ABSTRACT:
The delivery of clarithromycin (CRL) to its site of action in bronchial/alveolar epithelial cells (EC), bronchial epithelial lining fluid (ELF), and bronchoalveolar lavage cells (BALC) may be influenced by CYP3A4 and the drug transporters, ATP-binding cassette (ABC) B1 and ABC2, and organic anion-transporting polypeptides (OATPs), which can be modulated and/or up-regulated via the nuclear pregnane X receptor (PXR) by rifampicin (RIF). Therefore, we evaluated the disposition and pulmonary distribution of CLR (7.5 mg/kg b.i.d., 21 days) and expression of ABCB1, ABC2, OATP1A2, and OATP2B1 in EC and BALC before and after comedication of RIF (10 mg/kg b.i.d., 11 days) in nine healthy foals (41–61 days, 115–159 kg) in which the genetic homology of drug transporters is close to that of their human analogs. After RIF comedication, relative bioavailability of CLR decreased by more than 90%. Concentrations in plasma (29.8 ± 26.3 versus 462 ± 368 ng/ml), ELF (0.69 ± 0.66 versus 9.49 ± 6.12 μg/ml), and BALC (10.2 ± 10.2 μg/ml 264 ± 375 μg/ml; all P < 0.05) were lowered drastically, whereas levels of the metabolite 14-hydroxyclarithromycin were not elevated despite higher 4β-hydroxycholesterol/cholesterol plasma concentration ratio, a surrogate for CYP3A4 induction. In the presence of CLR, ABC2 and PXR mRNA contents were significantly and coordinately (r² = 0.664, P < 0.001) reduced in BALC after RIF. In EC, mRNA expression of OATP1A2 increased but that of OATP2B1 decreased (both P < 0.05). RIF interrupts oral absorption and decreases CRL plasma levels below the minimal inhibitory concentration for eradication of Rhodococcus equi. Evidence that RIF influences the cellular uptake of CLR in bronchial cells and the PXR expression in BALC in the presence of high CLR concentrations exists.

Introduction
Efficacy of drugs is dependent on the availability of active concentrations at the site(s) of the desired pharmacodynamic effect. In the case of infectious lung diseases, for instance, the minimal inhibition concentrations (MICs) for antimicrobial agents must be exceeded in the environment of the respective bacteria, i.e., in the bronchial and alveolar epithelial cells (EC), in the bronchial epithelial lining fluid (ELF), and in bronchoalveolar lavage cells (BALC) of which approximately 80% are alveolar macrophages. Thus, the frequently prescribed macrolide antibiotics penetrate into these pulmonary spaces to reach drug levels many times greater than the concurrent plasma concentrations at steady-state (BALC > ELF > plasma) (Conte et al., 1995). The mechanisms by which macrolides accumulate in pulmonary cells are poorly understood. So far, trapping of the basic compounds in the acidic compartment of alveolar macrophages (i.e., lysosomes and endosomes) is the only plausible rationale. However, BALC/plasma gradients of 50 to 100:1 and steeper and accumulation in the alveolar/bronchial ELF are not solely explainable by base trapping. There is ample evidence that unidirectional penetration of drugs from the blood throughout the vascular and the alveolar/bronchial epithelium into the alveolar/bronchial ELF and from there into the BALC is achieved by the coordinate interplay of multidrug transporters. At present, it is know that the cell membranes along the pulmonary penetration route are equipped with 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 along the intestinal/hepatic absorption route of the drugs (Chan et al., 2004; Bosquillon, 2010).

