum was found to lower (by 16-fold) the MIC$_{90}$ for tulathromycin. More detailed time-killing kinetics studies with some individual *A. pleuropneumoniae* strains confirmed the positive impact of
serum supplementation in the incubation medium on tulathromycin activity. Data shown here for strain 776 (Figure 7) indicate a shortening of the time to exert bactericidal activity (defined as a 99.9% reduction in bacterial cell count) in the presence of serum.

The mechanism for serum enhancement observed for *H. somni* and *A. pleuropneumoniae* is undetermined, and its relevance to the pharmacodynamics of tulathromycin requires further investigation. The phenomenon may be associated with stabilization of pH in the in vitro system or with other unknown factors, possibly immunologic, that have not been destroyed by the heat-inactivation process. Serum enhancement of tulathromycin potency in vitro has also been

**Figure 7.** Killing kinetics of tulathromycin against *A. pleuropneumoniae* (strain 776; standard MIC, 32 µg/ml) when cultured without (A) and with (B) serum and carbon dioxide in the incubation medium.

**TABLE 4. Summarized Pharmacokinetic Parameters of Tulathromycin in Plasma and Lung following SC Administration in Cattle and IM Administration in Swine**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cattle</td>
<td>Swine</td>
</tr>
<tr>
<td>Time to maximum concentration (hr)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Maximum concentration (µg/ml)</td>
<td>-0.5</td>
<td>-0.6</td>
</tr>
<tr>
<td>Half life (h)</td>
<td>-90</td>
<td>-91</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (ng x h/ml)</td>
<td>16,700</td>
<td>12,200</td>
</tr>
<tr>
<td>Volume of distribution at steady state (L/kg)</td>
<td>11</td>
<td>13.2</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>91</td>
<td>88</td>
</tr>
<tr>
<td>Lung AUC:Plasma AUC</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

AUC = area under concentration–time curve.
Figure 8. Lung and plasma concentrations of tulathromycin in cattle after a single SC dose (A) and swine after a single IM dose (B), both at the rate of 2.5 mg/kg body weight. *n = six/time point.

PHARMACOKINETICS

The efficacy of any antimicrobial is determined by both its pharmacokinetic and pharmacodynamic properties. The pharmacokinetics of tulathromycin in cattle is characterized by rapid absorption from the injection site, extensive distribution into tissue, and slow elimination, which collectively provide high, prolonged lung tissue concentrations.

Several studies have been conducted to determine the pharmacokinetic behavior of tulathromycin in cattle and swine following a single administration at the recommended dosage of 2.5 mg/kg body weight. The pharmacokinetic profiles for the drug in plasma and lung following SC administration in cattle and IM administration in swine are shown in Figure 8A and 8B, respectively; summarized pharmacokinetic parameters, based on a collection of separate studies, are shown in Table 4.

The maximum concentration ($C_{\text{max}}$) in plasma for both cattle and swine was quickly achieved within an hour after dosing (time of maximum concentration [$T_{\text{max}}$] < 1 hour). Drug concentrations in plasma are relatively low because tulathromycin is so rapidly and extensively distributed into tissue compartments. The apparent volume of distribution at steady state ($V_{\text{ss}}$; determined after IV administration) was approximately 11 L/kg in cattle and more than 13 L/kg in swine, which is consistent with the high tissue penetration of tulathromycin. The bioavailability of tulathromycin after SC (cattle) and IM (swine) administration was approximately 91% and 88%, respectively.

The high and extended concentrations of tulathromycin in lung tissue are a characteristic feature of Draxxin and are presumed to underpin the high levels of efficacy that have been observed from a single dose. Lung concentrations are many times higher than plasma concentrations,
with the lung:plasma area under concentration–time curve (AUC) ratios reaching 74 for cattle and 61 for swine. Tulathromycin is metabolized slowly, and the majority of drug is excreted unchanged in feces and urine. These characteristics, together with intrinsically slow release from cells, are thought to account for the prolonged drug levels in lung and other tissue. Actual lung half-life values for cattle and swine were calculated as 184 and 142 hours, respectively.\textsuperscript{10,11}

### PHARMACODYNAMICS

In common with most macrolide antibiotics, existing pharmacodynamic models seem to be poor predictors of clinical efficacy for tulathromycin. Concentration-dependent models are probably inappropriate, but sole consideration of time-dependent activity (i.e., duration of drug concentrations above MIC) also fails to adequately predict observed efficacy of tulathromycin in target animals. More recently, it has been recognized that for some modern macrolides (e.g., azithromycin), assessments of total drug exposure to the pathogen may be more predictive.\textsuperscript{15} In such codependent (time and concentration) cases, the ratio of AUC:MIC may be useful since this measure incorporates elements of both time and concentration. Because tulathromycin rapidly distributes from plasma into tissue, it is possible that the AUC in lung, rather than plasma, should be considered in the search for pharmacodynamic predictors of clinical efficacy for this triamidile antibiotic.

