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Clarithromycin increases linezolid exposure in multidrug-resistant tuberculosis patients

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Abstract

The use of linezolid for the treatment of multidrug-resistant tuberculosis is limited by dose- and time-dependent toxicity. Recently, we reported a case of pharmacokinetic drug-drug interaction between linezolid and clarithromycin resulting in increased linezolid exposure. The aim of this prospective pharmacokinetic study is to quantify the effect of clarithromycin on the exposure of linezolid.

Subjects were included in an open-label, single-center, 1-arm, fixed-order pharmacokinetic interaction study. All subjects received 300mg linezolid twice daily during the entire study, consecutively co-administered with 250mg and 500mg clarithromycin once daily. Steady-state serum curves of linezolid and clarithromycin were analyzed using validated methods and differences between pharmacokinetic parameters were calculated.

Linezolid exposure increased by a median of 44% (interquartile range: 23-102%, P=0.043) after co-administration of 500mg clarithromycin (n=5) compared to baseline, whereas 250mg clarithromycin had no statistically significant effect. Co-administration was well tolerated by most patients: none experienced severe adverse effects. One patient reported Common Toxicity Criteria Grade 2 gastro-intestinal adverse events.

In this study, we showed that clarithromycin significantly increased linezolid serum exposure after combining clarithromycin with linezolid in multidrug-resistant tuberculosis patients. The drug-drug interaction is possibly P-glycoprotein mediated. Due to large inter-patient variability, therapeutic drug monitoring is advisable to determine individual effect size.

Introduction

Multidrug-resistant tuberculosis (MDR-TB) is an infectious disease of major concern, especially in high TB burden countries [1-3]. Treatment of MDR-TB poses challenges such as designing an effective second-line anti-tuberculosis regimen, entailing a combination of multiple drugs and a long duration of treatment [4]. This translates in an intensive phase of at least 8 months and total treatment duration of at least 20 months as recommended by the WHO [5]. In the intensive phase, treatment of MDR-TB should consist of at least four second-line anti-tuberculosis drugs likely to be effective. Additional drugs from Group 5, such as linezolid and clarithromycin, may be used, but their efficacy in the treatment of MDR-TB is unclear [5]. Unfortunately, knowledge on the efficacy in the treatment of MDR-TB with these drugs is scarce.

Linezolid is a promising antimicrobial agent for the treatment of MDR-TB. However, evidence on the treatment of MDR-TB with linezolid is limited. Efficacy against *Mycobacterium tuberculosis* has been shown *in vitro* [6], in animals [7], and in patients [8-10]. A recent meta-analysis confirms this efficacy, but shows the necessity of caution in the prescription of linezolid due to toxicity; almost 60% of all analyzed patients experienced adverse events [11]. Adverse events, such anemia (38%), peripheral neuropathy (47%), gastro-intestinal side effects/symptoms (17%), optic neuritis (13%), and thrombocytopenia (12%), have all been reported and limit the use of linezolid [11]. Reducing the dose of linezolid has been evaluated in an attempt to reduce toxicity [12]. A dose of ≤600mg linezolid daily resulted in lower frequency of adverse events than a dose of >600mg daily (respectively 47% vs 75%), thereby enabling longer treatment duration [11].

Clarithromycin has a less prominent place in the treatment of MDR-TB. The minimal inhibitory concentration (MIC) of *Mycobacterium tuberculosis* was thought to be well in excess of achievable serum concentrations based on 12 strains of *Mycobacterium tuberculosis* [13]. However, lower MICs have been observed (<2mg/L) and clarithromycin shows concentrations in epithelial lining fluid that are often higher than in serum, enabling clarithromycin to be added to treatment regimens [14]. Several Group 5 drugs, e.g. linezolid and clarithromycin, may need to be combined in a single MDR-TB treatment regimen, albeit that little is known on drug-drug interactions of these agents. Drug-drug interactions could compromise the efficacy of treatment regimens or could increase toxicity through reduced or increased exposure respectively.

