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Interchangeability and Comparative Effectiveness between Micronized and Non-micronized Products of Glibenclamide Tablets

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Abstract:

Background: During the last few years there was wide debate about the interchangeability and effectiveness between circulated products containing Glibenclamide in the market.

Objectives: This study aimed to compare the effectiveness of this product "non-micronized" to the originator's product of Glibenclamide tablets "of micronized" sulfonylurea.

Methods: 12 volunteers received a dose of 5mg of Glibenclamide (from test and standard products) under fasting conditions in two separate sessions using randomized crossover design. Blood glucose level for the volunteers was monitored to avoid the development of hypoglycemia. Plasma samples were collected over 24 hours and analyzed using HPLC.

Results: The maximum concentration Cmax for the test and reference products were 2.508 ± 0.104 and 3.526 ± 0.118 (µg/ml) respectively and the area under the curve AUC0-∞ were 3.511 ± 0.153 4.572 ± 0.202 (µg.h/ml) for these products respectively, with a difference of about 24% between the test and reference products in its AUC.

Conclusions: The results indicate that the test product is not bioequivalent to reference product. The difference in formulation between micronized product and non-micronized product of Glibenclamide tablets has impact on clinical outcomes.

Key words:sulfonylurea,Blood glucose,hypoglycemia.

The chemical content of pharmaceutical products was no longer the most important indicator for the quality measure or quality judgment about generic drugs. Some evidences showed the importance of other important indicators (e.g. bioequivalence, relative potency, etc) which should be considered while evaluating the quality of pharmaceutical products[(1)]. The combined interpretation of quality control results with clinical data or therapeutic outcomes for any drug could be of potential importance. The dosage form should also have the capability of delivering this amount into the systemic circulation of the patient to ensure the achievement of desired effects (2).

Glibenclamide is a sulfonylurea derivative (also known as Glyburide) that is very widely used in the treatment of type II diabetes mellitus. The availability of large number of authorized products in different markets, lack of information about these products' bioavailability data and lack of patients' information and education programs; highlights many problems regarding the interchangeability of available products in the markets and its effectiveness (3).

Recent discussion was raised among scientists in Sudan about quality evaluation of authorized products of Glibenclamide tablets. The debate was mainly focused on the comparative bioavailability characteristics of certain product compared to other formulations (bioequivalence). It was noticed that among patients with diabetes, and mainly for economic reasons, the patients tends to use one of the products that is non-micronized formulation as it is much cheaper in price. However, patients complain about the effectiveness of this product.

Objectives:

to investigate factors affecting the quality of clinical outcomes of Glibenclamide in

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Khartoum using different investigation methods and to evaluate bioequivalence of a single dose of test formulation (containing Glibenclamide 5 mg tablet, manufactured by company C06 - Sudan) and to compare it with a single dose of reference formulation (containing Glibenclamide 5 mg tablet, manufactured by company C09 - Germany) under fasting conditions.

Patients and methods:

Study design: Comparative in-vivo bioavailability (bioequivalence) study, in which Glibenclamide concentration in plasma was measured for bioavailability and bioequivalence.

Methods: Single dose study was applied with a two-period, two-sequence crossover design (as recommended for this kind of studies). This was applied as two phases of treatment separated by 14 days as washing period.

The volunteers received a dose of 5 mg of Glibenclamide (of test and reference products) under fasting conditions in two separate sessions using a randomized crossover design. Plasma samples were obtained at selected times over 24 hours and stored frozen until analyzed using basic HPLC technique. Pharmacokinetic parameters were compared using the analysis of variance for a cross-over design and ratios of AUC24h and Cmax, 90% confidence intervals were obtained for summery of the results. Results were considered positively if the confidence intervals did not exceed the limits of acceptance (80--120%) for AUC24h and Cmax.

