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Review

TULATHROMYCIN – A SEMI-SYNTHETIC MACROLIDE ANTIBIOTIC. I. CHARACTERISTICS AND ANTIBACTERIAL ACTIVITY

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Summary

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This article reviews the available literature on the characteristics and antimicrobial activity of the semi-synthetic macrolide antibiotic tulathromycin from the triamilides subclass. The product has a high activity against Gram-negative respiratory pathogens and desirable pharmacological characteristics for high and persistent tissue levels in domestic animals. Representatives of the macrolide and lincosamide group are investigated and the similarities and differences from tulathromycin are outlined. It is emphasised that at the background of increasing bacterial resistance to a number of antimicrobial agents, only few tulathromycin-resistant strains of *P. multocida* and *M. haemolytica* have been reported so far. Tulathromycin is considered highly effective against common bacterial agents of respiratory diseases in large ruminants, pigs and sheeps, and may be an alternative for control of resistant bacterial pathogens.

Key words: activity, antibiotics, effectiveness, macrolides, resistance, tulathromycin

CHEMICAL CHARACTERISTICS OF TULATHROMYCIN

Tulathromycin is a new, semi-synthetic macrolide antibiotic, from the sub-class triamilides, approved for treatment and prevention of bovine respiratory disease (BRD) and treatment and metaphylactics of swine respiratory disease (SRD) in the European Union (EU) and the United States of America (USA) (Evans, 2005).

As a result of the search for a new antibiotic that meets the requirement for high efficacy against BRD and PRD with single treatment, a new subclass of macrolides – triamilides was found to possess a high activity against Gram-negative respiratory pathogens and desirable pharmacological characteristics: high and prolonged tissue levels in domestic animals (Letavič, 2002; Nowakowski *et al.*, 2004).

Tulathromycin is a semi-synthetic macrolide antibiotic with unique chemical structure consisting of a regioisometric equilibrated mixture of 13-membered ring azalide (10%) and 15-membered ring azalide (90%), characterised by the presence of three amino groups. It is structurally related to erythromycin, which is monobasic, and to azithromycin, which is dibasic. The molecular formula of tulathromycin is: $C_{41}H_{79}N_3O_{12}$, and its molecular weight is 806.23. The drug molecule has three nitrogen/amino functional groups, and is the first member of a new subclass of macrolides known as "triamilides".

All macrolides have basic molecules with a dissociation constant (pKa) greater than 7. Tulathromycin molecule is more basic than the other macrolides and its pKa value can be from 8.6 to 9.6. This physicochemical feature allows the non-ionised fraction of the drug to attain easily the tissues and plasma and accumulate in acidic environment of some cells. This may explain the large volume of drug distribution and its accumulation in alveolar epithelial fluid and bronchoalveolar lavage cells (Evans, 2005; Villarino *et al.*, 2013).

TULATHROMYCIN PHARMACODYNAMICS

Mechanism of tulathromycin action

Macrolides and lincosamides have different chemical structures but are functionally similar drugs with bacteriostatic action. They are active against Gram-positive and some Gram-negative microorganisms and may be alsoeffective against intracellular pathogens. Macrolide resistance may develop with mutations of genes encoding ribosomal RNA or some ribosomal proteins. The microorganisms that have acquired resistance to one macrolide may be also resistant to others (Georgiev *et al.*, 2010).

According to the US Pharmacopoeia Convention (USPC, 2007), macrolides have been evaluated as bacteriostatic in therapeutic concentrations, but they can behave also as slow bactericidal agents, in particular against streptococci. Their bactericidal action has been described as time-dependent. The antimicrobial activity of some macrolides increases parallelly to increasing the pH and is suppressed at low pH, which makes them from effective in abscesses and tissue necrosis (Villarino *et al.*, 2013).

The macrolide group includes:

- azithromycin is designed for use in human medicine. It is well absorbed in the digestive tract and can be active against extra- and intracellular pathogens. Azithromycin is more active *in vitro* than erythromycin and clarithromycin against some respiratory pathogens. It is the macrolide with the longest therapeutic concentration and good tolerance;
- clarythromycin has a higher activity than erythromycin against *Mycobacterium avium, Toxoplasma gondii, Chlamidophyla felis* and *Mycobacterium leprae.* It is applied i.v. in dogs and orally in dogs, foals and donkeys at a daily dose of 10 mg/kg. In rabbits, high doses could result in intrauterine loss of foetuses (USPC, 2007; Georgiev et al, 2010).
- erythromycin is an antibiotic with activity against Gram-positive bacteria such as *Staphylococcus aureus, Rhodococcus equi* and *Streptococcus* spp., as well as against some Gram-negative microorganisms such as *Haemophilus* and *Pasteurella* sensitive to erythromycin;