Although standard methods reveal the high levels of tulathromycin achieved in lung tissue, the amount of active drug available to fight bacteria is unknown, and further research is warranted in this area. One factor that can contribute positively to drug levels in the vicinity of target pathogens is the accumulation, and subsequent release, of antibiotic in phagocytic cells, which are attracted to inflamed infection sites. This phenomenon has been demonstrated previously for other macrolides, such as azithromycin,\textsuperscript{16} and it also appears to be a powerful attribute of tulathromycin, with the drug accumulating in blood polymorphonuclear leukocytes or neutrophils (PMNs) and alveolar macrophages, and then being slowly released from these cell types.\textsuperscript{17} For the tulathromycin studies, PMNs were prepared from peripheral blood of healthy donor calves and incubated at 39°C in radio-labeled [\textsuperscript{14}C] tulathromycin or N-[methyl-\textsuperscript{14}C]-erythromycin (as a positive control macrolide). Uptake of drug was monitored for 4 hours, with cellular accumulation expressed as the ratio of intracellular:extracellular (I:E) drug concentration. Efflux of tulathromycin from bovine PMNs was investigated separately by incubating drug-loaded

![Figure 9. Accumulation of tulathromycin and erythromycin in bovine neutrophils.](image)
cells in clean (antibiotic-free) medium and monitoring efflux into the medium over a 4-hour period.

Tulathromycin was found to accumulate steadily in bovine neutrophils over the incubation period (Figure 9). The I:E concentration ratio for tulathromycin after 4 hours was significantly greater (26-fold; $P < .001$) than for erythromycin (threefold). In addition, when drug-loaded cells were placed into antibiotic-free media, efflux occurred for both tulathromycin and erythromycin, although the rate was slower for tulathromycin (Figure 10), suggesting that phagocytic cells can function as a reservoir for controlled release of tulathromycin into the extracellular environment. Tulathromycin also accumulates in bovine and porcine alveolar macrophages and in porcine neutrophils, although the extent appears to be lower than for bovine neutrophils.\textsuperscript{17} I:E ratios after a 4-hour incubation period using these cells were as follows: bovine alveolar macrophages, 19-fold; porcine alveolar macrophages, eightfold; and porcine neutrophils, 17-fold.

Taken together, the available data suggest that drug concentrations in infected lungs may be influenced by a tissue-targeting phenomenon. Tulathromycin is taken up by immune cells that migrate to lung tissue in response to the inflammation caused by bacterial infection. Tulathromycin is not permanently sequestered within these phagocytic cells; it is slowly released into the extracellular environment, where it would potentially be available to attack respiratory pathogens. Further enhancement of drug availability may occur as a result of the leukotoxin production by gram-negative organisms, such as \textit{M. haemolytica}, which would induce lysis of drug-loaded phagocytic cells. Finally, the actual potency of tulathromycin at the host–pathogen interface within the lung microenvironment may be underestimated by standard in vitro testing methodology, because bacteriostatic and bactericidal activity are both influenced by such factors as pH, carbon dioxide, and serum, which have significant potential relevance in vivo.

**CLINICAL EFFICACY AND SAFETY**

Clinical assessment of tulathromycin in cattle and swine has been accomplished in comprehensive multisite programs conducted in Europe and North America. These studies reveal that the efficacy of a single dose of tulathromycin for treating cattle with BRD, and for preventing BRD in animals at high risk of contracting respiratory disease, is significantly greater for tulathromycin than for the comparator products, tilmicosin and florfenicol.\textsuperscript{12,13,18,19} High levels of
efficacy have also been observed in the swine clinical programs,14,20 in which cure rates obtained with a single administration of Draxxin were compared with those obtained from multiple doses of ceftiofur sodium (Naxcel, Pfizer Animal Health) or tiamulin (Tiamulin, Novartis Animal Health). Tulathromycin provides a high initial cure rate in cattle (peaking within 1–3 days) and an extremely low subsequent relapse rate because of the extended lung tissue kinetics and prolonged antibacterial activity. No serious adverse events were noted in the clinical development programs. Furthermore, at elevated doses (up to 10 times the commercial dose rate; administered as a component of the target safety evaluation program), the most significant findings were associated with the injection site itself. These adverse effects included pain on injection, probably associated with administering large volumes of product, and self-resolving histopathologic lesions at the injection site.

SUMMARY

Tulathromycin is the first representative of a novel macrolide class, the triamilides, possessing physicochemical attributes that promote strong activity against gram-negative respiratory pathogens and extended pharmacokinetics in the lungs of cattle and swine. Draxxin, an aqueous solution of tulathromycin, offers convenient low-volume dosing and high levels of efficacy against BRD and SRD from a single, parenterally administered dose.

ACKNOWLEDGMENTS

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REFERENCES