To evaluate whether and how multidrug transport proteins influence absorption and pulmonary distribution of macrolides, we initi-
ated a multiple-dose drug interaction study with clarithromycin (CLR) and rifampicin (RIF) in healthy foals. CLR is a substrate of cytochrome P450 3A4, of ABCB1 (P-glycoprotein), and probably of ABCC2 (multidrug resistance-associated protein 2) and of OATPs (Suzuki et al., 2003; Seithel et al., 2007; Garver et al., 2008; Lan et al., 2009; Munić et al., 2010). The efflux carriers ABCB1 and ABCC2 and several OATPs are modulated in the presence of RIF (Geick et al., 2001; Vavricka et al., 2002). After chronic treatment, however, RIF can up-regulate gene expression of intestinal and hepatic CYP3A4, ABCB1, ABCC2, and OAP1A2 via the nuclear pregnane X receptor (PXR) pathway (Vavricka et al., 2002; Zong and Pollack, 2003; Lau et al., 2006; Tirona, 2011). We have recently shown that RIF may also regulate pulmonary ABCB1 and ABCC2 of healthy foals (Venner et al., 2010). Therefore, the overall changes in absorption and pulmonary distribution of CLR may be caused by competitive effects in the presence of RIF and by the chronic effects as caused by PXR-type transporter induction.

We have chosen foals for our mechanistic drug interaction study because of the clinical challenge in horse-breeding to eradicate Rhodococcus equi, which resists innate macrophage defense in adult horses but causes severe seaseous, necrotizing lung infection in foals with high mortality rate of up to 80% (Hillidge, 1987). Combined antimicrobial therapy with macrolides and RIF has become the most effective treatment protocol to increase the survival rate from 20% to nearly 90% (Hillidge, 1987). In a retrospective study, it was shown that the combination of RIF with CLR is superior to combinations with erythromycin or azithromycin (Giguère et al., 2004). Furthermore, we have chosen the animal model because bronchoscopy and bronchoalveolar lavage are accepted diagnostic techniques in foals for sampling of biomaterial. Finally, the sequence homology of the equine drug transporters is distinctly closer to that of the human analogs (ABCB1, 92%; ABCC2, 82%; OATP1A2, 85%; and OATP2B1, 89% on protein basis) than, for instance, the homology of the transporter of rats, which are often used to predict the situations in human beings (Abcb1a, 86%; Abcb1b, 79%; Abcc2, 77%; Oatp1a5, 72%; Oatp2b1, 76% on protein basis, http://www.ncbi.nlm.nih.gov, http://www.ebi.ac.uk; Hagenbuch and Meier, 2004).

Materials and Methods

Study Protocol. Animals. The drug interaction study was performed after approval by the State Authority of Mecklenburg-Vorpommern (reference code: LALLF M-VTSD/72213-1.1-066/08). Nine foals (four females and five males, aged 41–61 days, body weight 115–159 kg) of warm-blooded horses of the Oldenburgian breed were included after confirmation of good health by physical examinations including sonography of the lungs and routine clinical and hematological screenings. The animals were kept at natural light rhythm on paddocks together with their mares and had free access to equine milk, hay, oats, and tap water. All clinical examinations were done in individual stalls that were covered with straw. Before the study, the foals did not receive any other medication.

Study design. The drug interaction study was performed under steady-state conditions within a study period of 21 days. Initially, the foals were treated orally with 7.5 mg/kg CLR (Abbott, Wiesbaden, Germany) twice daily for 6 days. A last dose was given on the morning of the 7th study day. On treatment days 9 to 19, the animals received 7.5 mg/kg CLR and 10 mg/kg RIF (Gruententhal GmbH, Aachen, Germany) twice daily. Administration of CLR was continued until the morning of the 21st day, whereas RIF was not given on the 20th day or on the morning of the 21st day to avoid competitive interferences with the pharmacokinetics of CLR after the last administration. RIF tablets suspended in 30 ml of water and the commercial CLR suspension were sprinkled in the mouth using a syringe to ensure complete swallowing by the foals.