Recently, we reported a pharmacokinetic drug-drug interaction between linezolid and clarithromycin that resulted in increased linezolid exposure [15]. Increased serum linezolid concentrations could lead to toxicity, such as time- and dose-dependent severe myelosuppression and polyneuropathy. In a meta-analysis comparing a cohort treated with

>600mg linezolid per day with a cohort treated with ≤600mg, there was a higher probability of anemia (60% vs 23%), leucopenia (17% vs 2%) and gastrointestinal symptoms (29% vs 8%) in the cohort that received >600mg linezolid [11]. Such toxicity could lead to the need to cease treatment with linezolid, severely limiting the treatment options left. Therefore, the aim of this prospective pharmacokinetic study was to quantify the effect of clarithromycin on the exposure of linezolid in adult MDR-TB patients hospitalized at the Tuberculosis Center Beatrixoord (Haren, the Netherlands).

Method

Study design

This study was an open-label, prospective single-center, 1-arm, fixed-order, interventional pharmacokinetic interaction study. The study was performed at the Tuberculosis Centre Beatrixoord (University of Groningen, University Medical Center Groningen, Haren, The Netherlands). All study subjects received standard care for their MDR-TB and co-morbidities. Treatment of MDR-TB was based on the World Health Organization (WHO) guideline [5] individualized for each included patient.

The primary objective was to quantify linezolid area under the concentration-time curve from 0 to 12 hours ($AUC_{0\text{-}12h}$) without clarithromycin and with 250mg and 500mg clarithromycin once daily. Secondary objectives were to compare pharmacokinetic parameters of linezolid and clarithromycin between different dosing combinations and to describe tolerability and safety of co-administration of clarithromycin and linezolid in MDR-TB patients.

All patients gave written informed consent. The study protocol was approved by the Medical Ethical Review Committee of the University Medical Center Groningen (University of Groningen, Groningen, the Netherlands). The study was registered at clinicaltrials.gov (NCT01521364).

Subjects

All study subjects were aged ≥18 years and were diagnosed with MDR-TB, confirmed with standard microbiological culture methods. The criteria for exclusion were based on the contraindications and known drug-drug interactions as mentioned in the Summary of Product Characteristics of linezolid and clarithromycin [16,17]. Subjects were excluded from the study if they were pregnant or lactating; had previously shown hypersensitivity to linezolid, any macrolide antibiotics, or any of the excipients of linezolid or clarithromycin; had

hypokalemia; or concomitantly received P-glycoprotein modulators.Drug sensitivity testing (DST) was performed at the Dutch National Mycobacterial Reference Laboratory (National Institute for Public Health and the Environment [RIVM], Bilthoven, The Netherlands) using the Middlebrook 7H10 agar dilution method.

Treatment

All patients received linezolid 300mg every 12 hours. In previous studies, we showed that this dose resulted in seemingly effective serum concentrations with a median $AUC_{0\text{-}12h}$ of 57.6 mg*h/L (IQR: 38.5-64.2 mg*h/L) and $AUC_{0\text{-}24h}$/MIC ratios of 452 (IQR: 343-513) [12]. Clarithromycin was added to therapy in a dose of 250mg and 500mg once daily consecutively during two weeks in a fixed order (figure 1). From three cases at the Tuberculosis Center Beatrixoord, of which one case is published [15], it was expected that 500mg clarithromycin would result in an approximately doubled linezolid exposure, matching the exposure of linezolid resulting of labeled dose of 600mg twice daily.

Full linezolid pharmacokinetic curves were recorded at baseline (after one week of linezolid without clarithromycin), after receiving linezolid with 250mg clarithromycin, and after linezolid with 500mg clarithromycin for two weeks (figure 1). A trough sample was obtained after a washout period of one week, during which the patients only received linezolid besides their standard treatment, but no clarithromycin.

Sample size was derived from AUCs in a previous study in MDR-TB patients [12] and from the relative large increase of exposure observed in three cases ([15]; two cases unpublished). To reach a desired power of 80%, a sample size of at least 5 patients was calculated using G*Power 3.1 (Heinrich Heine Universitat, Dusseldorf, Germany). A drop-out rate of 15% was estimated based on previous studies at the Tuberculosis Center Beatrixoord (Haren, the Netherlands). To compensate for this estimated drop-out, seven patients were included.

Experimental procedures

The baseline linezolid pharmacokinetic curve and the trough after a one-week washout period were obtained at steady state, which is reached after approximately three days [16]. Pharmacokinetic curves after co-administration of linezolid and clarithromycin were assessed at steady state after two weeks, allowing the pharmacokinetic interaction to develop fully [18]. Blood samples were collected before and 1, 2, 3, 4, 8, and 12 hours after intake of medication. The second dosage of linezolid was given directly after this last blood sample. The patients did not receive standardized meals, but were allowed to eat a regular

breakfast, reflecting common clinical practice, since food does not influence the linezolid exposure [19]. Adherence was ensured through a directly observed inpatient treatment program.

