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Intestinal and hepatic contributions to the pharmacokinetic interaction between gamithromycin and rifampicin after single-dose and multiple-dose administration in healthy foals

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Summary

Background: Standard treatment of foals with severe abscessing lung infection caused by *Rhodococcus equi* using rifampicin and a macrolide antibiotic can be compromised by extensive inhibition and/or induction of drug metabolising enzymes (e.g. CYP3A4) and transport proteins (e.g. P-glycoprotein), as has been shown for rifampicin and clarithromycin. The combination of rifampicin with the new, poorly metabolised gamithromycin, a long-acting analogue of azithromycin and tulathromycin with lower pharmacokinetic interaction potential, might be a suitable alternative.
Objectives: To evaluate the pharmacokinetic interactions and pulmonary distribution of rifampicin and gamithromycin in healthy foals, and to investigate the cellular uptake of gamithromycin in vitro.
Study design: Controlled, four-period, consecutive, single-dose and multiple-dose study.
Methods: Pharmacokinetics and lung distribution of rifampicin (10 mg/kg) and gamithromycin (6 mg/kg) were measured in nine healthy foals using LC-MS/MS. Enzyme induction was confirmed using the 4β-OH-cholesterol/cholesterol ratio. Affinity of gamithromycin to drug transport proteins was evaluated in vitro using equine hepatocytes and MDCKII-cells stably transfected with human OATP1B1, OATP1B3 and OATP2B1.
Results: Rifampicin significantly ($P<0.05$) increased the plasma exposure of gamithromycin (16.2 ± 4.77 vs. 8.57 ± 3.10 μg × h/mL) by decreasing the total body clearance. Otherwise, gamithromycin significantly lowered plasma exposure of single- and multiple-dose rifampicin (83.8 ± 35.3 and 112 ± 43.1 vs. 164 ± 96.7 μg × h/mL) without a change in metabolic ratio and half-life. Gamithromycin was identified as an inhibitor of human OATP1B1, OATP1B3 and OATP2B1 and as a substrate of OATP2B1. In addition, it was extracted by equine hepatocytes via a mechanism which could be inhibited by rifampicin.
Main limitations: Influence of gamithromycin on pulmonary distribution of rifampicin was not evaluated.
Conclusion: The plasma exposure of gamithromycin is significantly increased by co-administration of rifampicin which is most likely caused by inhibition of hepatic elimination.

Keywords: horse; rifampicin; gamithromycin; drug-drug interaction; lung distribution; healthy foals; *Rhodococcus equi*

Introduction

The treatment of pulmonary infections caused by *Rhodococcus equi* (*R. equi*) in foals follows the 'watch-and-wait' strategy. In mild to moderate cases, antimicrobial treatment is only initiated after life-threatening deterioration of clinical signs (e.g. abscess score ≥10 cm, leucocyte count ≥13.000 cells/μL) [1]. Macrolide antibiotics are a major part of the standard therapy [2] as they widely distribute into pulmonary compartments, which are the survival and reproduction site of *R. equi* [3]. The combination of macrolides with rifampicin is recommended to achieve substantial synergism in efficacy [4]. However, the pharmacodynamic benefits can be limited by adverse pharmacokinetic drug-drug interactions (DDIs), as has recently been shown for clarithromycin [5,6].

DDIs between macrolide antibiotics and rifampicin can result from the mutual influence of drug metabolising enzymes and transporters involved in drug disposition. Some macrolides are substrates of the efflux transporters P-glycoprotein (P-gp) and the multidrug resistance-associated protein 2 (MRP2) [7] and of the organic anion transporting polypeptides (OATP) 1A2, 1B1 and 1B3 [8,9]. The analogue of human OATP1B1 and 1B3 in horses is OATP1B4. Furthermore, macrolides are metabolised by cytochrome P450 (CYP) 3A4 (analogue in horses: CYP3A89) and inhibit P-gp, OATPs [10,11] and CYP3A4 [12]. Otherwise, rifampicin is a substrate and inhibitor of P-gp and OATPs [13,14], and a ligand of the nuclear pregnane X receptor (PXR) which regulates gene transcription of P-gp, MRP2 and CYP3A4 [15]. Therefore, rifampicin should preferably be combined with macrolides with low pharmacokinetic interaction potential such as the novel 15-membered macrolactone gamithromycin which is currently approved for the treatment of swine and bovine respiratory disease [16].

Gamithromycin is rapidly and widely distributed (V_{ss} ~ 25 L/kg) and penetrates into the epithelial lining fluid (ELF) and bronchoalveolar lavage cells (BALCs) from which it is slowly eliminated with half-lives of 63.6 h (ELF) and 70.3 h (BALCs) respectively [17]. The major route of systemic elimination of gamithromycin in different species (e.g. rats, dogs, pigs and cattle) is by biliary excretion of the parent compound (≤90%) [18,19]. So far, little is known about drug transport proteins involved in the distribution and hepatic elimination of gamithromycin. Studies investigating the potential pharmacokinetic interactions between rifampicin and gamithromycin in foals are also missing so far.

To evaluate the interaction potential, we investigated the cellular uptake of gamithromycin into primary equine hepatocytes and measured its affinity to human OATPs. Finally, we conducted a clinical study in healthy foals to evaluate potential DDIs with rifampicin.

