scientific reports

Check for updates

OPEN

Pharmacokinetics of extended-release clarithromycin in patients with *Mycobacterium ulcerans* infection

Sandor-Adrian Klis[1], Ymkje Stienstra[1,2], Kabiru M. Abass[3], Justice Abottsi[3], Samuel O. Mireku[3], Jan-Willem Alffenaar[4,5,6] & Tjip S. van der Werf[1,7]✉

Clarithromycin extended-release (CLA-ER) was used as companion drug to rifampicin (RIF) for *Mycobacterium ulcerans* infection in the intervention arm of a WHO drug trial. RIF enhances CYP3A4 metabolism, thereby reducing CLA serum concentrations, and RIF concentrations might be increased by CLA co-administration. We studied the pharmacokinetics of CLA-ER at a daily dose of 15 mg/kg combined with RIF at a dose of 10 mg/kg in a subset of trial participants, and compared these to previously obtained pharmacokinetic data. Serial dried blood spot samples were obtained over a period of ten hours, and analyzed by LC–MS/MS in 30 study participants—20 in the RIF-CLA study arm, and 10 in the RIF-streptomycin study arm. Median CLA C_{max} was 0.4 mg/L—and median AUC 3.9 mg*h/L, following 15 mg/kg CLA-ER. Compared to standard CLA dosed at 7.5 mg/kg previously, CLA-ER resulted in a non-significant 58% decrease in C_{max} and a non-significant 30% increase in AUC. CLA co-administration did not alter RIF C_{max} or AUC. Treatment was successful in all study participants. No effect of CLA co-administration on RIF pharmacokinetics was observed. Based on our serum concentration studies, the benefits CLA-ER over CLA immediate release are unclear.

Buruli Ulcer Disease (BUD) is a neglected tropical disease caused by infection with *M. ulcerans*[1]. Prolonged infection causes necrotic skin lesions, through the secretion of mycolactone, a class of toxins secreted by *M. ulcerans*[2,3]. BUD can effectively be managed by antimicrobial therapy, in combination with appropriate wound care[4–8]. Under the guidance of WHO, a prospective randomized single blinded study was conducted between 2012 and 2018, in five different trial sites in Ghana and Benin; this study was first registered August 6, 2012 (clinicaltrials.gov, identifier: NTC 01659437). Before this study was completed, the WHO recommendation for antibiotic treatment of BUD was 8 weeks of intramuscular streptomycin (SM) and oral rifampicin (RIF). Daily injections with SM are a burden to the patient and health care worker. In addition, both the risk for injection-related infections and the nephro-, acoustic and vestibular toxicity of aminoglycosides are problematic; hence, this prospective randomized study aimed to establish an all-oral treatment regimen to replace the injection-based regimen[9]. Indeed, clarithromycin (CLA) and RIF combination therapy had earlier been shown to be a promising alternative to the SM-RIF therapy that was the standard at the time the WHO trial was conducted[5–7].

There is a potentially significant drug-drug interaction between CLA and RIF. CLA plasma levels are reduced by RIF-induced activation of cytochrome P450 enzymes (e.g., CYP 3A4, involved in the elimination of CLA), while CLA is known to inhibit the enzyme activity of CYP 3A4[10,11]. The P-glycoprotein (Pgp) efflux transporter is also affected, as RIF induces Pgp while at the same time, RIF is a substrate of Pgp; and CLA inhibits Pgp[12,13]. Therefore, RIF-CLA co-administration might lead to increased RIF concentrations and decreased CLA concentrations.

In a previous pharmacokinetic study of RIF and CLA combination therapy in BUD, conducted in the context of an earlier randomized controlled trial, the maximum concentration (C_{max}), time above the minimal inhibitory

[1]Department of Internal Medicine–Infectious Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. [2]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK. [3]Agogo Presbyterian Hospital, Agogo, Ghana. [4]The University of Sydney Institute for Infectious Diseases, Sydney, NSW, Australia. [5]Faculty of Medicine and Health, School of Pharmacy, The University of Sydney, Sydney, NSW, Australia. [6]Westmead Hospital, Sydney, NSW, Australia. [7]Department of Pulmonary Diseases & Tuberculosis, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. ✉email: t.s.van.der.werf@umcg.nl

concentration (MIC), and area under the concentration–time curve (AUC) of CLA were less than expected, and there was a 59% non-significant increase of the RIF AUC in the RIF-CLA arm compared to the RIF-SM arm[14]. Although that trial revealed no significant differences in clinical response between the study participants in CLA and non-CLA arms, treatment failure was observed slightly more frequently in the CLA-arm[6]. Therefore, there were some concerns that CLA-RIF treatment may be inferior to SM-RIF treatment due to rapid, RIF-induced metabolism.

