Expert Opinion on Investigational Drugs

Review

Anti-infectives

Macrolide antibiotics in food-animal health

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Several 14- and 16-membered-ring macrolide antibiotics have acquired important roles in the modern production of food animals. Macrolide antibiotics exhibit many similar antimicrobial properties whether used in veterinary or human medicine. In addition to their direct inhibitory action on micro-organisms, macrolides exert a variety of subinhibitory concentration (sub-MIC) effects that are being increasingly recognised as important factors in the explanation of therapeutic results. Macrolides achieve wide tissue distribution and high intracellular concentrations that contribute prominently to their efficacy. Another important factor governing efficacy is the complex interaction between macrolides, micro-organisms, and phagocytes that may enable the host defence system to enhance the antibiotic's inhibitory action. A potential role for macrolides in modulating inflammatory processes has also been recognised. In both sub-MIC effects and interactions with the host immune system, different macrolides exert different responses that may reinforce or oppose each other. This complexity of responses requires additional studies in appropriate disease states and animal species in order to elucidate a more comprehensive understanding and explanation of *in vivo* outcomes.

Keywords: *aivlosin, cattle, erythromycin, food animals, growth enhancer, host defence, intracellular, macrolide antibiotics, macrolides, phagocytes, pigs, poultry, spiramycin, sub-MIC effects, tilmicosin, tylosin*

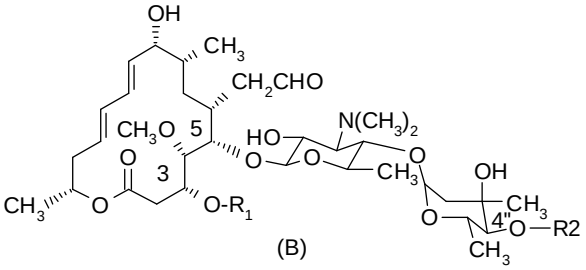
Exp. Opin. Invest. Drugs (1997) ***6****(2):103-117*

1. Classification of macrolide antibiotics

Macrolide antibiotics constitute a large class of fermentation-derived compounds whose structures consist of highly substituted macrocyclic lactones to which are attached neutral and/or amino sugars [1]. The lactone component lacking the sugar substituents is termed an aglycone, and the ring size of the aglycone forms the basis on which macrolides are classified. For the traditional macrolide antibiotics covered in this review, the lactone is a monocyclic 12- to 16-membered ring. Relatively few 12-membered-ring macrolides have been discovered. The prototype for this small family is methymycin, a compound that has been isolated from cultures of soil micro-organisms such as *Streptomyces venezuelae* [2]. None of the 12-membered-ring macrolides has yet been found to possess any commercial utility.

2. 14-Membered-ring macrolide antibiotics

The most important 14-membered-ring macrolide is erythromycin A, whose relatively broad antimicrobial spectrum, clinical efficacy, and good safety profile have made it the most widely used macrolide antibiotic in human medicine [3,4]. It is also employed in veteri-



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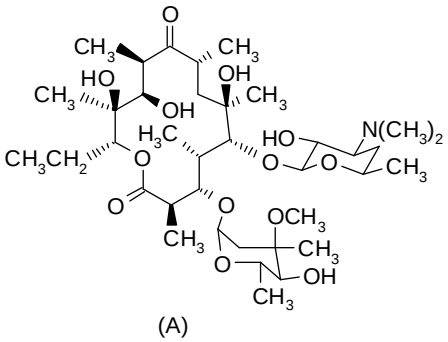
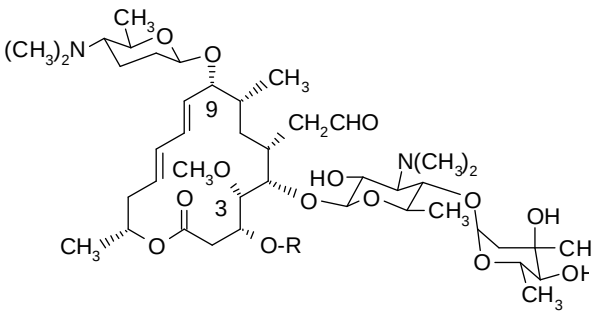


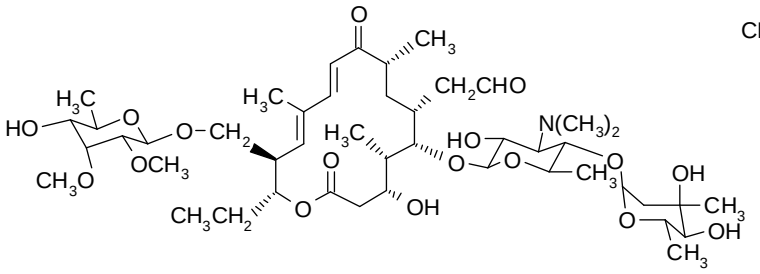
(A)

Leucomycin factors: R1=H or acetyl; R2=short-chain acyl group
Leucomycin A3 (Josamycin): R1=acetyl; R2=isovaleryl