Materials and methods

Chemicals and reagents

Gamithromycin (Zactran®, 150 mg/mL)[a] for i.v. injection (6 mg/kg) was dissolved in 50 mL sterile water. Rifampicin was purchased as tablets (Eremfat®)[b]. For oral administration, rifampicin (10 mg/kg) was dissolved in 20 mL water and administered orally to the foals using a syringe (Soft Ject®)[c].

Study protocol

Animals: The study was performed in 12 healthy warm-blooded foals (7 males, 5 females, age 42–70 days, body weight 100–180 kg) of the Oldenburg breed. The mares and foals were vaccinated against influenza, tetanus and equine herpes virus 1 and 4, and received regular anthelmintic therapy. None of the subjects received any medication prior to or during the course of the study. Good health was confirmed by physical examination, lung ultrasonography and blood biochemistry and haematological screening. The animals were kept at a natural light rhythm on paddocks together with the mares, and had free access to mare's milk, hay, oats and water. The time of food intake relative to drug administration could therefore not be standardised. All clinical examinations were done in individual stables bedded with straw. The per protocol population consisted of 9 subjects (5 female, 4 male, age 45–63 days, body weight 100–177 kg). Two foals had to be withdrawn during the course of the study due to severe dyspnoea and symptoms of interstitial pneumonia, which appeared on the 5th and 19th study day respectively. One foal was excluded because of technical problems in blood sampling and storage.

Study protocol: Pharmacokinetics of gamithromycin and rifampicin respectively, were measured on 4 days within the total clinical study period of 50 days (Fig 1). On the first study day, the pharmacokinetics of rifampicin was evaluated after a single-dose administration of 10 mg/kg. Venous blood was sampled before and 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36 and 48 h after oral administration. Seven days later, the animals were treated with gamithromycin i.v. for 6 weeks (6 mg/kg, once weekly), that is, on study days 8, 15, 22, 29, 36 and 43. Gamithromycin was injected via a cephalic vein within 2–3 min using an indwelling catheter (VasoVet®, G14)[d]. On the 22nd study day, pharmacokinetics of gamithromycin at steady-state was evaluated by blood sampling before and 0.5, 1, 3, 6, 12, 24, 48, 72, 120 and 168 h after the third i.v. administration. Venous blood (each sample 5–7 mL) was collected from a jugular vein using a venous catheter (VasoVet®, G16)[d]. On the 29th and 43rd study day respectively, the mutual interactions between single-dose and multiple-dose rifampicin (10 mg/kg, once daily) and chronic gamithromycin (4th and 6th intravenous dosing) were evaluated by blood sampling before and 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 120 and 168 h after administration of rifampicin. The dosing of rifampicin on the 30th study day was omitted to accurately evaluate the existing half-lives. In total, co-medication was continued up to study day 50.

A bronchoscopy with bronchoalveolar lavage (BAL) was performed 24 h after the respective i.v. gamithromycin dosing on the 22nd and 43rd study day to obtain ELF and BALC [20]. Plasma was obtained by centrifugation of the blood samples at 2000× ***g*** for 10 min. Aliquots of 5×10^6 BALC and of the lavage fluid were immediately snap frozen using liquid nitrogen and were stored as plasma at −80°C until analysis. The plasma 4β-OH-cholesterol/cholesterol (4β-OH-CHOL/CHOL)-ratio, a biomarker for induction of microsomal CYP450 enzymes in humans [21], was determined before and after 2 weeks of daily treatment with rifampicin.

Quantitative assays for drugs and biomarkers

Gamithromycin, decladinosyl gamithromycin (declad-gamithromycin), rifampicin, 25-O-desacetyl rifampicin (DAc-rifampicin), CHOL and 4β-OH-CHOL) were quantified in plasma, lavage fluid and BALC respectively, using validated LC-MS/MS methods as recently described [22–24]. The lower limits of quantification (LLOQ) were 1 and 2 ng/mL for gamithromycin and declad-gamithromycin, 2.5 ng/mL for rifampicin and DAc-rifampicin, 50 and 5 ng/mL for CHOL and 4β-OH-CHOL respectively. Within-day and between-day accuracy of the assays ranged for all analytes within −11.8% to 15.0% and −12.7% to 13.0% respectively, of the nominal concentrations. Within-day and between-day precision was 1.10–11.2% and 0.60–14.4% of the respective means (coefficient of variation). Concentrations of gamithromycin, declad-gamithromycin, rifampicin and DAc-rifampicin in the ELF were assessed by normalising to the concentration ratio of urea in plasma over BAL-fluid. The BALC concentrations were normalised to a mean alveolar cell volume of 1.2 μL/10^6 cells in foals [25,26]. Urea was quantified using the kit LT-UR 0010[e].

Cells and cell culture

Madin-Darby canine kidney (MDCKII) cells were purchased from the European Collection of Cell Cultures (Salisbury, United Kingdom). Cells were grown in Dulbecco's modified Eagle's medium[f] (DMEM) supplemented with foetal bovine serum[f] (FBS), L-glutamine[f] and nonessential amino acids[f] (NEAA) at 37°C, 95% humidity, and 5% CO_2. MDCKII-cells stably transfected with human OATP1B1, OATP1B3 and OATP2B1, and the respective vector-transfected control cells were established as previously described [27]. Equine hepatocytes were isolated from the liver of a female 8-month-old foal, which had to be sacrificed because of a progressive, inoperable knee joint disease and retinal detachment in both eyes. For isolation, the same protocol as for the human liver was used [28]. The hepatocytes were plated on collagen-coated 24-well plates with a density of 10^6 cells/well and cultured for 2 days using human hepatocyte maintenance medium[g] (HHMM) before further use.