Serum concentrations

Blood samples were drawn and after centrifuging serum samples were stored at -20^{o}C until analysis. Linezolid and clarithromycin serum concentrations were analyzed using validated high performance liquid chromatography tandem mass-spectrometry methods [20,21].

Tolerability and safety

The patients were clinically observed by nurses and attending physicians. Routine checks including blood tests were carried out at least weekly as part of continued standard care including monitoring for hyperlactatemia, haematological abnormalities such as thrombocytopenia and anemia. All patients received epoetine alpha (Eprex®) pre-emptively in a dose of 2000 IE twice a week to prevent anemia as part of standard care. Gastro-intestinal side effects were determined using the Common Toxicity Criteria (CTC) and were scored Grade 0 to 4 [22]. Routine testing of neurotoxic adverse events through electromyogram (EMG) or vibration sense monitoring are not carried out during the study of 6 weeks, since these effects have been reported to occur after a median of 16 weeks (range 10-111 weeks) [23]. In case of clinical suspicion of peripheral neuropathy, a neurologist was consulted as is common practice at the Tuberculosis Center Beatrixoord. Furthermore, patients receiving linezolid were examined by an ophthalmologist once monthly, which is also common practice in this Center.

Pharmacokinetic and statistical analysis

The main study parameter, linezolid $AUC_{0\text{-}12h}$ and secondary study parameters clearance (CL), elimination constant (k) and elimination half life ($t_{1/2}$) are calculated using trapezoidal rule in the Kinfit software (MWPharm 3.60; Mediware, Groningen, The Netherlands) [24]. Pharmacokinetic parameters of linezolid and clarithromycin are described. C_{max} was defined as the highest observed serum concentration and C_{min} was defined as the concentration before intake of medication.

The hypothesis that the median of differences of $AUC_{0\text{-}12h}$ of linezolid at baseline compared to $AUC_{0\text{-}12h}$ after co-administration with either 250mg or 500mg clarithromycin equals zero was tested using the related-samples Wilcoxon Signed Rank test. Secondary pharmacokinetic parameters from the three curves were compared using the same related-samples Wilcoxon Signed Rank test. The non-parametric analysis of variances (ANOVA)

Friedman test was used to test dose dependency of an effect of clarithromycin on linezolid exposure. All statistical evaluations were performed using SPSS 20 (SPSS, Chicago, IL, USA).

Results

Patient characteristics

From December 2011 to October 2012, 16 patients with possible MDR-TB were admitted to the Tuberculosis Center Beatrixoord (Haren, the Netherlands). Two of these 16 patients were <18 years old, one patient was pregnant, one patient was participating in another study, for one patient the planned period of admission was too short, leaving eleven patients for formal screening. Four patients were not included in the study for various reasons. In one patient DST revealed normal sensitivity, in another patient venous blood samples were not obtained due to venous access problems, rendering the collection of three full pharmacokinetic curves impossible; a third patient was included in another study; and the last patient was deemed psychologically too unstable to comply with the study protocol. Seven hospitalized patients were included in the study, five of whom were suitable for evaluation. One of the included patients dropped out of the study in the fourth week due to medical reasons. The patient had a fever and was nauseous, probably due to an infected venous access port and possibly combined with side effects of clarithromycin and other anti-TB medication such as moxifloxacin. Another patient could not be evaluated due to a logistical problem with the study medication. The two patients that dropped out of the study were excluded from all analyses.

Patient baseline demographics and results from the drug susceptibility testing are presented in table 1. The mean age of included subjects was 35 years (range 23 - 65 years) and the mean weight was 66.8 kg (range: 55.2 – 78.5 kg). One of the patients was HIV positive and was treated with emtricitabin/tenofovir and raltegravir. Three patients originated from Somalia, one from Turkey, and one from the Netherlands.