(C)

Spiramycin factors: R=H, acetyl, or propionyl



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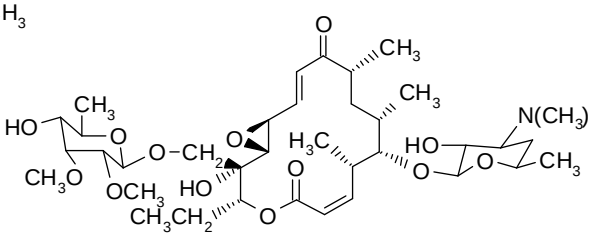

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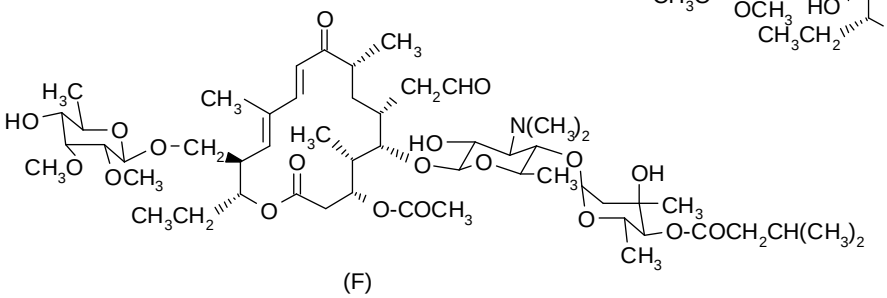

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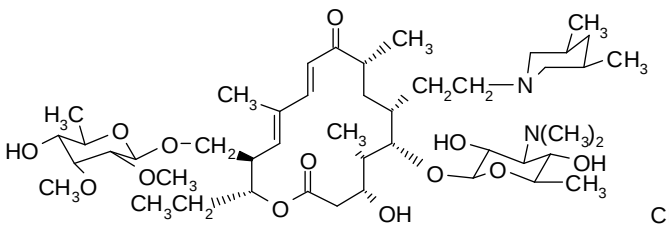

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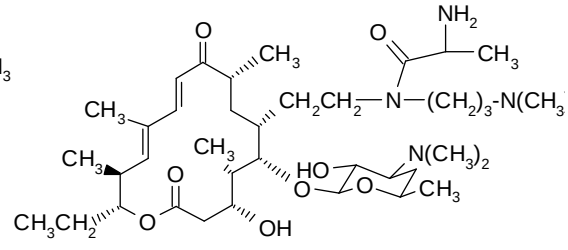

(H)

nary medicine as an antibiotic to treat a variety of infections in animals. Erythromycin A is the principal component obtained from fermentation of *Saccharopolyspora erythraea* (formerly classified as *Streptomyces erythrea)* whose structure is comprised of an aglycone (erythronolide A), an amino sugar (β-D-desosamine), and a neutral sugar (α-L-cladinose) (**A**) [5]. During the decades since its discovery, many commercially important semisynthetic derivatives and formulations of erythromycin A have been prepared and tested in order to overcome the chemical instability and low oral bioavailability of the parent compound. This has been carried out in order to expand its antimicrobial spectrum, and to improve its pharmacokinetic features [6]. Some of these derivatives are pro-drugs of erythromycin, such as acid-addition salts, 2′– esters, and ester-salt combinations, which improve oral delivery of the antibiotic and then regenerate erythromycin after being absorbed. Other derivatives involve structural changes in the aglycone, which enhance its stability toward acid-catalysed decomposition [6]. Formulations such as enteric-coated capsules and tablets are also employed to protect the antibiotic from acid-catalysed degradation in the stomach during oral administration. The water-soluble gluceptate and lactobionate salts of erythromycin are available for intravenous administration, but severe irritation and pain on injection limit usage by intramuscular administration. Although many other 14-membered-ring macrolides have been discovered [1], none of them except oleandomycin has yet achieved any commercial significance.

