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Bioequivalence Evaluation of Two Brands of Tamoxifen 10 mg Tablets (Tamophar & Nolvadex) in Healthy Human Volunteers

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Abstract

Objective To evaluate the bioequivalence of two oral formulations of 10 mg tamoxifen, *Tamophar* tablets (Julphar, UAE) and *Nolvadex* tablets (Zeneca Limited, U.K.) in 24 healthy human volunteers by statistical analysis of the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} as recommended by US *FDA*.

Design Single dose, two-sequence, cross-over randomized design at International Pharmaceutical Research Centre (IPRC), Amman, Jordan.

Methods Both test and reference tablets were administered to each subject after an overnight fasting on two treatment days separated by 50 days washout period; blood samples were collected up to 20 days (480 hours) and analyzed for tamoxifen using a validated LC-MS/MS method at Cartesius Analytical Unit, Institute of Biomedical Sciences – USP, Sao Paulo, Brazil. Various pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, and λ_Z were determined.

Results Pharmacokinetic parameters for both formulations were found to be in good agreement with reported values. AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence interval (100.1-107.6% for AUC_{0-t}, 99.8-108.7% for $AUC_{0-\infty}$; 99.9-109.6% for C_{max}) for test/reference ratio of these parameters were found within FDA acceptance range of 80 – 125%.

Conclusion *Tamophar* was found bioequivalent to *Nolvadex* and interchangeable in medical practice.

Introduction

Bioequivalence of two formulations of the same drug is concluded based on the lack of difference in the rate (C_{max}) and extent of absorption (AUC) especially in conventional drug formulations.[1] In the present study bioequivalence of two tamoxifen tablets was evaluated by comparing those pharmacokinetic parameters derived from plasma concentration of tamoxifen.

Tamoxifen is a non-steroidal antiestrogenic agent with combined partial-estrogen-agonist activity. Additional properties of tamoxifen including antioxidant properties, membrane-modulating activities, and modulation of intracellular messenger systems, are recognized and may contribute to the anticancer as well as other effects of tamoxifen.[2,3]

Tamoxifen can be used as primary therapy for metastatic breast cancer in both men and postmenopausal women. Tamoxifen has been shown to decrease the incidence of breast cancer in women who are at high risk for developing the disease. Tamoxifen binds to estrogen receptors (ER) and induces conformational changes in the receptor such as it stimulates ER in bone and may actually prevent postmenopausal osteoporosis and has antiestrogenic effects on the breast tissue.[4,5,6,7]

Tamoxifen is absorbed well orally;[3] a dose of 20 mg tamoxifen produced an average peak plasma concentration of 40 ng/ml (range 35 to 45 ng/ml) occurred approximately 5 hours after dosing[4]. Protein binding is approx. 99%.[4] Tamoxifen is extensively metabolized after oral administration. N-desmethyl tamoxifen is the major metabolite with similar activity of tamoxifen. 4-Hydroxytamoxifen and a side chain primary alcohol derivative of tamoxifen have been identified as minor metabolites in plasma.[3,4] Tamoxifen is excreted slowly in the faeces, mainly as conjugates, small amounts are excreted in urine; it appears to undergo enterohepatic circulation.[3,8] The decline in plasma concentrations of tamoxifen is biphasic with an initial half-life of 7-14 hours and terminal elimination half-life of about 5 to 7 days.[4] After initiation of therapy, steady state concentrations for tamoxifen are achieved in about 4 weeks and steady state concentrations for N-desmethyl

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tamoxifen are achieved in about 8 weeks, suggesting a half-life of approximately 14 days for this metabolite.[4]

Materials and Methods

Study Products

Test Product: *Tamophar* - Tamoxifen 10 mg tablet

Batch No.: 0003, Expiry Date : 06/2002

Gulf Pharmaceutical Industries - Julphar, United Arab Emirates

Reference Product: *Nolvadex* - Tamoxifen 10 mg tablets

Batch No.: AB 872, Expiry Date 06/2005 Zeneca Limited, U.K.

Study Subjects

Twenty-four (24) healthy adult male volunteers participated in this comparative study at Al-Mowasah Hospital, Amman, Jordan, as joint venture with International Pharmaceutical Research Center (IPRC). Amman, Jordan. Their mean age was 24.3 ± 4.21 years; mean body weight was 72.8 ± 9.93 kg and mean body height was 171.3 ± 4.72 cm. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastro-intestinal and haematological diseases, as determined by their medical history, physical examination, and routine laboratory tests (haematology, blood biochemistry, and urine analysis). This study was performed according to the revised Declaration of Helsinki for bio-medical research involving human subjects and the rules of Good Clinical Practices. The study protocol was approved by Institutional Review Board (IRB) of Al-Mowasah Hospital, Amman, Jordan.