To yield higher CLA plasma levels increasing the dosage of CLA would have been an option. In the previous pharmacokinetic study, a dosage of 7.5 mg/kg/day was given. In healthy adult volunteers, increasing the dosage from 500 mg (approximately 7.5 mg/kg/day) to 1000 mg (approximately 15 mg/kg/day) increased the C_{max} proportionally[15]. Both in healthy volunteers and in various patient groups (*M. avium* complex infection in HIV patients, *Legionella pneumophila* pneumonia), CLA has been demonstrated to be safe and effective in a dosage of 1000 mg[16,17], and we expected that it is at least as effective against BUD at a dosage of 500 mg. In order to minimize side effects and create a more favorable dosing scheme, immediate release CLA can be replaced with its extended-release form (CLA-ER). In several infectious diseases, CLA-ER has been demonstrated to be equally effective as immediate release CLA (CLA-IR) in the same dosage, and in general, while fewer side effects (especially gastrointestinal events) were reported in CLA-ER treated individuals compared to CLA-IR[18,19]. In healthy volunteers, CLA-ER has a comparable area under the concentration–time curve (AUC) for both the original substance and the 14-OH form to that of CLA-IR, but peak concentrations are lower[15]. In patients with advanced HIV infection, treated for *M. avium* complex infection, dosages of 1000 mg per day of CLA-ER and CLA-IR had similar pharmacokinetics in terms of C_{max}, and AUC, and no differences in tolerability or adverse events were found[17].

However, the pharmacokinetics of CLA-ER have not been studied in combination with RIF, and it is unknown how an extended-release formulation of CLA is affected by RIF-induced metabolism. Here we report on the pharmacokinetics of RIF and CLA-ER combination therapy in BUD.

Results

Study subjects

A total of 30 patients were enrolled as participants in this study, 20 patients receiving CLA-ER and RIF, and 10 patients receiving SM and RIF. Age, gender, body weight, body mass index, and mean drug doses per kg are presented in Table 1; the baseline characteristics did not differ substantially between the two treatment arms. Twenty-nine out of 30 lesions were confirmed by PCR to be caused by *M. ulcerans* infection. All of these patients healed completely (complete epithelialization of the affected skin) without recurrence within one year after starting antimicrobial therapy. The lesion that was not PCR-confirmed disappeared after 3 weeks of treatment, and therapy was discontinued. None of the patients mentioned any side effect, and audiometric, electrocardiogram, and hepatic enzyme monitoring did not show any drug-induced abnormalities.

Pharmacokinetics

At the time of blood sampling, the patients had received the study medication for a median (interquartile range; IQR) of 39 (28;50) days. The rifampicin concentration time profiles are shown in Fig. 1 and $AUC_{0\text{-}10\,h}$, C_{max} and T_{max} values in Table 2. The median RIF C_{max} was 29% higher in the CLA group compared to those without CLA (8.0 vs. 6.2 mg/L) (Fig. 1A and B), but this difference was not statistically significant. The CLA and hydroxyclarithromycin concentration time profiles are shown in Fig. 1C and D, respectively and $AUC_{0\text{-}10\,h}$, Cmax and Tmax values in Table 2. The median (IQR) ratio of the $AUC_{0\text{–}10\,h}$ of CLA to 14-OH-CLA was 0.52 (0.39; 0.83). The actual CLA dose per kg did not significantly predict AUC and C_{max} by linear regression ($p = 0.277$, and $p = 0.153$ respectively) (Table 3).

CLA concentrations reached a theoretical MIC of 0.25 mg/L in all patients, with a median (IQR) time above MIC of 465 (300; 540) min. An MIC of 0.50 mg/L was only obtained in 9 out of 20 study participants at any one point in time, yielding a median time above MIC of zero. For RIF, an MIC of 2 mg/L was reached in 29 out of 30 participants, with a median (IQR) time above MIC of 240 (225;300) min. No differences in time above MIC were found between study groups. Detectable CLA and RIF concentrations at baseline, due to drug intake on the day preceding the study were found in 2, and 3 participants respectively. RIF and CLA concentrations and AUCs that were 4–fivefold higher than the mean were reached in one participant. This was a 37-year-old female

	SM-RIF (N = 10)	CLA-ER-RIF (N = 20)
Age (years)	22.8 (16)	31.0 (16)
Gender (F/M)	6/4	11/9
Body weight (kg)	45.2 (18.8)	52.1 (12.5)
BMI (kg/m^2)	19.9 (5.1)	20.8 (3.6)
Mean RIF dose per kg	9.8 (2.0)	9.7 (.84)
Mean CLA dose per kg	–	14.4 (3.1)

Table 1. Mean (SD) Patient characteristics and drug doses per kilogram. All between group differences of the mean non significant by t-test.

