Bioequivalence of Two Lithium Formulations in Healthy Volunteers

Márcia Cristina Guilherme[1], Dárcio Gomes Pereira[1], Miriam Prado Galuppo[1], Gustavo D. Mendes[1,2], José Luiz Donato[1], and Gilberto De Nucci[1,2]

[1] Galeno Research Unit, St. Campinas, Sao Paulo (Brazil)
[2] Department of Internal Medicine, State University of Campinas, Campinas/SP (Brazil)

Corresponding author: Prof. Gilberto De Nucci, Ph. D., 415 Jesuino Marcondes Machado Ave., 13092-320, Campinas/SP (Brazil); fax: +55 19 3252 1516; e-mail: denucci@dglnet.com.br

Summary

Objective: **The purpose of this study was to compare the maximum exposure and extent of bioavailability of two lithium carbonate (CAS 554-13-2) containing 300 mg tablet formulations (test and reference) for oral administration.**

Method: **This bioequivalence study was conducted in a 2-period crossover design with a washout phase of 7 days. Plasma samples were obtained by blood sampling over 72 h in each period. Twenty-four healthy volunteers of both genders participated in the trial. Samples were analyzed by a flame atomic absorption spectrometer. Resulting Li^+ concentrations were used for determination of the pharmacokinetic parameters AUC_{last}, AUC_{inf} and C_{max}.**

Results: **90 % confidence intervals for AUC_{last}, AUC_{inf} and C_{max} were 96.81–107.44 %, 98.44–109.54 % and 98.60–111.33 %, respectively.**

Conclusion: **All 90 % and 95 % confidence intervals were inside the limits defined by the FDA Guidance for Industry (80 %–125 %) and thus stated that test and reference formulation may be accepted as bioequivalent, with regard to both, maximum exposure and extent of bioavailability.**

Key words

- **CAS 554-13-2**
- **Lithium, bioavailability, bioequivalence**
- **Mood-stabilizing drug**

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Zusammenfassung

Bioäquivalenz zweier Lithium-Formulierungen bei gesunden Probanden

Zielsetzung: **Ziel dieser Studie war der Vergleich der maximalen Aufnahme und des Ausmaßes der Bioverfügbarkeit zweier 300 mg Lithiumkarbonat (CAS 554-13-2) enthaltender Tablettenformulierungen (Test und Referenz) zur oralen Anwendung.**

Methode: **Diese Bioäquivalenz-Studie verfolgte ein 2-Perioden-Crossover-Design mit einer Auswaschphase von 7 Tagen. Plasmaproben wurden nach Blutabnahmen über 72 h in jeder Studienperiode gewonnen. 24 gesunde Probanden beider Geschlechter nahmen an der Studie teil. Die Proben wurden mittels Flammen-Atom-Absorptions-Spektrometer analysiert. Die resultierenden Li^+-Kon-**

zentrationen wurden zur Bestimmung der pharmakokinetischen Parameter AUC_{last}, AUC_{inf} und C_{max} herangezogen.

Ergebnisse: **Die 90 %-Konfidenzintervalle von AUC_{last}, AUC_{inf} und C_{max} lagen bei 96.81–107.44 %, 98.44–109.54 % bzw. bei 98.60–111.33 %.**

Schlußfolgerung: **Alle 90 %- und 95 %-Konfidenzintervalle lagen innerhalb der durch die FDA Guidance for Industry vorgegebenen Grenzen von 80 %–125 %, und somit können Test- und Referenz-Formulierung im Hinblick auf maximale Aufnahme und Ausmaß der Bioverfügbarkeit als bioäquivalent angesehen werden.**

1. Introduction

Lithium salt (Li^+) is a mood-stabilizing drug frequently used for treatment and prophylaxis of bipolar disorder [1]. Its therapeutic properties have been repeatedly verified since initially reported by Cade in 1949 [2], but the mechanism of action of Li^+ is still not well understood. [3]. Commercially, Li^+ is available as immediate-release capsules and tablets containing 300 and 450 mg (as Li^+ free base) for oral administration. Those dosage forms make the drug immediately available for absorption, with peak plasma concentrations occurring between 0.5 and 3 h [4].