In vitro transport studies

Functional testing of the cells was carried out using radiolabelled estrone 3-sulphate[h] (E_1S, 1 μmol/L) for OATP2B1 and bromosulphthalein[h] (BSP) for OATP1B1 and OATP1B3 at final concentrations of 0.05 and 1 μmol/L respectively. Time-dependent uptake was measured in MDCKII-cells stably

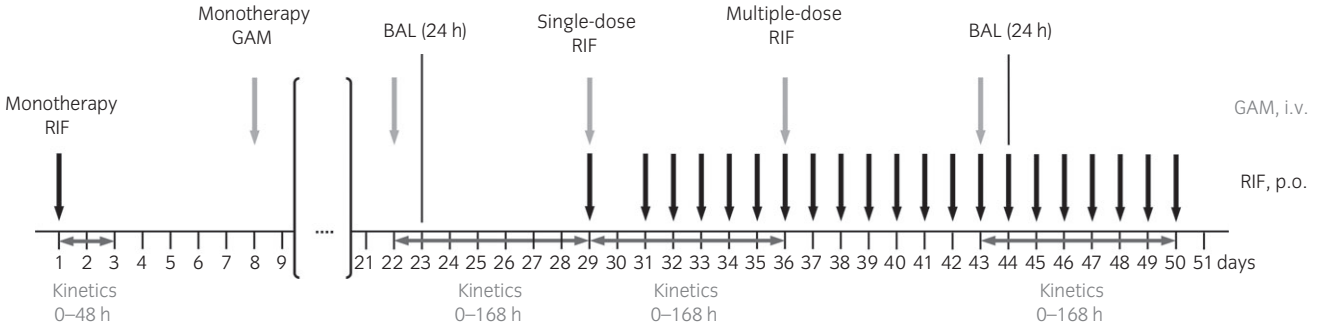

Fig 1: Study schedule of the controlled, four-period, consecutive, single-dose and multiple-dose study. The four periods are as follows: monotherapy of rifampicin (RIF), monotherapy of gamithromycin (GAM), combination therapy of GAM with single-dose RIF (acute interaction) and multiple-dose RIF (chronic interaction). Oral administration of rifampicin (RIF) alone is followed by blood sample collection (0−48 h). For each gamithromycin (GAM) treatment, blood sample collection was extended until 168 h. A bronchoalveolar lavage (BAL) was performed after chronic monotherapy of gamithromycin and gamithromycin with multiple-dose rifampicin, 24 h after the last respective gamithromycin administration on study days 23 and 44 respectively.

transfected with OATP1B1, OATP1B3 and OATP2B1 in comparison to vector control cells. Cells were incubated with 50 μmol/L gamithromycin (pH buffer: 7.3) for 5, 10 and 30 min respectively, washed three times with ice-cold PBS[f] followed by cell lysis using 600 μL of 0.2% SDS[i] and 5 mmol/L EDTA[i]. The cells were subsequently frozen at −80°C, followed by re-thawing the next day and manual cell disruption using ultrasound. Time-dependent uptake of 50 μmol/L gamithromycin and 0.05 μmol/L radiolabelled BSP was also measured in equine hepatocytes after incubation for 10, 30 and 60 min in the absence or presence of rifampicin. Uptake of radiolabelled BSP in MDCKII-OATP2B1, 1B1 and 1B3 was measured in the presence of different concentrations of gamithromycin (0.1–31.6 μmol/L) to determine the half maximal inhibitory concentration (IC_{50}). Data analysis and curve fitting (nonlinear regression) was carried out using the software GraphPad Prism 5 (Version 5.01)[j].

Biometrical evaluation

The pharmacokinetic evaluation and statistical analysis was performed using the software Phoenix (Build 6.4.0.768)[k]. The pharmacokinetic parameters were assessed by noncompartmental analysis. Maximum (C_{max}) and minimum (C_{min}) plasma concentrations and the time of C_{max} (T_{max}) of rifampicin were taken from the plasma concentration-time curves. The area under the plasma concentration-time curve during an administration interval (gamithromycin: $AUC_{0-168\ h}$; rifampicin: $AUC_{0-24\ h}$) was assessed with the measured data points using the trapezoidal method. The $AUC_{0-\infty}$ after single-dose administration of rifampicin was calculated using the measured data points up till the last quantifiable concentration (AUC_{0-t}) and extrapolated to infinity. Terminal elimination half-life ($T_{1/2}$) was estimated by log-linear regression analysis of the terminal slope. Mean residence time (MRT) was calculated as the quotient of the area under the moment curve (AUMC) and AUC. Total body clearance (CL_{tot}) was calculated as the quotient of dose and the respective AUC and the volume of distribution (V_{ss}) by dose × $AUMC/AUC^2$. The metabolic ratio (R_{met}, %) was calculated as $AUC_{metabolite}/(AUC_{mother\ substance} + AUC_{metabolite})$ after molecular mass correction with $AUC_{metabolite}$ as AUC of $DAc\text{-}_{rifampicin}$. Mean ± standard deviation (s.d.) values are shown. For multiple sample comparison the Friedman's test was carried out with post-hoc analysis ($P \leq 0.05$ as level of statistical significance) using IBM SPSS Statistics (Version 22)[l].