3. 16-Membered-ring macrolide antibiotics

The 16-membered-ring macrolide antibiotics are the other large and more important family of macrolide antibiotics. These are traditionally divided into subfamilies based upon the substitution patterns of their aglycones [1]. The principal prototypes of this family can be represented by leucomycin, spiramycin, and tylosin. The leucomycin complex is a multi-component fermentation product produced by *Streptoverticillium kitasatoensis* (formerly classified as *Streptomyces kitasatoensis)* whose generalised structure consists of an aglycone (leuconolide), a disaccharide [4-0-(α-L-mycarosyl)-β-D-mycaminosyl] attached to the 5-hydroxyl group of the aglycone, and short chain acyl substituents attached to the 3-hydroxyl group of the aglycone and the 4″-hydroxyl group of mycarose (**B**) [7-9]. Many structurally related 16-membered-ring macrolide complexes have been discovered that differ in their acyl substituents and the oxidation patterns of their aglycones [1]. The spiramycin complex, isolated from cultures of *Streptomyces ambofaciens*, is structurally related to the leucomycins, but is distinguished by the additional amino sugar, β-D-forosamine, attached to the 9-hydroxyl group of the aglycone (**C**) [8]. The other major sub-family of 16-membered-ring macrolides is represented by tylosin, the fermentation product of *Streptomyces fradiae*, which possesses a more highly substituted aglycone (tylonolide) and a third saccharide substituent *(*β-D-mycinose) in addition to the disaccharide attached to the 5-hydroxyl group (**D**) [8-10]. In addition to the abundance of naturally occurring 16-membered-ring macrolides that have been discovered, several semisynthetic derivatives within this family have been commercially developed, two of which have been introduced as new veterinary antibiotics [11]. A series of 3,4″–ester derivatives of tylosin that are structurally analogous to some of the leucomycin factors was initially prepared by bioconversion methods [12]. From this series, 3-O-acetyl-4″–O–isovaleryl-tylosin (Aivlosin, AIV-tylosin, **F** was selected for commercial development as a new veterinary antibiotic to treat *Mycoplasma* pneumonia in swine and poultry [13]. The second new macrolide, tilmicosin, originated from a SAR study of tylosin derivatives that had exhibited improved oral efficacy and bioavailability [14,15]. Tilmicosin is synthesised from tylosin by sequential hydrolysis of mycarose and reductive amination of the aldehyde in demycarosyltylosin (desmycosin) with 3,5-dimethylpiperidine (**G**) [16]. This modification enhanced antimicrobial activity against Gram-negative bacteria such as *Pasteurella* spp. and *Actinobacillus* spp., increased oral efficacy and bioavailability, and provided higher and more persistent concentrations of antibiotic activity in serum, tissues, and intracellular environments. Among the many 16-membered-ring macrolide derivatives that have been prepared and evaluated over several decades of research, a few, such as miokamycin and rokitamycin, have been recently introduced into human medicine. However, tilmicosin and AIV-tylosin appear to be the only semisynthetic 16-membered ring macrolides developed exclusively for veterinary medicine [11]. More recently, an extensive series of semisynthetic derivatives has been prepared by reductive amination of rosaramicin and repromicin, leading to compounds such as CP-163505 (**H**), which were optimised for activity against *Pasteurella* species [17]. The efficacy and performance of these new semisynthetic macrolides under commercial field conditions have yet to be established.

4. Uses of macrolide antibiotics

Over the past decade, macrolides have experienced a remarkable renaissance in their use and importance in human medicine [18,19]. Their renewed significance has been partly due to the discovery and commercial development of several semisynthetic derivatives that have expanded the antimicrobial spectrum and efficacy of the parent compounds and have provided greater chemical and *in vivo* stability, higher and more persistent serum and tissue concentrations of antibiotic, better oral bioavailability, and shorter and less frequent dosing schedules [6,11]. The macrolide renaissance has also been sparked by the prominence of several human pathogens that are effectively treated by macrolide antibiotics, such as species of *Legionella, Chlamydia, Helicobacter*, and, for some of the newer agents, pathogens not traditionally covered by macrolides such as certain species of mycobacteria [20]. Macrolide antibiotics are used in veterinary medicine to treat and control a variety of susceptible organisms and diseases in companion animals such as dogs, cats, and horses [21-27]. Some of the newer semisynthetic derivatives may also find utility in the companion animal market when their antimicrobial spectrum, efficacy, pharmacokinetic parameters, and safety profiles are further investigated. Space does not permit a detailed review of the numerous and diverse veterinary uses of macrolide antibiotics, but the literature references cited above provide some specific information about registered compounds and their approved indications, dosage amounts, formulations, etc.

Macrolide antibiotics are used in food-producing animals in two important ways: as therapeutic agents to treat and control infectious diseases, and as growth enhancing agents, both of which result in reduced mortality and better health of animals, substantial savings in the costs of food production, and consequently, greater availability, higher quality, and, lower prices of food for consumers [28]. Antibacterial agents constitute a very large and important segment of the animal pharmaceutical market [29,30]. The naturally occurring macrolides that are most widely used for therapeutic applications in animals are erythromycin, tylosin, and spiramycin [21,23]. The new semisynthetic derivative, tilmicosin, has established itself for the treatment of bovine respiratory disease (BRD) and is administered by a single subcutaneous injection due to its long *in vivo* half-life and its high and persistent concentrations of antibiotic activity in lung tissues and fluids [31-33]. More recently, it has received regulatory approval in several countries as a feed additive for control of respiratory diseases in pigs [34,35]. Other macrolides that are sometimes used in selected markets and certain countries, particularly in Europe and Asia, include: kitasamycin and turimycin (leucomycin-type complexes, josamycin (identical to leucomycin A_3: **B**), AIV-tylosin (**F**) and mirosamicin (mycinamicin II, **E**) [36-39]. Although this review will focus only on conventional macrolide antibiotics, some non-traditional macrolides have veterinary applications, such as the 17-membered macrolide sedecamycin and its semisynthetic derivative terdecamycin, which are used to treat swine dysentery [40,41], and several members of the avermectin- milbemycin family, which are widely used to treat and control many important parasitic diseases [42-45].