Drug administration and sample collection

After an overnight fasting (10 hours) subjects were given single dose of either formulation (reference or test in a randomized fashion) of tamoxifen (2 x 10mg) tablet with 240mL of water; fasting was continued until 5 hours after ingestion of the dose. Approximately 10 mL of blood samples for tamoxifen assay were drawn into evacuated heparinized glass tubes through indwelling cannula before (0 hr) and at 0.5, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 8.0, 12.0, 24.0, 48.0, 96.0, 144.0, 192.0, 264.0, 336.0, and 480.0 hours after dosing. Blood samples were centrifuged at 3500 rpm for 10 minutes; plasma was transferred to 5 mL plastic tubes and stored frozen at –20°C pending drug analysis. After a period of 50 days study was repeated in the same manner to complete the crossover design.

Chromatographic conditions

The LC-MS-MS was consisted of liquid chromatograph, Agilent 1100 series, Model G1312A, degasser, Agilent 1100 series, Model G1322A, an auto-injector CTC Analytics, Model MXY01-01B, column oven, Shimadzu, Model CTO 10Avp and a Micromass Quattro LC with electrospray ionisation source mass spectrometer (Micromass, UK); integration was done using Masslynx software version 3.5 (Micromass, UK). Chromatographic separation was performed using Genesis C18 (4.0 µm) (100 x 2.1mm) column from Jones Chromatography, UK. The mobile phase consisted of 0.05% formic acid in deionised water/acetonitrile (25/75) and eluted at flow rate of 0.2 mL/min; oven temperature was set at 30°C. Detection was done at MRM of 372.23 >72.01and 406.23>100.123 for tamoxifen and internal standard (Clomiphene) respectively. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by Masslynx software. The method was validated by following international guidelines.[9]

Sample preparation for HPLC injection

50 µL of internal standard (clomiphene 80 ng/mL) was added to 500 µL plasma sample and vortexed for 10 seconds. 4.0 mL mixture of diethyl ether and hexane (80/20) was added and vortexed for 1 minute. Sample was then frozen for 10 minute at –70°C; organic layer was transferred to another tube and evaporated to dryness in water bath at 37°C under nitrogen. The residue was reconstituted with 200 µL of acetonitrile/water (50/50), vortexed for 30 seconds and transferred to microcentrifuge tube (1.5mL) and centrifuged at 13200 rpm for 2 minutes. 20 µL aliquot was injected to column where tamoxifen and internal standards were separated from endogenous plasma substances.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic analysis was performed by means of model independent method using Kinetica™ 2000 computer program.[10] To assess the bioequivalence between two formulations, AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were considered as the primary variables. Two way analysis of variance (ANOVA GLM procedure; Kinetica™ 2000 Computer program[10]) for crossover

design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. Difference between two related parameters was considered statistically significant for p-value equal to or less than 0.05. Parametric 90% confidence intervals[11] based on the ANOVA of the mean test/reference (T/R) ratios of *AUCs* and C_{max} were computed.

Results and Discussion

Tamoxifen was well tolerated by the volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all volunteers who started the study continued to the end and were discharged in good health. The described analytical method was proven sensitive and accurate for determination of tamoxifen in plasma.

Both formulations were readily absorbed from the gastrointestinal tract and tamoxifen was measurable at the first sampling time (0.5 hour) in all of the volunteers. The mean concentration-time profile of the two formulations is shown in the Figure 1. Peak concentrations were attained at 5.63 (Test) and 5.54 (Reference) hours after dose administration and were detectable till last sample (480 hours). All calculated pharmacokinetic parameter were in good agreement with reported values.[3,4,8]

Table 1 shows the pharmacokinetic parameters for the two brands of tamoxifen 10mg tablets. The relative bioavailability of *Tamophar* was 104.3% for AUC_{0-t}, 104.9% for $AUC_{0-\infty}$, and 105.5% for C_{max}.

The mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two products did not differ significantly, suggesting that the plasma profiles generated by *Tamophar* are comparable to those produced by *Nolvadex*. Table 2 shows statistical results; analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having p value greater than 0.05. 90% confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ or C_{max} of the two formulations lie within the FDA acceptable range of 80 – 125%.[11]

For T_{max} the parametric point estimate of difference (test – reference) was 0.09 h, and found to be within the acceptance limits (± 20% of reference mean).