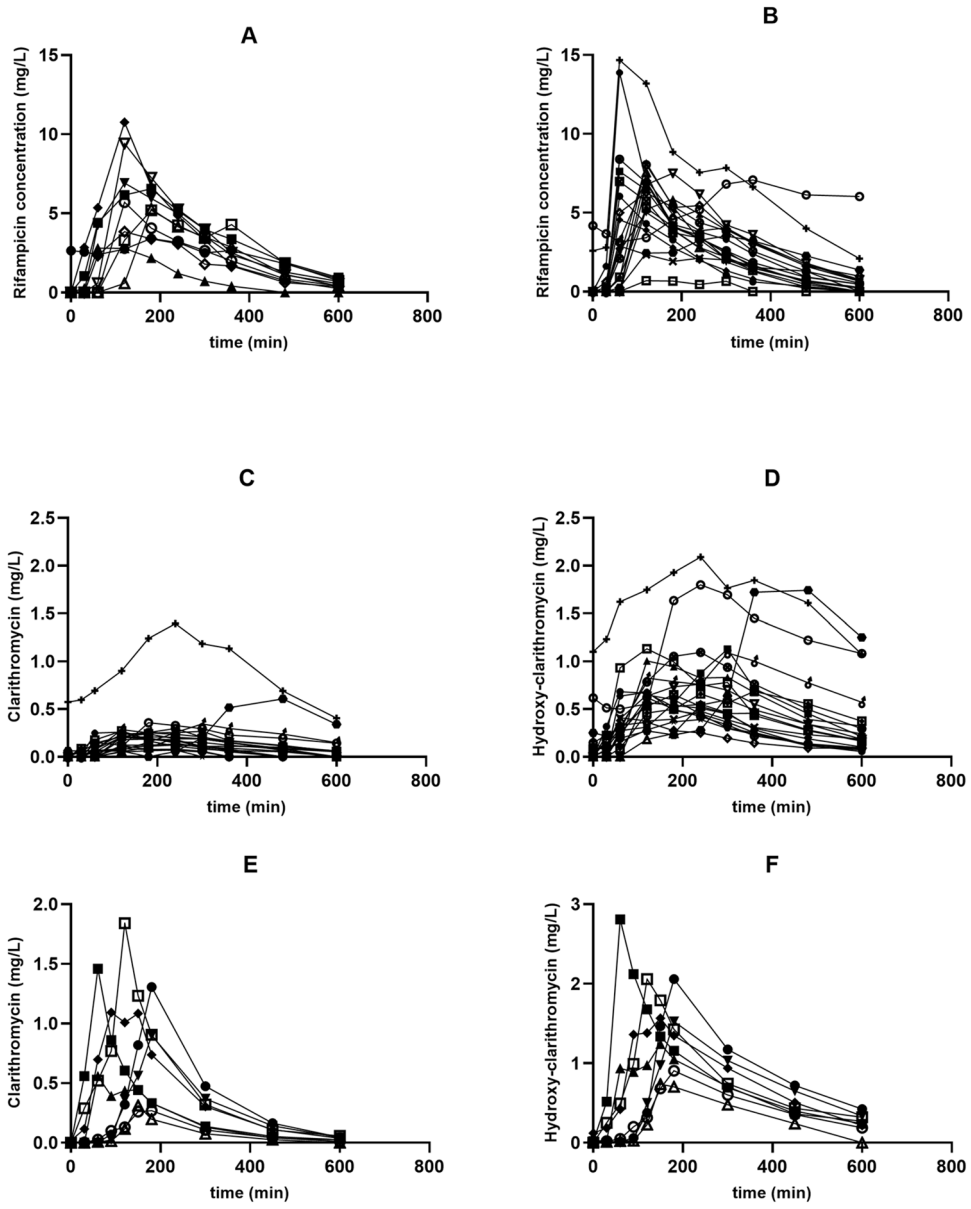

Fig. 1. Concentration–time curves of rifampicin and clarithromycin (extended and immediate release tablets). (**A**) rifampicin (SM-RIF), (**B**) rifampicin (CLA-ER-RIF), (**C**) clarithromycin (CLA-ER-RIF), (**D**) hydroxy-clarithromycin (CLA-ER-RIF), (**E**) clarithromycin (CLA-IR-RIF[14]), (**F**) hydroxy-clarithromycin (CLA-IR-RIF[14]). SM = streptomycin, RIF = rifampicin, CLA-ER = clarithromycin extended release, CLA-IR = clarithromycin immediate release.

