Clarithromycin is absorbed by an intestinal uptake mechanism which is sensitive to major inhibition by rifampicin – results of a short-time drug interaction study in foals

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List of abbreviations:
ABCB1, P-glycoprotein; ABCC2, multidrug resistance related protein (MRP) 2, CLR, clarithromycin; BALC, broncho-alveolar lavage cells; BAL, broncho-alveolar lavage; BEC, broncho-epithelial cells; OATP, organic anion transporting protein; ELF, epithelial lining fluid; PXR, pregnane-X receptor; RIF, rifampicin; 14OH-CLR, 14-hydroxy-clarithromycin; DAc-RIF, 25-O-desacetylrifampicin; 4βOH-C, 4-β-hydroxycholesterol; MIC₉₀, minimal inhibitory concentration
Abstract
Pulmonary penetration of clarithromycin (CLR) in epithelial lining fluid (ELF) and broncho-alveolar lavage cells (BALC) can be influenced by CYP3A4, ABCB1 and to our hypothesis by a member of the OATP-family for which rifampicin (RIF) is inhibiting in single doses but inducing after long-term co-medication. To assess the partial inhibitory effect, we measured absorption and pulmonary distribution of CLR after short-term (2.5 days) co-medication of RIF after which up-regulation is still not expected. The drug interaction study was performed with five doses (12h interval) of CLR (7.5 mg/kg) and RIF (10 mg/kg) in nine healthy foals; horse transporters are very similar in protein sequence and transcriptional regulation to the human analogues. RIF equally distributed into ELF but reached half the plasma levels in BALC. The de-acetylated metabolite accumulated 1.4-6-fold in ELF and 8-60-fold in BALC. CLR did not significantly influence distribution of RIF. CLR and 14-hydroxy-clarithromycin (14OH-CLR), accumulated approximately 20-40-fold and 1.5-4.5-fold in ELF as well as 300-1,800-fold and 25-90-fold in BALC, respectively. By RIF, plasma levels of CLR dropped down by more than 70% without changing 14OH-CLR formation, half-lives of CLR and 14OH-CLR and the 4-ß-hydroxycholesterol/cholesterol ratio, a surrogate for CYP3A4 induction. CLR was an inhibitor of OATP1B3 (IC50=9.50±3.50µM), OATP1B1 (IC50=46.0±2.27µM), OATP1A2 (IC50=92.6±1.49µM) and OATP2B1 (IC50=384±5.30µM) but not a substrate for these transporters in transfected HEK cells. In conclusion, despite no significant inducing effects, RIF lowers plasma levels of CLR below the MIC90 of pathogenic bacteria most likely by inhibition of an unknown intestinal uptake transporter.
Introduction

The invasion of orally administered drugs to the sites of pharmacodynamic action, e.g. in the lung, can be influenced by the activity of drug metabolizing enzymes and transport proteins in the intestinal wall, the liver and in pulmonary compartments such as the broncho-epithelial cells (BEC), the epithelial lining fluid (ELF) and luminal cells that can be sampled by broncho-alveolar lavage (broncho-alveolar-lavage cells, BALC) (Giacomini et al., 2010; van der Deen et al., 2005). Major factors known to change the function of these enzymes and transporters are genetic polymorphisms, drug interactions and diseases.

Thus, we have recently evaluated oral absorption and pulmonary distribution of clarithromycin (CLR) at steady-state before and after long-term co-medication of rifampicin (RIF) in foals because CLR is a substrate for cytochrome P450 (CYP) 3A4 and for the efflux transporters ABCB1 (and likely for ABCC2) which are expressed in enterocytes, hepatocytes, BEC and BALC and which can be induced by chronic treatment with the pregnane-X receptor (PXR) ligand RIF (Peters et al., 2011; Urquhart et al., 2007). Pharmacokinetic studies in foals are suitable to predict transporter variability in human beings because of their body size enabling non-invasive experimental procedures, because protein homology and expression of drug transporters in horses is similar to rodents and man and that protein expression is regulated at a transcriptional level (Tyden et al., 2009; Tyden et al., 2010)(www.ncbi.nih.gov, www.ebi.ac.uk). CLR and RIF are components of treatment protocols for eradication of Rhodococcus equi which causes severe necrotisizing pneumonia with high mortality rate in foals (Giguère et al., 2004). While information about CLR pharmacokinetics and pulmonary distribution exists to a certain extent
only little is known about RIF pharmacokinetics and its distribution into pulmonary compartments.