Among the infections for which macrolide antibiotics are used in animals are respiratory tract diseases, enteric diseases, genito-urinary tract infections, bacterial mastitis, eye infections, and foot and leg infections; the principal species of food animals that are treated include cattle, sheep, pigs, and poultry. Depending on the antibiotic, animal species, and microbial infection, the route of administration may range from injectable forms (iv., im., sc.) to intramammary infusions, topical applications and incorporation into an animal's feed or drinking water. In aquaculture, erythromycin is one of the most effective agents for treating bacterial kidney disease (BKD) in salmonids, a serious infection caused by a fastidious Gram-positive bacterium that has proven difficult to control [46,47]. A few macrolides have been authorised or approved for use in aquaculture in certain countries [48]. A detailed compilation of all world-wide therapeutic claims and indications for macrolide antibiotics is well beyond the scope of this review, but many references are available that provide much of this information [21-27,49].

It has long been recognised that the regular feeding of certain non-nutrient compounds results in a significant increase in an animal's weight gain and an improvement in the efficiency with which it utilises its feed for growth [50]. Several different classes of antibiotics are presently used in this manner, in which tylosin is the most widely used macrolide [28,29,51]. Many different terms have been used to denote these effects on improved weight gain and feed efficiency; 'growth promoter' is most frequently used in the USA, but, due to potential confusion of antibiotics with hormones, the alternative terms 'digestive enhancer' and 'growth permitter' are used in Europe to distinguish them from hormonal products. The detailed mechanisms by which antibiotics enhance growth in animals are complicated by many variable parameters such as the animal's age, nutrition, environmental surroundings, and management practices. Despite this, it is likely that part of an antibiotic's effectiveness arises from preventing or controlling sub-clinical infections and providing prophylaxis against infectious diseases, thereby keep-

ing the animals in a state of good general health in which they can grow more efficiently and expend less energy to combat various pathogenic organisms. This explanation for positive growth enhancing effects in animals is strengthened by the growing knowledge (see below) of the antimicrobial effects exerted by antibiotics on microbes at sub-MIC concentrations and the beneficial effects that may be exerted by antibiotics on the host animal. Effects in the gastrointestinal tract permit better absorption and utilisation of nutrients, and actions on certain components of the host's immune system strengthen host defences against pathogenic organisms. Antibiotics may also improve an animal's overall nutrition by affecting the intestinal microflora and its interactions with the host in a variety of complex ways that ultimately produce beneficial results to the animal. Examples by which antibiotics may affect gut micro-organisms to the benefit of the host include altering the composition of microbially derived short-chain fatty acids, decreasing formation of methane, reducing microbial degradation of protein and other important nutrients, and sparing metabolism of glucose. All of these diminish the conversion of feed components into forms that are less available or less efficiently utilised by the animal, thus allowing it to utilise the energy and nutrients contained in its feed more efficiently.

5. *In vitro* antimicrobial features of macrolides

Although the antimicrobial properties of the macrolide class have been primarily learned from *in vitro*, animal and clinical studies on those macrolide antibiotics used in human medicine, many generalisations can be made about the class that also apply to their uses in food animals. Macrolides generally possess potent inhibitory activity against many Gram-positive bacteria, certain nonenteric Gram-negative and anaerobic bacteria, and *Mycoplasma* species [3,4,52]. They readily penetrate and accumulate within many types of cells where they can inhibit intracellular pathogens. Recent studies have shown that they penetrate the microbial periplasmic space through porin channels from which they cross the inner membrane by passive diffusion [53]. Macrolides are usually bacteriostatic agents, although bactericidal activity can be measured against some micro-organisms under certain conditions and concentrations [3,52,54]. They inhibit bacterial growth by penetrating the microbial cytoplasm and inhibiting ribosomal protein synthesis by binding to the 50S ribosomal sub-unit, thereby preventing the peptide bond formations necessary for elongation of growing peptide chains on the ribosome [55-57]. As a result, the next amino acid is not added, extension of a growing peptide chain is prevented, and an incompletely formed peptide can prematurely detach from the ribosome [58]. The contribution to overall antimicrobial activity by both inhibition of ribosomal protein synthesis and drug uptake has been recently delineated for a series of tylosin-related macrolides [59]. All macrolides, as well as members of the lincosamide and streptogramin B families, bind to the ribosome at common or overlapping sites [57,60]. This degree of similiarity between the mechanisms of action of structurally dissimilar compounds has resulted in the designation of 'MLS_B' (or more simply 'MLS') for the group of macrolide-lincosamide-streptogramin B antibiotics (MLS antibiotics).

