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Bioequivalence Study of Two Hydrochlorothiazide Formulations after Oral Administration in Healthy Chilean Volunteers

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Abstract: The study was carried out in healthy Chilean volunteers in order to compare the pharmacokinetics (rate and extent absorption) of two commercial oral formulations of 50 mg hydrochlorothiazide. Thirty nine subjects were administered hydrochlorothiazide tablets of test (*T*) and reference (*R*) formulation in a single blind, randomized, fasting, 2 × 2 crossover study, seven washout days. Blood samples were taken during a 48 h period after drug administration. Plasma concentrations were quantified by HPLC-MS/MS. The primary parameters log–transformed C_{max} (maximum plasma concentration), $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ (area under the plasma concentration-time curve from zero to the last time and zero to infinity) were tested for bioequivalence considering the ratios of geometric means (test/reference); whereas t_{max} (the time of maximum plasma concentration) was analyzed nonparametrically. The 90% confidence intervals for the geometric mean values of test/reference ratios for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were 95.37%, 93.59% and 96.34%, respectively, and were located within the bioequivalence acceptance range of 80~125%, as were t_{max} and elimination constants. Together, we conclude that the test formulation of the hydrochlorothiazide 50 mg tablet is bioequivalent to the reference product and suitable for generic prescription.

Key words: Bioequivalence, hydrochlorothiazide, interchangeable.

1. Introduction

The term thiazide diuretics is used here to refer to all members of the class of Na^+/Cl^- symporter inhibitors [1]. Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-enzothiadiazine-7-sulf onamide 1,1-dioxide) is a potent thiazide diuretic that enhances natriuresis which leads to a reduction in plasma volume and cardiac output [2]. Thiazide diuretics are used for the treatment of edema associated with heart (congestive heart failure), liver (hepatic cirrhosis), and renal (nephrotic syndrome, chronic renal failure, and acute glomerulonephritis) disease [1, 2]. Therefore, hydrochlorothiazide is widely used alone or in combination with other antihypertensive drugs for the treatment of hypertension and congestive heart failure [3]. This drug has been used for a long time and is regarded as a highly safe medication although some common and frequent adverse effects are hypokalemia, hyperuricaemia, decreased glucose tolerance, hyperlipidaemia, impotence and lethargy [1, 2].

Hydrochlorothiazide is readily absorbed from the gastrointestinal tract and its absolute bioavailability following oral administration is approximately 60~80% of the dose [1, 3]. The peak plasma concentration is

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achieved around 2 h after oral administration and the elimination half-life is approximately 10 h [4]. The diuretic effect persists 6~12 h and the hypotensive effect persists for up to one week after therapy withdrawal [4]. The bioavailable dose fraction is excreted almost completely in the urine as unchanged drug, and 50~70% of the oral dose is excreted during the next 24 h after oral administration [3, 4].

Thiazide diuretics were the first tolerated efficient antihypertensive drugs that significantly reduced cardiovascular morbidity and mortality in placebo-controlled clinical studies [5]. Today, these drugs are still considered a fundamental therapeutic tool in the clinical arsenal for treatment of hypertensive patients. However, a high correlation between oral dose and plasma concentration is important to guarantee the quality of the medication and ensure an optimal therapeutic response [5].

Generic drug medication is fundamental for the general population, especially those in the low economic sector of the public health system, in order to have access to quality drugs with lower costs [6]. In most cases, the innovator formulation is usually too expensive for the majority of patients. Therefore, the use of certified generic drugs is essential for the population to have equitable access to treatment with the same quality of innovators, but with reduced costs.

Interchangeability of drugs is established by bioequivalence studies comparing the plasma concentration versus time curves for the generic and the innovator formulations. They are considered bioequivalent when the rate and extent of bioavailability of the active ingredient in the two products are not significantly different under suitable test conditions [6, 7].

The objective of this study was to perform a bioequivalence analyses of two 50 mg tablet formulations of hydrochlorothiazide after a single oral administration under fasting conditions in healthy volunteers. In addition, our study provides the Chilean population with a new certified low cost therapeutic alternative; and also characterizes the pharmacokinetic parameters of Chilean subjects to this type of drug.