Astonishingly for a well established clinical drug combination, we recognized in our long-term study, that the plasma levels of CLR fall by more than 90% even below the minimum inhibitory concentration required to inhibit the growth of 90% (MIC\textsubscript{90%}) of \textit{Rhodococcus equi} (Jacks et al., 2003). The distribution of CLR into the ELF in absolute measures was decreased while, interestingly, it was nearly two-fold increased relative to the (lowered) plasma levels after RIF. A further interesting finding was, that RIF comedication resulted in decreased OATP1A2 mRNA but increased OATP2B1 mRNA expression levels in BEC, whereas the expected up-regulation of the pulmonary efflux transporters ABCB1 and ABCC2 in BEC and BALC did not occur. However, it was unknown whether CLR is a substrate for OATPs. Prior work of Lan et al. showed that CLR is able to inhibit the uptake of estrone-3-sulfate in COS-cells overexpressing OATP1A2 and OATP2B1 even though with low potency (IC\textsubscript{50} >700 µM for OATP1A2, >1400 µM for OATP2B)(Lan et al., 2009). It was also unknown, whether RIF reaches pulmonary compartments in concentrations known to induce PXR-regulated transporter gene expression in-vitro (Meyer zu Schwabedissen et al., 2008; Kast et al., 2002; Geick et al., 2001). Furthermore, we could not clarify whether the nearly abolished CLR absorption resulted entirely from induction of intestinal efflux via ABCB1/ABCC2 or whether a so far unknown intestinal uptake transporter susceptible to inhibition by RIF was involved, most likely a member of the OATP-family for which RIF is a strong inhibitor (Vavricka et al., 2002).

To get deeper insights into the mechanisms behind our major findings with CLR after long-term treatment with RIF, further research was needed, i.e. to measure the
exposure of RIF in lung compartments, to evaluate affinity of CLR to OATPs and to quantify that portion of the absorption deficit that is most likely caused by inhibition of an intestinal uptake transporter for CLR. Therefore, we initiated a cross-over drug interaction study with CLR and RIF at steady-state following short-term repeated dosing by which expression of drug metabolizing enzymes and drug transporters is not expected to be up-regulated in a significant manner. Furthermore, we evaluated in vitro whether CLR is a substrate and/or an inhibitor of OATP1A2, 2B1, 1B1 and 1B3 which are expressed along the oro-hepatic uptake route of CLR into lung compartments.
Material and methods

Animal experimental study

Animals: The pharmacokinetic CLR-RIF interaction study was performed in healthy warm-blooded foals (N=9, 6 females, 3 males, age 6-10 weeks, body weight 102-167 kg) of the Oldenburger trait after approval of the State Authority of Mecklenburg-Vorpommern, Germany. Good health of the animals was confirmed by physical examination including sonography of the lung and routine clinical-chemical and hematological screenings. The animals were housed at natural light rhythm and free access to equine milk, standard pellets, hay, oats and tap water. All clinical examinations were performed in individual stables covered with straw. The foals did not receive any other medication for at least 4 weeks prior to the study.

