Is Rifampin necessary for the treatment of Rhodococcus equi infections in Foals?

Authors: to be decided

Rifampin is a broad-spectrum, concentration-dependent, bactericidal and/or bacteriostatic antibiotic, with activity against many gram-positive and some gram-negative aerobic bacteria as well as facultative anaerobic organisms (Wilson et al., 1988). It is a complex macrocyclic semisynthetic antibiotic derived from rifamycin B. The principle use of rifampin in equine medicine is for combination treatments against Rhodococcus equi infections in foals (Plumb, 2011). It can also be used to treat proliferative enteropathy caused by Lawsonia intracellularis in foals. However, rifampin is not specifically approved for veterinary use. In Europe, rifampicin is listed by Federation of Veterinarians of Europe (FVE) on the essential substance list for horses (Commission Regulation (EC) No 1950/2006).

Rifampin enters bacteria and works by forming stable complexes with the β-subunit of bacterial DNA-dependent RNA polymerase, while not affecting mammalian polymerase at therapeutic doses (Frank, 1990). This binding results in inactivation of the enzymes and inhibition of RNA synthesis by preventing chain initiation only (Spoor & Riviere, 1995). Rifampin appears to penetrate the outer membrane of gram-positive bacteria more easily than that of gram-negative bacteria (Frank, 1990). This is reflected in the generally lower minimum inhibitory concentrations (MIC) required for gram-positive bacteria (0.01 mcg per mL of serum) compared with gram-negative bacteria (8 to 32 mcg per mL) (Frank, 1990). Thus, most gram-negative bacteria should be considered resistant or to have unpredictable susceptibilities (Thornsberry & Hill, 1983).

Rifampin is highly lipid-soluble and widely distributed in the body (Frank, 1990; Kohn et al., 1993), and thus active against extracellular as well as against susceptible intracellular organisms (Zak et al., 1985), including intra-leukocytic organisms (Lobo & Mandell, 1972). Rifampin can enter neutrophils and macrophages to kill intracellular bacteria, while not interfering with phagocytosis (Lobo & Mandell, 1972; Frank, 1990). The ability of rifampin to reach intracellular bacteria can make it difficult to predict in vivo therapy results based on in vitro sensitivity tests (Zak et al., 1985). Rifampin has been shown to have in vitro activity against equine Corynebacterium pseudotuberculosis, Rhodococcus equi, Staphylococcus species, Streptococcus equi, S. equisimilis, and S. zooepidemicus isolates (Wilson et al., 1988; Kohn et al., 1993).

The pharmacokinetics of rifampin have been well-studied in horses (Kohn et al., 1993; Wilson et al., 1988; Burrows et al., 1985) and minimal side effects have been reported in foals (Knottenbelt, 1993; Hillidge, 1987; Sweeney et al., 1987). Rifampin is absorbed rapidly after oral administration to horses (Frank, 1990), although bioavailability is not high in adult horses. Administration with food can prolong the time-to-peak serum concentration in adult horses (Frank, 1990; Burrows et al., 1992a). For example, single dose of 10 mg per kg of body weight (mg/kg) resulted in 48.8% oral bioavailability (Kohn et al., 1993), compared to 39.5%, with a single dose of 10 mg/kg, administered in the feed (Burrows et al., 1985). An unpublished study of horses receiving a dose of 5 mg/ kg found a bioavailability of 68% when rifampin was administered one hour before feeding and 26% when it was administered 1 hour after feeding (Baggot, 1992). Protein binding is high in
horses (e.g. 78%), with serum concentrations of 2 to 20 micrograms per milliliter (mcg/mL) (Kohn et al., 1993).

The biotransformation and elimination of rifampin in animals is not well defined. Induction of hepatic enzymes occurs in response to administration of rifampin (Adachi et al., 1985; Burrows et al., 1992b; Benedetti & Dostert, 1994), but major metabolites of the parent drug in most animals have not yet been traced (Kohn et al., 1993). Rifampin is metabolized in the liver to a deacetylated form (e.g. 25-desacetylrifampin), which is bioactive (Frank, 1990). Both parent and metabolite compounds can be excreted in bile, where enterohepatic circulation occurs for both (Spoo & Riviere, 1995). The rate of excretion in foals is lower than adult horses, due to biliary excretion mechanisms being less developed. For example, foals given 10mg/kg of rifampin orally had a mean serum half-life of 17.5 hours, which was significantly longer than that found in adult horses (4.2 ± 1.2 hours, with a dose of 10 mg/kg, orally, with food - Burrows et al., 1985) (Spoo & Riviere, 1995).