Pharmacokinetic and statistical analysis

From all patients suitable for evaluation (n=5), three full pharmacokinetic curves in serum were available. The mean plasma concentration-time curves are shown in figure 2. The baseline median $AUC_{0\text{-}12h}$ of linezolid of 36.3 mg*h/L [IQR 33.2-46.3] in patients with a mean body weight of 66.8 kg (range: 55.2 – 78.5 kg), is lower than the $AUC_{0\text{-}12h}$ of linezolid of 57.6 mg*h/L (IQR: 38.5-64.2 mg*h/L) from a previous study with patients with a body weight of 58.3 kg (IQR: 52.7-62.8 kg) [12]. Linezolid concentrations in serum increased after co-

administration of clarithromycin compared to baseline, but display a large standard deviation. There appears to be no effect on time of C_{max}, i.e. $t_{max.}$

Pharmacokinetic parameters of linezolid and clarithromycin are presented in table 2. Compared to baseline, the median AUC_{0-12h} of linezolid increased statistically significantly after co-administration with 500mg clarithromycin (P=0.043), but not after co-administration with 250mg clarithromycin (P=0.686). After co-administration of linezolid with 500mg clarithromycin, the median AUC_{0-12h} of linezolid increased by 44% (IQR: 23-102%) compared to baseline. Furthermore, administration of 500mg clarithromycin statistically significantly increased the C_{max} of linezolid by median 48% (IQR: 35-103%, P=0.043), but not the C_{min} of linezolid by median 50% (IQR: 44-189%, P=0.080) compared to baseline. There was no statistically significant difference in linezolid half life after co-administration of 500mg clarithromycin with linezolid compared to linezolid alone (P=0.138). Linezolid clearance and elimination constant decreased statistically non-significant when linezolid and 500mg clarithromycin are co-administered compared to baseline (both P=0.08). No dose-dependent effect of clarithromycin on the linezolid exposure could be detected using the Friedman test (P=0.091).

Safety / tolerability

Co-administration of linezolid and clarithromycin was well tolerated by most patients. None of the patients experienced severe adverse events, such as anemia, peripheral neuropathy, optic neuritis, or thrombocytopenia. One patient experienced CTC Grade 2 gastro-intestinal side effects three days after the start of administration of 500mg clarithromycin once daily.

Discussion

In this study, we showed that clarithromycin significantly increased linezolid serum AUC_{0-12h} after combining clarithromycin with linezolid in MDR-TB patients. After two weeks of co-administration of linezolid with clarithromycin 500mg once daily, the C_{max} of linezolid increased significantly by approximately 50%. Combining linezolid with clarithromycin in a dose of 500mg once daily resulted in a significantly higher AUC_{0-12h} of linezolid with a median of 44%. None of the patients experienced any severe adverse events. However, it should be noted that patients pre-emptively received epoetine alpha as part of standard care, potentially obscuring anemia as a side effect.

Besides our recent report on the interaction between clarithromycin and linezolid, there are no other reports on this pharmacokinetic drug-interaction. In fact, one of the few known drug interactions of linezolid to date is with rifampicin. Rifampicin, a well-known inducer of P-glycoprotein and cytochrome P 450 enzymes, decreases linezolid serum levels in critically ill

patients [25]. Another study confirmed this finding in healthy volunteers [26,27]. Gebhart *et al* suggest the interaction to be mediated by P-glycoprotein, since an *in vitro* study has shown that linezolid is not metabolized by cytochrome P450 enzymes [28]. The interaction of linezolid and clarithromycin could also be mediated by P-glycoprotein, since clarithromycin is a well-known cytochrome P450 3A4 inhibitor and a potent inhibitor of P-glycoproteins [29]. P-glycoprotein is a membrane efflux transporter enzyme that is highly expressed in a variety of tissues including the intestine, liver, and kidney [30]. Inhibition of the P-glycoprotein efflux pump by clarithromycin could result in the increased levels of linezolid, possibly a P-glycoprotein substrate, through inhibition of P-glycoprotein at the intestinal site as well as the renal site. P-glycoprotein polymorphism could explain some of the inter-patient variation that we observed. However, in a recent study Gandelman *et al* refer to unpublished data on file from Pfizer suggesting linezolid is not a P-glycoprotein substrate [27]. Their hypothesis for the observed interaction between linezolid and rifampicin is that a large increase in expression of cytochrome P450 3A (CYP3A) that typically has a small contribution (0.7-10.5%) to linezolid clearance, could cause a small decrease in linezolid exposure [27]. Further research on the exact mechanism of the drug-drug interaction is needed.

