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Research Article

BIOAVAILABILITY OF TWO FORMULATIONS OF BICALUTAMIDE 50 mg

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ABSTRACT

Background: The purpose of this study was to evaluate the bioequivalence of a generic formulation of bicalutamide 50 mg tablets (test°) *vs.* the available branded formulation (reference*).

Methods and findings: A single-dose, randomized-sequence, open label, 2-period crossover study was conducted in 30 healthy male volunteers. A single oral dose of 50 mg of the test or reference formulation was followed by a 45 day washout period after which subjects received the alternative formulation. Blood samples were collected before dosing (Time 0) and at 5.0, 7.0, 9.0, 11.0, 14.0, 18.0, 20.0, 30.0, 48.0, 72.0, 120.0, 168.0, 336.0, 504.0, and 672.0 h after dosing. A very close follow-up helped subjects not to miss the second phase. Plasma samples were assayed for bicalutamide using a selective and sensitive HPLC method with a UV detector at a wave length of 272 nm. The C_{max}, t_{max}, AUC_{0-t}(672 h), $AUC_{0-\infty}$, and $t_{1/2}$ were determined from the plasma concentration-time profiles of both formulations.

Conclusions: The formulations were considered bioequivalent since 90% confidence intervals of the geometric mean ratios for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the regulatory range of 80-125%.

*Casodex® AstraZeneca, S.A. de C.V. °Apo-Bicalutamide® Apotex Inc.

Keywords: Bioequivalence; Bioavailability; Bicalutamide; Healthy Mexican volunteers

INTRODUCTION

Sexual hormones are important for many physiological processes in human growth and some diseases. In men, the androgen receptor is a ligand-inducible hormone receptor that plays a role in development and regulation of sexual secondary characteristics, spermatogenesis, bone, muscle mass, and is essential for the development and growth of a normal prostate; on the other hand, the androgen receptor is also responsible for the development of benign prostatic hyperplasia and probably prostate cancer (Singh, *et al*. 2005).

There are currently a number of possible management options for prostate hyperplasia or cancer. Some patients are offered radical radiotherapy, radical prostatectomy or androgen deprivation therapy. Agents that block the actions of endogenous androgens as testosterone are highly effective and routinely used for the treatment of prostate cancer. Nonsteroidal ligands are more favourable for clinical applications because of the lack of crossreactivity with other steroid receptors (e.g., progesterone receptor) and improved oral bioavailability. The first nonsteroidal antiandrogen, flutamide (Eulexin) was approved for prostate cancer in 1989 and the structurally related compounds, bicalutamide (Casodex) and nilutamide (Nilandron), were later launched in 1995 and 1996, respectively (Bohl, *et al*. 2005).

Bicalutamide has a long plasma half-life that allows once-daily dosing compared with three times daily dosing for flutamide (Sarosdy, 1999). In addition, bicalutamide is more selective for the peripheral androgen receptor and has less activity at the central androgen receptor on the hypothalamic-pituitary axis (Furr, *et al*. 1996) Bicalutamide is most effective when combined with Luteinizing Hormone-releasing Hormone (LHRH) agonists such as leuprolide acetate (Akaza , *et al*. 2004).

Bicalutamide competitively inhibits the action of androgens by binding to cytoplasmic androgen receptors, primarily in the prostate (Masiello, *et al*. 2002) Bicalutamide is a race mate composed with the enantiomers R and S. The antiandrogenic activity of this drug is located almost in the enantiomer R, since both enantiomers have different pharmacokinetic properties.