The generally accepted steady-state therapeutic range of plasma Li^+ concentrations is about 4.17–8.33 mg/L in affective disorders [4, 5] although many patients will respond to lower concentrations (2.08–4.86 mg/L) [6]. Li^+ serum levels lower than 4.17 mg/L appear to be associated with a risk of relapse, while toxic effects begin at concentrations above 10.41 mg/L [7]. This constricted therapeutic range places Li^+ into the classification of a narrow therapeutic index (NTI) drug, in which slight fluctuations in plasma concentrations may result either in inadequate clinical response or in significant adverse effects [8]. In order to guarantee that different Li^+ formulations produce similar clinical responses, bioequivalence studies must be conducted to evaluate the relative rate and extent of absorption.

In order to monitor Li^+ concentration present in plasma and other biological fluids, a number of methods have been developed such as flame photometry [9], graphite furnace atomic absorption spectrometry [10], ion-selective electrode technique [11] and flame atomic absorption spectrophotometry (FAAS) [12]. However, since FAAS is a widespread method used to determine individual elements in biological samples, and since it has high sensitivity to permit the accurate determination of Li^+, the FAAS method was chosen for this study.

The aim of this study was to compare the pharmacokinetic profiles and to evaluate the bioequivalence of two Li^+ carbonate (CAS 554-13-2) oral tablets (300 mg) in 24 human volunteers: test formulation versus reference.

2. Materials and methods

2.1. Clinical protocol

The study began with 26 healthy volunteers. One volunteer dropped out of the study due to personal reasons and another volunteer dropped out of the study before the second period of the administration due to the decision of the clinical investigator, since the clinical laboratory test for hemoglobin was outside the normal range. Twenty-four volunteers completed the clinical study.

The volunteers of both sexes (12 males and 12 females) selected for the study were between 21 and 45 years old (32.29 ± 7.10, mean ± SD), between 1.50 and 1.81 meters in height (1.64 ± 0.10, mean ± SD), with body weights (63.78 kg ± 10.51, mean ± SD) and body mass index equal to or greater than 19 and equal to or lower than 27.

All subjects signed informed consent forms, and the Campinas State University Ethics Committee approved the clinical protocol. All volunteers were healthy as assessed by physical examination, ECG and the following laboratory tests: hemoglobin, hematocrit, red and white blood count, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), routine urinalysis, total cholesterol, triglycerides, total proteins, albumin, uric acid, total bilirubin, alkaline phosphatase, gamma-glutamyl transpeptidase (γ-GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine and fasting blood glucose. All subjects were negative for human immunodeficiency virus (HIV), hepatitis B virus (HBV) (except for serological scar) and human coronavirus (HCV).

The study was conducted in an open randomized two-period crossover balanced design with a one-week washout period between the doses. During each period, the volunteers were hospitalized and had a dinner at 19:00 h. After an overnight fast, they received a 300 mg dose of the Reference formulation or a 300 mg tablet of the test formulation. The tablets were given at 7:00 h directly into the volunteer's mouth followed by 200 ml of tap water. All volunteers had fasted for 2 h after drug administration, when a xanthine-free standard breakfast was consumed. Standard meals were provided 5, 8 and 11 h after dosing. No other food was permitted during the "in house" period. Liquid consumption was permitted ad libitum after lunch, but xanthine-containing drinks including tea, coffee, and cola were prohibited.

Systolic and diastolic arterial pressure (measured non-invasively with a sphygmomanometer) and heart rate were recorded before and after drug administration.

2.2. Formulations

The following test formulation was employed: lithium carbonate 300 mg (batch number 243/04, expiration date 07/2007) made by Cristália Produtos Químicos Farmacêuticos Ltda., Itapira City, Sao Paulo State (Brazil).