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Resistance to rifampin can develop quickly, especially when used alone; therefore, it is most often used in combination with other antimicrobials (Thornsberry & Hill, 1983). Resistant mutants may be concentration-sensitive and contain RNA polymerases with one of a variety of sensitivities to rifampin (Wehrli, 1983). Resistance may occur as a single-step mutation of the β-subunit of bacterial DNA-dependent RNA polymerase; therefore, initial susceptibility can diminish rapidly as small populations of resistant cells soon out-number susceptible cells. One case of the development of resistant Rhodococcus equi in a foal treated with erythromycin and rifampin has been reported (Kenny et al., 1994). Cross-resistance to other antibiotics or transfer of resistance to other local microorganisms has not been reported (Frank, 1990).

In equine medicine, rifampin is used in combination with macrolides (e.g. erythromycin, clarithromycin) for the treatment of pneumonia caused by Rhodococcus (Corynebacterium) equi infection in foals (Knottenbelt, 1993; Hillidge, 1987; Sweeney et al., 1987). These drugs have the advantage of being available in oral dosage forms. Although the lung appears to be most common site for Rhodococcus equi infection, in some cases susceptible foals have been found to have abdominal or subcutaneous abscesses, bacterial endocarditis, diskospondylitis, gastrointestinal infections, osteomyelitis, or septicemia (Nay, 1996; Chaffin et al., 1995; Kenney et al., 1994; Collatos et al., 1990; Desjardins and Vachon, 1990; Perdrizet and Scott 1987). In many, but not all of these cases, the foal has an accompanying pneumonia. Rhodococcus equi resists innate macrophage defenses in horses and causes a severe caseous, necrotizing lung infection in foals with
high mortality rate of up to 80% (Hillidge, 1987). *R. equi* are susceptible *in vitro* to erythromycin alone, and erythromycin alone has been effective in the treatment of this infection (Sweeney & Sweeney, 1987; Prescott, 1991; Giguère & Prescott, 1997; Verville *et al*., 1994). However, no studies have been performed to compare the efficacy of erythromycin alone or with the combination of erythromycin and rifampin in foals. The *in vitro* evidence suggests a synergistic activity for the combination of macrolides and rifampin against *R. equi* (Prescott & Nicholson, 1984) and the volume of case reports supporting the efficacy of the combination results in combination therapy more commonly recommended for this indication than monotherapy alone (Prescott & Sweeney, 1985). For example, with combination treatment protocols, survival rates have increased from 20% to nearly 90% (Hillidge, 1987). In a retrospective study, it was shown that the combination of rifampin with clarithromycin maybe superior to combinations with erythromycin or azithromycin (Giguère *et al*., 2004).

Although the efficacy is not established, rifampin is used in combination with erythromycin in the treatment of Potomac horse fever (equine ehrlichial colitis) (Palmer and Benson, 1992). It is as effective as oxytetracycline in the resolution of clinical signs, with the exception that rifampin and erythromycin will not reduce fever as quickly as oxytetracycline, taking up to 12 hours longer to return the body temperature to normal (Palmer and Benson, 1992).

The combination treatment of rifampin and macrolides is not without risk. Severe enterocolitis has also been reported in mares whose foals are treated with erythromycin. The exact pathogenesis has yet to be fully elucidated but is thought to occur by disrupting the mare’s normal colonic microflora after ingestion of small amounts of active drug during coprophagia or by contamination of feeders or water buckets with drug present on the foal’s muzzle. This complication appears to be rare in some countries and more common in others. Enterocolitis in mares has been reproduced experimentally by administration of sub-therapeutic doses of erythromycin (Gustafsson *et al*., 1997). In most cases, this fatal enterocolitis in the mares of treated foals is associated with *Clostridium difficile* (Baverud *et al*., 1998).