- josamycin is active *in vivo* against *Mycoplasma* and Gram-positive cocci: *Staphylococcus* spp., *Streptococcus* spp. and *Diplococcus* spp. In some countries but not in the EC, it is used in chickens and pigs for the prevention and treatment of respiratory diseases, sinusitis and arthritis;
- tylosin has a spectrum of action against *Streptococcus* spp., *Staphylococcus* spp., *Erysipelothrix* spp., *Corynebacterium* spp., *B. hyodysenteriae, Spirochaeta* spp., *Campylobacter coli*, *Pasteurella* spp. and *Brucella* spp.;
- tilmicosin is a semi-synthetic derivative of desmicosin. *In vitro*, it is effective against Gram-positive microorganisms and *Mycoplasma* and some Gram-negative bacteria, such as *H. somni, M. haemolytica,* and *P. multocida*. It is recommended for cattle, pigs and birds (Reese & Betts, 2000; USPC, 2007; Yordanov, 2009; Georgiev *et al.*, 2010; Dimitrova *et al.*; 2014; 2019; Petkova & Yordanov, 2016). The *E. coli* strains isolated from pigs in 2015 are resistant to many of used antibiotics, including tylosin, tilmicosin and lincomycin, with share of resistant strains from 51 to 100% (Petkova, 2017).

Tulathromycin is the first member of a new macrolide class: triamilides, designed for systemic veterinary use and approved for treatment and prevention of BRD and PRD in more than 30 countries in America, Europe, Oceania and Asia (Villarino *et al.*, 2013). Triamilides are semi-synthetic derivatives of the natural product erythromycin. In its crude form, tulathromycin is a white or off-white crystalline powder that is well soluble in water at pH 8.0 or lower. It is chemically stable when stored at room temperature (up to 25 °C) for 36 months. As a solution, tulathromycin is a 9:1 stable medicine of two isomers that remain in equilibrium. The proportion of isomers in biological fluids and tissues also remains in equilibrium (Letavič *et al.*, 2002; USPC, 2007).

The three-basic structure of tulathromycin makes it able to penetrate Gram-negative pathogens, which are the commonest causes of BRD and SRD. Like other macrolides, tulathromycin binds to 50S subunits of bacterial ribosomes, thereby inhibiting protein synthesis leading to inhibition of cell division and cell death. Tulathromycin exhibits mixed bacteriostatic and bactericidal activity. It differs from other macrolides in that it has a longer duration of action. This feature, together with the slow release from the cells, is a likely explanation for the long-term retention of the drug in the lungs and other tissues. The value of the current pulmonary half-life for calves is 184 and for pigs: 142 hours (Evans, 2005; Georgiev *et al.*, 2010; Villarino *et al.*, 2013).

The minimum bactericidal concentration (MBC) was found to be similar to the minimum inhibitory concentration (MIC) of 70% of *M. haemolytica* and *P. multocida* strains isolated from cattle and pigs in North America. Tulathromycin metabolises slowly, with much of the drug being excreted unchanged in faeces and urine (Evans, 2005; Godinho *et al.*, 2005a).

The special feature of this new drug class is increased penetration into Gram-negative bacterial pathogens, resulting in an increased drug potency (Nowakowski *et al.*, 2004; Villarino *et al.*, 2013).

TULATHROMYCIN PHARMACOKINETICS

Tulathromycin is concentrated noticeably in some areas, including lung epithelial lining fluid, macrophages, and neutro-

phils, but its distribution and accumulation are found to vary in extent and duration (Cox *et al.*, 2010; Villarino *et al.*, 2013).

This feature, along with slow release from cells is the probable explanation for the long-term retention of the drug in the lungs and other tissues (Evans, 2005; Georgiev *et al.*, 2010; Villarino *et al.*, 2013).

In cattle, the pharmacokinetics of tulathromycin is characterised by rapid absorption from the injection site, prolonged distribution to the tissues, with a maximum plasma concentration of 0.5 μg/mL, which is reached approximately 30 minutes after dose administration and slow elimination. The apparent elimination half-life ($t_{1/2}$) is 90 hours in plasma. Significant concentrations of tulathromycin in neutrophils and alveolar macrophages have been demonstrated. The magnitude of local accumulation and prolonged persistence of the drug in the target tissues of the lungs result in an appropriate treatment regimen, as a single administration and a positive clinical outcome of the respiratory disease (Cox *et al.*, 2010).

In pigs, the pharmacokinetic profile of tulathromycin after intramuscular administration of a single dose of 2.5 mg/kg was also characterised by rapid and extensive absorption, rapid distribution and slow elimination. The maximum plasma concentration was 0.6 μg/mL, reached approximately 30 minutes after injection. The levels of tulathromycin in the lung homogenates were significantly higher than those in the plasma. The elimination half-life ($t_{1/2}$) was 90 hours. The bioavailability of tulathromycin after intramuscular administration in pigs was about 88% (Waag *et al.*, 2008).