Results

The i.v. administration of gamithromycin was well tolerated; three foals suffered from a mild or moderate form of diarrhoea, which was self-limiting. One foal showed restlessness, skin swelling in the area of the nostrils, mouth and eyes, itching around the puncture site and increased sweating. This foal received methylprednisolone and recovered within one hour.

The plasma concentration of gamithromycin after i.v. administration decreased in the typical manner of a multi-compartment open model (Fig 2). The drug was rapidly and widely distributed (V_{ss} ~ 20 L/kg). Within 24 h, the drug penetrated into pulmonary compartments to reach ~1.40 μg/mL in the ELF and ~ 180 μg/mL in BALCs, that is, approximately 23- and >3000-fold respectively, of the measured plasma levels. Gamithromycin underwent only minor biotransformation by hydrolytic cleavage of the cladinose moiety. The drug was slowly eliminated with a terminal half-life of ~40 h. The total systemic clearance accounted for ~13 mL/min/kg (Table 1).

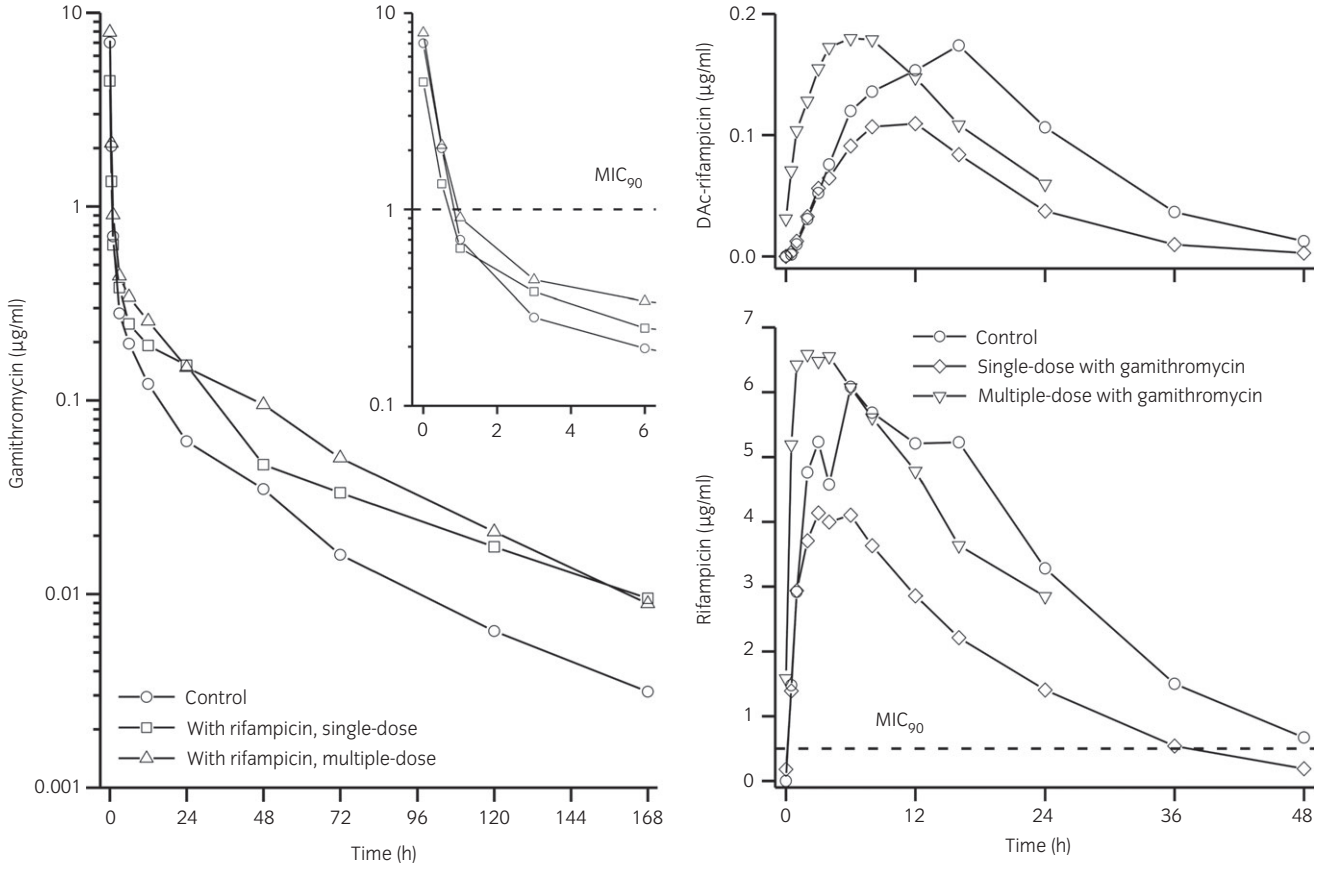

Fig 2: Left: Mean plasma concentration-time curves of gamithromycin) at steady-state (6 mg/kg, i.v., once weekly; control).Right: Mean plasma concentration-time curves of rifampicin after single-dose administration (10 mg/kg, p.o.) without gamithromycin (control), and during co-medication with gamithromycin following single-dose and multiple-dose administration. The study was performed in nine healthy foals. The dashed line indicates the MIC_{90}-value for *Rhodococcus equi*.