To evaluate the pharmacokinetics of CLR on treatment days 7 and 21, venous blood was collected via an indwelling jugular vein cannula (VYGON, Aachen, Germany) before and 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 36, and 48 h after administration. Plasma was separated by centrifugation at 2000xg for 10 min and stored at least at −80°C until further analysis. To measure drug distribution in the ELF and BALT, a bronchoalveolar lavage was performed 12 h after the last administrations of CLR of each study period as described recently (Venner et al., 2010). In brief, after anesthesia with ketamine (Sermwerke, Bernburg, Germany) and diazepam (CT Arzneimittel, Berlin, Germany), a flexible fiberscope (Karl Storz, Tuttinglen, Germany) was advanced through the nose into the trachea to take two biopsy specimens from the bronchial epithelium behind the carina. Then, the endoscope was advanced and wedged in a second-generation bronchus. The lavage was performed by repeated instillation of 100 ml of phosphate-buffered saline (pH 7.4, temperature approximately 37°C). The first aspirate was discarded to remove the excess of epithelial cells. Three additional aliquots were combined, filtered through a layer of gauze (Lohmann and Rauscher, Neuwied, Germany), and centrifuged at 400xg for 10 min. The BALT pellet consisted of 77 to 84.5% alveolar macrophages, 11.5 to 18.5% lymphocytes, and 2.5 to 8.5% mast cells as confirmed by May-Grunwald staining. For mRNA analysis of ABCB1, ABCC2, and PXR, the biopsy specimens and aliquots of 5 × 10⁶ BALT were incubated overnight with RNAlater buffer (4°C) (QIAGEN, Hilden, Germany) before freezing. For drug analysis, aliquots of BALT and of the supernatant were shock frozen using dry ice and stored at −80°C until further use.

Quantitative Assays for Clarithromycin and 4-Hydroxycholesterol. CLR and 14-hydroxyclarithromycin (14-OH-CLR) were quantified in plasma, lung lavage fluid, and BALT using a validated liquid chromatography-tandem mass spectrometry method as described recently (Oswald et al., 2011a). The limit of quantification for all matrices was 2.5 ng/ml. The within-day accuracy of the assay ranged from −1.1 to 9.6% of the nominal concentrations, and precision was 1.6 to 11.2% of means (coefficient of variation). Between-day accuracy was −11.7 to 8.6% of the nominal concentrations and precision was 3.7 to 10.0% of the respective mean control values. CLR and 14-OH-CLR concentrations in ELF were assessed by normalizing to the concentration ratio of urea in plasma over bronchoalveolar fluid and in BALT to a mean macrophage cell volume of 1.2 µm³ cells in foals (Rennard et al., 1986; Jaks et al., 2001). Urea was quantified using a LT-UR 0010 kit (Labotechnick Eberhard Lehmann, Berlin, Germany).

Plasma concentrations of 4-hydroxycholesterol (4-OH-C) were assayed using gas chromatography-mass spectrometry for an isotope dilution method with [26,26,26,27,27,27-2H₆]4-OH-C as an internal standard as described previously (Tomalik-Scharte et al., 2009). The lower limit of quantification 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.

Quantitative mRNA Expression of Drug Transporters and PXR. The bronchial biopsy specimens and BALT were homogenized using a dismembrator (Braun, Melsungen, Germany), total RNA was prepared by applying a NucleoSpin RNAII Kit (Macherey-Nagel, Düren, Germany), and reverse transcription of 100 ng of RNA was performed using a SuperScript VILO cDNA Kit (Invitrogen, Karlsruhe, Germany) according to the protocols of the manufacturer. The quantitative real-time RT-PCR analysis for ABCB1, ABCC2, and PXR was conducted using primers selected for equine mRNA (PrimerDesign, Southampton, UK). The sequences were as follows: ABCB1_SE, 5'-AGAGATGTCTTGTTGG-TATCTTCA-3'; ABCB1_AS, 5'-GACATTGTTGGTTGCTGAT-3'; ABCC2_SE, 5'-ACCTCAAGT-CCACCAACATCTCC-3'; ABCC2_AS, 5'-CACCTGCTAATCCCAAGAG-3'; PXR_SE, 5'-CGATTTGCAAAATTTGATTAATTCAGC-3'; PXR_AS, 5'-CGGAGGCGCATTAG-GAATAGTAGAAT-3'. Sequences for the probes for ABCB1, ABCC2, and PXR were 5'-FAM-CAATCAGAACCTGATTTCCTGCGCC-3'; 5'-FAM-CCATA-GGGCGCAACTCTTTGACCTTCTC-3'; and 5'-FAM-CTCTTGCACCA-CATCTCATACGG-3', respectively. For mRNA quantification of OATP1A2 and OATP2B1, we used equine primers according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA). The RT-PCR was performed using the TaqMan method with the human IIS RNA (Applied Biosystems) as reference because of the high homology between the equine and human genes. Each experiment was conducted in duplicate. Gene expression was quantified using the ΔΔCt method.