Bicalutamide is administered orally and Co-administration with food has no clinically significant effect. The drug does not cross the blood-brain barrier and is therefore devoid of CNS effects. R-bicalutamide is slowly absorbed from the gastrointestinal tract and reaches a

maximum concentration at 31.3 h. Bicalutamide is 96% protein-bound in the plasma. The active R-enantiomer of bicalutamide is metabolized mainly by oxidation followed by glucuronidation. The S-enantiomer is rapidly cleared relative to the R-enantiomer, accounting for about 99% of total plasma levels (Cantarini, *et al.* 2006*). In vitro* studies have shown that R-bicalutamide is an inhibitor of hepatic cytochrome P450 3A4 with lesser inhibitory effects on CYP 2C9, 2C19, and 2D6 activity. Both the parent and metabolite glucuronides are eliminated in the urine and feces. The half-life of the R-enantiomer is roughly 5.9 days for adults without liver disease. This half-life of nearly 6 days affords once-daily dosing. Plasma concentrations are linearly related to doses up to 150 mg (Cockshott, 2004). The liver extensively metabolizes bicalutamide, and limited data suggest that in subjects with moderate to severe hepatic disease, excretion could be delayed, leading to accumulation (Lee, *et al.* 2009).

The purpose of this study was to compare the relative biodisponibility and the tolerability to one dose of a new generic formulation of bicalutamide 50 mg and the reference formulation (CASODEX®) in healthy Mexican volunteers (Lu, *et al.* 2012).

MATERIAL AND METHODS

30 healthy Mexican male volunteers aged 18 to 50 years were enrolled in the study. Clinical history, physical examination, clinical laboratory tests (blood chemistry, clinical chemistry, immunological tests for HIV, hepatitis B and C, and urinalysis), 12-lead EKG, and drug abuse tests were obtained from all volunteers. Exclusion criteria included medically documented conditions, such as cardiovascular, respiratory, renal, hepatic or gastrointestinal disorders, history of hypersensitivity to bicalutamide, history of alcohol or drug abuse, and volunteers participation in another trial within the last 3 months. Subjects did not smoke, drink beverages with xanthine derivatives, nor use any medication within one week of study initiation. The study purpose and procedures were explained to the volunteers before initiating the study, and a written informed consent was obtained from all subjects. The study protocol and the informed consent form were reviewed and approved by an Independent Review Board, registered and authorized by Mexican Health authorities (Secretary of Health). All procedures were conducted in accordance with the principles of the Declaration of Helsinki, good clinical practices, and guidelines of the General Health Law of Mexico.

Study Design

The present study was carried out from August to November, 2009. The study was an open-label, randomized-sequence, single dose, two-period crossover design, with a 45-day washout period. In the first period, subjects were randomly assigned to receive the test (batch number HP1777; expiration date, December 2009) or the reference formulation (batch number B90144; expiration date, January 2013), and in the second period, after the washout time (45 days), volunteers received the alternative formulation. In both periods, subjects were admitted to the CEB clinical trial centre at 6 pm the day before study drug administration. They had a standardized dinner at 8 pm and no food was allowed after dinner. Next day at 7 am, volunteers received a single 50 mg bicalutamide tablet of the test or reference formulation along with 250 mL of water. Food was prohibited for 4 h after dosing as well as water during the first two hours after dosing. While subjects stayed at the centre, they had a standardized breakfast (11 am), lunch (3 pm), and dinner (7 pm) (3000 Kcal/day). Consumption of other food or beverages besides those allowed during the study was prohibited.

Blood Sampling

Serial venous blood samples were obtained from arm veins through an indwelling catheter in a forearm vein or by direct venepuncture. The times selected for taking blood samples were 0.0 before drug administration, and 5.0, 7.0, 9.0, 11.0, 14.0, 18.0, 20.0, 30.0, 48.0, 72.0, 120.0, 168.0, 336.0, 504.0, and 672.0 hours after drug administration in each period. Blood was collected in vacutainer tubes containing sodium-heparin which were inverted gently and placed on ice until centrifugation at 4000 rpm for 5 minutes at 4°C. Separated plasma was divided and collected in two Eppendorf tubes and stored at -70°C until analysed.