2. Materials and Methods

Hydrochlorothiazide in plasma samples were analyzed using a validated method involving HPLC (Agilent 1200 Series, Agilent Technologies, Waldbronn, Germany) with MS/MS detection (API 4000, MDS Sciex, Ontario, Canada) in AGQ Labs, Santiago, Chile. The developed method was validated with respect to the following validation parameters: selectivity, linearity and linear working range, limits of detection and quantitation, recovery, accuracy, precision, and stability. The validation follows the FDA (US Food and Drug Administration) guideline for bioanalytical methods. The stability evaluation showed that Hydrochlorothiazide was stable in plasma at least 40 days when stored at −75 °C. Hydrochlorothiazide and the internal standard (Losartan) were isolated from 0.3 mL plasma samples by protein precipitation using acetonitrile. Aliquots of plasma extracts (10 μL) were subjected to chromatographic separation using an Agilent Poroshell 120 EC-C18 column (2.7 um 3.0 × 50 mm) thermostated at 40 °C. The mobile phase was (1) water with 0.2% acetic acid, ammonium acetate 10 mM and (2) acetonitrile HPLC-grade (B/A; 60/40 v/v%). The separation was performed under isocratic conditions with a constant flow rate of 0.3 mL/min. LC-MS/MS experimental conditions utilized the multiple reaction monitoring, and detection of hydrochlorothiazide and internal standard (Losartan) was performed in the negative ESI mode for their respective $[M\text{-}H]^-$ ions. Calibration curves were linear in the range of 10~450 ng/mL with a coefficient of correlation of $r \geq 0.9969$ and a lower limit of quantification of 10 ng/mL. Recovery of hydrochlorothiazide ranged from 94.5% to 100%. Inter-assay precision (expressed as a coefficient of variation) was ≤ 9.29%; and inter-assay accuracy was ≤ 9.14%. All solvents were of HPLC (high-pressure

liquid chromatography) grade.

2.1 Subjects

The study sample size was calculated based on a within-subject CV% of $AUC_{0-\infty}$ and C_{max} [8, 9]. Forty-eight healthy male and female adult volunteers were enrolled in this study, which was performed in the Guillermo Grant Benavente Hospital, Concepcion, Chile. The age range of the subjects was 18~30 years, with a weight range of 52~89 kg and a height range of 154~185 cm (Table 1). All subjects gave written informed consent and the protocol form was approved prior to the start of the study by the Scientific and Ethic Committee of the Health Service of Concepcion (CEC), and National Public Health Authority (Instituto de Salud Pública de Chile) of Chile. The study was conducted in accordance with Good Clinical Practice guidelines and according to the Revised Declaration of Helsinki [10].

Table 1 Demographic data of healthy volunteers participant on the study.

Volunteer	Sex	Age (years)	BMC	Sequence
1	M	25	21.6	AB
2	M	25	25.1	BA
3	M	20	29.6	AB
4	F	27	27	BA
5	F	24	20.8	AB
6	F	20	21.1	BA
9	M	27	26.9	BA
10	F	24	22.4	AB
12	F	24	23.4	AB
14	F	24	20.8	AB
16	M	23	25.1	BA
17	M	37	26	AB
18	F	24	23.4	AB
19	M	20	23.4	AB
20	M	21	27.5	BA
21	M	20	24.1	AB
22	M	23	25.4	BA
23	M	24	22.2	AB
24	M	21	23.7	BA
25	F	21	23.4	AB
26	M	23	29.1	BA
27	F	23	18.3	AB
28	M	22	20.9	BA
30	M	18	19.6	BA
32	F	26	21.1	AB
33	F	19	18.6	BA
34	M	26	29.4	AB
35	F	19	26	BA
36	M	19	19.5	AB
37	F	23	19.3	AB
38	M	23	23.1	BA
39	F	23	19.5	AB
40	F	23	22.8	BA
41	M	22	26.5	AB
44	M	21	20.6	AB
45	M	27	26.2	BA
46	M	21	22.6	AB
47	M	22	21.6	BA
48	M	20	23.5	AB
Mean ± SD		22.9 ± 3.3	23.4 ± 3	

F: female; M: male; SD: standard deviation; AB: test-reference; BA: reference-test.