Parameter	CLA-ER-RIF (*N* = 20)	SM-RIF (*N* = 10)	*p*
$AUC_{0\text{-}10}$ (mg x h/L)	23.5 (2.5–70.6)	24.0 (9.8–35.5)	1.0
C_{max} (mg/L)	8.0 (5.3–9.0)	6.2 (4.1–8.6)	0.35
T_{max} (h)	2 (1–2.8)	2 (2–3)	0.13

Table 2. Pharmacokinetic analysis of rifampicin. The $AUC_{0-10\,h}$ is geometric mean (range). All other results are in IQR. *p* = *p*-value for the test of significant difference between treatment arms of RIF parameter by Mann–Whitney U-test. CLA-ER = clarithromycin extended release, RIF = rifampicin.

Parameter	CLA-ER (*N* = 20)	CLA-IR (*N* = 8)	*p*
AUC_{0-24} (mg × h/L)	3.9 (2.3–5.4)	3.2 (2.3–5.2)	0.11
C_{max} (mg/L)	0.40 (0.40–0.50)	0.53 (0.42–0.71)	0.15
T_{max} (h)	2 (2–3)	2 (2–2)	0.64

Table 3. Pharmacokinetic analysis of clarithromycin. IR-CLA data obtained from Alffenaar et al.[14]. The $AUC_{0-10\,h}$ is geometric mean (range). All other results are in median (IQR). *p* = *p*-value for the test of significant difference between IR-CLA and ER-CLA parameter by Mann–Whitney U-test. CLA = clarithromycin, CLA-ER = extended release clarithromycin, CLA-IR = immediate release clarithromycin.

patient with no known co-morbidities, no laboratory signs of renal or hepatic impairment, no ECG abnormalities, and she did not experience any side effects.

The plasma concentration–time curves of CLA-IR and hydroxy CLA-IR—from our previous study[14]—are shown in Fig. 1E and F respectively.

Discussion

In this study we report on the pharmacokinetics of CLA-ER in combination with RIF. The clinical response of BUD to this regimen was satisfactory, as all patients in this sub-study were cured[20]. However, it appeared that CLA-ER was more affected by RIF induced metabolism than CLA-IR[14]. In healthy volunteers, a dose of 1000 mg ER-CLA (corresponding to a dose of 15 mg/kg in adults), yielded a mean C_{max} of 2.59 mg/L, and a mean AUC of 42.1 mg*h/L[15]. This implies that the interaction with RIF decreased the mean CLA-ER C_{max} and AUC by 79% and 88% respectively. Compared to the previous pharmacokinetic study in which a dose of 7.5 mg/kg CLA-IR was used, increasing the dose to15 mg/kg CLA-ER lowered the C_{max} by 58%, and yielded only a 30% increase in AUC (3.9 vs. 2.9 mg*h/L). Perhaps, in an extended-release formulation, continuously shedding low quantities of CLA results in less CYP3A4 enzyme saturation, and thus results in more efficient metabolism of CLA into 14-OH-CLA. In a study using physiologically based pharmacokinetic modelling, a decrease in AUC of ER formulations compared to IR formulations for a number of CYP3A4 substrates was demonstrated[21].

Contrary to the previous pharmacokinetic study, where a non-significant 60% increase of RIF $AUC_{0-10\,h}$ was found in the CLA group, we observed no effect of CLA-ER on RIF concentrations[14]. This might be due to lower CLA peak concentrations, leading to reduced Pgp efflux transporter inhibition.

