Bioequivalence evaluation of glibenclamide 5-mg tablets: diabenil® and daonil® (in 24 healthy volunteers).

Evaluation de la bioequivalence entre deux formulations de glibenclamide: diabenil ® et daonil ® (chez 24 volontaires sains).

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RÉSUMÉ

Prérequis: Il est admis actuellement que la prescription des médicaments génériques est un moyen efficace pour une meilleure gestion des dépenses de santé. L'étude de bioéquivalence sans être systématiquement demandée, est reconnue comme la procédure la plus adaptée de comparaison par rapport à un médicament princeps pour l'évaluation de la qualité et de l'efficacité thérapeutique d'une spécialité générique. Favoriser la prescription des génériques est un choix stratégique en Tunisie.

Objectif: étudier la bio équivalence entre un produit générique le DIABENIL* (glibenclamide) du laboratoire tunisien Dar Essaidaly et un produit de référence le DAONIL* (glibenclamide) des laboratoires Aventis pharma.

Méthodes : Il s'agit d'un essai randomisé croisé en double aveugle. 24 volontaires sains ont participé à cette étude. Au total, 15 prélèvements sanguins ont été réalisés pour chaque sujet, lors de chaque période. Le dosage du Glibenclamide a été fait par chromatographie liquide haute performance. La bioéquivalence des paramètres pharmacocinétiques AUC0t, AUC0 , Cmax et Tmax a été testée en utilisant respectivement des tests paramétrique et non paramétrique.

Résultats: Pour tous les paramètres, l'analyse statistique a généré des intervalles de confiance classiques à 90% des rapports des moyennes des deux formulations inclus dans l'intervalle de bioéquivalence de [0.8, 1.25].

Conclusions : Ainsi, les deux formulations du glibenclamide: le DAONIL* et le DIABENIL* ont été jugées bioéquivalentes.

Mots-clés

Glibenclamide, bioéquivalence, pharmacocinétique, chromatographie liquide haute performance.

SUMMARY

Background: Prescription of generic products is a way to reduce health expense. Bioequivalence is the most appropriate procedure to evaluate the quality and therapeutic efficacy of a generic product. Generic prescriptions are a strategic choice in Tunisia.

Objective: We expose in this work, a bioequivalence study witch compare a generic (test) product: DIABENIL* manufactured by a Tunisian pharmaceutical industry Dar Essaidaly to the innovative (reference) product: DAONIL* of Aventis pharma laboratories.

Methods: The bioequivalence of two glibenclamide 5-mg tablets was determined in healthy human, adult volunteers after a single dose in a randomized cross-over in double blind study. Test and reference were administered to twenty-four healthy volunteers of both sexes after overnight fasting. In total, 15 Blood samples were collected before and following the administration of the drug. Serum concentrations of glibenclamide were determined by validated HPLC method. The pharmacokinetic parameters AUC0t, AUC0 , Cmax and tmax were tested for bioequivalence.

Results: All parameters showed bioequivalence between both formulations as their confidence intervals were within the bioequivalence acceptable range of 0.80-1.25 limits. Conclusion: We conclude that the two formulations exhibited comparable pharmacokinetic profiles and that the two products can be considered interchangeable in medical practice.

Key-words

Glibenclamide, bioequivalence, pharmacokinetics, high-performance liquid chromatography.

The rising cost of health care has motivated governments around the world to examine methods to decreasing costs without compromising health care services. One of the methods to reduce costs, employed by Tunisia, is the passing of regulations that encourage the use of generic pharmaceuticals. This can reduce the cost of pharmaceutical care by about 50% [1, 2]. Pharmaceutical products essentially similar to an “innovator” product are usually designated as “generics” or “branded generics”. A product is an 'innovator' product if its marketing authorization has been obtained on the basis of dossier with full clinical documentation. “Generic” product is considered to be bioequivalent to an “innovator” product when their concentrations versus time profiles are so similar that they are unlikely to produce clinically relevant differences in therapeutic and or adverse effects [3, 4, 5]. Bioequivalence assessment is usually the most important quality control tool as a surrogate for therapeutic efficacy [6].

Sulphonylurea drugs have been used for over 50 years in the treatment of type 2 diabetes [7]. The most commonly employed agent is Glibenclamide. Glibeclamide is effective at very low concentration [8]. It acts by stimulating insulin release in normal and diabetes type 2 subjects [8]. Following oral administration, peak plasma concentrations are attained 2 to 6 hours after ingestion in the fasting state [7, 8, 9]. Food does not alter either the rate or completeness of absorption of Glibenclamide [7]. It is almost completely metabolized in the liver [7]. At our knowledge, our bioequivalence study is the first study in our region.