2.2 Clinical Protocol

The study was a single-dose, 2-way randomized crossover design with a one week washout period between the doses. The subjects were instructed to abstain from taking any medication for at least one week before and during the study period. No subject was alcoholic or a smoker and no alcohol was allowed 48 h before each study period and until after the last sample in each period had been collected. The subjects were also not allowed to drink grapefruit juice or beverages or food containing xanthines, such as tea, coffee and cola. Screening at the beginning of the study included physical examination, medical history, laboratory safety tests (hemoglobin, hematocrit, total and differential white cell count, creatinine, alkaline phosphatase, Alanin Trans Aminase/ALT, total bilirubin, albumin and total protein, routine urinalysis) and vital signs. During the enrollment process and each of the clinical phases, all volunteers were tested for drug abuse consumption. All clinical laboratory tests were performed at Guillermo Grant Benavente Hospital, Concepcion, Chile.

The subjects fasted for 10 h prior to drug administration. On the morning of the study phase, volunteers were given a single dose of reference

formulation (Clorana®, Sanofi Adventis Laboratory), or test formulation (Hydrochlorothiazide®, Laboratory Chile) of a 50 mg hydrochlorothiazide tablet. General instructions were provided to all volunteers. Briefly, no food was allowed for 2 h after dosing, food was given according to the time schedule and nutritionist recommendations, and the volunteers were instructed to stay in bed for 5 h after drug administration. Blood pressure, pulse, and adverse events were monitored and recorded in the CRF (Case Report Form) by our clinical staff. The samples for 24 h, 36 h and 48 h were taken under ambulatory conditions.

2.3 Drug Administration and Sample Collection

The drugs (test or reference) were given to the volunteers with 250 mL of water. Blood sample volumes were 5.0 mL at each sampling time (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24, 36 and 48 h after dosing). Samples were centrifuged at 4,000 g for 5 min immediately after collection, and the plasma was stored at −20 °C until assay. After seven days (washout period), the study was repeated under the same conditions to complete the crossover design. Counter samples were stored at −80°C in the Physiology Department at the University of Concepcion.

2.4 Pharmacokinetic Analysis

Pharmacokinetic parameters were determined and calculated using a Winnolin version 6.3 computer program. The data was described by arithmetic mean, standard deviation and 95% confidence intervals. The elimination rate constant (Ke) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration-time profile. The hydrochlorothiazide C_{max} and the corresponding T_{max} were determined by the individual drug serum concentration-time profiles. The elimination half-life ($T_{1/2}$) was calculated as 0.693/Ke. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t$/Ke, where C_t was the last measurable concentration. Bioequivalence was accepted if the calculated 90% confidence intervals were within 0.80~1.25 for AUC_{0-t} and C_{max}. Analysis of C_{max} and AUC was carried out by ANOVA (analysis of variance) using Prism 5.0.1, GraphPad Software Inc. To improve the normality of the distribution, logarithmic transformation was applied prior to the analyses. The 90% CIs (confidence intervals) were constructed for the ratio of geometric means of tested and reference products and were compared to the reference intervals (0.8~1.25) as suggested by the FDA [11].