TABLE 1: Pharmacokinetic characteristics of gamithromycin at steady-state in nine healthy foals after repeated i.v. administration (6 mg/kg, once weekly; control) and in the presence of rifampicin (10 mg/kg, p.o.) after the first oral dose (single-dose rifampicin) and after multiple-dose co-medication (once daily for 15 days; multiple-dose rifampicin). Mean ± s.d. values are given

			Co-medication with rifampicin	
		Control	Single-dose rifampicin	Multiple-dose rifampicin
$AUC_{0-168\ h}$	(μg × h/mL)	8.57 ± 3.10	11.8 ± 1.77	16.2 ± 4.77*
$AUC_{0-168\ h,\ declad\text{-}gamithromycin}$	(μg × h/mL)	0.25 ± 0.10	0.62 ± 0.12*	0.68 ± 0.11*
R_{met}	(%)	2.52 ± 1.22	4.27 ± 0.91	3.57 ± 0.95
V_{ss}	(L/kg)	21.0 ± 9.75	21.1 ± 6.61	15.0 ± 5.79
$C_{min,\ ss}$	(μg/mL)	0.004 ± 0.002	0.011 ± 0.005*	0.015 ± 0.012*
$T_{1/2}$	(h)	33.9 ± 2.80	42.9 ± 8.59	38.4 ± 5.98
CL	(mL/min/kg)	12.9 ± 3.89	8.72 ± 1.69*	6.67 ± 1.91*
MRT	(h)	25.8 ± 7.82	39.7 ± 5.48*	36.8 ± 6.98*
$C_{24\ h}$	(μg/mL)	0.06 ± 0.01	Not assessed	0.15 ± 0.05
$ELF_{24\ h}$	(μg/mL)	1.40 ± 0.59	Not assessed	3.47 ± 1.34
$BALC_{24\ h}$	(μg/mL)	179 ± 117	Not assessed	212 ± 78.5

$AUC_{0-168\ h}$, area under the curve in the dosing interval 0–168 h; $BALC_{24\ h}$, concentration in bronchoalveolar lavage cells 24 h after gamithromycin administration; CL, Clearance; $C_{min,\ ss}$, minimum plasma concentration under steady-state conditions; $C_{24\ h}$, plasma concentration 24 h after gamithromycin administration; declad-gamithromycin, gamithromycin without cladinose; $ELF_{24\ h}$, concentration in the epithelial lining fluid 24 h after gamithromycin administration; MRT, mean residence time; R_{met}, metabolic ratio; $T_{1/2}$, terminal elimination half-life; V_{ss}, volume of distribution.
*Compared with control, P≤0.05 (Friedman's test with post-hoc analysis).

Multiple-dose treatment with rifampicin and gamithromycin was associated with a moderate PXR-type enzyme induction (plasma 4β-OH-CHOL/CHOL-ratio: 1.7 ± 0.4 vs. 1.0 ± 0.6; P<0.001). Single-dose co-administration of rifampicin and, to a much greater extent, the enzyme-inducing multiple-dose treatment significantly increased the plasma exposure of gamithromycin ($AUC_{0-168\ h}$). The CL_{tot} of gamithromycin was significantly reduced in the presence of rifampicin, and the MRTs were prolonged accordingly. Terminal elimination half-life of gamithromycin was not influenced by rifampicin. Penetration of gamithromycin into the ELF increased in parallel to the plasma exposure, however, without being of statistical significance. Penetration into BALCs did not follow this trend. Interestingly, seven of nine healthy foals responded with lower V_{ss} of gamithromycin after multiple-dose treatment with rifampicin.

Multiple-dose administration of gamithromycin slightly, but not significantly, reduced the steady-state plasma exposure of rifampicin. Rifampicin exposure after single-dose administration was halved by gamithromycin co-administration, however, without any changes in elimination half-life. The metabolic ratios were also not significantly influenced by gamithromycin (Table 2). The T_{max}-values of rifampicin were generally reduced by gamithromycin co-medication. The steady-state trough-levels of rifampicin in plasma, ELF and BALCs amounted to 2.85 ± 1.13 μg/mL, 3.42 ± 1.37 μg/mL and 5.41 ± 3.33 μg/mL, respectively.

In vitro experiments showed that BSP and gamithromycin were extracted by equine hepatocytes via a transport mechanism that could be inhibited by rifampicin (Fig 3). Gamithromycin was also significantly taken up in MDCKII-cells overexpressing human OATP2B1. Furthermore, gamithromycin inhibits the BSP-uptake by human OATP2B1, OATP1B1 and OATP1B3. The IC_{50}-values were 0.79 μmol/L (0.61 μg/mL; 95 % CI: 0.50–1.26 μmol/L, 0.39–0.98 μg/mL), 0.65 μmol/L (0.51 μg/mL; 95 % CI: 0.32–1.32 μmol/L, 0.25–1.03 μg/mL) and 0.65 μmol/L (0.51 μg/mL; 95 % CI: 0.33–1.29 μmol/L, 0.26–1.00 μg/mL) for OATP2B1, OATP1B1 and OATP1B3 respectively.