Determination of Plasma Concentrations

Determination of plasma bicalutamide concentrations was performed at Qually Corporation, S.A. de C.V., and Analytical Laboratory. Plasma concentrations were determined by HPLC. Bicalutamide was extracted by protein precipitation with 50 µL of 5% zinc sulfate and 500 µL of acetonitrile. The sample was stirred and then centrifuged at 13 000 rpm for 10 minutes at 4°C. Afterwards, 25 µL were injected into the HPLC system where a column, SB-C8, 4.6 × 50 mm, with a particle size of 5 µm was used. The mobile phase consisted of water pH 3: Acetonitrile (58:42 v/v) at a flow rate of 1 mL/min, with UV detection at 272 nm. The linear response ranged from 50.450 to 1513.485 ng/mL. The quantification limit was 47.604 ng/mL and the detection limit was 25.225 ng/mL.

Pharmacokinetic Analysis

Pharmacokinetic parameters were determined by non-compartmental assessment of data using WinNonlin Version 5.2., Pharsight Corporation, Mountain View, CA, USA. Concentration-time profiles were used to determine maximum plasma concentrations (C_{max}) and time to reach the maximum concentration (T_{max}). The area under the plasma concentration-time curve from t to the time point of the last quantifiable plasma concentration ($AUC_{0\text{-}tlast}$) was calculated by the linear trapezoidal rule. The elimination rate constant λ was estimated by log-linear regression of concentrations observed during the terminal phase of elimination. $AUC_{0\text{-}\infty}$ was calculated by extrapolating AUC0-tlast to infinity according to the equation $AUC_{0\text{-}infinity} = AUC_{0\text{-}tlast} + Clast / \lambda$.

Additionally, the plasma elimination constant λ and the terminal plasma elimination half-life ($t_{1/2}$) were determined.

Statistical Evaluation

Statistical analysis was also carried out using WinNonlin software. To achieve a better approximation to a normal distribution, pharmacokinetic parameters ($AUC_{0\text{-}\infty}$, $AUC_{0\text{-}tlast}$, and C_{max}) data of bicalutamide were log-transformed (base e) before analysis. Also, parametrical tests of data were performed for statistically significant differences by analysis of variance and determination of 90% confidence intervals (ANOVA, 2 one-sided t-tests procedure).

The 2 one-sided hypotheses at the α=0.05 level of significance were tested by constructing 90% confidence intervals for the ratios of the geometric means of test versus reference formulation. The 90% confidence intervals were calculated by retransformation of the shortest confidence interval for the difference of ln-transformed mean values.

Bioequivalence was demonstrated if 90% confidence intervals for the mean ratios of the treatments were fully contained within the acceptance limit of 80%-125% for all three pharmacokinetic parameters mentioned above.

The parameter T_{max} was statistically tested by non-parametric analysis applying 2 one-sided Wilcoxon tests. Results of non-parametric analysis only served as supporting data.

RESULTS

Thirty healthy Mexican male subjects participated in the study. Mean (standard deviation, SD) age, height, weight and body mass index were 25.33 (5.49) years, 171 (5.0) cm, 70.30 (6.98) kg, and 24.20 (2.2) kg/m^2, respectively. There were no significant differences in demographic characteristics between groups, suggesting an adequate subject randomization in the groups studied. Bicalutamide administration did not produce clinical significant changes on vital signs measured throughout the study. In each period, vital signs (blood pressure, heart rate, respiratory rate, and body temperature) were measured before drug administration, time 0, and 5.0, 7.0, 9.0, 11.0, 48.0, 72.0, 120.0, 168.0, 336.0, 504.0, and 672.0 h after drug ingestion. Adverse events were collected during each study period based on direct questioning and spontaneous report. Adverse event relationship with the study medication was assessed as none, unlikely, possible, and probable or certain. Also, adverse event intensity was classified as mild, moderate, or severe. There were no adverse events in period I, however, in period II, 3 adverse events were present in 2 subjects and its relation with the drug was classified as possible. Two study subjects presented dizziness, starting 6 hours after drug administration in one subject, and after 8 h in the other. This symptom had duration of 5 minutes and 1 hour respectively. One of these subjects also presented nausea, 6 h after drug administration with duration of 45 minutes. All adverse events subsided spontaneously after the time mentioned, and every case reported as "mild" intensity.