CLA-ER concentrations remained above an MIC of 0.25 mg/L almost twice as long as CLA-IR concentrations. However, an MIC of 0.5 mg/L was only reached in a small group of patients. The clinical relevance of these findings depends on several factors. First, CLA concentrations above the MIC might not be needed, as CLA is given in combination with RIF. Sub MIC concentrations might suffice to reduce *M. ulcerans* metabolism, perhaps inhibiting mycolactone production, and preventing the development of RIF resistance[22]. Second, for CLA to be effective against *M. ulcerans*, it needs to exert its effects mainly at the site of the infection, and CLA is known to accumulate in tissues. Tissue concentrations of up to 10 times the serum concentration have been measured in human lung tissue and alveolar macrophages[23,24], and in mouse footpads infected with *M. leprae*[25]. As CLA might well accumulate in Buruli ulcer lesions, future studies should measure CLA in these lesions, for example by homogenizing punch biopsies. If present in tissue in high concentrations, serum based pharmacokinetic parameters might not be a relevant measure for the antimicrobial effect of CLA on *M. ulcerans*.

There are some logistical disadvantages to CLA-ER compared to CLA-IR. The dosing by body weight needs to be rounded off to the nearest 500 mg, compared to 250 mg for CLA-IR, causing a larger variation in actual CLA per kg dosed. The CLA-ER formulation is also about four times more expensive than the regular CLA-IR tablet, and it has not been registered for use in most BU endemic countries. A limitation of our study is that it was a nested PK study and not a formal randomized cross over bioequivalence study, directly comparing CLA exposure after intake of the ER and IR release tablets on top of RIF. Logistically, this would be challenging due to long time required for RIF induction and washout in a cross over design. Despite this limitation, our study provides valuable information for clinicians who have to make a decision on the selection of antimicrobial treatment for patients with Buruli ulcer disease. The clinical response to all-oral CLA-RIF combination therapy was excellent, as demonstrated in the drug trial[20].

This is the first time a clinical pharmacokinetic study was carried out using a dried blood spot (DBS) sampling technique for the determination of CLA and RIF in a rural setting. This technique was previously validated in a clinical setting in the Netherlands[26,27]. In practice, it yields a considerable advantage. Rifampin serum samples require immediate freezing to − 80 °C, and transport on dry ice, both costly and not readily available in less affluent settings. The DBS cards were stored at room temperature, and could be shipped with regular mail. One potential limitation to this study is that we compare our results with previous data that were obtained using serum samples. However, the methods have been demonstrated to be equivalent in a Dutch clinical setting, and the RIF pharmacokinetic parameters found were comparable to those found in the previous study using serum samples, providing an indication of usefulness and feasibility of the DBS method[14,21]. Therefore, DBS may help to facilitate pharmacokinetic studies in poorly resourced settings[22].

Conclusions

A CLA-induced increase of RIF concentrations was not observed. RIF-induced metabolism appeared to be increased in CLA ER-compared to CLA IR-, and so the benefits of CLA-ER over CLA-IR in the treatment of BUD are unclear, and require further study although the clinical response to the all-oral treatment using CLA-ER was excellent.

Materials and methods

This pharmacokinetic study was conducted as a sub-study nested in a multi-center randomized controlled trial (Clinicaltrials.gov identifier NCT01659437; first submitted 2012-08-02; first posted 2012-08-07; last update posted 2019-09-26; last verified 2019-9) that was completed in 2018, and reported in 2020[20]. In this trial, patients with early (duration < 6 months), limited (cross-sectional diameter < 10 cm) BUD were randomized to receive either SM-RIF or CLA-ER-RIF for 8 weeks. For the current sub-study, that was conducted in one of the five study sites—Agogo Hospital, Ghana—between 17th July, 2013 and 11th April, 2014, the trial registration—Clinicaltrials.gov identifier NCT01659437—was adapted on 16/1/2013.

Ethics

The protocol for this pharmacokinetic side-study was approved in Dec 2012, by the Commission on Human Research, Publication and Ethics, Kwame Nkrumah University of Science and Technology (CHRPE/AP/040/12). All participants were given at least 24 h to consider participation, after which written and verbal informed consent was obtained. For participants below 18 years of age, informed consent was obtained from their legal guardian while the minor-age participants themselves had to provide informed assent. All participants and their company were offered a reimbursement of their transportation costs, 3 meals, and an incentive in cash and kind of approximately $20. All research was performed in accordance with relevant guidelines/regulations, in accordance with the Declaration of Helsinki. For this pharmacokinetic sub-study, no additional funding was obtained; the funders had no role in trial design, data analysis, writing of the manuscript, or decision to publish.