Discussion

The treatment of abscessing lung infections in foals is highly challenging due to the escalating colonisation of mares by *R. equi*, the growing resistance rate of the bacterium against standard antimicrobial drugs, and the lack of alternatives for efficient eradication (e.g. vaccination). The clinical efficacy of the widely accepted treatment protocols, a combination of a macrolide antibiotic (e.g. clarithromycin, azithromycin) and the ansamycin antibiotic rifampicin, however, can be compromised by severe adverse DDIs as has recently been shown for the combination with clarithromycin. In the latter case the systemic exposure of the macrolide antibiotic is substantially lowered even to levels which are below the MIC_{90} for *R. equi* [5,6]. Therefore, treatment protocols with a lower pharmacokinetic interaction potential should be evaluated and put into clinical practice.

We have shown that the combination of rifampicin and the azalide antibiotic gamithromycin is associated with significant pharmacokinetic DDIs. Co-medication with rifampicin leads, in contrast to the above-mentioned combination of clarithromycin and rifampicin, to an increased exposure of gamithromycin during chronic treatment. The reason for the rifampicin-gamithromycin interaction was most likely due to inhibition of hepatic uptake leading to a reduction in the total body clearance of gamithromycin by rifampicin. The major route of elimination of gamithromycin in various species (e.g. cattle, pigs, rats and dogs) is biliary excretion [18,19]. In cattle, ~50% of gamithromycin is eliminated via faeces and only ~15% via urine [18]. To the best of the authors' knowledge, the route of elimination of gamithromycin in horses has so far not been investigated. However, the total body clearance of gamithromycin after intravenous administration was 12.9 ± 3.89 mL/min/kg, which accounts for ~50% of the BSP clearance (~24 mL/min/kg) in resting, adult horses [29]. The BSP-clearance is a suitable measure to quantify hepatic transporter functions because BSP is primarily extracted from the sinusoidal blood by members of the OATP-family, and secreted into the bile by MRP2 [30]. We have also shown in vitro that gamithromycin is extracted by equine hepatocytes via a transport mechanism which can be inhibited by rifampicin. Furthermore, there was evidence that gamithromycin is a substrate of the human OATP2B1 for which rifampicin is a strong inhibitor [13]. In addition, it should be mentioned that macrolides are substrates of P-gp which is also inhibited by rifampicin [31]. Therefore, we hypothesise that rifampicin interferes primarily with the hepatic uptake and probably biliary secretion of gamithromycin, which is in turn the rationale for elevation of gamithromycin plasma exposure.

We also observed significant DDIs with regard to the combination partner rifampicin. An unexpected novel finding was that i.v. co-medication of gamithromycin impairs rifampicin plasma exposure by nearly 50% after single-dose, and by ~ 30% after multiple-dose administration. In contrast, the half-life and metabolic ratio were not influenced. In both dosing schedules, the rate of rifampicin absorption was increased (T_{max} (control) > T_{max} (single-dose rifampicin) > T_{max} (multiple-dose rifampicin)) which is most likely caused by the known prokinetic effect of the macrolides [32]. It should be noted that this study was designed as a controlled, four-period, consecutive study without randomisation and

TABLE 2: Pharmacokinetic characteristics of rifampicin (rifampicin, 10 mg/kg, p.o.) in nine healthy foals before (control) and during multiple-dose intravenous co-medication with gamithromycin (6 mg/kg once weekly, steady-state conditions) after administration of the first oral dose of rifampicin (single-dose rifampicin), and after chronic, enzyme-inducing treatment with rifampicin (multiple-dose rifampicin). Mean ± s.d. values are given

			Co-medication with gamithromycin	
		Control	Single-dose rifampicin	Multiple-dose rifampicin
AUC	(µg × h/mL)	[a]164 ± 96.7	[a]83.8 ± 35.3*	[b]112 ± 43.1
$AUC_{DAc\text{-}rifampicin}$	(µg × h/mL)	[a]4.10 ± 1.64	[a]2.12 ± 1.01*	[b]3.01 ± 1.03
R_{met}	(%)	2.80 ± 0.98	2.43 ± 0.82	2.76 ± 1.08
C_{max}	(µg/mL)	7.03 ± 3.69	4.82 ± 2.09	7.68 ± 3.12[†]
C_{min}	(µg/mL)	–	–	2.85 ± 1.13
T_{max}	(h)	7.66 ± 5.14	5.56 ± 4.37	4.23 ± 3.77*
$T_{1/2}$	(h)	10.4 ± 1.37	8.99 ± 2.10	–
MRT	(h)	20.2 ± 3.21	16.1 ± 2.75*	22.7 ± 5.77[†]

AUC, area under the plasma concentration-time for rifampicin; $AUC_{DAc\text{-}rifampicin}$, area under the plasma concentration-time for 25-O-desacetyl rifampicin; C_{max}, maximum plasma concentration, steady-state conditions for multiple-dose rifampicin; C_{min}, minimum plasma concentration, steady-state conditions for multiple-dose rifampicin; DAc-rifampicin, 25-O-desacetyl rifampicin; MRT, mean residence time; R_{met}, metabolic ratio; $T_{1/2}$, terminal elimination half-life.
[a]$AUC_{0-\infty}$ after single oral administration
[b]$AUC_{0-24\ h}$ after repeated oral dosing.
*Compared with control, P≤0.05 (Friedman's test with post-hoc analysis).
[†]Compared with single-dose rifampicin during chronic gamithromycin, P≤0.05 (Wilcoxon signed-rank test).