Efficacy of these antibiotics is dependent on the availability of active unbound concentrations at the target site(s). For the lung tissue, the minimal inhibition concentrations (MICs) for antimicrobial agents must be exceeded in the environment of the respective bacteria (i.e., bronchial and alveolar epithelial cells (EC), bronchial epithelial lining fluid (PELF), and bronchoalveolar lavage cells (BAL). Macrolides are known to penetrate lung and achieve drug levels many times greater than the concurrent plasma concentrations at a steady-state (BAL > ELF > plasma) (Conte *et al*., 1995). The mechanisms by which macrolides accumulate in pulmonary spaces are poorly understood. Ion-trapping of the basic compounds in the acidic compartment of alveolar macrophages is part of the explanation. There is also likely a co-ordinated interplay of pulmonary multi-drug transporters. At present, it is known that the cell membranes of lung tissue possess uptake carriers of the organic anion-transporting polypeptide (OATP), organic cation transporter (OCT) and peptide transporter families and with efflux carriers of the ATP-binding cassette (ABC) family, which are also expressed in other organs (e.g. intestines, liver) (Chan *et al*., 2004; Bosquillon, 2010). In particular, the OATP1A2 and OATP2B1 are knwon in *Equus caballus*, and OATP1A2 is expressed in EC and OATP2B1 is expressed in EC and BALC. These efflux carriers ABCB1 and ABCC2 and several OATPs are modulated in the presence of rifampin, able to exhibit inhibitory effects on some types of transporters and induction of others (Geick *et al*., 2001; Vavricka *et al*., 2002). For example, equine OATP2B1 in EC has been found to be regulated by rifampin (Venner *et al*., 2010; Peters *et al*., 2011; Meyer zu Schwabedissen *et al*., 2008).
Recently, it has been found in foals that co-medication with rifampin (short and long-term) leads to a dramatic lowering of the average steady-state plasma concentrations of clarithromycin by more than 90% and in turn a similarly limited distribution into the PELF and BAL (Peters et al., 2011; Peters et al., 2012). This drug interaction with rifampin/clarithromycin combination likely also applies to other macrolides. Also, this same decrease in clarithromycin oral bioavailability after rifampin co-administration has been shown in other species including rats, with a reduction of 45% (Garver et al., 2008; Lan et al., 2009) and 30-40% in humans (Wallace et al., 1995; Taki et al., 2007). In healthy foals, clarithromycin oral bioavailability alone is incomplete (60%), due in part to pre-systemic “first-pass” metabolism by which the active metabolite 14-OH-clarithromycin is generated (Womble et al., 2006). This dramatic decrease in the clarithromycin plasma levels with rifampin co-medication was the consequence of nearly complete abolition of clarithromycin oral bioavailability since the plasma half-lives remained unchanged (Peters et al., 2011). The exact reasons for this inhibition is unknown. Peters et al. (2011) proposes two theories for this phenomenon: inhibition of a so far unknown important macrolide intestinal uptake transporter in the presence of rifampin, or an absorption deficit caused by either rifampin induction of ABCB1, or inhibition of the OATP family intestinal transporter. Other possible candidates might be the multidrug resistance-related protein 1 (ABCC1) and canalicular multispecific organic anion transporter 2 (ABCC3), because these transporters are highly expressed within the basolateral membrane of the enterocytes, acting there as efflux transporters and pumping their substrates towards the blood of the submucosal vessels. Therefore, inhibition of ABCC1 and ABCC3 by rifampin would result in decreased bioavailability of clarithromycin (Oswald et al., 2006; Giacomini et al., 2010).

Consequently, this major decrease in clarithromycin bioavailability could be improved if rifampin and clarithromycin were administered at different times of the day, but this treatment strategy has yet to be investigated. In other words, clarithromycin absorption appears to be affected via a mechanism that is susceptible to direct competition with rifampin (Taki et al., 2007). Nevertheless, based on this new information then there are doubts, from a pharmacokinetic point-of-view, that combination therapy of clarithromycin with rifampin might not be superior to other treatment protocols (Giguère et al., 2004). For example, the average steady-state of clarithromycin plasma concentrations dropped to levels below the MIC (90%) of 0.12g/ml for R. equi (Jacks et al., 2003).

This drug interaction between rifampin and clarithromycin - and probably all macrolides - resulting in reduced bioavailability of clarithromycin raises another important issue. Previously, it has been found that fatal enterocolitis can occur in mares whose foals are treated with erythromycin, likely due to ingesting small amounts of the active drug during coprophagia or by contamination of feeders or water buckets that is shared by the mare and foal. If clarithromycin oral bioavailability is inhibited strongly by co-medication with rifampin, then clearly more clarithromycin (e.g. macrolides) would be present in the foal’s feces then from mono-therapy with clarithromycin. Thus, perhaps an important risk factor for macrolide-induced fatal enterocolitis in mares, secondary to foal Rhodococcus equi treatments, is the concurrent co-medication with rifampin.