The objective of this study was to compare pharmacokinetic profiles of two immediate released formulations of 5-mg Glibencamide tablets in healthy volunteers.

METHODS

Study Products

Reference product: DAONIL® - glibenclamide 5-mg tablet. Manufacturer: Laboratories Aventis. Daonil® is mentioned in the WHO list of comparator products [10].

Test product: DIABENIL® – glibenclamide 5-mg tablet. Manufacturer: Laboratories Dar Essaidaly Sfax, Tunisia.

Subjects

Twenty-four adult volunteers (18 males and 6 females) were participated in the study. Their mean (+ SEM) age was 29,3 + 7,87 years with a range of 20 to 45 years, body weigh of 68,6 + 9,55 kg with a range of 54 to 93 kg and height of 172 + 7 cm with a range of 154 to 180 cm.

On the basis of medical history, clinical investigation and laboratory analysis, no subject had an history or evidence of diabetes mellitus, cardiac, renal, hepatic, gastro-intestinal disease or drug allergy. The volunteers were asked to abstain from taking any drug for at least one week prior to the study and until after the study was completed. No alcohol or xanthine containing was consumed for 48 hours prior to dosing and after the last blood sample was collected. Informed consent of subjects was obtained after explaining the nature and the purpose of the study. The local ethical committee approved the study protocol.

Study design

All subjects received on two occasions, separated by a one week wash-out period, in a cross over design, two formulations of Glibenclamide 5-mg tablets. Considering the reported pharmacokinetic data of glibenclamide, considering d=0.05, and the bioequivalence range (0.8 – 1.25), a total number of 24 volunteers is expected to be sufficient to obtain a statistical power greater than 80% [11, 12, 13, 14, 15, 16].

Procedure

Following an overnight fast of at least 10 hours, subjects received a single dose (5mg) of the test or reference product with 240 ml of a 20% glucose solution in water. Then, the subjects were given 60 ml of a 20% glucose solution in water every 15 min for 4 hours. The subjects were fasted 4 hours after administration of the treatment. All meals were standardized during the study, and the same meals were served during the both phases of the study. Blood pressure and pulse rate were monitored during the first four hours of the study. Subjects were asked for unusual symptoms observed during the study.

Blood sampling

Blood samples were collected before administration of the drug and at the following intervals after wards: 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 30 and 36 hours as recommended by FDA guidelines [16]. Each sample was centrifuged for 15 min at 5°C and the separated serum was frozen at (-20) °C until analysis.

Analytical methods

Glibenclamide in serum samples was analyzed by HPLC as previously described [17]. The method was validated by following the Société Française des sciences et techniques pharmaceutiques (SFSTP) guidelines [18]. The limit of quantification for glibenclamide was 25ng/ml. No interfering peaks were detected in the control human samples or in samples from subjects receiving Glibenclamide. The calibration curves were linear in the range of 25-300 ng/ml. Accuracy of the method was determined by processing spiked plasma samples at four concentrations: 25, 50, 100 and 200 ng/ ml. The accuracy profile was obtained by connecting the lower and the upper limits of confidence for eatch concentration level. All concentration levels were inside the acceptance limits of 80-120%. This method provided adequate specificity and accuracy for the bioequivalence study.

Pharmacokinetic analysis

Pharmacokinetic parameters of Glibenclamide after oral administration of both the test and reference formulation were derived from a model independent method. The maximum glibenclamide serum concentration (Cmax) and the corresponding peak times (tmax) were determined by inspection of the individual data and individual drug serum concentration-time profiles. The elimination rate constant (k) was obtained from the least square fitted terminal log-linear portion of the serum concentration-time curve. At least three or four terminal points were included in the regression analysis for k estimation. The area under the serum concentration-time curve to the last sampling time (AUC0t) was obtained by the linear trapezoidal rule. The area under the serum concentration-time curve extrapolated to infinity (AUC0) was calculated by adding the quotient cp/k, where cp is the serum concentration at the last sampling time.