Bacteria have evolved several different mechanisms of resistance to the action of macrolides, including alteration of their target binding site, enzymatic inactivation, reduced intracellular uptake, and active efflux of antibiotic from cells [61-65]. Alteration of the ribosomal binding site is most commonly caused by specific methylation of ribosomal RNA, an event that reduces the macrolide's affinity for binding to its antimicrobial target [61,64]. Because this event confers resistance to the entire MLS group of antibiotics, it is denoted as 'MLS resistance'. MLS resistance can be either inducible or constitutive. 14-Membered-ring macrolides generally act as inducers of resistance, whereas 16-membered-ring macrolides usually do not induce resistance; consequently, one advantage of 16-membered-ring macrolides is their ability to inhibit inducibly resistant bacteria. However, once resistance has been induced, the organisms are resistant to all MLS antibiotics, as are the constitutively MLS-resistant bacteria [61-64]. Enzymatic inactivation of macrolides may occur *via* hydrolysis of their aglycone by esterases and either phosphorylation or glycosylation of the 2′-hydroxyl group of their amino sugar [63,65-67]. Although these structural modifications are relatively specific for particular macrolides and their closely related analogues, the ultimate result of each type of inactivation is to convert the macrolide into a derivative having greatly reduced or no antimicrobial activity. The ribosomes of many Gram-negative bacteria are fully susceptible to inhibition by macrolides. Despite this, the organisms are not susceptible because their outer membrane effectively acts as a barrier to prevent the lipophilic macrolides from penetrating in sufficient amount to achieve inhibitory concentrations inside the cell [63,65]. In addition to preventing uptake, bacteria can also reduce intracellular concentrations of antibiotic *via* active efflux pumps that remove whatever amount of antibiotic that does successfully penetrate, thereby keeping antibiotic concentrations below inhibitory levels [67,68]. This latter mechanism provides one explanation for bacterial strains that are not com-

pletely cross-resistant to all MLS antibiotics ('partial' MLS resistance) [63,67,68].

The debate surrounding growth enhancing agents has recently resurfaced due to questions about whether their use plays any significant role in the cause, spread, and impact on human microbial resistance, thereby compromising antibiotic therapy [23,69-74]. Due to the recent emergence of microbial resistance to glycopeptide antibiotics, the fear of untreatable infections caused by multiple-resistant bacteria has injected a critical urgency into the debate along with personal biases and assumptions. From the time when antibiotics were first introduced, relatively rapid development of microbial resistance to a given antibiotic has usually followed its introduction into human therapy, and the serious negative impacts of this recurring problem on human antibiotic therapy have long been recognised [75-82]. Despite this long history, recommended strategies and practices that attempt to prevent or minimise the development and spread of resistant organisms derived from direct human antibiotic usage are only now becoming more seriously considered and implemented [78-85].

Over the past decade, macrolide antibiotics have substantially expanded rather than lost their importance in human medicine, having undergone their remarkable renaissance. This has, therefore, increased the number of clinical macrolides, their therapeutic utility and applications, and the size of the market that they have established. Although microbial resistance is clearly a potentially serious problem that warrants continuous monitoring, several recent *in vitro* studies related to veterinary micro-organisms have reconfirmed that susceptibility of bacterial strains to 16-membered-ring macrolides generally still remains close to previous levels [36-39,86,87]. Resistance to tylosin has been found to be low in human pathogenic bacteria [88]. Most importantly, these macrolides have generally remained efficacious in both their growth-enhancing and therapeutic roles even after several decades of continuous successful use [89,90]. For a better understanding of the mechanisms involved in both of the above, the effects of macrolides both on micro-organisms at sub-inhibitory concentrations and on interactions of these antibiotics with host defence systems appear to be particularly relevant.

6. Subinhibitory concentration antimicrobial effects of macrolides

It is becoming well recognised that sub-MICs of antibiotics can exert a wide variety of potentially lethal effects on bacteria, such as altering microbial physiology and cell structural integrity, modifying some factors involved in microbial virulence and pathogenicity, reducing production of microbial toxins and degradative enzymes, and reducing the micro-organism's capability to adhere to or colonise host cells [91]. Such sub-MIC effects serve to weaken the capability of pathogenic organisms to cause or maintain infections and strengthen the ability of the host animal to withstand and eradicate infectious organisms. A caveat must be noted that broad generalisations about this subject are tenuous because some sub-MIC effects appear to be somewhat specific for individual antibiotics against certain organisms rather than broadly applicable for many antibiotics against many organisms. Since most studies to date have been performed *in vitro*, follow-up studies need to be conducted with individual macrolides under conditions of their actual use in order to define better the true role of sub-MIC effects on *in vivo* outcomes in both humans and animals.

