

PHARMACOKINETIC INTERACTION BETWEEN LINEZOLID AND GRAPEFRUIT JUICE IN RATS

RODICA CUCIUREANU^{1*}, L. VLASE², DANA MUNTEAN²,
MAGDALENA CUCIUREANU³

University of Medicine and Pharmacy "Gr.T.Popa", Iasi, 16, Universității street, 700115, Romania

¹*Department of Environmental and Food Chemistry*

³*Department of Pharmacology*

²*University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj-Napoca, 13 Emil Isac street, 400023, Romania Department of Pharmaceutical Technology and Biopharmaceutics,*

**corresponding author: rcuciur@yahoo.com*

Abstract

Grapefruit juice has been reported to increase the oral availability of many cytochrome P450 substrates, mainly by inhibiting the first-pass metabolism of the drugs metabolized by cytochrome P450 3A in the gut wall rather than in liver. Linezolid is the first of a new class of antimicrobial drugs, is indicated for the treatment of nosocomial pneumonia and complicated skin infections. It was developed a fast and simple liquid chromatography/ mass spectrometry/ mass spectrometry (LC/MS/MS) method able to quantify linezolid in rat plasma after oral administration. Finally, the developed and validated method was applied for the investigation of the pharmacokinetic interaction between linezolid and grapefruit juice.

Rezumat

Sucul de grapefruit poate determina creșterea biodisponibilității unor medicamente administrate pe cale orală în principal prin inhibarea activității citocromului P₄₅₀ (CYP), izoforma CYP_{3A4} la nivel intestinal. Linezolidul face parte din clasa oxazolidinonelor - o nouă clasă de antibiotice utilizate în tratamentul pneumoniilor nosocomiale și a infecțiilor țesuturilor moi. Lucrarea descrie o metodă LC/MS/MS simplă și rapidă de a cuantifica concentrația linezolidului în plasma de șobolan alb Wistar, după administrare orală. Această metodă a fost validată și aplicată în studierea interacțiunii farmacocinetice între linezolid și sucul de grapefruit.

Keywords: linezolid, grapefruit juice, pharmacokinetic interaction

Introduction

Linezolid, Zyvox® [(S)-N-[[3-[3-Fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl] methyl]acetamide, (Figure 1) is the first substance on the market belonging to a new class of antibiotics, the oxazolidinones.

This drug is a synthetic oxazolidinone antimicrobial agent that has been proven to be effective against nosocomial and community-acquired pneumonias, and skin infections [3,5]. Linezolid inhibits bacterial protein

synthesis through a mechanism of action different from that of other antibacterial agents; therefore, cross-resistance between linezolid and other classes of antibiotics is unlikely.

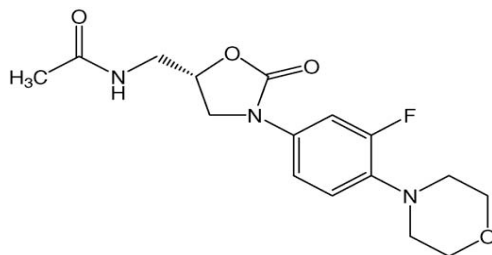


Figure 1
Chemical structure of linezolid

This drug is a synthetic oxazolidinone antimicrobial agent that has been proven to be effective against nosocomial and community-acquired and pneumonias, and skin infections [3,5]. Linezolid inhibits bacterial protein synthesis through a mechanism of action different from that of other antibacterial agents; therefore, cross-resistance between linezolid and other classes of antibiotics is unlikely. Preclinical animal studies have shown that linezolid is completely absorbed after oral administration, with an absolute bioavailability of 100%; maximum plasma concentrations are reached approximately 1 to 2 hours after dosing. Plasma protein binding is 32%. Linezolid is primarily metabolized by oxidation of the morpholine ring, which results in two inactive ring-opened carboxylic acid metabolites (the aminoethoxyacetic acid metabolite, and the hydroxyethyl glycine metabolite). The formation of hydroxyethyl glycine metabolite is mediated by a non-enzymatic chemical oxidation mechanism *in vitro* [5]. Linezolid is not an inducer of cytochrome P450 (CYP) in rats.

Grapefruit juice have been reported to exhibit pharmacokinetic interaction with drugs when administered together [1]. Some of the most known drugs that interact with grapefruit juice are: calcium channel blockers, antihistamines, hidroxy-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, immunosuppressants, anxiolytics, protease inhibitors, macrolide (especially troleandomycin and erythromycin) and quinolone (enoxacin, pefloxacin and ciprofloxacin) [1, 2]. Grapefruit juice can produce several-fold increase in the levels of particular drugs (augmenting therapeutic or toxic effects) by decreasing pre-systemic metabolism through significant inhibition of:

- gut wall CYP3A4 isoenzymes;

- P-glycoprotein (P-gp) (efflux membrane transporter pump belonging to the adenosine triphosphate-binding cassette family of proteins which is located in the apical brush border of enterocytes);
- multidrug resistance protein-2 (MRP2) efflux protein closely related to P-gp in terms of its expression and function;

After uptake by the enterocyte, the drugs are either metabolized by CYP3A4 or pumped back out into the lumen by the P-gp transporter - processes that increase blood levels of the drug substrate.