Study protocol: The drug interaction study was performed controlled, three-period, cross-over with wash-out phases of 11 days between the study periods. In the study periods, the foals received 5 times either 10 mg/kg RIF (Rifampicin, Gruenenthal GmbH, Aachen, Germany), 7.5 mg/kg CLR (Klacid, Abbott, Wiesbaden, Germany) or 10 mg/kg RIF in combination with 7.5 mg/kg CLR in intervals of 12 hours according to the randomization list. For administration, RIF tablets suspended in 30 ml of water and the commercially available CLR suspension were sprinkled in the mouth using a syringe to ensure complete swallowing by the foals. Pharmacokinetics was measured after last administration of the respective study medication in the morning of the 3rd treatment day. Venous blood was collected via an indwelling jugular vein catheter before and 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 12 hours after administration; and after 16, 24, 36 and 48 hours to calculated elimination half-lives correctly. Plasma was separated by centrifugation at 2.000 x g for 10 min and stored at least at -80°C until further analysis. 12 h after administration, a BAL was performed as described.
recently to measure drug distribution in the ELF and BALC (Peters et al., 2011). The BALC pellet consisted of 42-82.5% alveolar macrophages, 16.5-57.0% lymphocytes, 0-1.5% mast cells and 0-2.5% neutrophil granulocytes as confirmed by May-Gruenwald staining.

**Assays for drugs, major metabolites, 4β-hydroxycholesterol and cholesterol**

CLR, 14-hydroxy-clarithromycin (14OH-CLR), RIF and 25-O-desacytilyrifampicin (DAc-RIF) in plasma, lavage fluid and BALC were quantified by LC-MS/MS as recently described (Oswald et al., 2011). The lower limit of quantification (LLOQ) for all matrices was 2.5 ng/ml. Within-day accuracy ranged for all analytes between -6.1 and 13.7% of the nominal concentrations and within-day precision was 1.6 to 12.6% of means (coefficient of variation). Between-day accuracy was -3.6 to 9.1 % of the nominal concentrations and precision was 3.4 to 13.3% of mean controls. Drug concentrations in ELF were assessed by normalizing to the concentration of urea in lavage fluid over the concentrations in plasma and in BALC to a mean macrophage cell volume of 1.2 µl/10⁶ cells in foals (Jacks et al., 2001; Rennard et al., 1986). Plasma concentrations of cholesterol and its metabolite 4β-hydroxycholesterol (4βOH-C) were assayed using GC-MS for an isotope dilution method with [26.26.26.27.27-2H₆] 4βOH-C as an internal standard as described previously (Tomalik-Scharte et al., 2009). The LLOQ was 3.0 ng/ml for plasma. Between-day and within-day precision was 2.1 and 2.7%, respectively, of the mean values and between-day and within-day accuracy was between 2.9 and 3.3% of the nominal values.

**Biometrical evaluation**

Maximum (C_max) and minimum (C_min) plasma concentrations and the time of C_max (t_max) at steady-state were taken from the plasma concentration-time curves. The
area under the plasma concentration-time curve (AUC\textsubscript{0-12h}) was calculated using the trapezoidal rule and the average plasma concentration (C\textsubscript{av}) was derived (AUC/dosing interval). Terminal elimination half-life (t\textsubscript{1/2}) was estimated by log-linear regression analysis of the terminal slope. Arithmetic means ± standard deviations (mean±SD) are given. Differences between two groups were evaluated using the nonparametric Wilcoxon and Mann-Whitney test as appropriate.

**Cellular uptake by HEK-OATPs**

*Chemicals:* CLR, estrone-3-sulphate (E\textsubscript{3}S) and bromosulfophthalein (BSP) were obtained from Sigma-Aldrich (Taufkirchen, Germany). [\textsuperscript{3}H]-BSP (14 Ci/mmol) was purchased from Hartmann Analytic (Braunschweig, Germany) and [\textsuperscript{3}H]-E\textsubscript{3}S (50 Ci/mmol) was acquired from Biotrend (Cologne, Germany).