Subinhibitory concentrations of antibiotics are well known to alter the physical appearance of bacteria [92]. The altered physical appearance may be indicative of damage to the cell's structural integrity, thus rendering it more susceptible to lysis or phagocytosis [93]. In a susceptible strain of *Pseudomonas aeruginosa*, erythromycin and azithromycin, but not josamycin altered outer membrane proteins and lipopolysaccharide, which correlated with enhanced serum sensitivity of the strain [94]. In contrast, macrolides did not cause structural changes in *Klebsiella pneumoniae*, a Gram-negative bacterium not susceptible to macrolides [95]. Structural changes were found to persist even during the post-antibiotic phase for four macrolides against *Staphylococcus aureus* [96]. Post-antibiotic effects (PAE) by macrolides against Gram-positive bacteria were observed in other studies, some of which also noted sub-MIC post-antibiotic effects [97-99]. One explanation for these observations might be that the additional time conferred by the PAE is needed for bacteria to overcome the macrolide's inhibition of bacterial protein synthesis, a factor that may also be contributing to some extent in sub-MIC effects even when microbial growth has not been completely inhibited [96]. Because an antibiotic is usually administered at a dosage that gives antibiotic concentrations above the minimum inhibitory concentration (MIC), which is then followed by a period where concentrations fall below the MIC, considerations regarding both the PAE and the extended sub-MIC PAE can provide a rationale for longer dosing intervals for some macrolides [97].

Virulence factors produced by microbial pathogens are regarded as important elements that allow a pathogen to colonise an animal and establish an infection, such as bovine pneumonic pasteurellosis [100]. Several stud-

ies have found that different factors associated with virulence and pathogenicity of bacteria have diminished after exposure to sub-MICs of macrolides, rendering the organisms more susceptible to eradication by components of the host defence system. For example, roxithromycin repressed formation of a pneumococcal polysaccharide, a virulence factor of *Streptococcus pneumoniae,* and resulted in greater ingestion of that bacterium by phagocytes, whereas no effect was noticed against *S. aureus or Streptococcus pyogenes* [101]. Another study found that three of the newer erythromycin derivatives suppressed other virulence factors produced by *S. aureus* [102]. Although *P. aeruginosa* is intrinsically resistant to macrolides, sub-MICs suppressed expression of several virulence factors at concentrations that did not inhibit growth [103-107]. These results were recently found to be caused by inhibition of protein synthesis during long-term exposure to macrolides [108]. Sub-MIC effects are considered to account partially for the degree of effectiveness shown by macrolides against *P. aeruginosa* in human respiratory diseases such as diffuse panbronchiolitis [109]. Sub-MICs of macrolides diminished adhesion of Gram-positive bacteria to several types of host cells, possibly by interfering with the microbial biosynthesis of materials such as adhesins and thus making the bacteria more susceptible to phagocytosis [110-112]. Macrolides have shown similar effects in reducing adhesiveness of some Gram-negative bacteria as well as suppressing biofilm formation of *P. aeruginosa* at subinhibitory concentrations [113-117]. In all of these sub-MIC studies, it must be noted that different macrolides have often shown different degrees of effectiveness in each assay, so the specific effect for any individual macrolide against a particular bacterium must be measured by appropriate experiments. Nevertheless, these examples indicate that sub-MICs of macrolides can exert measurable effects on both Gram-positive and Gram-negative bacteria that may reduce their ability to cause and maintain infections and may assist the host in eradicating infectious organisms. Although these examples have demonstrated some correlations between data from *in vitro* and *ex vivo* experiments with results in animals and humans, further definitive *in vivo* studies proving the relevance of these potentially useful sub-MIC effects to *in vivo* outcomes still need to be conducted.

7. Pharmacokinetic and pharmacological effects of macrolides

Macrolides are orally bioavailable agents, so oral formulations provide a common and convenient route for administration to animals *via* their feed and/or drinking water. One prominent feature of some of the newer semisynthetic derivatives in human medicine is their significantly greater oral bioavailability compared to their parent compounds [118,119]. In contrast to the 14-membered-ring macrolides, 16-membered-ring macrolides do not appear to elicit changes in gastrointestinal motility or to act as gastrointestinal prokinetic agents [120]. Tylosin and spiramycin may be given by intramuscular injection and tilmicosin by subcutaneous injection to cattle. However, pain on injection has limited usage of erythromycin by intramuscular administration [23]. Various formulations have been prepared for application in animals requiring intramammary or topical routes of administration, and water-soluble acid addition salts of erythromycin are available for intravenous administration.

Macrolides undergo metabolism to different extents, depending on the particular compound and the metabolically susceptible functional groups within its structure [121]. If metabolism of macrolides occurs, it generally does so predominantly in the liver where the cytochrome P450 system is involved; the unchanged parent and whatever metabolites that may be formed are mostly excreted *via* faeces. Serum concentrations of macrolides vary, again depending on the particular compound and animal species, although the older macrolides tend to have relatively lower serum concentrations and shorter *in vivo* half-lives. Among the principal advantages exhibited by some of the newer macrolides are their higher and more persistent serum concentrations of antibiotic activity.