Most of these interactions are modest in magnitude and clinically relevant only for drugs that have a narrow therapeutic range [2].

The aim of this study was to develop a fast and simple LC/MS/MS method able to quantify linezolid in rat plasma after oral administration. Finally, the developed and validated method was applied for investigation of pharmacokinetic interaction between linezolid and grapefruit juice.

Materials and methods

Reagents

Linezolid (batch no. 037K1588) was provided by Sigma Chemical Co. (Sigma Aldrich, Germany). Squeezed grapefruit juice was prepared from fresh *Citrus paradisi* fruits (using also the peel). The solvents and reagents for chromatography were purchased from Merck KgaA, Darmstadt, Germany) and the water was obtained with Ultra Pure Water System (Ultra Clear TWF, Germany).

Standard solutions

The stock solution of linezolid, with a concentration of 1.56 mg/mL was prepared by dissolving an appropriate quantity of reference substance in methanol. A working solution of 93600 ng/mL was prepared by diluting the specific volume of stock solution. Then, this solution was used to prepare eight plasma standards with the concentrations ranged between 468.0 and 29952.0 ng/mL. Quality control samples (QC) were prepared by diluting specific volumes of working solution with plasma and were used during clinical samples analysis.

Chromatographic and mass spectrometry systems and conditions

The HPLC system was a 1100 series model (Agilent Technologies) consisting of a binary pump, an in-line degasser, an autosampler, a column thermostat, and a 1100 Ion Trap SL mass spectrometer detector (Bruker Daltonics GmbH, Germany). Chromatograms were processed using QuantAnalysis Software. The detection of linezolid was performed using the ion trap mass spectrometer with electrospray ion source operated in

positive ionization. The ion transition monitored was m/Z 388 $\rightarrow$ m/Z 296. Chromatographic separation was performed at 40°C on a Zorbax SB-C18 100x3.0 mm, 3.5 μ m (Agilent Technologies), protected by an in-line filter. The mobile phase consisted in a mixture of water containing 0.05% trifluoroacetic acid (at pH 6.5 adjusted with ammonia) and acetonitrile (76:25v/v). The pump delivered the mobile phase at 1 mL/min.

Sample preparation

In a test tube of 1.5 mL, 0.2 mL plasma and 0.6 mL methanol were added. The tube was vortex mixed for 10 seconds and then centrifuged for 6 minutes at 5000 rpm. 0.1 mL of the supernatant was diluted 1/50 with water and then 0.15 mL were transferred in an autosampler vial, 5 μ L were injected into the HPLC system.

Method validation

As a first step of method validation, specificity was verified using six different plasma blanks obtained from rats who had not previously taken any medication.

The concentration of the analyte was determined automatically by the instrument data system. The calibration curve model was $y = ax + b$, weight 1/y linear response, where y-peak area and x-concentration. Distribution of the residuals (% difference of the back-calculated concentration from the nominal concentration) was investigated. The calibration model was accepted, if the residuals were within $\pm 20\%$ at the lower limit of quantification (LOQ) and within $\pm 15\%$ at all other calibration levels and at least 2/3 of the standards meet this criterion, including the highest and lowest calibration levels.

The lower limit of quantification was established as the lowest calibration standard with an accuracy and precision less than 20%. The within-and between-run precision (expressed as coefficient of variation, CV%) and accuracy (expressed as relative difference between obtained and theoretical concentration, bias%) of the assay procedure were determined by analysing on the same day five different samples at each of the lower, medium and higher levels of the considered concentration range and one different sample of each at five different occasions, respectively [6-12].

Experimental protocol on laboratory animals

Adult Wistar male rats having the weight ranging 180–220 g were obtained from the Laboratory Animal Center of Cantacuzino Institute of Research, Bucharest, Romania for the experiments.

They were placed in groups of three in plexiglas cages with the floor covered with sawdust. The rats were exposed to a controlled

environment with 12 h light/dark cycle and a temperature of $20 \pm 1^\circ\text{C}$ before and throughout the experimental period. These experiments were approved by the University's Committee for Bioethics and animal experimentation according to international rules of conduct.