Plasma levels may be used as surrogate parameters for

Table 1 Pharmacokinetic Parameters of Tamoxifen (2 x 10mg) Tablets (mean ± standard deviation, n = 24)

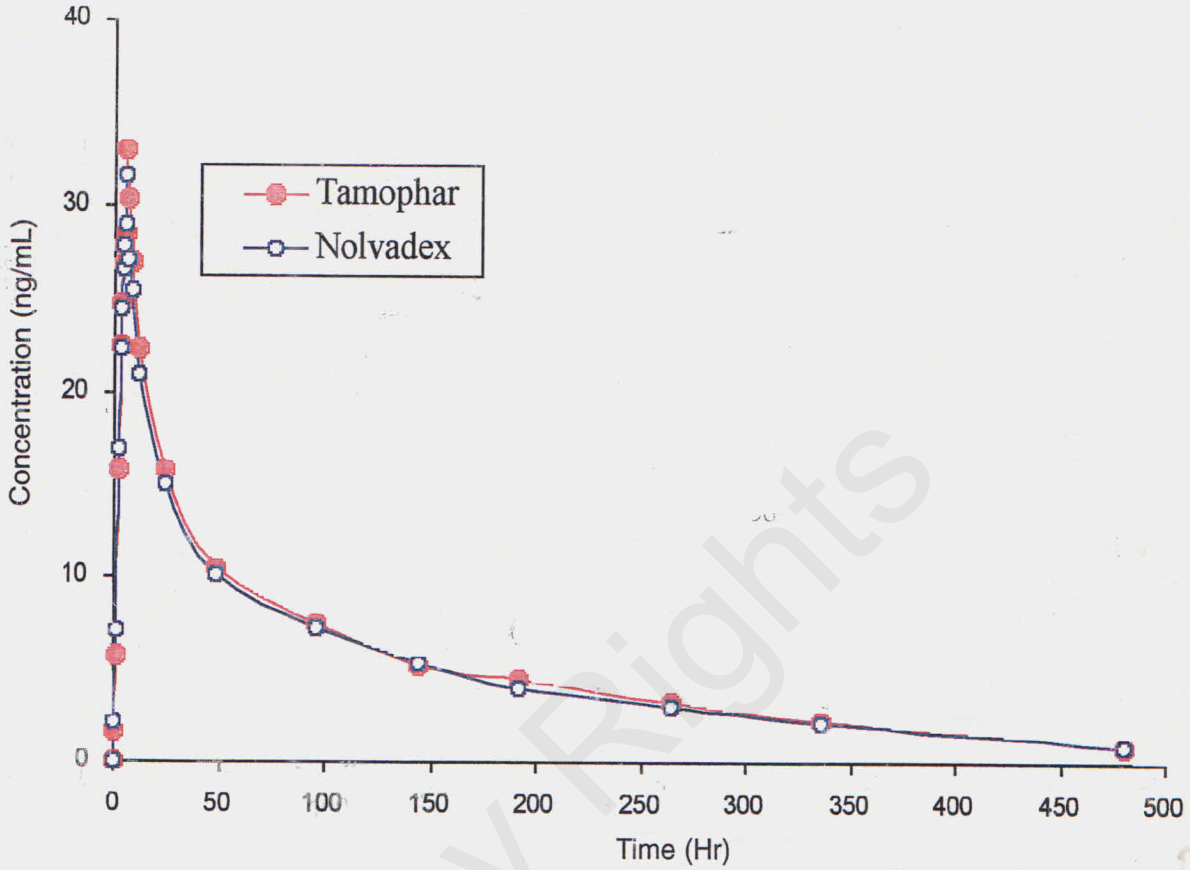
Pharmacokinetic Parameter	*Tamophar* (Test)	*Nolvadex* (Reference)
AUC_{0-t} (ng/mL.hr)	2497.20 ± 684.16	2408.54 ± 684.46
$AUC_{0-\infty}$ (ng/mL.hr)	2664.10 ± 746.21	2556.77 ± 726.78
C_{max}(ng/mL)	34.01 ± 8.26	32.27 ± 6.84
T_{max} (Hr)	5.63 ± 0.92	5.54 ± 0.62
$T_{1/2}$ (Hr)	114.88 ± 25.44	116.26 ± 20.12
λZ (/Hr)	0.0064 ± 0.0017	0.0062 ± 0.0012

Table 2 Statistical Analysis of Log-transformed data

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM (P-value)	0.0912 (0.3270)	0.1150 (0.1990)	0.1035 (0.5315)
90% CI	100.1– 107.6%	99.8 – 108.7%	99.9 – 109.6%

Parenthesis values indicate analysis for periods.

Figure 1 Mean Plasma Concentrations of Tamoxifen After Oral Administration of 2 x 10mg tablets to 24 Healthy Human Volunteers



clinical activity; therefore results of this study suggest equal clinical efficacy of the two brands of tamoxifen.

Conclusion

Statistical comparison of the AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} clearly indicated no significant difference between two studied brands in any of the calculated pharmacokinetic parameters. Based on the above we can conclude that *Tamophar*, manufactured by Gulf Pharmaceutical Industries, U.A.E. is bioequivalent to *Nolvadex*, manufactured by Zeneca Limited, UK, and that both products can be considered equally effective in medical practice.

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