Statistical analysis

Bioequivalence was assessed by means of an analysis of variance (kinetica 2000 software) for cross-over design and calculating standard 90% confidence intervals [19] of the ratio test/reference (T/R). The analysis of variance taking into account four factors: formulation, period sequence, subject (sequence), and was carried out on the individual ln-transformed Cmax, AUC0t and AUC0 values. The products were considered bioequivalent if the difference between two compared parameters was found statistically insignifiant (=0.05) and 90% confidence intervals for these parameters fell within 80-125%. The Anderson-huanck test [20] was applied which computes the probability in the two one sided t-test based on the null hypothesis. The individual tmax values were compared between formulations by a non-parametric test.

RESULTS

No significant changes in vital signs, blood pressure, heart rate or hypoglycemic manifestation had occurred.

Figure 1 shows the mean concentration-time profiles for the two brands of glibenclamide 5-mg tablet. The table 1 and 2 show the individual pharmacokinetic parameters obtained for both formulations. Table 3 shows the results of statistical analysis for AUC0t, AUC0 and Cmax values after ln-transformation. According to the mean serum levels of the 24 subjects, the relative bioavailability was found to be 103.4, 105.9 and 103.8% on the basis of mean AUC0t, AUC0 and Cmax, respectively. The results of ANOVA for all pharmacokinetic parameters show a significant difference in variances between subjects.

Table 1: Pharmacokinetic parameters of the reference product of glibenclamide tablet.

Reference product Subjects	tmax (h)	Cmax(ng/ml)	AUC0t	$AUC_0 \infty$
1	6	226.41	1532.05	1770.39
2	5	254.32	1737.47	1914.75
3	6	226.65	2113.53	2531.83
4	6	182.18	1287.33	1509.54
5	6	204.47	2065.31	2395.85
6	6	165.78	787.29	948.67
7	8	186.41	1754.07	1884.41
8	8	169.40	1215.92	1376.21
9	8	144.14	1126.4	1228.82
10	5	173.69	961.23	1003.88
11	2	196.40	1028.58	1326.38
12	1	161.90	1033.98	1229.16
13	6	115.20	661.92	971.25
14	5	169.84	986.95	1133.48
15	4	206.04	1073.03	1272.53
16	4	108.17	733.77	840.60
17	2	122.83	821.00	884.43
18	6	176.29	1177.40	1353.98
19	8	143.73	1535.31	1756.59
20	6	167.24	871.68	1037.75
21	5	139.27	638.39	786.39
22	5	169.66	1033.19	1200.10
23	3	165.76	980.45	1237.94
24	5	128.31	830.28	1084.95
N	24	24	24	24
Means	4.78	167.3	1102.2	1295.6
SD	+1.89	+36.32	+418.09	+464.56
CV%		21.71	37.93	35.86
MIN	1	108.17	638.39	786.39
MAX	8	254.32	2113.50	2531.80

Figure 1 : Mean serum Glibenclamide concentration-time profiles after intake of single dose of Daonil® 5-mg tablet (formulation A) and Diabenil ® 5-mg tablet (formulationB).

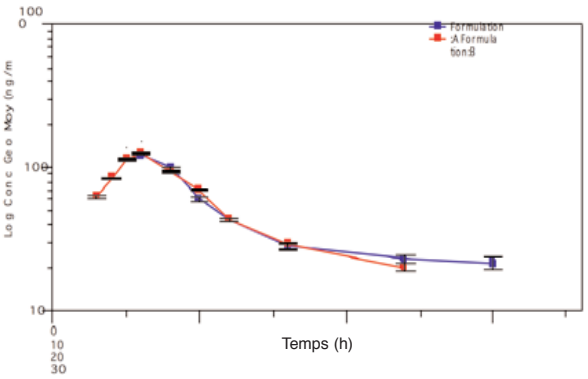

Table 2 : Pharmacokinetic parameters of the test product of glibenclamide tablet.

Test product Subjects	tmax (h)	Cmax (ng/ml)	AUC0t	AUC0 ∞
1	6	219.53	1248.38	1543.25
2	5	291.31	1518.74	1630.37
3	6	222.10	1587.61	2451.76
4	6	201.10	1125.12	1322.60
5	10	194.59	1708.63	2109.55
6	6	195.92	999.56	1056.13
7	5	188.45	1171.76	1426.39
8	6	187.09	1069.94	1160.77
9	6	159.90	860.00	1001.15
10	5	184.18	730.38	845.36
11	4	217.92	1306.05	1526.50
12	4	186.76	1119.98	1569.33
13	6	145.09	1334.97	1536.70
14	5	183.77	1648.38	1726.97
15	3	218.37	1095.90	1202.45
16	4	111.24	776.06	854.74
17	6	116.09	747.27	949.62
18	2	173.07	720.97	1230.94
19	4	149.78	1220.42	1544.34
20	6	165.82	1919.39	2197.91
21	8	127.03	969.04	1133.45
22	5	168.12	1435.06	1631.73
23	4	133.41	1035.99	1207.9
24	6	131.78	867.07	1214.05
N	24	24	24	24
Means	5.09	173.56	1132.1	1366.3
ET	+1.61	+41.02	+331.3	+412.55
CV%		23.63	29.23	30.19
MIN	2	111.24	720.97	845.36
MAX	10	291.31	1919.40	2451.80

Table 3: Statistical analysis of pharmacokinetic data.