Subjects: Healthy 12 Sudanese volunteers were recruited in this study. The detailed process of selecting these subjects followed the recommendations of Helsinki Declaration regarding the ethical principles for medical research involving human subjects[8]. All subjects were residents of Khartoum State; females enrolled were neither pregnant nor lactating. The range of age was (≤18 to 55 ≥ years). Health status of the subjects was evaluated based on information obtained from each volunteer regarding the following: free from history of diabetes mellitus (DM), no smoking history, no history of hospitalization within the last 12 months , no evidence of burns, no evidences for impaired renal or hepatic functions. ECG, clinical blood chemistry, blood pressure were obtained. As part of inclusion criteria, all of the volunteers were checked to ensure that they didn't take (during the study period or within 1 month before) any of the following medicines: Allopurinol, Captopril, Enalapril, Anticoagulants, coumarin or indandione derivative, Miconazole, Fluconazole, Appetite suppressants, Corticosteroids thiazide Diuretics, Barbiturates, Beta-adrenergic blocking agents, Cimetidine, Ranitidine, Fluoroquinolones, Quinine, Rifampin, Chloramphenicol, NSAIDs, Sulfonamides or Hyperglycemia-causing agents.

Selection of the volunteers:

The selection process of the volunteers was aimed to minimize the variations between the individuals participated in this study. Age, weight, gender and health status of the participants all were taken into considerations. The recruitment of the subjects in this study was on voluntary basis and all of the ethical considerations were taken into account. Volunteers received a welcoming package to orient them about the study (its objectives, methods, instructions for preparations, sample collection and other relevant information). Besides that, they received basic information sheet about them to fill.

Set up: The study was taken place in Soba University Hospital after getting the permission from the hospital administration.

Other variability aspects:

The factors that were expected to affect the study were controlled carefully during the study course. They include environment, diet, fluids intake, physical conditions and timing of blood sampling (day or night). Standardized information sheet was developed and distributed to all of the participants prior to the starting date. This aimed to ensure the consistency of all affecting factors to minimize the variations between subjects included and hence the results obtained.

Ethical considerations: This study wasconducted by independent professionals from the academic sector and was designed for scientific and academic purposes only. The investigators express no conflict of interest in selecting the products or suppliers under testing. The principal investigator was committed prior to start the study for his responsibility to ensure the protection of the rights, safety and well-being of subjects involved in this study. The ethical approval has been obtained from the National Board for Ethical Review of Health Research in Sudan Ministry of Health. In addition to that, the study team included certified medical doctor in order to monitor the subjects closely during their admission in the hospital. All study subjects, after receiving the information sheet and upon agreement to taken part of the study, were asked to sign a consent form before considering him/her as study subject. This was kept well with other confidential documents under this study.

Blood sampling scheme:

The purpose of sampling scheme includes mainly the half-life that play critical role in determining the elimination profile of Glibenclamide. Accordingly its dose response curve in addition to the elapsed time to reach the maximum concentration (absorption and elimination period) were the determinant factors for this sampling scheme[10]. The sampling period and schedule were determined to cover 24 hours following drug administration. Blood samples (14 samples) were collected at 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3, 3.5, 4, 4.5, 6, 12 and 24 hours after administration of the dose. Using 5 ml syringes, the samples were transferred to heparinised tubes after labeling. Each sample was then centrifuged for five minutes using regular centrifuge at 3000 rpm. Serum was separated and transferred into 3ml plain containers. The samples were re-labeled using the final codes, transported and frozen up to the time of analysis. Parallel with the collection of blood sample, sample collectors used small amount of blood (0.1 ml) to measure the glucose level in the sample using Accu-chek® Glucometer.

Samples preparation and analysis:

Basic HPLC techniques were used to measure targeted parameters. Analysis was done in collaboration with the Central Laboratory in Faculty of Science – University of Khartoum.