After a habituation period of one week, animals were randomized into three groups of 30 animals which received as follows:

- group I (control) – saline solution (single administration);
- group II – grapefruit juice 2 mL followed 30 min later by single administration of linezolid (40 mg/kgbw) p.o.;
- group III – linezolid (40 mg/kgbw) p.o.

The animals were fasted 18 hours before the experiment but had access to water *ad libitum*.

Blood samples were collected into ethylenediaminetetraacetic acid tubes at 0.33, 0.66, 1, 2, 3, 6, 8, 12, 24, 48 hours after drug administration. The blood was processed to plasma by centrifugation at 4500 rpm for 15 min. Plasma samples were stored at -34°C until the HPLC/MS analysis of linezolid.

Kinetica software (TermoLabSystems, SUA) was used for the pharmacokinetic analysis.

Pharmacokinetic analysis

The non-compartmental and compartmental pharmacokinetic analysis method was employed to determine the pharmacokinetic parameters of linezolid given alone or in combination with grapefruit juice. The maximal plasma concentration (C_{max} , ng/mL) and the time to reach the peak concentration (t_{max} , hr) were obtained directly by the visual inspection of each subject's plasma concentration-time profile.

The area under the concentration-time curve (AUC_{0-t}) has been estimated by integration using trapezoidal rule from time zero to the last measurable concentration at time t . The area was extrapolated to infinity ($\text{AUC}_{0-\infty}$) by addition of C_t / K_{el} to AUC_{0-t} where C_t is the last quantifiable drug concentration and k_{el} is the elimination rate constant [4,7,8,13,14]. The elimination rate constant K_{el} was estimated by the least-square regression of plasma concentration-time data points lying in the terminal region by using semilogarithmic dependence that corresponds to a first-order kinetics. The half-life ($T_{1/2}$) was calculated as $0.693/k_{\text{el}}$ [4, 7, 8, 13, 14]. The pharmacokinetic analysis was performed using Kinetica 4.0.2 (Thermo Labsystems, U.S.A.).

Results and discussion

No significant interference at the retention time of linezolid (1.45 min) was observed in different plasma blank samples chromatograms, due to the specificity of the selected signal (figure 2, 3).

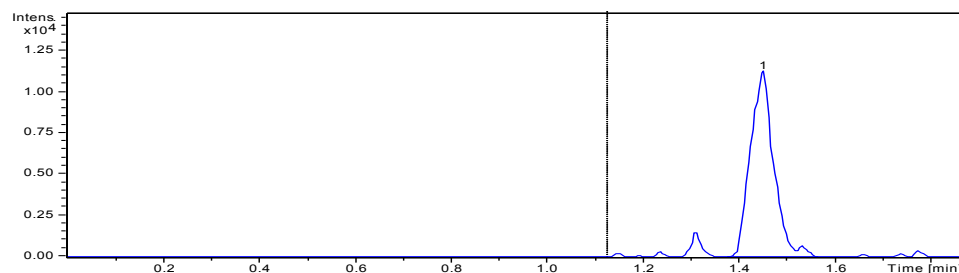
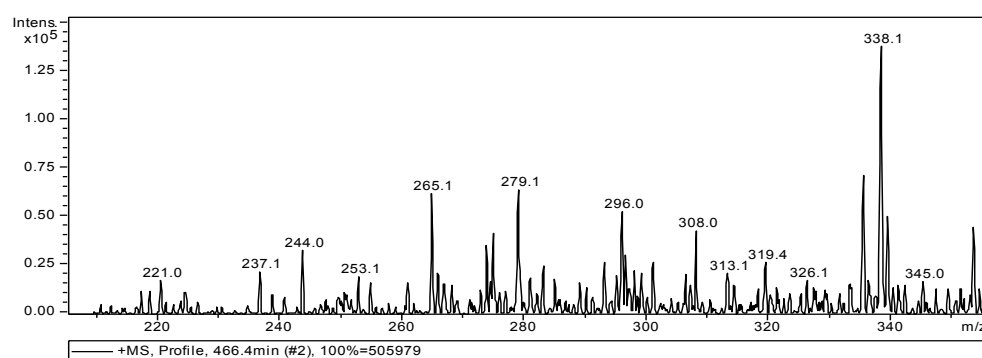
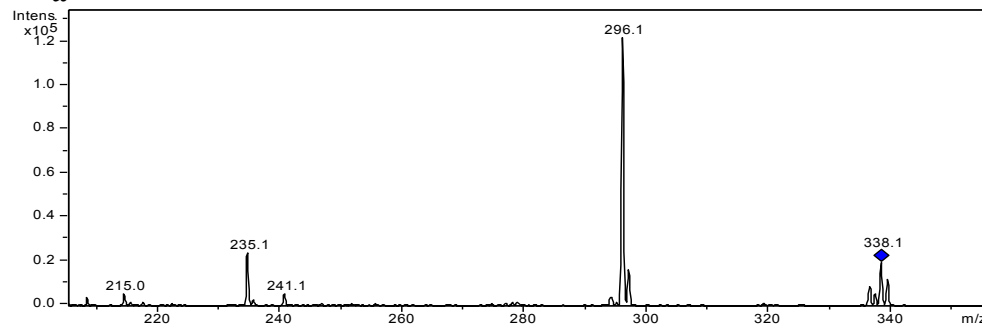