*Competitions assays with CLR:* HEK-OATP1A2, 1B1, 1B3, 2B1 and the respective vector-transfected control cells (HEK-VC) were established as previously described (Leonhardt et al., 2010; Mandery et al., 2010). For competition assays, the cells were seeded in 24-well plates (BD Biosciences, Heidelberg, Germany) in full growth medium (minimal essential medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine and 2 mM nonessential amino acids; PAA, Coelbe, Germany) for three days at an initial density of 200,000 cells per well. Before starting the experiments, the cells were washed with phosphate buffered saline (PBS, temperature 37°C). [\textsuperscript{3}H]-BSP and [\textsuperscript{3}H]-E\textsubscript{3}S were dissolved in incubation buffer and unlabeled substrates were added to reach final concentrations of 0.05 µM and 1.0 µM BSP for OATP1B1 and OATP1B3, respectively, and of 1.0 µM E\textsubscript{3}S for OATP1A2 and OATP2B1. After incubation at 37°C for 5 min in the presence or absence of CLR, the cells were washed three times with ice-cold PBS and lysed with 0.5% Triton-X-100 and 0.5% sodium deoxycholate. Aliquots were mixed with 2 ml of
scintillation cocktail (Rotiszint eco plus, Carl Roth, Karlsruhe, Germany) and the intracellular accumulation of radioactivity was measured using a scintillation beta counter (type 1409; LKB-Wallac, Turku, Finland). During the incubation time of 5 min, the cellular influx of BSP and E$_3$S was still in the linear range (data are not shown).

**Uptake assays with CLR:** To measure time-dependence of CLR-uptake, HEK-OATP1A2 and 2B1 were incubated with 0.1 mM CLR and HEK-OATP1B1 and 1B3 with 0.01 mM CLR for 60 min. Afterwards, the cellular uptake of CLR in OATP1A2 and 2B1 (3.9-500 µM) and in OATP1B1 and 1B3 (1.9-250 µM) was measured after incubation for 1 min. Cells were validated using E$_3$S (OATP1A2 and 2B1) or BSP (OATP1B1 and 1B3) as substrates and the respective K$_M$ and V$_{max}$ values were 32.5±4.0 µmol/l and 98.7±3.0 pmol/mg×min for OATP1A2, 14.7±4.5 µmol/l and 47.7±4.7 pmol/mg×min for OATP2B1, 4.2±0.7 µmol/l and 52.9±3.6 pmol/mg×min for OATP1B1 and 1.0±0.4 µmol/l and 11.3±1.0 pmol/mg×min for OATP1B3. Inhibition experiments were done using E$_3$S and naringin for OATP1A2 and observed IC$_{50}$ value was 23.6±1.1 µmol/l. For OATP2B1, 1B1 and 1B1 RIF was used as inhibitor and E$_3$S (OATP2B1) and BSP (OATP1B1 and 1B3) were used as substrates. IC$_{50}$ values were 3.83±0.69, 11.7±1.1 and 2.53±1.1 µmol/l for OATP2B1, 1B1 and 1B3 respectively. Positive control experiments with standard substrates and inhibitors were done in parallel to uptake experiments with CLR and revealed inhibitable uptake into transfected cells.

After adding 0.2% sodium dodecyl sulfate, CLR was quantified in cell lysates using LC-MS/MS as described before (Oswald et al., 2011). Protein concentrations were determined using the BCA Protein Assay Kit (Thermo Fisher Scientific Inc. Rockford, USA) according to the manufacturer’s instructions. All experiments were performed in triplicates.
In all experiments, the substrate uptake in OATP-transfected cells was corrected by the vector control uptake. In competition assays, 50% inhibitory concentration (IC\textsubscript{50}) was calculated by fitting the data to a sigmoidal dose-response regression curve using Prism 5 (GraphPad Software, San Diego, CA, USA). Means±SD are given.
Results

Pharmacokinetic interaction between CLR and RIF

RIF was slowly absorbed from the gastrointestinal tract and reached maximum plasma concentrations between 7.93 and 19.5 µg/ml after administration of the last maintenance dose (Figure 1). The parent drug was eliminated with half-lives between 9.48 and 19.8 hours. Formation of the de-acetylated metabolite DAc-RIF accounted for <1 % of the plasma exposure of total RIF. RIF distributed equally into the ELF but reached only half the plasma levels in BALC. In contrast, the more polar metabolite achieved concentration gradients of 1.3-6 in ELF and approximately 8-60 in BALC relative to plasma (Table 1, Figure 2). Co-medication of CLR did not significantly influence any pharmacokinetic characteristics and pulmonary distribution of RIF except formation of the de-acetylated metabolite which was numerically but not significantly decreased in presence of CLR.