Biometrical Evaluation. Maximum (Cmax) and minimum (Cmin) plasma concentrations and the time of Cmax (tmax) at steady state were taken from the
INTERACTION OF CLARITHROMYCIN WITH RIFAMPICIN IN FOALS

CLR was slowly absorbed during the chronic oral treatment to generate average plasma concentrations between 133 and 1300 ng/ml at steady state (Fig. 1; Table 1). The plasma exposure (AUC_{0–12 h}) of the metabolite 14-OH-CLR amounted to 33 ± 9% of the CLR exposure (metabolic ratio). CLR accumulated along the pulmonary distribution route to reach more than 30-fold higher levels in the ELF and 700-fold higher concentrations in BALC than in plasma at trough 12 h after administration. (Note in Table 1 that the concentrations for CLR and 14-OH-CLR in plasma are given in nanograms per milliliter and those in ELF and BALC are given in micrograms per milliliter.) 14-OH-CLR underwent similar accumulation although to a lower extent (ELF, approximately 5-fold; BALC, approximately 44-fold) (Table 2). CLR was eliminated in foals at steady-state with terminal half-lives between 4.7 and 7.3 h.

After comedication of RIF for 11 days, relative bioavailability of CLR decreased by more than 90%; accordingly, the C_{av} was significantly reduced and fell even below the MIC for _R. equi_ (Jacks et al., 2003). The elimination rate was not significantly influenced. The drastic decrease in CLR bioavailability did not result in a proportional increase in metabolite exposure, although relative 14-OH-CLR exposure increased more than 7-fold compared with the situation before RIF comedication (metabolic ratio 2.56 ± 0.97 versus 0.33 ± 0.09, _P_ < 0.05). Lower bioavailability of CLR after RIF was also associated with parallel lowering of CLR and 14-OH-CLR in ELF and BALC by more than 90 and 60%, respectively, compared with the levels reached by CLR monotherapy. A remarkable finding is that CLR penetrated significantly better into the ELF after RIF comedication as confirmed by the increase in the ELF/plasma concentration ratio (Fig. 2). RIF treatment was also associated with induction of systemic metabolic activity. The 4β-OH-Cl/cholesterol plasma concentration ratio significantly increased from 0.68 ± 0.34 to 1.05 ± 0.40 (_p_ = 0.004) (Fig. 3). However, the pulmonary mRNA expression of the efflux transporters ABCB1 and ABCC2 and of the nuclear PXR receptor was not significantly up-regulated by RIF; the bronchoalveolar mRNA content of _ABC2C_ and _PXR_ was even significantly reduced (Fig. 4). Nevertheless, we found a significant correlation between the decrease in _PXR_ mRNA and the decrease in _ABC2C_ mRNA expression in BALC (Fig. 5). _OATP1A2_ mRNA and _OATP2B1_ mRNA were expressed in EC. In BALC, only _OATP2B1_ was markedly expressed. RIF comedication significantly decreased the mRNA content of _OATP1A2_ in EC but increased that of _OATP2B1_ (Fig. 6). Content of both _OATP_ mRNAs was not significantly correlated to the content of _PXR_ mRNA.