Participants

A total of 20 patients randomized to receive CLA-ER-RIF and 10 patients randomized to receive CLA-IR-RIF were enrolled for the pharmacokinetic side-study. As our main interest was in obtaining pharmacokinetic data of CLA-ER in combination with RIF in comparison to CLA-IR from our previous study[14], no power calculation was done. We chose to include 20 participants to better capture individual differences in e.g. hepatic and renal function or body fat percentage, which can cause some variations in drug exposure. Exclusion criteria for the drug trial were HIV infection, pregnancy, known hypersensitivity to any of the study drugs, previous treatment with streptomycin, and significant comorbidities. For this pharmacokinetic study, the minimum age for inclusion was 10 years, and in order to obtain steady-state pharmacokinetics, all participants had to be taking the study drugs at the same dose for at least 7 days. Consecutive eligible study participants were screened, and informed consent and assent for minors were obtained; only two individuals refused participation; none of the study participants withdrew consent or assent.

Drug administration

Patients received CLA-ER at a dose of approximately 15 mg/kg, which was rounded to either 500 mg or 1000 mg as 500 mg is the lowest available dose for the extended-release formulation of CLA. RIF was administered at a dose of 10 mg/kg, which was rounded to the nearest 150 mg. As food intake can influence the absorption of RIF[28], the drugs were administered on an empty stomach, but patients were offered a standardized breakfast 1 h after drug administration. All drugs were taken under direct observation of the study team.

Pharmacokinetic assessment

On the day of the PK study, the patient was admitted to the hospital, and blood levels were obtained just before drug administration, and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 h after drug administration. At each time point, approximately 2 ml of blood was drawn from a venous cannula into an EDTA tube, from which 50 µl was pipetted onto dried blood spot (DBS) cards (Whatman DMPK-C) and dried for 2 h. In a previous study, DBS samples of RIF and CLA were proven to be stable at room temperature for 2 months[26]. In the current study, the cards were stored with desiccants in an air-conditioned room at 16 °C for a maximum of 4 weeks before they were transported to the Department of Clinical Pharmacy and Pharmacology of the University Medical Center Groningen, where they were frozen at -80 °C until analysis. CLA, 14OH-CLA, and RIF DBS concentrations were determined by a previously validated LC–MS/MS assay[26,27].

Pharmacokinetic analysis

A non-compartmental analysis was performed (MW/Pharm, version 3.60; Mediware, Netherlands)[29]. C_{max} was defined as the highest observed serum concentration, and T_{max} was the time corresponding to C_{max}. C_{min} was the serum concentration before intake of the dose. The AUC from time zero to 24 h (AUC_{0-24}) was calculated using the log-linear trapezoidal rule. The elimination half-life ($t_{1/2}$) was calculated by 0.693/kel. The apparent clearance of the drug (CL/F) was calculated as the dose/AUC_{0-24}, and the apparent volume of distribution (V/F) was calculated as the dose/concentration at steady state.

The known MIC of CLA for *M. ulcerans* differs between strains. Previous studies suggested that MICs range between 0.25 and 2 mg/L, with most strains having an MIC of 0.25 mg/L, and 95% of strains having an MIC of 0.50 mg/L or less[14,30,31]. The MICs of 14-OH-CLA range between 4 and 8 mg/L[14]. The MIC of RIF appears to

range between 0.12 and 4 mg/L, with 90% of strains having an MIC of 2 mg/L or less[31,32]. Checkerboard analyses show that MIC in drug combinations are even lower for individual drugs[31].

Ethical approval

Consent for publication—provided by the Commission on Human Research, Publication and Ethics, Kwame Nkrumah University of Science and Technology (CHRPE/AP/040/12; Dec 2012).

Data availability

JWA, YS or TSW should be contacted for unrestricted provision of all data; y.stienstra@umcg.nl; johannes.alffenaar@sydney.edu.au; t.s.van.der.werf@umcg.nl.

Received: 20 July 2023; Accepted: 22 August 2024
Published online: 28 August 2024