	AUC_{0t}	$AUC_0 \infty$	Cmax
90% confidence intervals	91.2-115.7%	96.2-115.6%	100.4-100.7%
Two one-sided t-test probability	>0.99	>0.99	>0.999

Area under the curve (AUC0t)

The mean of AUC0t was 1102.2 ng h/ml for the reference product and 1132.1 ng h/ml for the test product. ANOVA did not show any significant differences for periods effects and formulations. 90% confidence interval fell within the bioequivalence acceptance criteria. Two one sided t-test were also performed on the ratio (r) of mean AUC0t of test to mean AUC0t of reference. These test showed the p ($r<0.8$) < 0.01 and p ($r>1.25$) <0.01; so both tests were rejected and it was accepted that the probability for ratio (T/R) to lie within 0.8 and 1.25 was >0.99.

Area under the curve (AUC0 ∞)

The mean of AUC0 was 1295.6 ng h/ ml for the reference product and 1366.3 ng h/ml for the test product. ANOVA did not show any significant differences for periods effects and formulations. 90% confidence interval fell within the bioequivalence acceptance criteria. Two one sided t-test were also performed on the ratio (r) of mean AUC0 of test to mean AUC0 of reference. These test showed the p ($r<0.8$) <0.01 and p ($r>1.25$) <0.001; so both tests were rejected and it was accepted that the probability for ratio (T/R) to lie within 0.8 and 1.25 was >0.99.

Peak plasma concentration (Cmax)

The mean of Cmax was 167.3 ng/ ml for the reference product and 173.6 ng/ ml for the test product. ANOVA did not show any significant differences for periods effects and formulations. 90% confidence interval fell within the bioequivalence acceptance criteria. Two one sided t-test were also performed on the ratio (r) of mean Cmax of test to mean Cmax of reference. These test showed the p ($r<0.8$) < 0.001 and p ($r>1.25$)<0.001; so both tests were rejected and it was accepted that the probability for ratio (T/R) to lie within 0.8 and 1.25 was > 0.999. For tmax the parametric estimate point of difference (test-reference) was 0.31h, which showed an improved rate of bioavailability, though it was very close to acceptance limits (+ 20% of reference mean).

DISCUSSION

The bioavailability results demonstrate that DiabenilL® 5-mg tablet (generic product)
is bioequivalent to Daonil® 5-mg tablet (reference product).

All calculated pharmacokinetic parameter values were in good agreement with the previously reported values [7, 8, 9, 21, 22], assuring the lack of any unusual pharmacokinetics values for glibenclamide. For bioequivalence evaluation various statistical modules were applied to AUC0t, AUC0 and Cmax as per current FDA guidelines [15]. According to the mean serum levels of the 24 subjects, the relative bioavailability was found to be 103.4, 105.9 and 103.8% on the basis of mean AUC0t, AUC0 and Cmax, respectively. The results of ANOVA for Cmax, AUC0t and AUC0 values did not show any significant difference in variances between two sequences as well as two test periods which means that the cross-over test was successful [23, 24, 25, 26]. However the results of ANOVA for all pharmacokinetic parameters show a significant difference in variances between subjects. These results were in agreement to those reported previously following the oral administration of glibenclamide [9, 16, 21, 22].

Based on these results of the study, the two formulations exhibited comparable pharmacokinetic profiles and then the two products can be considered interchangeable in medical practice.

In Tunisia, regulations encourage the development of local pharmaceutical laboratories since 1989. To produce a generic in Tunisia, two things are essentials: a complete pharmaceutical procedure for pharmaceutical product development and bioequivalence study for some substances. Bioequivalence is an intersecting region for three primary factors: the research community of scientists and statisticians, the pharmaceutical industry, and regulatory agencies. Bioequivalence study respond, today, to a valid statistic method witch allow to take decision easily.

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