**Discussion**

By this drug interaction study, it was clearly shown that chronic comedication of RIF leads to a dramatic lowering of the average

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**TABLE 1**

Pharmacokinetic characteristics of clarithromycin and 14-hydroxylaritromycin in nine healthy foals at steady state after monotherapy of clarithromycin (7.5 mg/kg b.w.t. b.i.d. for 7 days) followed by comedication of rifampicin (10 mg/kg b.w.t. b.i.d. for 11 days) and concentrations of clarithromycin and 14-hydroxylaritromycin in plasma

<table>
<thead>
<tr>
<th></th>
<th>AUC_{0–12 h}</th>
<th>C_{max}</th>
<th>C_{min}</th>
<th>C_{av}</th>
<th>t_{max}</th>
<th>t_{1/2}</th>
<th>Plasma_{12 h}</th>
<th>ELF_{12 h}</th>
<th>BALC_{12 h}</th>
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<tr>
<td></td>
<td>µg × h/ml</td>
<td>ng/ml</td>
<td>ng/ml</td>
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<td>ng/ml</td>
<td>µg/ml</td>
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<td>Clarithromycin</td>
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<tr>
<td>Without RIF</td>
<td>5.54 ± 4.42</td>
<td>614 ± 365</td>
<td>262 ± 254</td>
<td>462 ± 368</td>
<td>3.73 ± 1.79</td>
<td>6.11 ± 0.83</td>
<td>301 ± 270</td>
<td>9.49 ± 6.12</td>
<td>264 ± 375</td>
</tr>
<tr>
<td>RIF</td>
<td>0.35 ± 0.31*</td>
<td>65.0 ± 51.5*</td>
<td>9.26 ± 9.63*</td>
<td>29.8 ± 26.3*</td>
<td>2.84 ± 1.79</td>
<td>6.88 ± 3.44</td>
<td>13.9 ± 15.8</td>
<td>0.69 ± 0.66*</td>
<td>10.2 ± 10.2*</td>
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<tr>
<td>14-Hydroxyclarithromycin</td>
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<tr>
<td>Without RIF</td>
<td>1.58 ± 1.03</td>
<td>161 ± 69.9</td>
<td>84.7 ± 68.1</td>
<td>132 ± 86.0</td>
<td>3.56 ± 2.59</td>
<td>8.11 ± 1.61</td>
<td>92.0 ± 64.3</td>
<td>0.41 ± 0.20</td>
<td>4.73 ± 5.49</td>
</tr>
<tr>
<td>RIF</td>
<td>0.72 ± 0.44*</td>
<td>109 ± 54.3*</td>
<td>21.0 ± 21.3*</td>
<td>60.4 ± 36.8*</td>
<td>2.89 ± 1.76</td>
<td>5.10 ± 0.88*</td>
<td>29.6 ± 26.7</td>
<td>0.13 ± 0.08*</td>
<td>1.71 ± 1.22*</td>
</tr>
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</table>

* _P_ < 0.05 (Wilcoxon test).
steady-state plasma concentrations of CLR by more than 90% and in turn to a similarly limited distribution into the bronchial ELF and BALC. RIF comedication was not associated with up-regulation of the pulmonary efflux transporters ABCB1 and ABCC2. Nevertheless, the distribution of CLR into the ELF in absolute measures was decreased, whereas it was nearly 2-fold increased relative to the (lowered) plasma levels after RIF. Of interest, RIF comedication was associated with decreased OATP1A2 mRNA but increased OATP2B1 mRNA expression levels in EC.