Co-administration of clarithromycin and linezolid resulted in a near statistically significant decrease of clearance and elimination constant of linezolid compared to baseline. This might suggest inhibition of CYP3A or renal or hepatic P-glycoprotein efflux transporter pumps. However, decreased clearance might not solely explain the observed increase of linezolid exposure. Unfortunately, due to the limited number of samples during the absorption phase, it is impossible to adequately compare data on absorption constant and T_{max}. Since patients did not receive intravenous linezolid, data on bioavailability is not available. It is therefore difficult to draw conclusions on involvement of inhibition of intestinal P-glycoprotein efflux transporters, which could result in increased absorption.

The increase of linezolid exposure after co-administration with clarithromycin has possible implications for clinical practice. The higher linezolid $AUC_{0\text{-}12h}$ could result in toxicity of linezolid, an agent that often causes adverse events, such as time- and dose-dependent severe myelosuppression and polyneuropathy. Severe adverse events often necessitate the cessation of effective anti-MDR-TB treatment, leaving few alternatives. Dose reduction of linezolid could prevent toxicity. However, care should be taken to assure adequate linezolid exposure and added information on whether linezolid exposure is too high, too low, or in the therapeutic range, could prove helpful. Therapeutic drug monitoring could help in assessing the linezolid dose after dose reduction [12], especially since the observed drug-drug interaction shows a large inter-patient variability. In limited resource settings, dried blood spot sampling could resolve logistical problems encountered with conventional therapeutic drug monitoring [31].

After evaluation of the combination of clarithromycin and linezolid in a larger population and during a longer period of time, clarithromycin could eventually even be used as a booster for linezolid, comparable to the use of low-dose ritonavir as a booster to improve protease inhibitor exposure in combined anti-retroviral therapy. The relatively cheap clarithromycin could reduce the dose of the expensive linezolid while the same exposure is maintained, thereby leaving the risk of toxicity unaltered. Since the highest prevalence of MDR-TB is found in countries with limited resources, such a booster strategy could make treatment with linezolid feasible for a larger group of patients. Such a cost reduction could even contribute to the call for making global MDR-TB control affordable [32]. Further research on WHO Group 5 drugs, such as linezolid besides evaluation of new drugs such as delaminid [33] or old drugs such as co-trimoxazole [34], is of great importance.

In conclusion, we showed a 44% increase of linezolid $AUC_{0\text{-}12h}$ after co-administration of linezolid with clarithromycin in a dose of 500mg daily in MDR-TB patients. The pharmacokinetic interaction between linezolid and clarithromycin is suggested to be P-gp mediated. Further research in a larger cohort is needed to provide insight in observed inter-patient variation, perhaps caused by P-gp polymorphism. Until effect size is predictable, possibly with help of P-gp polymorphism testing, therapeutic drug monitoring is advisable to determine individual effect size. The drug-drug interaction we showed in this study is an important step towards making the effective anti-TB drug linezolid available through cost reduction in less affluent settings where MDR-TB is highly prevalent.

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Table 1. Baseline demographics (n=5) and results from drug susceptibility testing.

Parameter	Value
Age - year[†]	35.0 (23 – 65)
Male sex – no. (%)	4 (80%)
Bodyweight - kg[†]	66.8 (55.2 – 78.5)
Height - m[†]	1.74 (1.67 – 1.82)
Body Mass Index[†¥]	22.1 (17.1 – 26.2)
Ethnicity – no.	
African	3
Caucasian	1
Asian	1
HIV positive – no.	1
Isolate resistant to drug based on DST – no./no. total	
Ethambutol	3/5
Isoniazid	5/5
Pyrazinamide[∫]	2/4
Rifampicin	5/5
Streptomycin	4/5
Capreomycin	1/5
Amikacin	0/5
Ciprofloxacin	1/5
Claritromycin[∫]	2/3
Clofazimin[∫]	0/3
Linezolid	0/5
Moxifloxacin	1/5
Protionamide[∫]	1/4
Rifabutin	4/5

[†] *data presented as mean (range)*
[¥] *Body-mass index is calculated by dividing weight in kilograms by the square of the height in meters*
[∫] *Drug susceptibility testing (DST) was not available for all isolates of the included patients*

Table 2. Pharmacokinetic parameters of linezolid and clarithromycin (n=5).