Treatment of many infections requires an antibiotic to reach effective concentrations at sites within the body beyond the vascular system. Macrolides are readily accumulated into many tissues, tissue fluids, organs, and extravascular sites, such as in the lung and bronchial secretions, where high local concentrations of antibiotic are desirable, e.g., for treatment of respiratory tract infections [122-124]. Tissue and fluid concentrations of antibiotic may exceed serum concentrations by 2- to > 100-fold, depending on the specific tissue or fluid being measured. For example, tylosin was found to achieve concentrations in lung tissue of both healthy pigs and pigs suffering from pneumonia that exceeded serum concentrations by several fold [125]. Macrolides are readily accumulated intracellularly where they achieve intracellular concentrations that once again often greatly exceed serum concentrations [126-129]. As a result, macrolides can inhibit many intracellular pathogens because the antibiotic penetrates and accumulates within cells to the extent that inhibitory concentrations are achieved at the intracellular site where the pathogen is located [126-132]. However, since the three-way interaction between antibiotic, pathogen, and cell is complex, high concentrations of antibiotic by themselves do not necessarily

suffice to inhibit a pathogen [130-132]. Among the various factors that may cause a lack of intracellular antimicrobial activity are differences in the specific intracellular locations of pathogen and antibiotic, reduction of the macrolide's antibiotic activity by protonation within acidic intracellular sites, and diminished intracellular growth of pathogens. The latter results in the reduction of their susceptibility to bacteriostatic antibiotics such as macrolides that exert their action by inhibiting microbial protein synthesis [130,131].

The high intraphagocytic concentrations achieved by macrolides coupled with the facile movement of phagocytic cells to sites of infection provide an unusual delivery mechanism that allows higher concentrations of macrolides to be transported to the sites where they are most needed by the body to combat pathogenic organisms [133-135]. Consequently, the facile penetration and high accumulation of macrolides into various cells and tissues, along with other important factors such as their long PAE and sub-MIC effects, must be accounted for when the *in vivo* efficacy of these agents is examined [136]. The simple combination of MICs determined under standard *in vitro* susceptibility conditions, along with measurements of serum concentrations of antibiotic, may not always suffice to explain the *in vivo* efficacy exhibited by some macrolides. One well known example where such alternative explanations are required is the 'spiramycin paradox' in which the *in vivo* effectiveness of spiramycin is not readily explained by its relatively modest *in vitro* activity [137,138]. A more recent example is the efficacy exhibited by tilmicosin in controlling pneumonia in pigs, which is greater than expected based solely upon MICs and easily measured serum and tissue concentrations of antibiotic [34,35,139].

8. Effects of macrolides on the host

In addition to their direct inhibitory action and sub-MIC effects on micro-organisms, macrolides may also affect the host in ways that modulate an animal's ability to withstand and overcome infections. Even with the deployment of antibiotics, it is well established that complete eradication of an infectious organism generally requires the active participation of a competent immune system in the host animal [140]. Consequently, many different ways to stimulate or enhance the immune system and thereby assist complete elimination of pathogens have been investigated. One of the more important interactions is the triangle between antibiotic, pathogen, and phagocytes, a complex interaction that is steadily becoming better recognised and more clearly defined. If the effect of a macrolide is a stimulation or enhancement of important functions of the immune system, this mechanism may contribute to the overall therapeutic efficacy of the antibiotic; conversely, a negative effect on parts of the immune system may work against the antimicrobial action of the antibiotic. However, there have been very few studies of this subject applied directly to veterinary uses of macrolides, and the caveat must again be noted that generalisations within this field are difficult to make because species differences may be critical, a macrolide may affect different components of the immune system in different and even opposite ways, and different macrolides often exert different effects on the same immune component or function [141-148]. In many studies, only one or a small number of macrolides and only one or a few components of the host's defence system have been examined. In addition, critical studies that conclusively demonstrate how these effects translate into clinical relevance are often missing [149-151]. Consequently, the many variations among individual macrolides and their diverse effects indicate that comprehensive comparisons or overall assessments cannot be made about the macrolide class regarding host defence mechanisms, but rather that individual compounds must be studied in specific infections and animal species. One example which highlights the need for *in vivo* studies is illustrated by the case where no advantage was observed when josamycin was combined with an immunostimulant to treat *Mycoplasma gallisepticum* in chickens [152]. In contrast, oral administration of tylosin tartrate to broiler chickens potentiated humoral and cellular immune responses [153].

Phagocytic cells, such as polymorphonuclear leukocytes (PMNs), blood monocytes, and macrophages, are responsible for ingesting and killing foreign micro-organisms and thus constitute one of the foremost crucial components of the host's defences against infectious organisms. Unfortunately, this subject is too broad and complex to allow for more discussion other than citing a few examples of ways in which some macrolides stimulate some of the antimicrobial functions of phagocytes. Macrolide antibiotics are readily taken into phagocytic cells by passive diffusion and achieve high intracellular concentrations, where they tend to localise within acidic compartments such as the lysosome [126-129]. This situation creates an equilibrium between extracellular drug and the two-compartment intracellular system of cytosol and lysosome, in which the acidity of the latter drives the uptake of macrolide into the lysosome and enables greater antibiotic persistence in the phagocyte [128,129]. The intracellular concentrations of antibiotic may be increased, decreased, or unchanged by the presence of intracellular bacteria [154-158]. The uptake mechanism of four macrolides into PMNs has been recently linked to the required presence of extracellular calcium ions [159].