CLR after short-term administration was faster absorbed than RIF; t\text{max} occurred approximately one hour earlier. Plasma exposure, accumulation in pulmonary compartments, formation of 14OH-CLR and elimination rates for CLR and the metabolite were quite similar to the data obtained in our recent long-time study which was performed with the same staff and in identical experimental environment (Peters et al., 2011). By co-medication of RIF, the plasma concentrations dropped down by more than 70 % without change of 14OH-CLR formation and without influencing half-lives of CLR and 14OH-CLR (Table 2, Figure 3). Relative to the lowered plasma levels, the accumulation of CLR in ELF was significantly increased and, consequently, the BALC/ELF-ratio decreased (Figure 4).

The 4βOH-C/cholesterol ratio was not significantly different in the treatment groups (CLR, 1.7±1.0; RIF, 1.8±0.8; CLR with RIF, 1.6±0.6).
Affinity of CLR to HEK-OATP1A2, 2B1, 1B1, 1B3

In competition assays with the model substrates BSP and E₃S, respectively, CLR was a strong inhibitor of the liver specific uptake transporters OATP1B3 (IC₅₀=9.50±3.50 µM) and OATP1B1 (IC₅₀=46.0±2.27 µM). OATP1A2 (IC₅₀=92.6±1.49 µM) and OATP2B1 (IC₅₀=384±5.30 µM) were inhibited with markedly lower potency (Figure 5). The uptake of CLR in all OATP-transfected cells was not significantly different from the uptake in vector control cells (data not shown).
Discussion

We provide for the first time data on penetration of RIF and of its major de-acetylated metabolite into pulmonary compartments of foals under steady-state condition. At distribution equilibrium, RIF permeated unrestricted into ELF but reached approximately 40-80% lower levels in BALC. Interestingly, the de-acetylated, more polar metabolite accumulated 1.4-6-fold in ELF and 8-60-fold in BALC thus accounting for approximately 2% of total drug exposure in ELF but 25% in BALC. Therefore, a significant part of the antimicrobial effect of RIF in BALC must be attributed to DAc-RIF which is as active as the parent compound (Acocella, 1978). However, the concentrations of unbound RIF in plasma and in all pulmonary compartments at steady state were in excess of the MIC90 for equine pathogens as β-hemolytic streptococci (<0.5 µg/ml), *Staphylococcus* spp. (1 µg/ml), *Pasteurella* spp. (1 µg/ml) and *Rhodococcus equi* (<0.5 µg/ml) (Jacks et al., 2003). Furthermore, the concentrations of RIF in plasma and in the lung compartments were also in the magnitude for which transcriptional regulation via the nuclear PXR-receptor pathway was shown in-vitro for CYP3A4 (Luo et al., 2002) and for the multidrug transporters ABCB1 (Geick et al., 2001), ABCC2 (Kast et al., 2002) and OATP1A2 (Meyer zu Schwabedissen et al., 2008) which are expressed in BEC and BALC of foals (Peters et al., 2011). Our steady-state data are more reliable to predict pharmacodynamic effects of RIF in the therapeutic in-vivo situation than the data of Ziglam et al. from a single dose study with arbitrary sampling at times points of non-equilibrium. They found in ELF of patients approximately one third but in BALC 16-fold the concentrations in plasma ~2-5 hours after a single 600 mg dose (microbiological assay) (Ziglam et al., 2002).
Contrary to our expectation, pharmacokinetics of RIF was not influenced by co-medication of CLR although RIF is a substrate of the liver specific uptake transporters OATP1B1 and 1B3 (Vavricka et al., 2002) which can be inhibited by CLR at concentrations that most likely occur in portal venous blood after oral administration of therapeutic doses (our study data and (Seithel et al., 2007)).