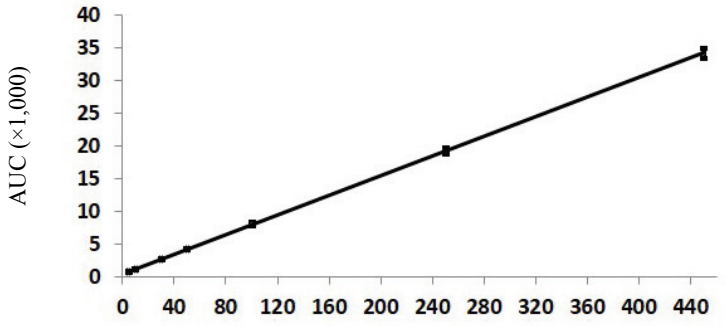
3. Results

The analytical method to quantify hydrochlorothiazide was developed in order to use a 0.3 mL of plasma sample, and losartan was used as the internal standard. The calibration curve for hydrochlorothiazide showed linearity between 10 and 450 ng/mL (Fig. 1a), with a confidence interval of 95% and two-freedom degree. Thus, we had optimal conditions for the quantification of all samples obtained from the clinical stages. Each sample was injected three times to make the quantification of each time of sampling. Additionally, all fortification levels showed a coefficient of variations under 15% for all parameters considered in this methodological validation: recovery, precision (intra and between days),

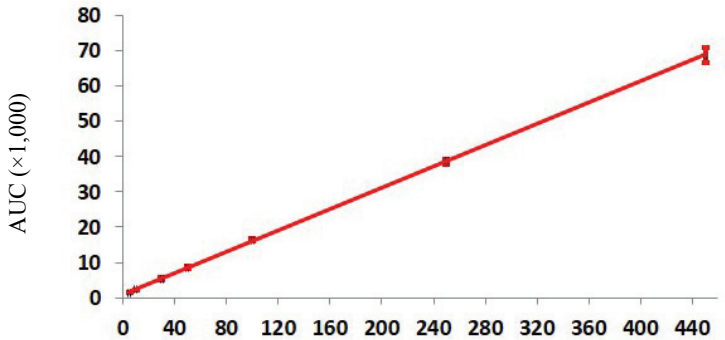
Table 2 Bioequivalence assessment summary.

Parameter[a]	Test/reference (%)	Reference	Test
C_{max} (ng/mL)	95.37	280.81 ± 1.05	267.81 ± 1.05
AUC_{0-t} (h*ng/mL)	95.59	1669.99 ± 1	1563.08 ± 1.05
$AUC_{0-\infty}$(h*ng/mL)	96.34	1730.39 ± 1.05	1667.10 ± 1.05
T_{max} (h)	105.98	2.07 ± 1.06	2.19 ± 1.06

[a] Pharmacokinetic parameters are given as mean (SEM).



(a)



(b)

Fig. 1 Calibration curve of hydrochrolothiazide: (a) linear regression of the calibration curve for hydrochlorothiazide between 10 and 450 ng/mL at injection time for each point; (b) linear regression of the calibration curve for losartan, used as an internal standard, at the same concentration range as the analyte (*n* = 3, for each point).

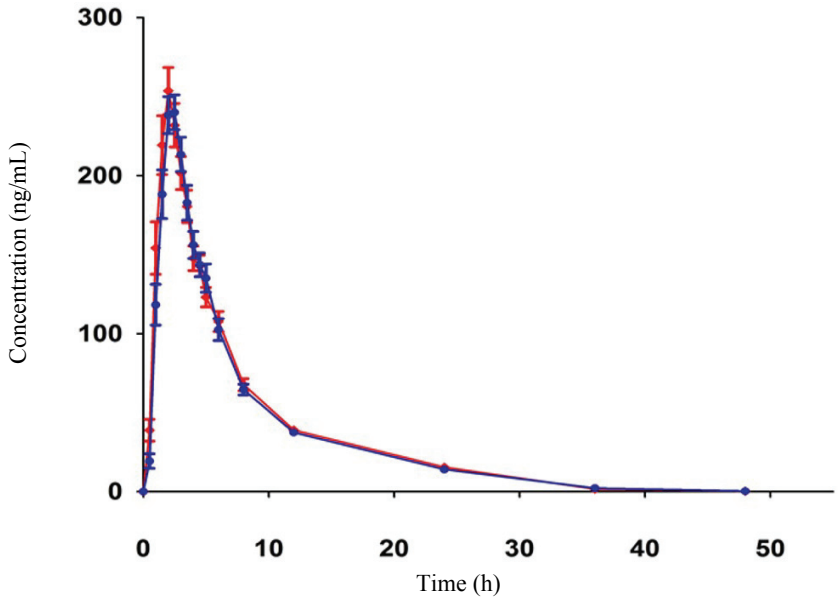


Fig. 2 Linear profile of the mean concentration versus time curve and variations in plasma concentration during the sampling time. The values represent the mean ± SEM of 39 volunteers monitored for 48 h. The blue line corresponds to the pharmacokinetics of the reference formulation, and the red line corresponds to the pharmacokinetics of the test formulation. No statistical differences were found (*n* = 39).

exactitude (intra and between days), and uncertainty (Table 2). In parallel, the internal standard losartan showed linearity in the same range of concentrations similar to the analyte (Fig. 1b).