Another interesting finding was a more intensive distribution of clarithromycin into the bronchial ELF despite markedly lower plasma concentrations after rifampin co-medication (Peters et al., 2011). For example, clarithromycin and 14-hydroxycalithromycin accumulated approximately 20- to 40-fold and 1.5- to 4.5-fold in ELF and 300- to 1800-fold and 25- to 90-fold in BAL, respectively (Peters et al., 2012). This could again be explained by the action of specific drug transporter proteins. For example, clarithromycin is a substrate of ABCB1 (and probably of ABC2), and
rifampin is a strong inducer of both. Both ABCB1 and ABCC2 are expressed in the apical membrane of the EC and in the cell membrane of BAL, and both may mediate active efflux of their substrates into the ELF and the environment of macrophages, respectively (Seral et al., 2003a,b; Bosquillon, 2010). Also, mRNA expression of ABCB1 and ABCC2 has been found to be up-regulated by rifampicin in foals (Venner et al., 2010).

The confusing aspects in trying to understand the complex drug interactions of rifampin and macrolides in relation to various drug transporter proteins is the case that within the gastrointestinal tract the rifampin is inhibitory against clarithromycin absorption, but in the case of pulmonary penetration, rifampin maybe complimentary for clarithromycin penetration. For example, OATPs are inhibited strongly in the presence of rifampin (Vavricka et al., 2002), and located both in the intestines and lungs. Peters et al. (2012) suggests that the explanation might the location of the drug transporter protein on the cell surface. If the pulmonary OATPs are localized to the basolateral site of EC, higher clarithromycin ELF/plasma ratios are explained by up-regulation of OATP2B1 by rifampin co-medication. For apical localization of the uptake carriers, the pharmacokinetic phenomenon in the presence of rifampin (down-regulation and or inhibition) is in line with delayed clarithromycin re-uptake from the bronchial ELF back to EC during systemic elimination of the drug. Therefore, information on the pulmonary localization of OATPs and quantitative data on the affinity of clarithromycin and rifampin to the uptake transporters and their regulatory elements are needed before this drug interaction can be fully understood (Peters et al., 2012).

Peters et al. (2012) found that the pharmacokinetic characteristics of rifampin were not influenced by co-administration of clarithromycin. They showed that the penetration of rifampin and its major deacetylated metabolite into pulmonary compartments of foals under steady-state conditions. At distribution equilibrium, rifampin permeated unrestricted into ELF but reached approximately 40 to 80% lower levels in BALCs. The deacetylated, more-polar metabolite accumulated 1.4- to 6-fold in ELF and 8- to 60-fold in BALCs, accounting for approximately 2% of total drug exposure in ELF but 25% in BALCs. Therefore, a significant part of the antimicrobial effect of rifampin in BAL is likely attributed to deacetylated-rifampin, which is as active as the parent compound (Acocella, 1978). Unbound rifampin in plasma and all pulmonary compartments at steady state were in excess of the MIC90 for equine R. equi (>0.5 µg/ml) (Jacks et al., 2003).

Thus, different scenarios likely take place in vivo, during the complex drug interactions between rifampin and macrolide drugs used for the treatment of equine rhodococcal infections. During the first week of treatment, the most likely main antimicrobial effect against pulmonary Rhodococcus equi infection is from rifampin, and 25-desacetyl rifampin metabolite to lesser extent, since there is strong inhibition in macrolide oral bioavailability with rifampin co-medication. Macrolide plasma and lung concentrations may barely achieve MIC levels during this time. After that, rifampin will have induced accelerated long-lasting activity of hepatic cytochrome P450 enzymes leading to increased hepatic biotransformation of both rifampin and macrolide drugs. Certain macrolides maybe more affected by this process than others. For example, According to the summary of product characteristics of the manufacturers for many macrolides, it states that strong enzyme inducers of the cytochrome P450 system (e.g. rifampin) may accelerate the metabolism of macrolides and, by this, decrease the plasma levels of the parent drug. In other words, post one week of treatment, the main antimicrobial effect against pulmonary Rhodococcus equi infection are most likely from the metabolites of rifampin and macrolide drugs (e.g. 25-desacetyl rifampin, 14-OH-clarithromycin) for which there is no information about MIC values against Rhodococcus equi, and little information about pharmacokinetics/pharmacodynamics in equine lung compartments.
Therapies, by coincidence, that border on rifampin monotherapy will lead to an increased development of rifampin resistance in horses.

References