The dramatic decrease in the steady-state levels is doubtlessly the consequence of nearly complete abolishment of CLR bioavailability because the plasma half-lives remained unchanged after comedication of RIF. In healthy foals, bioavailability of CLR after oral administration is incomplete (60%) as caused at least in part by presystemic “first-pass” metabolism by which the active 14-OH-CLR is generated (Womble et al., 2006). The results of in vitro studies using human liver microsomes and recombinant P450s suggested that CYP3A4 plays a major role in the overall metabolic clearance of CLR (Suzuki et al., 2003). Another reason for deficit bioavailability could be intestinal efflux transport. Macrolides are substrates of ABCB1 as confirmed by competition assays using Caco2, Madin-Darby canine kidney, and ABCB1-overexpressing MES-SA/Dx5 (American Type Culture Collection, Manassas, VA) human uterine sarcoma cells. From these in vitro studies, the overall impression that CLR has moderate affinity to ABCB1 can be derived (Pachot et al., 2003; Hughes and Crowe, 2010; Munic et al., 2010). There is also evidence for macrolides to be substrates of ABCC2 as shown for azithromycin by pharmacokinetic studies in Abcc2-deficient rats and in a drug interaction study with probenecid, an inhibitor of ABCC2 (Sugie et al., 2004). Therefore, the drastic lowering of the average CLR plasma concentrations by more than 90% may have resulted from induction of hepatic and intestinal CYP3A4 and intestinal ABCB1 and probably ABCC2.

### Table 2

<table>
<thead>
<tr>
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<th>ELFF/Plasma</th>
<th>BALC/Plasma</th>
<th>BALC/ELF</th>
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<tr>
<td>Clarithromycin</td>
<td></td>
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<tr>
<td>Without RIF</td>
<td>36.1 ± 14.8</td>
<td>757 ± 478</td>
<td>25.8 ± 23.0</td>
</tr>
<tr>
<td>RIF</td>
<td>62.0 ± 48.6</td>
<td>858 ± 449</td>
<td>14.3 ± 6.79</td>
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<tr>
<td>14-Hydroxyclarithromycin</td>
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</tr>
<tr>
<td>Without RIF</td>
<td>4.82 ± 1.86</td>
<td>44.2 ± 17.1</td>
<td>11.2 ± 9.14</td>
</tr>
<tr>
<td>RIF</td>
<td>4.88 ± 2.02</td>
<td>63.0 ± 20.0</td>
<td>14.2 ± 4.90</td>
</tr>
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</table>

* P < 0.05 (Wilcoxon test).

RIF is a prototype ligand for the nuclear PXR receptor that regulates many drug-metabolizing enzymes and multidrug transport proteins in the small intestine and the liver (Handschin and Meyer, 2003; Glaser et al., 2011; Oswald et al., 2011b; Tirona, 2011). Induction of hepatic CYP3A4 in our study was confirmed by a significant increase in the 4β-hydroxycholesterol/cholesterol plasma concentration ratio, an accepted endogenous metric for hepatic CYP3A4 activity in vivo (Yang and Rodrigues, 2010). Nevertheless, the plasma levels of 14-OH-CLR increased only to a limited extent and did not agree with the large loss in CLR bioavailability. Therefore, induction of intestinal...
efflux transport seems to be the major reason for lower bioavailability, which is also supported by the results of clinical studies in man. According to the summary of product characteristics of the manufacturers for CLR, strong enzyme inducers of the cytochrome P450 system may accelerate the metabolism of CLR and, by this, decrease the plasma levels of the parent drug in human patients by approximately 30 to 40% and increase the concentrations of the microbially active 14-OH-CLR by the same extent (e.g., Klacid; Abbott). Contrary to the product information, the results of two clinical studies in patients with pulmonary Mycobacterium avium complex infections showed a somewhat different feature. In all patients, the trough plasma concentrations of CLR were markedly lower when it was administered together with RIF (20% of the levels after monotherapy); however, the 14-OH-CLR levels were not different (Wallace et al., 1995; Taki et al., 2007). Of interest, the decrease in CLR exposure could be avoided if RIF and CLR were administered at different times; i.e., CLR seems to be absorbed by a mechanism that is susceptible to direct competition with RIF (Taki et al., 2007), but this mechanism has not been identified yet.