Thirty-nine healthy Chilean volunteers (25 men, 14 women) completed the study. The general demographic volunteer information and sequence are summarized in Table 1. The mean participant age was 22.9 years old, and the mean BCM (body corporal mass) was around 23. No serious or unexpected adverse events were observed; however, nine volunteers were excluded during the study due to difficulties with their venous access, personal reasons, or desertion, and were replaced. The safety profile was similar for the test and reference formulations and it was consistent with the summary of the product characteristics [5]. The most frequently observed adverse event was hypotension followed by dizziness and headache.

Variations in plasma concentration during the sampling time after a single-dose oral administration (Fig. 2) showed that the test formulation elicited a similar profile and pharmacokinetic parameters as the reference formulation (Table 2); and these values stayed inside the confidence interval. The 90% CIs for the test/reference LSM (least squares mean) ratios of the log-transformed data for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and T_{max} were 95.37, 95.59, 96.34 and 105.98, respectively. No treatment, study period, or sequence effects were found on ANOVA of mentioned parameters. The intrasubject variabilities (CVs%) for the log-transformed data of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and T_{max} were 18, 16.6, 18.3 and 33.2, respectively. The difference in T_{max} values between the two products was not statistically significant according to the Anderson Hauck Procedure test ($p = 0.0151$).

4. Discussion

Bioequivalent preparations are considered to be therapeutically equivalent if the drug products exhibit comparable rate and extent of absorption when administered in the same dosage and under similar experimental conditions [9]. Bioequivalence is accepted by international agencies, as well as the Chilean National Regularity Authority, if the 90% confidence intervals are achieved for the primary pharmacokinetic parameters $AUC_{0-\infty}$, AUC_{0-t} and C_{max}, allowing an acceptance range of 80~125% [9]. ANOVA (analysis of variance) showed no significant differences between preparations on any of the pharmacokinetic parameters for both hydrochlorothiazide formulations. For C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, ANOVA for confidence level 90.00 and power at 20% were 0.999737, 0.999932 and 0.999678, respectively. Also, no statistically significant period effect was detected. The intrasubject variability was characterized by the coefficient of variation derived from ANOVA. It is important to note that in our study, the number of volunteers was greater than calculated from intra-individual variability. Both formulations presented good tolerability. Four non-serious adverse events were reported, all of which were considered to be mild. In the post study safety analyses, no alteration in clinical parameters was observed, and the volunteers received a medical discharge by the medical team.

5. Conclusions

The purpose of this study was to assess the bioequivalence of the test tablets compared to the reference tablets, both containing 50 mg hydrochlorothiazide. The extent of absorption, as reflected by the values for $AUC_{0-\infty}$ and AUC_{0-t}, and the rate of absorption as reflected by C_{max} and T_{max} values, demonstrate bioequivalence since both extremes lie within the required 90% confidence intervals 80~125%. Therefore, it can be concluded that both hydrochlorothiazide preparations are bioequivalent and are therapeutically equivalent and exchangeable in the clinical setting. This is an important advance to provide to general population an equitative access to drugs with high quality and guaranteed therapeutic effects., and can place us the basis to build the initial knowledge to study, in the future, the pharmacogenetic profile of this

population, and the impact of metabolic capacities into the adverse reactions associated to this type of drugs or similar.

Certainly thiazide-like diuretics are less expensive antihypertensive agents and therefore are favored in terms of cost minimization. In terms of cost hydrochlorothiazide test turns out to be almost five times cheaper than the reference drug, becoming a real contribution to antihypertensive therapy without compromising quality.

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