2.3. Drug analysis

Blood samples (4 ml) from a suitable forearm vein were collected into heparin containing tubes before drug administration at the following time points: 0:10, 0:20, 0:30, 0:40, 0:50, 1:00, 1:10, 1:20, 1:30, 1:40, 1:50, 2:00, 2:15, 2:30, 2:45, 3:00, 3:30, 4:00, 4:30, 5:00, 6:00, 7:00, 8:00, 10:00, 12:00, 14:00, 16:00, 18:00, 24:00, 28:00, 32:00, 36:00, 48:00 and 72:00 h post-dosing of each

Li^+ carbonate formulation. The blood samples were centrifuged at 3,200 g for 10 min at 4 °C. Plasma was transferred to labeled tubes and stored at ≤ –15 °C until analysis.

For drugs analysis, 50 µl of purified water was added to a glass tube, followed by plasma sample (450 µl) and 2 ml of a 10 % trichloroacetic acid aqueous solution (Mallinckrodt Baker, NJ, USA, # Y52610). The tube was vortex-mixed for 10 s and then centrifuged at 3,200 g for 10 min at 4 °C. The supernatant was transferred to another glass tube. It was analyzed by a flame atomic absorption spectrophotometer with a Shimadzu (Tokyo, Japan) AA-6300 spectrophotometer and a flame mode analytical technique. A Li^+ hollow-cathode lamp from Hamamatsu (Iwata, Japan) was operated at 8 mA with a 0.7-nm slit. The wavelength was set at 670.8 nm. Readings were made in triplicate. An acetylene/air mixture was used as a fuel/oxidant gas system, set at a flow rate of 1.7 L min^{-1} and 15.0 L min^{-1}, respectively. The burner height was set at 5.0 mm and the burner angle, at 0 degree.

2.4. Calibration standards and quality control

Li^+ stock solutions were prepared by dissolving appropriate amounts of Li^+ carbonate in deionized water to give 100 mg/L of free Li^+. Standards solutions were prepared from the stock solution by sequential dilutions with deionized water to give eight concentrations: 0.7, 1.4, 2.1, 3.5, 7, 14, 35 and 70 mg/L.

Calibration standards were prepared by spiking control human plasma with the corresponding standard solutions.

The calibration standards and blanks were freshly prepared (in duplicate) for each assay and were processed along with plasma samples and quality controls in low (QCA), medium (QCB and high (QCC) concentrations.

2.5. Recovery

In order to evaluate the recovery, experiments were conducted with the method described above. The recovery (%) was calculated as the ratio of the absorbance values for processed blank plasma, spiked with QCA, QCB and QCC, relative to the absorbance values of the equivalent standard solutions, spiked after sample processing.

2.6. Stability

Quality control samples were subjected to three stability tests: short-term (21 h) at room temperature, three freeze-and-thaw cycles (–20 to 25 °C) and long-term (248 days). Five aliquots of each concentration (QCA, QCB and QCC) were processed for the desired tests and compared to five aliquots of freshly prepared samples in each concentration (reference values).

2.7. Pharmacokinetics and statistical analysis

The first-order terminal elimination rate constant (k_e) was estimated by linear regression from the points describing the elimination phase in a log-linear plot. The half-life was derived from this rate constant ($t_{1/2} = \ln(2)/k_e$). The maximum observed plasma concentration (C_{max}) and the time taken to achieve this concentration (t_{max}) were obtained directly from the curves. The areas under the Li^+ plasma concentration vs. time curves from 0–72 h (AUC_{last}) were calculated by applying the linear trapezoid rule. These areas to infinity ($AUC_{0\text{-}inf}$) were determined by adding the value C_{last}/k_e to the calculated AUC_{last} (where C_{last} = the last detectable concentration).

3. Results

The lower limit of quantification (LOQ), defined as the lowest concentration at which both precision and accuracy were less than or equal to 20 %, was 0.07 mg/L. Based on the LOQ value, three quality controls were defined respectively with low (QCA), medium (QCB) and high (QCC) concentrations: 0.2, 2 and 5 mg/L. Precision and accuracy were based on back-calculated values of quality control samples measured on three consecutive days, with eight samples at each concentration.

The Li^+ calibration graphs were linear over the concentration range of 0.07–7.0 mg/L, which provided typical linear regressions. Mean absolute recovery of Li^+ in plasma was 86.9 % at 0.2 mg/L; 94.3 % at 2.0 mg/L and 96.0 % at 5.0 mg/L.