Therefore, we were encouraged to provide an alternative hypothesis: inhibition of a so far unknown intestinal uptake transporter in the presence of RIF may have caused lower bioavailability of CLR, instead of or in addition to the absorption deficit caused by RIF-type induction of ABCB1. Candidates might be members of the OATP family, which are known to be modulated by RIF in vitro (Vavricka et al., 2002). First, CLR is a potent inhibitor of the taurocholate uptake in rat Oatp1a5-transfected Madin-Darby canine kidney cells, the nearest analog to human OATP1A2. Both rat Oatp2b1 and human OATP2B1 were not inhibited by clarithromycin (Garver et al., 2008; Lan et al., 2009). Second, oral bioavailability of CLR in rats was reduced by 45% in the presence of RIF. Third, RIF had no effect on body clearance of CLR and most likely did not cause induction of
metabolic enzymes and/or transporters after the short comedication time in this study (120–180 min) (Garver et al., 2008). Therefore, a member of the OATP family might be involved in intestinal absorption of CLR, although there is little evidence that rat Oatp1a5/human OATP1A2 or rat Oatp2b1/human OATP2B1 really is the candidate (Lan et al., 2009). Hence, alternative intestinal absorption pathways that are susceptible to inhibition by RIF must exist. In any case, in assuming competition of RIF with an intestinal uptake transporter for CLR, it must be considered that RIF also modulates ABCB1 and ABCC2 (Zong and Pollack, 2003; Lau et al., 2006), which are also expressed in the horse intestine (Tydén et al., 2009). Thus, the net effect resulting from modulation of intestinal OATP(s) and/or induction of ABCB1/ABCC2 must have overshadowed the extent of the modulating effects of RIF on the ABCB1/ABCC2-mediated efflux of CLR.

Whichever mechanism has influenced the first-pass route of CLR, the average steady-state plasma concentrations in our foals dropped down to levels below the MIC (90%) of 0.12 μg/ml for R. equi (Jacks et al., 2003). Although decreased manifold, the concentrations in the ELF and BALC were still greater than the desired MIC. Nevertheless, there are many doubts from a pharmacokinetic point of view that combination therapy of CLR with RIF might really be superior to other eradication protocols as suggested by the results of a retrospective clinical study in foals (Giguère et al., 2004). The absence of major drug interactions as shown in our recent pharmacokinetic study with tadalafil and RIF should be confirmed before a combination treatment is launched in clinical practice (Venner et al., 2010).

A second major finding in our study was the more intensive distribution of CLR into the bronchial ELF despite markedly lower plasma concentrations after RIF comedication. We have hypothesized that finding in advance because, as discussed above, CLR is a substrate of ABCB1 (and probably of ABCC2), and RIF 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 BALC, and both may mediate active efflux of their substrates into the ELF and the environment of macrophages, respectively (Serai et al., 2003a,b; Bosquillon, 2010). According to our recent preliminary data, mRNA expression of ABCB1 and ABCC2 (semiquantitative RT-PCR in pooled samples) can be up-regulated by RIF in foals (Venner et al., 2010). We also found that the trough BALC concentrations of RIF after administration of 10 mg/kg given twice daily (6.0 ± 1.3 μM, n = 9, Oldenburger trait; W. Siegmund, unpublished results) are on the same order of magnitude as the in vitro concentrations necessary to activate the nuclear PXR and to increase CYP3A4, ABCB1, and ABCC2 activity (2–10 μM) (Geick et al., 2001; Kast et al., 2002). In fact, our results did not suit our working hypothesis: ABCB1 expression remained unchanged, and ABCC2 was significantly down-regulated by RIF comedication. However, we must consider some methodological limitations because quantitative PCR was performed 48 h after the last administration of RIF. We cannot exclude the possibility that mRNA levels returned to their pretreatment levels, whereas expression of the transporter protein in the cell membrane was still high, causing an increase in CLR accumulation in the ELF.