Although high intracellular concentrations of antibiotic are necessary to inhibit intracellular organisms, they are only one of several factors that must be considered and by themselves do not necessarily lead to potent intracellular bactericidal activity. Problems that may diminish intracellular killing by a well-accumulated antibiotic include: its intracellular degradation or metabolism, sequestration in an ineffective intracellular location away from the pathogen, and negative effects on intracellular functions necessary for microbial killing within the phagocytic cell. The low efficacy of erythromycin against bovine mastitis caused by *S. aureus* has been attributed to some extent to ineffective killing of *S. aureus* inside PMNs [154,155]. In contrast, uptake of tilmicosin into mouse macrophages and human monocytes exceeded that of erythromycin and tylosin while intracellular killing of *Toxoplasma gondii* was demonstrated within bovine turbinate cells [160,161]. Josamycin more so than erythromycin exhibited some *in vitro* synergy with PMNs in killing assays against *S. aureus* and *P. aeruginosa*, and azithromycin increased the destruction of *S. aureus* by PMNs without increasing self-killing or damage to the phagocytes [162,163].

Macrolide antibiotics may affect, either positively or negatively, many different functions of phagocytic cells such as cell growth and proliferation, chemotaxis to foreign stimuli, cytokine production, phagocytosis of foreign materials, and intracellular antimicrobial processes such as the oxidative burst, all of which are involved in the ultimate goal of microbial killing by phagocytes. One example from recent publications is the case where azithromycin elevated production of soluble interleukin-2 receptors in lymphocytes although it had no effect on several other lymphocyte functions [164]. It also enhanced *ex vivo* phagocytosis of *S. aureus* by PMNs obtained from the blood of volunteers given multiple doses [165]. However, three macrolides in another *ex vivo* study suppressed phagocytosis and other neutrophil functions [166]. Dirithromycin was more effective than erythromycin in inducing degranulation of neutrophils as measured by the release of three intragranular enzymes [167]. Among five macrolides, roxithromycin was the only one to impair strongly the oxidative burst and chemotaxis of human neutrophils [168]. Unfortunately, the data are sufficiently fragmented and diverse at the present time such that broad conclusions and generalisations cannot be made about the immunomodulatory properties of macrolides as a class, so specific macrolides still need to be examined individually against each relevant micro-organism, disease state, and animal species.

A recent interesting phenomenon has been the anti-inflammatory effect exhibited by several macrolides in animal models of inflammation [169,170]. Studies of the mechanism underlying this activity have focused on the ability of macrolides to inhibit some inflammatory properties of neutrophils [171-174]. More recently, macrolides were discovered to increase concentrations of glucocorticoids [175]. Roxithromycin and erythromycin inhibited infiltration of interleukin-8-induced neutrophils, suggesting a possible application in the treatment of airway hypersecretion [176]. However, macrolides also inhibited α-dornase, a hydrolytic enzyme used to decrease sputum viscosity in respiratory tract infections for which macrolides are sometimes used due to their sub-MIC effects against *P. aeruginosa* [177]. A further complicating factor in comprehending this field is the microbial production of cytokines by both normal gut flora and infectious micro-organisms that may affect both the inflammatory and immune responses of the host animal [178,179]. However, this intriguing area of potential utility for macrolides as anti-inflammatory agents is likely to have implications for the veterinary use of macrolides in certain animal infections and disease states, as illustrated by the recent report of anti-inflammatory effects exerted by tilmicosin in limiting lung tissue damage in *Pasteurella*-infected calves [180].

9. Conclusions and future directions

Several macrolide antibiotics have established important roles in the modern production of food animals needed to provide sufficient amounts of quality food at acceptable prices to consumers. Many antimicrobial features of macrolides used in veterinary applications are common to the macrolide class and are analogous to the effects of macrolides used in human medicine that have been much more widely studied. In addition to their direct inhibitory action on micro-organisms, macrolides exert a variety of sub-MIC effects that are being increasingly recognised as important factors for explaining many therapeutic results. Other properties, such as the broad tissue distribution and high intracellular concentrations achieved by macrolides, contribute prominently to their efficacy. Several other important factors which govern efficacy involve the extensive interactions between macrolides, micro-organisms, and phagocytic cells, which sometimes allow the host's immune system to enhance the antimicrobial activity of the antibiotics. More recently, the role of macrolides in modulating some inflammatory processes has been recognised. In regard to sub-MIC effects and interactions with the host immune system, a diverse variety of both positive and negative responses have been found, creating a complex matrix of results whose full understanding will require many additional studies with individual macrolides in appropriate ani-

mal species and disease states. Such studies may provide important additional information to help manage the wise and prudent use of macrolide antibiotics in the production of food animals.

Acknowledgements

The author expresses his sincere thanks and appreciation to Ms LW Crandall for her numerous literature searches and Drs Shryock TR, McGruder ED, Allen NE, Kennington AS, Martin BW, Walters JW, Langley MR, Lawrence K, Jeffers TK, and Wold JS, for their reading and helpful critiques of the manuscript.

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