The stability tests results presented indicate no significant differences in human plasma Li^+ concentrations.

The mean Li^+ plasma concentrations of the 24 volunteers after a 300 mg oral dose for both Li^+ formulations are shown in Fig. 1.

The respective mean pharmacokinetic parameters are shown in Table 1. Li^+ peak plasma concentrations

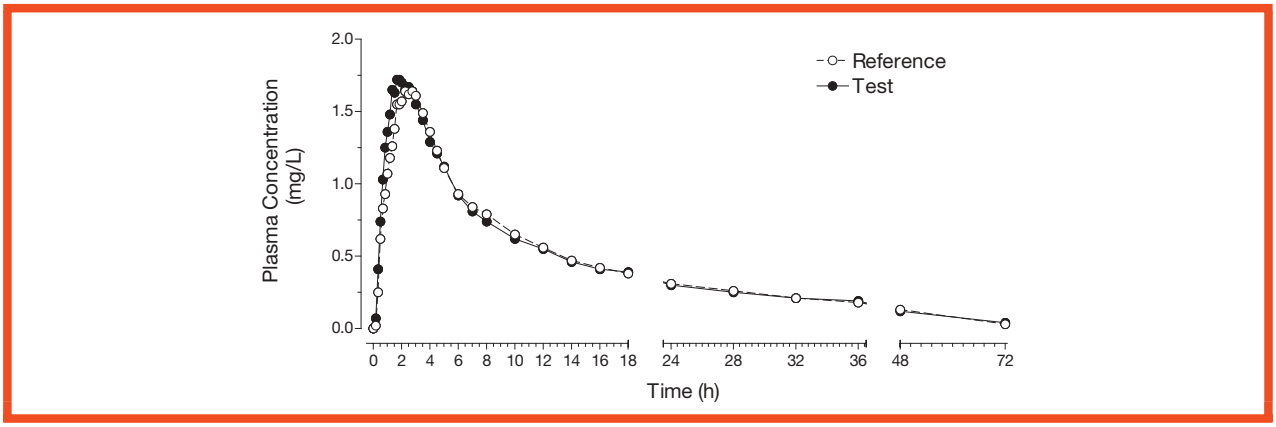

Fig. 1: Mean plasma concentrations as a function of time profile, obtained after single oral administrations of both Li^+ carbonate formulations (n = 24).

Table 1: Mean pharmacokinetic parameters obtained from 24 volunteers after administration of each Li^+ carbonate formulation.

Variable	Unit	N	Mean	Median	SD	SE	Min	Max	CV%
				Reference					
AUC_{all}	([mg* h]/L)	24	22.43	23.24	4.76	0.97	12.58	29.71	21.24
AUC_{inf}	([mg* h]/L)	24	25.22	24.93	5.71	1.17	13.81	35.78	22.65
AUC_{last}	([mg* h]/L)	24	21.60	21.50	4.99	1.02	11.98	29.71	23.11
C_{last}	(mg/L)	24	0.12	0.12	0.03	0.01	0.07	0.17	26.47
C_{max}	(mg/L)	24	1.98	1.94	0.43	0.09	1.34	2.82	21.52
k_e	(1/h)	24	0.04	0.03	0.01	0.00[a)]	0.02	0.08	38.04
$T_{1/2}$	(h)	24	20.87	20.33	7.19	1.47	9.18	34.48	34.46
T_{last}	(h)	24	51.34	48.00	12.72	2.60	36.00	72.00	24.77
T_{max}	(h)	24	2.13	1.92	0.93	0.19	0.50	4.00	43.61
				Test					
AUC_{all}	([mg* h]/L)	24	22.72	22.61	4.56	0.93	14.23	31.83	20.07
AUC_{inf}	([mg* h]/L)	24	26.01	24.83	5.27	1.08	15.37	36.14	20.26
AUC_{last}	([mg* h]/L)	24	21.93	21.57	4.78	0.98	13.29	31.83	21.80
C_{last}	(mg/L)	24	0.12	0.11	0.04	0.01	0.07	0.20	31.29
C_{max}	(mg/L)	24	2.06	2.04	0.37	0.07	1.46	2.82	17.73
k_e	(1/h)	24	0.03	0.03	0.01	0.00[a)]	0.01	0.06	38.55
$T_{1/2}$	(h)	24	25.18	24.37	13.21	2.70	12.24	74.16	52.48
T_{last}	(h)	24	54.38	48.00	13.06	2.67	36.00	72.00	24.02
T_{max}	(h)	24	1.96	1.92	0.71	0.15	0.67	3.50	36.35