Chromatographic conditions:

The method of analysis adopted for this process was developed by SD Rajendran and others with some minor modifications as needed[10]. The HPLC system consisted of a Shimadzu LC-10AT liquid chromatographic pump, SIL-10a manual injector and SPD-10A UV/Vis UV absorbance detector (Shimadzu, Kyoto, Japan). Data collection, integration and calibration were accomplished using Class VP Chromatography Data System Version 6.14 computer software (Shimadzu, Kyoto, Japan). The chromatographic separations of Glibenclamide and internal standard (glimepride) were accomplished using a 150 mm×4.6 mm ID Shim-Pack VP-ODS analytical column (SHIMADZU). A Guard-Pak precolumn module (Phenomenex, USA) containing an ODS cartridge insert was placed serially just before the analytical column. The mobile phase consisted of acetonitrile: 25 mM phosphate buffer (pH: 3.5) in a combination of 80:20 v/v. Before use the mobile phase was degassed by passing it through a 0.22µm filter. The mobile phase was pumped at an isocratic flow rate of 1 ml/min at room temperature. The UV detection wave length was set at 253 nm. The wave-length of 236 nm represented the UV maximum of Glibenclamide in acetonitrile: water in 1:1 ratio.

Assay procedure:

In which a stock solution representing 100µg/ml of Glibenclamide was prepared in acetonitrile: water in 1:1 ratio. These solutions were stored at -20^{o} until use. The working standard solutions were prepared prior to use from the stock solution by sequential dilution with a combination of acetonitrile: water in 90:10 ratio to yield final concentrations of 50, 100, 200, 400, and 500 ng/ml of Glibenclamide. The internal standard stock solution was prepared by dissolving 1mg of glimepride in 100ml of acetonitrile: water in 1:1 ratio. This solution was stored at

-20° until use. In a 2ml microcentrifuge tube, 500µl of serum was added along with 500µl of internal standard solution. The serum was precipitated by the addition of 500µl of methanol, and then the tubes were vortexed for 30 sec and centrifuged at 5000 g for 8min. The supernatant was transferred to a clean, similarly labeled tube and was subsequently re-centrifuged for 2min. The resulted solution was injected in to the HPLC.

Assay parameters:

The extraction efficiency of the samples was determined by comparing the peak area of known amounts Glibenclamide (unextracted) in mobile phase that was directly injected to peak area of samples containing the same amounts of Glibenclamide in plasma after extraction. Quantification was based on calibration curves constructed using peak area ratios of drug to internal standard versus the nominal concentration. The procedure was repeated on three separate days to allow the determination of inter-day precision and accuracy. Intra-day accuracy was estimated based on the mean percentage error, and the inter-day accuracy was calculated as the mean of the intra-day accuracy determinations. The precision, which was expressed as percentage, was evaluated by calculating the intra- and inter-day relative standard deviations. The standard drug solutions in varying concentrations ranging from 50ng/ml to 500ng/ml were examined by the assay procedure. The peak area was calculated. The calibration acurvewas plotted using peak area vs. oncentration of the standard solutions.

Data management and analysis:

General analysis was done manually and by using computer programs e.g. SPSS and MS Excel.

Pharmacokinetic analyses:

The following parameters will be presented and discussed below: (1) maximum plasma concentration (Cmax) as indicator for absorption rate; (2) time to reach the maximum plasma concentration (Tmax) as indictor for the elimination rate; (3) area under of curve (AUC) as it described the total amount of drugs available in plasma after the administration of the dose; and (4) elimination half life time (T½) as indicator for the elimination.

Area Under the Curve (AUC):

The area under the curve (AUC0-t) was calculated using the following formula:

$$AUC0\text{-}t = \sum_{i=1}^{t}\left(\frac{Ci + Ci-1}{2}\right)(ti - ti - 1)$$

Pharmacokinetic parameters were calculated as indicted before. Based on these parameters the elimination rate constant (KE) was obtained using the following formula:

$$(KE) = 0.693 / T½$$

The area under the curve to the last measurable concentration (AUC0-t) was estimated by the linear trapezoidal rule. The area under the curve extrapolated to infinity (AUC0-∞) was calculated by equation below:

$$(AUC0\text{-}\infty) = AUC0\text{-}t + Ct / kE$$

* Where Ct is the last measured concentration

Paired samples analysis was used to compare the results of Tmax of the test and the reference products. The results were considered statistically significant for a P value of less than 0.05. The 90% confidence intervals of parameters under testing were also estimated. The inter-subject variation of AUC0-t, AUC0-∞, and Cmax parameters was also obtained by calculating the coefficient of variation (CV).