In sheep, following an intramuscular administration of a single dose of 2.5 mg/kg, a maximum plasma concentration of 1.19 μg/mL was attained after approximately 15 minutes. The elimination half-life ($t_{1/2}$) was 69.7 hours. The bioavailability of tulathromycin after intramuscular administration in sheep is 100% (Benchaoui *et al.*, 2004; Nowakowski *et al.*, 2004; Evans, 2005; Nanjiani *et al.*, 2005; USPC, 2007).

Tulathromycin is recommended for use in large ruminants (s.c.), in pigs (i.m. and i.v.) and in sheep (i.m.), at 2.5 mg/kg, as a single dose. Co-administration with other macrolides and lincosamides is not recommended. The withdrawal periods for meat and internal organs from tulathromycin-treated animals were as followed: for cattle – 22 days; for pigs – 13 days and for sheep – 16 days (Evans, 2005; USPC, 2007; Waag *et al.*, 2008; Villarino *et al.*, 2013).

SPECTRUM OF ANTIBACTERIAL ACTIVITY

Tulathromycin has a spectrum of activity similar to that of erythromycin, but is much more active against *Mycoplasma spp*. It penetrates well into Gram-negative bacteria, respiratory pathogens in calves (*M. haemolytica, H. somni, P. multocida* and *M. bovis*) and pigs (*A. pleuropneumoniae, B. bronchiseptica, P. multocida, H. parasuis* and *M. hyopneumoniae*). It is used for both treatment and metaphylaxis. According to Reese *et al.* (2004) its antimicrobial activity against *H. somni* and other fastidious microorganisms is dependent on the pH and pCO_2 of the surrounding environment. MIC was reported to increase as pH decreases and pCO_2 increases (Evans, 2005; Gudinho *et al.*, 2005a; Hart *et al.*, 2006; Villarino *et al.*, 2013).

Lees *et al.* (2016) tested the antibacterial activity of tulathromycin against *M. haemolytica* and *P. multocida.* Three antibacterial activity parameters were studied *in vitro* - MIC, MBC and time-killing curve for each organism, as each index was measured in two nutrient media: Mueller-Hinton broth (MHB) and calf serum. It was concluded that pharmacodynamic data from biological fluids such as serum will be used to determine the therapeutic dose of tulathromycin.

Activity against Gram-negative pathogenic bacteria

Tulathromycin is highly potent against Gram-negative respiratory pathogens and has prolonged pharmacokinetics in bovine and porcine lungs. Aqueous tulathromycin solution is administered at low volume doses and exhibits high efficacy levels against BRD and SRD via a single parenterally administered dose. The drug is bactericidal (from 4× and 8× MIC) against *M. haemolytica, A. pleuropneumoniae* and *P. multocida* (Evans, 2005; Godinho *et al.*, 2005a, b).

Sweeney *et al.* (2008) conducted a study on the activity of tulathromycin and ceftiofur either alone or in combination, and in combination with one of the 7 antibacterial agents used to treat BRD against bovine *P. multocida* and *M. haemolytica* isolates to establish synergism, antagonism or indifference. Most *in vitro* data indicated that tulathromycin and ceftiofur, in combination each with the other or with 7 other antibacterial products, produced a predominantly indifferent response, undetectable synergism and rarely detectable antagonism.

Waag *et al.* (2008) tested the prophylactic efficacy of tulathromycin administered once daily, intramuscularly (i.m.) at 2.5 mg/kg on the 11th, 9th, 7th, 5th and 3rd days before the intranasal inoculation of pigs with a highly pathogenic strain of *A. pleuropneumoniae (App)* from serotype 5. After inoculation, health of all pigs was monitored throughout the experimental period. It was reported that with the exception of day 11, the amount of pigs dropped in the tulathromycin group was significantly lower than in the control group ($P<0.05\%$).

Of the 237 swine lung samples studied, Zutič *et al.* (2008) isolated 13 bacterial species from 198 samples (83.5%). Of all bacterial species, *P. multocida* was the most dominant with 32.64%, followed by *App* with 29.01%. The susceptibility of isolated *App* strains to the antibiotics used in practice was tested by disc-diffusion method DDM (Oxoid). All *App* strains tested were sensitive to tulathromycin but showed high resistance to tetracycline (53%).

Activity against Gram-positive pathogenic bacteria

Tulathromycin, like other macrolides, is considered to be bacteriostatic when tested against *Staphylococcus aureus*. Macrolides have been evaluated as bacteriostatic at therapeutic concentrations, but they may also be slow bactericides, in particular against *Streptococcus spp.* (USPC, 2007).