[a)] Exact values: 0.003 for reference and test.

Table 2: Geometric mean of the individual AUC_{last}, $AUC_{0\text{-}inf}$ and C_{max} ratios (test/reference formulation) and the respective confidence intervals (CI).

Test vs. reference (T/R) 300 mg	Statistical analysis				
	Power	% Geom. mean	90 % CI	95 % CI	Intra-subject CV%
C_{max} (n = 24)	0.9998	104.7707	98.6008–111.3266	97.3653–112.7393	11.68
AUC_{last} (n = 24)	1.0000	101.9869	96.8089–107.4419	95.7665–108.6113	10.30
$AUC_{0\text{-}inf}$ (n = 24)	1.0000	103.8439	98.4386–109.5461	97.3514–110.7694	10.36
C_{max} (n = 12) – Male	0.9945	101.3311	93.9404–109.3033	92.3228–111.2185	10.10
AUC_{last} (n = 12) – Male	0.9870	98.6546	90.7421–107.2572	89.0186–109.3338	11.45
$AUC_{0\text{-}inf}$ (n = 12) – Male	0.9998	100.3898	95.0996–105.9741	93.9261–107.2982	7.29
C_{max} (n = 12) – Female	0.9246	108.3269	97.3432–120.5501	94.9852–123.5427	13.33
AUC_{last} (n = 12) – Female	0.9951	105.4317	97.8349–113.6184	96.1712–115.5840	9.58 %
$AUC_{0\text{-}inf}$ (n = 12) – Female	0.9460	107.4170	97.0693–118.8678	94.8401–121.6617	12.74 %

were 1.98 mg/L for the reference formulation and 2.06 mg/L for the test formulation.

Table 2 presents the ratios and the respective confidence intervals for the statistical analysis of this bioequivalence study.

4. Discussion

With widespread availability of generic drugs in recent years, bioavailability and bioequivalence have received increased attention [23]. Particularly in bioequivalence studies, the issue of narrow therapeutic index (NTI) drugs in which minor oscillations in drug serum levels could result in either under-medication or intoxication has become an even more pressing concern. Consequently, since Li^+ is one such NTI drug, the bioequivalence of two different Li^+ formulations is a highly critical issue.

In order to evaluate the bioavailability, two pharmacokinetics parameters are considered: (1) the absorbed drug amount represented by the area under the curve (AUC) and (2) the maximum drug concentration in plasma (C_{max}) that is a function of both rate and extent of absorption [24]. As can be seen in Fig. 1 and Table 1, both Li^+ formulations showed similar rate and extent of absorption, i.e. equivalent bioavailability, since there were no significant differences in the mentioned parameters. Furthermore, all 90 % and 95 % confidence intervals shown in Table 2 are in accordance with the 80–125 % interval proposed by the US Food and Drug Administration [25].

The observed Li^+ half-life ($t_{1/2}$) values after oral administration of a 300-mg dose were within the reported range of 8–24 h [26]. In addition, peak plasma concentration (C_{max}) and the time taken to achieve these values (t_{max}) were similar to those reported in the literature [4, 20].

Based on the intra-subject percent coefficient of variation (CV%) 11.68 for C_{max} and 10.30 for AUC_{last}

(Table 2), we propose using 16 volunteers for future studies.

The method described here to quantify Li^+ concentration in human plasma is simple, reproducible and presents appropriate sensitivity for this ion determination. The validation results of the analytical method meet the requirements for bioanalytical procedures prescribed by international regulatory guidelines [22, 27].

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