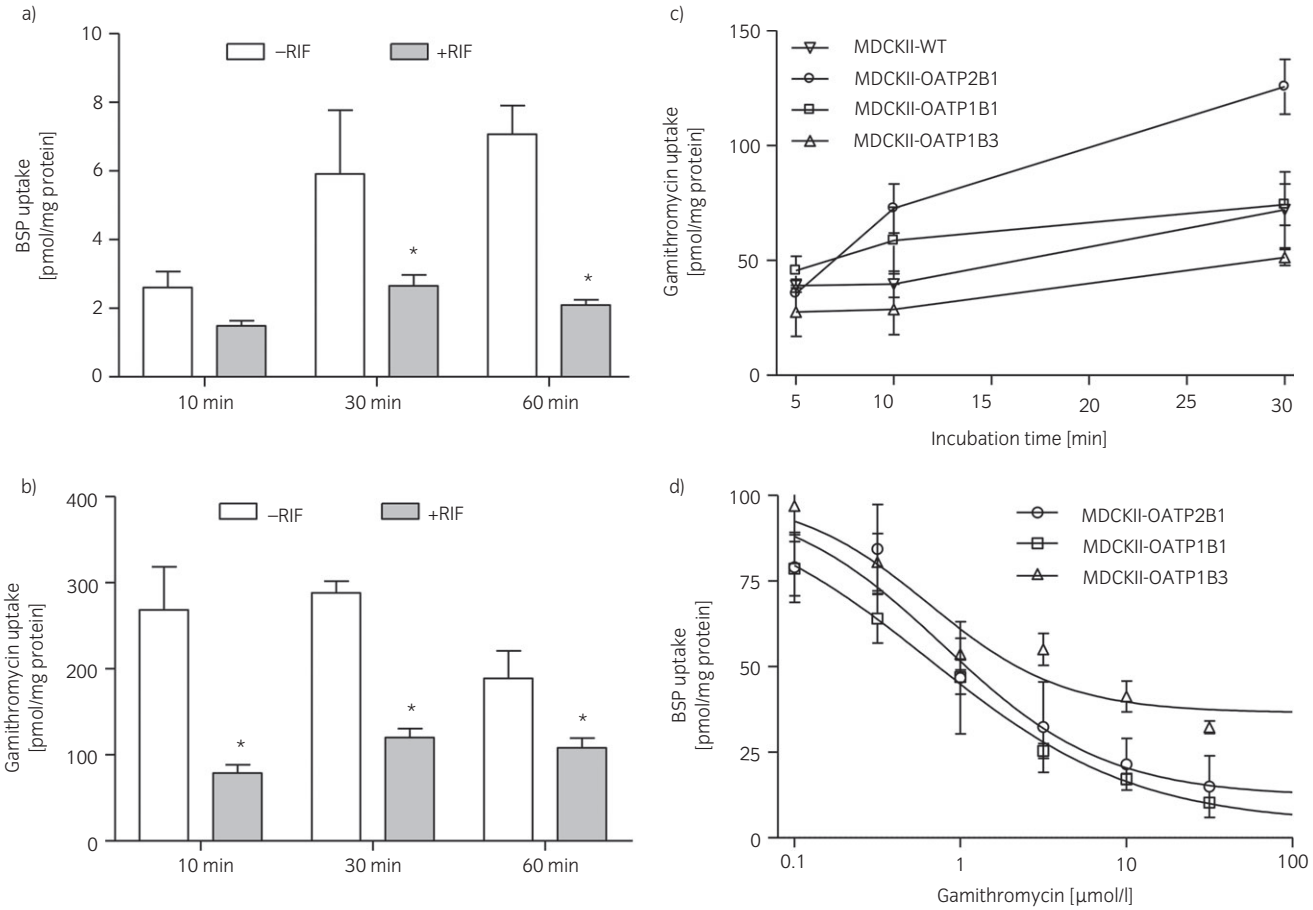

Fig 3: a) Uptake of [^{3}H]-bromosulphthalein (BSP, 0.05 µmol/L) and b) gamithromycin (50 µmol/L) in equine hepatocytes in the absence or presence of rifampicin (RIF; 100 µmol/L). Mean ± s.d. values are given; *P≤0.05 vs. -RIF (One-Way ANOVA with Bonferroni post-hoc test). c) Time-dependent uptake of gamithromycin (50 µmol/L) in MDCKII-cells stably transfected with the human organic anion transporting polypeptides (OATP) 1A2, 2B1, 1B1 and 1B3. d) Concentration-dependent uptake inhibition of [^{3}H]-BSP (0.05 µmol/L) by gamithromycin (0.1–31.6 µmol/L) after 5 min incubation in stably transfected MDCKII-OATP2B1, MDCKII-OATP1B1 and MDCKII-OATP1B3 cells. Mean ± s.d. values are given for three experiments performed in triplicates.

cross-over. Accordingly, it is conceivable that sequence effects, that is, the duration of gamithromycin treatment in particular (4 vs. 6 weeks gamithromycin treatment), might have influenced the study results. The introduction of wash-out periods of sufficient length (≥1 week) between the different sequences would minimise this effect on the one hand, but would also considerably extend the total length of the study on the other. Furthermore, the influence of gamithromycin on disposition of rifampicin seems to be very complex and too challenging to be satisfyingly explained with the currently available experimental evidence. The following issues should be considered:

Firstly, rifampicin is a substrate and PXR-type (auto)-inducer of intestinal and hepatic CYP3A4 and P-gp as confirmed in this study by elevation of the 4β-OH-CHOL/CHOL-ratio [21]. Typically, systemic exposure of rifampicin is decreased by ~60% after chronic oral treatment [33] which is caused by up-regulation of its presystemic ("first-pass") and systemic elimination. It is possible that gamithromycin might be a weak inhibitor of CYP3A4 and P-gp in a similar manner to its analogue azithromycin. Therefore, the expected major reduction in rifampicin exposure by PXR-type auto-induction could be reduced by co-medication with gamithromycin. The 14-membered macrolactones erythromycin or clarithromycin can even significantly increase rifampicin exposure as strong inhibitors. It should be noted that the metabolic ratio was not influenced by gamithromycin, whereby only DAc-rifampicin as the main biologically active metabolite is considered in this ratio. Therefore, interaction of gamithromycin with multiple-dose rifampicin via CYPs and P-gp seems to be of minor influence. The influence on other metabolic pathways of rifampicin was not measured (e.g. formylation, glucuronidation) in the present study [34].

Secondly, there is some evidence that rifampicin is absorbed from the gut via a specific transport mechanism instead of via simple nonionic diffusion. The zwitter ionic rifampicin, with pK_a-values of 1.7 and 7.9 for the acidic C1 and C8 hydroxy groups and the basic piperazine-nitrogen moiety, shows dose-dependent permeability in the jejunum and regio-selective permeability at pH 6.8 [35] which cannot solely be explained by abundance of P-gp along the gastrointestinal tract [36]. Therefore, we hypothesise that gamithromycin lowers intestinal absorption of rifampicin by inhibition of an as yet unknown specific uptake mechanism, for instance by an apical uptake transporter (e.g. OCT) or/and a basolateral efflux carrier (e.g. MRP3) for rifampicin transport in enterocytes. However, consideration must be given to the fact that, after oral administration of 10 mg/kg, an intestinal uptake mechanism might be saturated as a consequence of the high drug concentrations entering the intestinal transporter site after gastric emptying (~1.0 to 1.8 g in this study).

Thirdly, rifampicin is a substrate for the human liver-specific uptake transporters OATP1B1 and 1B3 [13] for which gamithromycin is an inhibitor, as was confirmed in our competition assays. The equine analogue of human OATP1B1/1B3 is OATP1B4 [37]. Therefore, the hepatic extraction of rifampicin via the equine OATP1B4 could be impaired by gamithromycin associated with reduced metabolic disposition, and in turn with increased plasma exposure. Gamithromycin is rapidly and widely distributed (V_{ss} ~ 25 L/kg) in cattle preferentially into the liver, followed by the lung, kidney, fat and muscle, from which it is slowly eliminated. Ten days after administration of 6 mg/kg, the concentration in the bovine liver was still 2790 μg/kg (3.6 μmol/kg) [18]. Therefore, the hepatic uptake of gamithromycin via OATP1B1 and 1B3 can compete with the uptake of rifampicin via the liver specific transporters, at least within the timeframe following parenteral administration when the concentrations in the sinusoidal blood are close to the in vitro IC_{50}-values. Inhibition of hepatic extraction of rifampicin by gamithromycin can therefore be the rationale for the relatively small increase in the plasma 4β-OH-CHOL/CHOL-ratio (1.7-fold) compared with the major increase (~3.1- to 5.8-fold) observed in previous studies [6,33].

Finally, we assume that oral absorption of rifampicin may be influenced in the presence of gamithromycin by competition with an as yet unknown intestinal uptake mechanism for rifampicin. The result is a reduced rifampicin plasma exposure, which can theoretically be intensified by CYP3A4 and P-gp auto-induction after multiple-dose administration of rifampicin. In the complex in vivo situation, the expected drop in rifampicin plasma exposure seems to be substantially reduced by gamithromycin-mediated inhibition of hepatic extraction, and probably by inhibition of intestinal and/or hepatic CYP3A4 and P-gp. The overall outcome of the DDI for rifampicin plasma exposure, however, was still undesired and of statistical significance.

In conclusion, the plasma exposure of gamithromycin during chronic treatment is significantly increased by co-administration of rifampicin, which is most likely caused by inhibition of hepatic elimination. On the other hand, gamithromycin influences the plasma exposure of rifampicin by a complex mechanism. It should be evaluated in future randomised clinical trials whether gamithromycin is a promising alternative to other macrolides in treatment protocols with rifampicin.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The study was approved by the State Authority of Mecklenburg-Vorpommern, Germany (reference code: 7221.3-1-053/14). The owners gave their consent to the inclusion of their foals.

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Authorship

M. Venner contributed to the study design and study execution. M. Grube contributed to the study design. W. Siegmund contributed to the study design and preparation of the manuscript. S. Berlin contributed to the study execution, data analysis and interpretation, and preparation of the manuscript. S. Wallstabe contributed to the study execution. D. Wegner, E. Scheuch, S. Oswald, M. Hasan and A. Ullrich contributed to the data analysis and interpretation. All authors gave their final approval of the manuscript.

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