Plasma concentrations were above the lower limit of quantification at all-time points after administration. Figure 1 depicts the mean plasma concentration-time profiles of a single oral dose of two formulations of bicalutamide. Whereas Table 1 shows the main pharmacokinetic parameters of bicalutamide after oral administration of the studied formulations, test and reference.

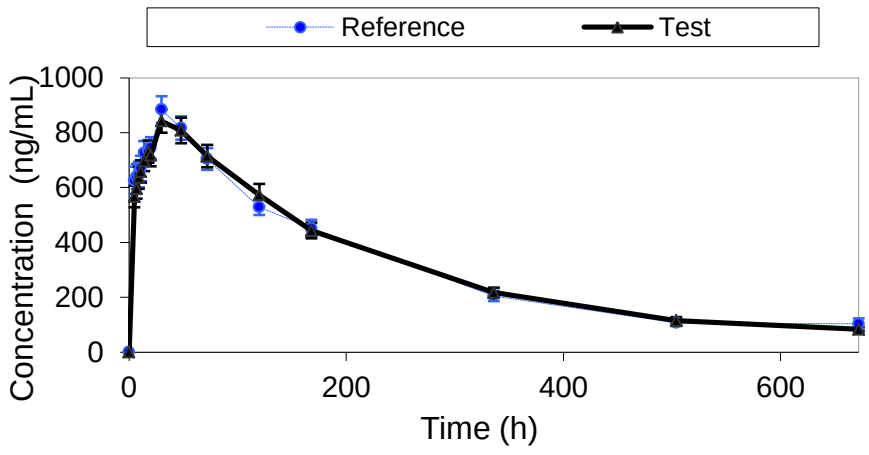

Figure 1: Mean plasma concentration of bicalutamide in 30 healthy male subjects after a single oral administration of two formulations (Reference and Test). Each time point represents the mean ± standard error of 30 subjects.

Table 1: Pharmacokinetic parameters of the two formulations.

PARAMETERS		
	TEST	REFERENCE
T_{max} (h)	37.13 ± 17.14	30.83 ± 14.10
C_{max} (ng/mL)	899.50 ± 255.28	929.86 ± 270.63
$T_{1/2}$ (h)	143.51 ± 32.04	147.58 ± 44.41
AUC_{0-t} (ng*h/mL)	182964.02 ± 76385.70	191726.04 ± 75778.35
$AUC_{0-\infty}$ (ng*h/mL)	207477.82 ± 77073.92	212908.15 ± 84562.38

Table 2: Results of statistical comparisons between test and reference formulations of bicalutamide (B is the test drug and A is the reference).

VARIABLE	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
B/A ratio	96.45	93.65	97.55
90% confidence limits	91.84-101.28	86.85-100.98	91.83-103.63
%CV intra-subject	11.17	17.29	13.57

Table 2 shows the results of the bioequivalence analysis of the main pharmacokinetic parameters (C_{max}, $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}infinity}$), all of them were entirely within the bioequivalence acceptance limits.

DISCUSSION

All 30 subjects completed the two phases of the study. Both formulations appeared to be well tolerated since only 3 adverse events were reported in the second period, in 2 subjects and all of "mild" intensity. During the study, vital signs were not altered by drug administration of the test formulation nor the reference.

The observed values of bicalutamide pharmacokinetic parameters after oral administration in fasting subjects agreed with those reported previously. The small differences observed with the report of Lu *et al.* 2012 might be due to a different analytical evaluation. Based on the

pharmacokinetic and statistical results of this study, the conclusion reached is that both studied products are bioequivalent.

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