References

1. Portaels, F., Silva, M. T. & Meyers, W. M. Buruli ulcer. *Clin. Dermatol.* **27**, 291–305. https://doi.org/10.1016/j.clindermatol.2008.09.021 (2009).
2. van der Werf, T. S., Stinear, T., Stienstra, Y., van der Graaf, W. T. & Small, P. L. Mycolactones and *Mycobacterium ulcerans* disease. *Lancet* **362**, 1062–1064. https://doi.org/10.1016/S0140-6736(03)14417-0 (2003).
3. George, K. M. *et al.* Mycolactone: A polyketide toxin from *Mycobacterium ulcerans* required for virulence. *Science* **283**, 854–857 (1999).
4. Chauty, A. *et al.* Promising clinical efficacy of streptomycin-rifampin combination for treatment of Buruli ulcer (*Mycobacterium ulcerans* disease). *Antimicrob. Agents Chemother.* **51**, 4029–4035. https://doi.org/10.1128/aac.00175-07 (2007).
5. Chauty, A. *et al.* Oral treatment for *Mycobacterium ulcerans* infection: Results from a pilot study in Benin. *Clin. Infect. Dis.* **52**, 94–96. https://doi.org/10.1093/cid/ciq072 (2011).
6. Nienhuis, W. A. *et al.* Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: A randomised controlled trial. *Lancet* **375**, 664–672. https://doi.org/10.1016/S0140-6736(09)61962-0 (2010).
7. Phillips, R. O. *et al.* Clinical and bacteriological efficacy of rifampin-streptomycin combination for two weeks followed by rifampin and clarithromycin for six weeks for treatment of *Mycobacterium ulcerans* disease. *Antimicrob. Agents Chemother.* **58**, 1161–1166. https://doi.org/10.1128/AAC.02165-13 (2014).
8. Sarfo, F. S. *et al.* Clinical efficacy of combination of rifampin and streptomycin for treatment of M*ycobacterium ulcerans* disease. *Antimicrob. Agents Chemother.* **54**, 3678–3685. https://doi.org/10.1128/AAC.00299-10 (2010).
9. Klis, S. *et al.* Long term streptomycin toxicity in the treatment of Buruli Ulcer: Follow-up of participants in the BURULICO drug trial. *PLoS Negl. Trop. Dis.* **8**, e2739. https://doi.org/10.1371/journal.pntd.0002739 (2014).
10. Gurley, B. *et al.* Assessing the clinical significance of botanical supplementation on human cytochrome P450 3A activity: Comparison of a milk thistle and black cohosh product to rifampin and clarithromycin. *J. Clin. Pharmacol.* **46**, 201–213. https://doi.org/10.1177/0091270005284854 (2006).
11. Benedetti, M. S. Inducing properties of rifabutin, and effects on the pharmacokinetics and metabolism of concomitant drugs. *Pharmacol. Res.* **32**, 177–187 (1995).
12. Eberl, S. *et al.* Role of p-glycoprotein inhibition for drug interactions: Evidence from in vitro and pharmacoepidemiological studies. *Clin. Pharmacokinet.* **46**, 1039–1049. https://doi.org/10.2165/00003088-200746120-00004 (2007).
13. Chen, J. & Raymond, K. Roles of rifampicin in drug-drug interactions: underlying molecular mechanisms involving the nuclear pregnane X receptor. *Ann. Clin. Microbiol. Antimicrob.* **5**, 3 (2006).
14. Alffenaar, J. W. *et al.* Pharmacokinetics of rifampin and clarithromycin in patients treated for *Mycobacterium ulcerans* infection. *Antimicrob. Agents Chemother.* **54**, 3878–3883. https://doi.org/10.1128/AAC.00099-10 (2010).
15. Guay, D. R. *et al.* Pharmacokinetics and tolerability of extended-release clarithromycin. *Clin. Ther.* **23**, 566–577 (2001).
16. Grau, S. *et al.* Impact of rifampicin addition to clarithromycin in *Legionella pneumophila* pneumonia. *Int. J. Antimicrob. Agents* **28**, 249–252. https://doi.org/10.1016/j.ijantimicag.2006.03.029 (2006).
17. Jacobson, M. A., Nicolau, D. P., Sutherland, C., Smith, A. & Aweeka, F. Pharmacokinetics of clarithromycin extended-release (ER) tablets in patients with AIDS. *HIV. Clin. Trials.* **6**, 246–253. https://doi.org/10.1310/9FEX-MHQQ-74L6-GGCJ (2005).
18. Adler, J. L. *et al.* Phase III, randomized, double-blind study of clarithromycin extended-release and immediate-release formulations in the treatment of patients with acute exacerbation of chronic bronchitis. *Clin. Ther.* **22**, 1410–1420 (2000).
19. Murray, J. J. *et al.* Phase III, randomized, double-blind study of clarithromycin extended-release and immediate-release formulations in the treatment of adult patients with acute maxillary sinusitis. *Clin. Ther.* **22**, 1421–1432 (2000).
20. Phillips, R. O. *et al.* Rifampicin and clarithromycin (extended release) versus rifampicin and streptomycin for limited Buruli ulcer lesions: A randomised, open-label, non-inferiority phase 3 trial. *Lancet* **395**(10232), 1259–1267. https://doi.org/10.1016/S0140-6736(20)30047-7 (2020).
21. Olivares-Morales, A., Kamiyama, Y., Darwich, A. S., Aarons, L. & Rostami-Hodjegan, A. Analysis of the impact of controlled release formulations on oral drug absorption, gut wall metabolism and relative bioavailability of CYP3A substrates using a physiologically-based pharmacokinetic model. *Eur. J. Pharm. Sci.* **67**, 32–44. https://doi.org/10.1016/j.ejps.2014.10.018 (2015).
22. Marsollier, L. *et al.* Isolation of three *Mycobacterium ulcerans* strains resistant to rifampin after experimental chemotherapy of mice. *Antimicrob. Agents Chemother.* **47**, 1228–1232 (2003).
23. Togami, K., Chono, S. & Morimoto, K. Distribution characteristics of clarithromycin and azithromycin, macrolide antimicrobial agents used for treatment of respiratory infections, in lung epithelial lining fluid and alveolar macrophages. *Biopharm. Drug Dispos.* **32**, 389–397. https://doi.org/10.1002/bdd.767 (2011).
24. Fish, D. N., Gotfried, M. H., Danziger, L. H. & Rodvold, K. A. Penetration of clarithromycin into lung tissues from patients undergoing lung resection. *Antimicrob. Agents Chemother.* **38**, 876–878 (1994).
25. Gelber, R. H., Siu, P., Tsang, M. & Murray, L. P. Activities of various macrolide antibiotics against *Mycobacterium leprae* infection in mice. *Antimicrob. Agents Chemother.* **35**, 760–763 (1991).
26. Vu, D. H. *et al.* Simultaneous determination of rifampicin, clarithromycin and their metabolites in dried blood spots using LC-MS/MS. *Talanta* **121**, 9–17. https://doi.org/10.1016/j.talanta.2013.12.043 (2014).
27. Vu, D. H., Alffenaar, J. W., Edelbroek, P. M., Brouwers, J. R. & Uges, D. R. Dried blood spots: A new tool for tuberculosis treatment optimization. *Curr. Pharm. Des.* **17**, 2931–2939. https://doi.org/10.2174/138161211797470174 (2011).
28. Zent, C. & Smith, P. Study of the effect of concomitant food on the bioavailability of rifampicin, isoniazid and pyrazinamide. *Tuber. Lung Dis.* **76**, 109–113. https://doi.org/10.1016/0962-8479(95)90551-0 (1995).
29. Proost, J. H. & Meijer, D. K. MW/Pharm, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Comput. Biol. Med.* **22**, 155–163 (1992).