In available literature, no other reports from antibacterial studies and the use of tulathromycin as a therapeutic or prophylactic antimicrobial agent, against animal diseases caused by other Gram-positive pathogenic bacteria were found out.

Activity against Mycoplasma

In an *in vitro* test, the sensitivity of 44 *M. hyopneumoniae* strains originating from Hungary, Slovakia and the Czech Republic to 15 different antibacterial drugs was

tested. They were found to be effective against most of the strains, but extremely high MICs of tilmicosin, tulathromycin and lincomycin – greater than 64 μg/mL, were also obtained. Against another strain (MycSu18) the following MIC values were found out: gamithromycin – 64 μg/mL; tylosin – 32 μg/mL; tylvalosin – 2 μg/mL. Both groups of macrolide agents designated as № 16 (tylosin, tilmicosin and tylvalosin) and No 15 (tulathromycin and gamithromycin) were effective against the tested strains, but the MIC data of tulathromycin against the typical strains were three times higher than those reported in the literature (USPC, 2007; Felde *et al.*, 2018).

RESISTANCE OF MICROBIAL PATHOGENS AGAINST TULATHROMYCIN

The resistance of microorganisms to antibiotics is a problem for both veterinary and human medicine. Antimicrobials used in productive animals in Europe are often the same or belong to the same classes as those used in human medicine. Therefore, antimicrobial resistance (AMR) is a major, undesirable, side effect of antimicrobial drugs (AMD) use in humans and animals (Turnidge & Paterson, 2007; Popova, 2009; Zaharieva & Vassileva, 2019).

In all cases, the use of antibacterial agents should be based on the proven sensitivity of the isolated pathogenic strains (Dimitrova, 2009; Lyutskanov, 2013; Dimitrova *et al.*, 2016; Yordanov *et al.*, 2016; Petkova, 2017). According to Mos *et al.* (2010) the disk diffusion method (DDM) is reliable in antibiotic testing, easily feasible and effective, and its results are comparable to those of broth dilution (MIC).

Information on isolates resistant to tulathromycin is limited. Since tulathromycin was first approved in the USA and Europe, only two tulathromycin-resistant *P. multocida* strains have actually been reported. One isolate was from a pig in Germany in 2004 (Kaspar *et al.*, 2007) and the other from a BRD calf at a Nebraska fattening farm in 2005 (Kadlec *et al.*, 2011).

Other studies evaluating the *in vitro* susceptibility of targeted bovine and porcine respiratory pathogens have been conducted in various European countries after the drug was approved for use (Godinho, 2008). The study included 170 bovine and 133 porcine isolates. The sensitivity results were compared with those obtained prior to the launch of tulathromycin on the market and showed no change in the sensitivity of the key target species.

Alexander *et al.* (2013) tested of *M. haemolytica* isolates obtained from calves over a 3-year period for resistance to tulathromycin. For this purpose, nasopharyngeal samples were taken from 5,814 calves at loading and from the same animals after 60 days of fattening. *M. haemolytica* was isolated from 796 (13.7%) and from 1,038 (20.6%) of the samples, respectively.

Therapeutic concentrations of tulathromycin, tilmicosin, or tylosin-tartrate, respectively were administered in *M. haemolytica* positive calves (18.5, 2.9, and 2.4%). As a result, no *M. haemolytica* isolates resistant to tulathromycin were detected in the first and second year, while in the third year 5 isolates (0.4%) were resistant. The isolates were obtained from 3 calves descending from one box, all of serotype 1 and genetically related in origin. These 5 isolates were multiresistant, but genes specific for macrolide resistance have not been identified. The results of this study indicated that *M. haemolytica* resistance to tulathromycin,

of the total population of calves for fattening in Western Canada, was low and did not change over the 3-year tulathromycin treatment period.

CONCLUSIONS

Tulathromycin is a new, triamilide, semisynthetic macrolide antibiotic approved for use in veterinary medicine. The pharmacokinetics of drug is characterised by rapid absorption from the injection site and good distribution to the peripheral tissues. The high and prolonged concentration in the lung tissue and alveolar macrophages and the slow release from the cells explain its high clinical efficacy from administration of a single dose. Tulathromycin metabolises slowly, with much of the drug being excreted unchanged in faeces and urine. Tulathromycin is active against Gram-negative and some Gram-positive microorganisms and intracellular pathogens. It is highly effective against common bacterial agents of respiratory diseases (*M. haemolytica, M. bovis, H. somni* and *P. multocida* in calves and *A. pleuropneumoniae, P. multocida, H. parasuis, B. bronchiseptica* and *M. hyopneumoniae* in pigs). Tulathromycin is active also against *S. aureus, C. pseudotuberculosis* and *Streptococcus* spp.

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