A second finding of our short-time study was the extreme loss of CLR bioavailability by more than 70% in the presence of RIF and, in parallel, the lower penetration in ELF and BALC by approximately 65% and 80%, respectively (Table 2). The average plasma concentrations of CLR which were in absence of RIF (~0.40 µg/ml) already quite near to the MIC90 for β-hemolytic streptococci (<0.06 µg/ml), Staphylococcus spp. (0.25 µg/ml), Pasteurella spp. (1 µg/ml) and Rhodococcus equi (0.12 µg/ml), dropped down to inactive levels in presence of RIF (~0.10 µg/ml) (Jacks et al., 2003).

In contrast to our hypothesis, these results were rather similar to the data of our long-term CLR-RIF interaction study (initial loading with 7.5 mg/kg CLR b.i.d. for 7 days followed by 7.5 mg/kg CLR plus 10 mg/kg RIF b.i.d. for 11 days) (Peters et al., 2011). Our findings were also in good agreement with data from rats published by Garver et al., showing a similar decrease in CLR bioavailability after CLR coadministration (Garver et al., 2008).

The short-term study was initiated to achieve steady-state condition for RIF and CLR without significant up-regulation of CYP3A4, ABCB1 and ABCC2 which are involved in disposition of CLR (Munic et al., 2010; Suzuki et al., 2003). A two-day treatment is unlikely to produce significant because substantial changes in activity take longer to develop than two days, although increased transcription is evident much earlier (Zhou et al., 2006). Evidences for absence of significant enzyme induction in our short-term study were the following: Firstly, the plasma 4βOH-C/cholesterol ratio was...
not significantly elevated as in our former study after chronic administration of RIF (Peters et al., 2011). In man, induction of CYP3A4 by RIF leads to a significant increase of the 4ßOH-C/cholesterol ratio after one week; the maximum (4-fold) elevation is reached after 14 days. Significant changes are not expected after two days even if there is a considerable induction (>10-fold) (Diczfalusy et al., 2009; Kanebratt et al., 2008). However, a certain degree of induction cannot be fully excluded because the 4ßOH-C/cholesterol ratio is relatively insensitive to rapid changes in enzyme expression (Yang and Rodrigues, 2010), presumably because of the long half-life of the metabolite (~17 days). Secondly, a five-day treatment with RIF was found to be insufficient for RIF-type induction in horses/foals (Burrows et al., 1992). Thirdly, the terminal elimination of 14OH-CLR by CYP450 mediated enzymes (N-demethylation, hydroxylation) was unchanged after short-term co-medication of RIF (7.28±1.35 h versus 6.60±1.76 h, n.s.) but significantly lowered after long-term treatment (8.11±1.61 h versus 5.10±0.88 h, p<0.05) (Peters et al., 2011; Suzuki et al., 2003). Therefore, the increased proportion of 14OH-CLR exposure (AUC0-12h) relative to the AUC of total CLR (0.52±0.13 vs. 0.21±0.06, p<0.01) is most likely not caused by induction of CYP3A4 hydroxylation of CLR (Suzuki et al., 2003) and, in turn, induction of presystemic metabolism of CLR is not the major reason for >70% lowering of bioavailability (please notice the discussion below on alternative reasons). Because of the short RIF exposure, we also assume that intestinal efflux via ABCB1 and ABCC2 was most likely also not significantly up-regulated although the plasma concentrations of RIF were sufficient to regulate CYP3A4, ABCB1 and ABCC2 in-vitro. Therefore, the loss of bioavailability must have been entirely or in its major portion resulted from inhibition of intestinal uptake mechanisms for CLR.
Potential intestinal uptake carriers for CLR might be members of the OATP-family because CLR inhibits the uptake of taurocholate in OATP-transfected MDCK cells and because its oral absorption in rats can be markedly reduced by co-medication of RIF (Garver et al., 2008; Lan et al., 2009). In our in-vitro studies using transfected HEK-cells, CLR was also an inhibitor with potency for OATP1B > OATP1B1 > OATP1A2 > OATP2B1 similar to previous results of Seithel et al. and Lan et al. (Seithel et al., 2007; Lan et al., 2009). However, CLR is not a substrate for OATP1A2, 1B1, 1B3 and 2B1 as clearly shown by the data of the in-vitro part of our study.