Figure 2

Chromatogram of LOQ plasma standard containing 468 ng/mL linezolid



a



b

Figure 3

Mass spectra of linezolid: full-scan spectrum (a) and MS/MS spectrum (b)

The applied calibration curve model proved to be accurate over the concentration range 468-29952 ng/mL, with a correlation coefficient greater than 0.995.

The method had within- and between-run accuracy and precision in agreement to international regulations regarding bioanalytical methods validation. The lower limit of quantification was established at 468 ng/mL, with an accuracy and precision less than 20%.

The recovery was consistent and reproducible for the analyte. It proved to be stable under various conditions, the bias% of the found concentration being less than 15%, the maximum accepted value for the method's accuracy.

The mean plasma levels of linezolid administered alone or in combination with grapefruit juice, in rats, are presented in figure 4. At the same dose of linezolid, there are higher plasma levels of drug when administered with grapefruit juice.

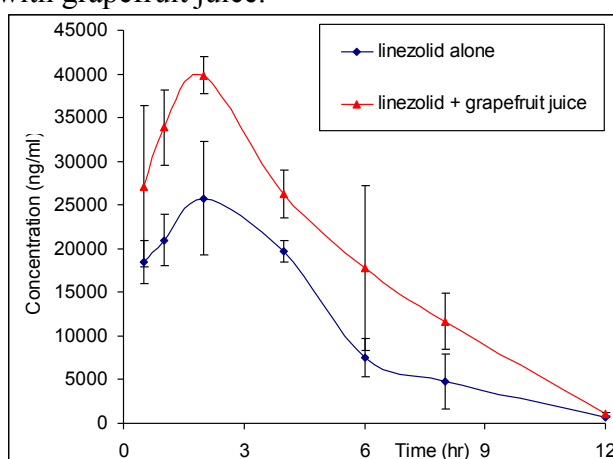


Figure 4

The mean plasma levels of linezolid administered alone or in combination with grapefruit juice

The calculated pharmacokinetic parameters of linezolid [4,7-10] administered alone or in combination with grapefruit juice are presented in Table I.

Table I

The pharmacokinetic parameters of linezolid administered alone or in combination with grapefruit juice

Parameter	Linezolid alone	Linezolid + grapefruit juice
C_{max} (ng/mL)	25755	39834
T_{max} (hr)	2	2
AUC_{0-t} (ng/mL*hr)	133611	220973
$AUC_{0-\infty}$ (ng/mL*hr)	135579	224304
% AUC_{extra}	1.45	1.48
$T_{1/2}$	1.73	1.69
Mean residence time (MRT) (hr)	3.65	3.97

C_{max} of group II (linezolid + grapefruit juice) is significantly higher than C_{max} of group III (linezolid) (39834 ng/mL vs. 25755 ng/mL) (Table I) data which proves the pharmacokinetic interaction between linezolid and grapefruit juice. As C_{max} is correlated both with the amount of absorbed substance and the absorption speed, the precise mechanism or site of the interaction cannot be established. Also AUC increases from 135579 ng/mL*hour in group III to 224304 ng/mL*hour in group II which means that relative bioavailability is 165.4%, data which also emphasizes the interaction between linezolid and grapefruit juice.

Non-significant differences regarding T_{max} , $T_{1/2}$ and mean residence time (MRT) indicate that there is no interaction between linezolid and grapefruit juice at the elimination level.

The above-mentioned data (the existence of pharmacokinetic interaction and the absence of interaction at level of elimination) suggest that bioavailability changes are due to the effect of first intestinal or liver passage (figure 4), through mechanisms that have not been clearly elucidated.

As linezolid is not detectably metabolized by human cytochrome P450 and it does not inhibit the activities of clinically significant human CYP isoforms indicate that glycoprotein P inhibition may be involved.

Conclusions

The proposed HPLC/MS/MS method is simple and sensitive and provides accuracy and precision in the determination of linezolid in rat plasma. The lower limit of quantification was established at 468 ng/mL linezolid, with an accuracy and precision less than 15%. The MS/MS detection and the simple sample preparation method allowed a specific and efficient analysis of a large number of rat plasma samples from the clinical study in a very short time.

The present study demonstrates that grapefruit juice significantly influences the pharmacokinetics of linezolid and increases its bioavailability in rats.

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