30. Portaels, F., Traore, H., De Ridder, K. & Meyers, W. M. In vitro susceptibility of *Mycobacterium ulcerans* to clarithromycin. *Antimicrob. Agents Chemother.* **42**, 2070–2073 (1998).
31. Arenaz-Callao, M. P. *et al.* Triple oral beta-lactam containing therapy for Buruli ulcer treatment shortening. *PLoS Negl. Trop. Dis.* **13**(1), e0007126. https://doi.org/10.1371/journal.pntd.0007126 (2019).
32. Ji, B. *et al.* In vitro and in vivo activities of rifampin, streptomycin, amikacin, moxifloxacin, R207910, linezolid, and PA-824 against *Mycobacterium ulcerans*. *Antimicrob. Agents Chemother.* **50**, 1921–1926. https://doi.org/10.1128/AAC.00052-06 (2006).

Acknowledgements

We acknowledge all study participants for their willingness to contribute voluntarily.

Author contributions

Conception and study design: J.W.A., T.S.V.D.W., Y.S.; enrollment and study procedures: S.A.K., K.M.A., J.A., S.O.M.; laboratory tests: J.W.A.; data analysis, and draft of the manuscript: S.A.K., J.W.A., T.S.V.D.W., Y.S.; all authors contributed to subsequent versions of the manuscript, and saw and approved the final draft of the manuscript.

Funding

Buruli ulcer Foundation, based in Groningen, the Netherlands, provided travel- and reimbursement costs; Sandor Klis was funded by a Young Investigators' reward (Junior Scientific Master Class, University of Groningen); this study is a sub-study of the multicenter clinical study (ClinicalTrials.gov Identifier: NCT01659437) sponsored by WHO, ALM, EU grants, and several other NGOs. Funders had no role in study design, data analysis, data interpretation, writing or approving the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to T.S.W.