Interestingly, we and others found that plasma exposure with 14OH-CLR (AUC, trough levels) is nearly not influenced by RIF, neither after short-time nor after long-term co-medication although CLR absorption was dramatically reduced (Peters et al., 2011; Taki et al., 2007; Wallace, Jr. et al., 1995); the lower AUC0-12h in our chronic study is explained by shortening the half-life of 14OH-CLR following induction of its terminal metabolism (Ferrero et al., 1990). It might be, that the proximal intestine is the major site of CLR hydroxylation in horses, where CYP3A-metabolic activity is highly expressed (Tyden et al., 2004). If intestinal metabolism is not influenced or even induced by RIF, than the uptake carrier for CLR must be located along the “first-pass” route beyond the place of intestinal biotransformation, i.e. in the basolateral membrane of enterocytes. Possible candidates might be ABCC1 and ABCC3 as these transporters are highly expressed within the basolateral membrane of the enterocytes, acting there as efflux transporters pumping their substrates towards the blood of the submucosal vessels. Inhibition of ABCC1 and/or ABCC3 by rifampicin is therefore expected to result in lower bioavailability of CLR (Giacomini et al., 2010; Oswald et al., 2006). In addition there exists evidence that basolateral transporters
have an impact on the absorption of orally administered drugs (Torii et al., 2002). In conclusion, to discover intestinal uptake carriers for macrolides, further research particularly directed to efflux transporters in the basolateral membrane of enterocytes is needed.
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Authorship Contributions

Participated in research design: Peters, Venner and Siegmund.

Conducted experiments: Peters, Eggers, Block, Lämmer

Contributed new reagents or analytic tools: Oswald and Lütjohann

Performed data analysis: Peters, Eggers and Oswald.

Wrote or contributed to the writing of the manuscript: Peters, Oswald, Venner and Siegmund.
Reference List


vitro: comparison between the identification from disappearance rate and that from formation rate of metabolites. Drug Metab Pharmacokinet 18:104-113.


Footnotes

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Conflict of interest: The authors declare that they have no conflict of interest.
Legends for Figures

**Figure 1:** Plasma-concentration time curves of rifampicin and 25-O-desacetylrifampicin after last administration of 10 mg/kg rifampicin alone and after co-medication with 7.5 mg/kg clarithromycin in nine healthy foals.

**Figure 2:** Penetration of rifampicin (upper figures) and 25-O-desacetylrifampicin (lower figures) in bronchial epithelial lining fluid (ELF) and broncho-alveolar lavage cells (BALC) after last administration of 10 mg/kg rifampicin alone (-CLR) and after comedication of 7.5 mg/kg clarithromycin (+CLR).

**Figure 3:** Plasma-concentration time curves of clarithromycin and 14-hydroxy-clarithromycin (14OH-clarithromycin) after last administration of 7.5 mg/kg clarithromycin alone and after co-medication with 10 mg/kg rifampicin in nine healthy foals.

**Figure 4:** Accumulation of clarithromycin (upper figures) and 14-hydroxy-clarithromycin (lower figures) in bronchial epithelial lining fluid (ELF) and broncho-alveolar lavage cells (BALC) after last administration of 7.5 mg/kg clarithromycin alone (-RIF) and after comedication of 10 mg/kg rifampicin (+RIF).