Pulmonary penetration of drugs is extremely complex, not well understood, and not mediated by efflux transporters only. In humans, the uptake carriers peptide transporter 2 and OCTN2 are expressed in the apical membrane of the EC. In alveolar macrophages, OCTN1 and OATP2B1 are expressed. In the lung, mRNA transcripts were also identified for OCT3, OCTN1, OATP1A2 and OATP2B1 (Endter et al., 2009; Bosquillon, 2010; Moreau et al., 2011). OATP1A2 and OATP2B1 are highly conserved in *Equus caballus* (mRNA homology compared with that of human genes: OATB1A2, 88%; OATP2B1, 84%; http://www.ncbi.nih.gov, http://www.ebi.ac.uk). From our data in foals, OATP1A2 is expressed in EC and OATP2B1 is expressed in EC and BALC; the exact cellular localization is unknown so far.

It is rather speculative to find a conclusive rationale for higher ELF/plasma ratios of CLR after RIF comedication because of the complexity of parallel processes. In our study, equine OATP2B1 in EC was regulated by RIF. Others also found OATP1A2 to be induced by RIF via a PXR response element (Meyer zu Schwabedissen et al., 2008). On the other hand, OATPs are inhibited in the presence of RIF (Vavricka et al., 2002). If the pulmonary OATPs are localized to the basolateral site of EC, higher ELF/plasma ratios are explained by up-regulation of OATP2B1 by chronic RIF comedication. For apical localization of the uptake carriers, the pharmacokinetic phenomenon in the presence of RIF (down-regulation and or inhibition) is in line with delayed CLR reuptake 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 CLR and RIF to the uptake transporters and their regulatory elements are needed before the results of the drug interaction study can be rationally discussed.

A third interesting and, to our knowledge, new finding in our study was the coordinate down-regulation of *ABCC2* mRNA expression in significant correlation with the *PXR* mRNA content (r = 0.815) in BALC after comedication of RIF; a tendency for lower *PXR* mRNA levels was also observed in EC. Note also that the down-regulation of PXR occurred in the presence of high cellular CLR concentrations (approximately 350 mM). PXR-activating concentrations of RIF in presence of high CLR concentrations obviously inhibit mRNA expression of PXR, whereas the interaction between PXR and other nuclear receptors and transcriptional factors that regulate the ABCC2 transcription was not observed in parallel studies in foals. The authors thank Dr. Christiane Vavricka for the gift of the *RIF*-responsive cell lines, the Department of Computer Science, University of Regensburg, for the software for the statistical analysis, the Animal Research Institute of the University of Veterinary Medicine Hanover, for the foals, and the four anonymous referees, whose comments substantially improved the manuscript. This study was supported by the German Federal Ministry for Education and Research (BMBF) under grant no. 01CU0118/4 and the Bavarian Ministry for Science, Research and the Environment (StMWK) under grant no. 050-FAK-11-123.

**Fig. 6.** Expression of OATP1A2 mRNA and OATP2B1 mRNA (relative to 18S rRNA) in EC and BALC before (−RIF) and after (+RIF) treatment with rifampicin (10.0 mg/kg b.i.d., 11 days) in nine healthy foals.
target gene seemed not to be disrupted by Rif/CLR as shown for ketoconazole and CYP3A4 (Lim et al., 2009). Further research is required to elucidate the mechanism behind our finding in BALC of foals.

In conclusion, chronic comedication of Rif in foals leads to an unexpectedly strong decrease in the bioavailability of CLR. By this undesired drug interaction, the plasma levels fall below the MIC for eradication of *R. equi*. Evidence that Rif influences the cellular uptake of CLR in bronchial cells and the PXR expression in BALC in presence of high CLR concentrations exists.

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Authorship Contributions

Participated in research design: Peters, Grube, Kroemer, Venner, and Siegmund.

Conducted experiments: Peters, Block, Freyer, Lämmir, and Venner.

Contributed new reagents or analytic tools: Oswald and Lütjohann.

Performed data analysis: Peters, Block, and Oswald.

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

References


Address correspondence to: Prof. Dr. Werner Siegmund, Department of Clinical Pharmacology, Ernst Moritz Arndt University, Friedrich-Loefftler-Str. 23d, D-17487 Greifswald, Germany. E-mail: siegmwu@uni-greifswald.de