	Linezolid + 0mg clarithromycin	Linezolid + 250mg clarithromycin	p-value	Linezolid + 500mg clarithromycin	p-value[†]
Linezolid					
$AUC_{0\text{-}12u}$ (mg*h/L)	36.3 [33.2-46.3]	61.0 [34.6-63.9]	0.686	67.2 [66.9-76.0]	0.043
C_{max} (mg/L)	6.0 [5.1-6.4]	8.0 [5.5-10.9]	0.104	9.4 [8,9-10.5]	0.043
C_{min} (mg/L)	1.2 [0.9-1.6]	2.1 [0.9-2.2]	0.686	2.6 [2,4-3.9]	0.080
CL (L/h)	7.0 [5.4-8.0]	4.0 [3.5-7.8]	0.686	3.5 [2.7-3.5]	0.080
k_{el} (/h)	0.17 [0.17-0.19]	0.14 [0.12-0.18]	0.785	0.13 [0.11-0.13]	0.080
$t_{1/2}$ (h)	4.1 [3.6-4.2]	4.9 [3.8-5.7]	0.686	5.4 [5.4-6.5]	0.138
Clarithromycin					
$AUC_{0\text{-}12u}$ (mg*h/L)	N/A	8.2 [5.8-9.8]	N/A	20.1 [14.0-23.6]	0.043[¥]

Data are presented as median [interquartile range].
[†] *p-values comparing parameters from co-administration of linezolid with 500mg clarithromycin to baseline*
[¥] *p-value comparing $AUC_{0\text{-}12h}$ of 500mg with 250mg clarithromycin*

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Figure 1

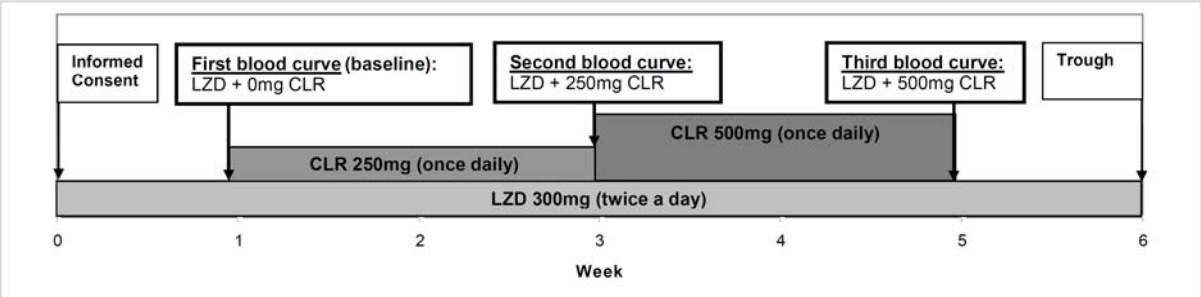
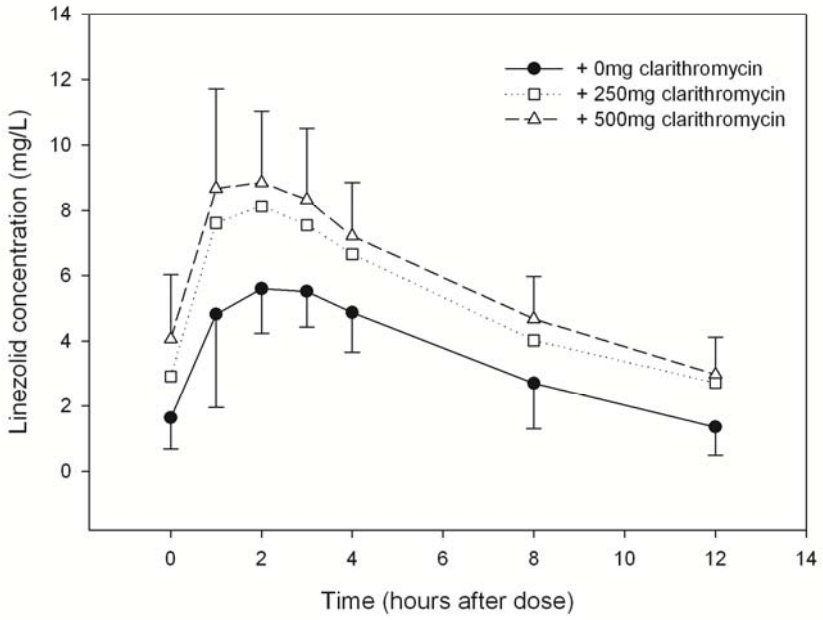


Figure 2


Linezolid concentration (mg/L)
Time (hours after dose)
+ 0mg clarithromycin
+ 250mg clarithromycin
+ 500mg clarithromycin
0
2
4
6
8
10
12
14