**Figure 5:** Competition of clarithromycin with the uptake of estrone-3-sulphate (E\textsubscript{3S}, 1.0 µM) in HEK cells transfected with OATP1A2 and OATP2B1 (upper figures) and with bromosulfophthalein (BSP) in cells transfected with OATP1B1 (0.05 µM) and OATP1B3 (0.1 µM), respectively (lower figures). Means±S.D. are given for three experiments each performed in triplicate.
**Table 1**: Pharmacokinetic characteristics and distribution of rifampicin in pulmonary epithelial lining fluid (ELF) and broncho-alveolar lavage cells (BALC) after mono-therapy with 10 mg/kg rifampicin and after co-medication of 7.5 mg/kg clarithromycin (CLR) in nine healthy foals. Mean ± SD are given.

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<td>11.0±5.01</td>
<td>3.42±2.58</td>
<td>14.5±5.01</td>
<td>8.12±5.86</td>
<td>6.65±3.66††</td>
</tr>
<tr>
<td><strong>25-O-desacyl rifampicin</strong></td>
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</tr>
<tr>
<td>without CLR</td>
<td>0.91±0.42</td>
<td>0.10±0.05</td>
<td>0.08±0.05</td>
<td>3.86±2.87</td>
<td>12.1±4.05</td>
<td>0.19±0.10††</td>
<td>1.44±0.62††</td>
</tr>
<tr>
<td>with CLR</td>
<td>0.75±0.22</td>
<td>0.08±0.02</td>
<td>0.07±0.02</td>
<td>5.34±3.48</td>
<td>10.1±1.85</td>
<td>0.18±0.07††</td>
<td>1.73±1.42††</td>
</tr>
</tbody>
</table>

*p<0.05 versus without clarithromycin (Wilcoxon); †<0.05, ††<0.01 versus plasma<sub>12h</sub> (Mann Whitney)
Table 2: Pharmacokinetic characteristics and distribution of clarithromycin in pulmonary epithelial lining fluid (ELF) and broncho-alveolar lavage cells (BALC) after mono-therapy with 7.5 mg/kg and after co-medication of 10 mg/kg rifampicin (RIF) in nine healthy foals. Mean ± SD are given.

<table>
<thead>
<tr>
<th></th>
<th>AUC$_{0-12h}$ (µg·h/ml)</th>
<th>C$_{max}$ (µg/ml)</th>
<th>C$_{min}$ (ng/ml)</th>
<th>t$_{max}$ (h)</th>
<th>t$_{1/2}$ (h)</th>
<th>ELF$_{12h}$ (µg/ml)</th>
<th>BALC$_{12h}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>clarithromycin</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>without RIF</td>
<td>4.76±3.50</td>
<td>0.61±0.37</td>
<td>0.21±0.23</td>
<td>2.44±0.65</td>
<td>7.17±1.92</td>
<td>9.95±19.9††</td>
<td>116±137††</td>
</tr>
<tr>
<td>with RIF</td>
<td>1.17±0.93*</td>
<td>0.18±0.14*</td>
<td>0.05±0.06</td>
<td>2.35±1.67</td>
<td>7.25±2.11</td>
<td>3.34±5.54††</td>
<td>24.8±29.0††</td>
</tr>
<tr>
<td><strong>14-hydroxy-clarithromycin</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>without RIF</td>
<td>1.09±0.64</td>
<td>0.13±0.06</td>
<td>0.06±0.05</td>
<td>2.48±0.48</td>
<td>7.28±1.35</td>
<td>0.16±0.23†</td>
<td>1.90±1.17††</td>
</tr>
<tr>
<td>with RIF</td>
<td>0.99±0.66</td>
<td>0.12±0.07</td>
<td>0.05±0.04</td>
<td>2.72±2.62</td>
<td>6.60±1.76</td>
<td>0.08±0.10*</td>
<td>1.15±0.78††</td>
</tr>
</tbody>
</table>

*p<0.05 versus without rifampicin (Wilcoxon); †<0.05, ††<0.01 versus plasma$_{12h}$ (Mann Whitney)
Figure 2

- **ELF / plasma**
  - p = 0.767

- **BALC / plasma**
  - p = 0.214

- **BALC / ELF**
  - p = 0.038

- **ELF / plasma**
  - p = 0.953

- **BALC / plasma**
  - p = 0.594

- **BALC / ELF**
  - p = 0.678