Results:

The volunteers:

Twelve healthy volunteers; 6 men and 6 women, aged between 20 years and 36 years, and with a range of weight between 65 kg and 94 kg; were enrolled to participate in this study.

Based on the results obtained:

(KE) Average slope for test product	0.104
(KE) Average slope for reference product	0.194

Accordingly the elimination half life (T1/2) in hours for each product:

T½ of test product	6.663
T½of reference product	3.572

The results indicated that the mean “C max”

of the test product was 2.508 compared to 2.526 the reference product. On other hand the "AUC0 - ∞" of test product was 3.511 compared to 4.572 the reference product.

Table 1: Main pharmacokinetic parameters for test and reference products

Parameters	Test product Mean ± SD	Reference product Mean ± SD	Intra- subject CV%
C max - (µg/ml)	2.508 ± 0.104	3.526 ± 0.118	2.8%
T max - (hrs)	1.639 ± 1.024	2.167 ± 1.275	63.9%
AUC0-12 - (µg.h/ml)	0.490 ± 0.188	0.638 ± 0.252	55.7%
AUC0 - ∞ - (µg.h/ml)	3.511 ± 0.153	4.572 ± 0.202	2.4%

The table below show the time interval for each of the test and reference products to reach the maximum concentration, the numbers showed the frequency of the volunteers.

Time interval (h)	Test	Reference
0,0 – 0,99	3	2
1,0 – 1,99	1	1
2,0 – 2,99	2	4
3,0 – 3,99	3	3
4.0 – 4.99	3	2

The AUC0-6, AUC0-∞, Cmax, and Tmax, for each pair of products (test vs. reference) in this study were statistically different ($P<0.05$), suggesting that the serum profiles generated by reference tablets were relatively higher than those produced by the test product (Table 10).

Moreover, 90% confidence intervals of the AUC0-6, AUC0-∞, and Cmax of the two formulations in the study were not found to be within the relative bioavailability acceptable range of 80-125% (Table 1). Wilcoxon signed rank test showed distinct difference between the untransformed values of Tmax of the test compared to the reference products. The intra-subject CV for AUC0-6, AUC0-∞, and Cmax appeared to be varied and relatively large for some AUC0-6 and Tmax. With a difference of about 24% between the test product and the reference product, the results indicate that the test product is not bioequivalent to reference product. The results indicated that, the probability that the true ratios with respect to Cmax and AUC are not acceptable in bio-equivalence range (which is 80% - 120%). It is therefore clear that test product cannot be considered bio-equivalent to the reference product with respect to the extent of absorption as measured by AUC. The fact that the two products also differed with respect to Cmax which is probably due to differences in the extent of absorption rather than the difference in the rate of absorption. No hazardous side-effects or adverse reactions were noticed during the observation of the volunteers; however, several of them experienced unpleasant symptoms relevant to hypoglycemia. This was noticed in 2 volunteers after the test product and in 3 volunteers after the reference product. The figure below showed the serum glucose level versus time for test and reference products.

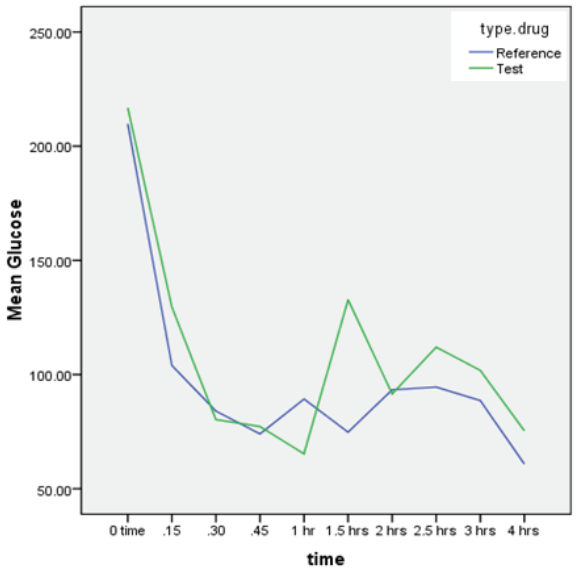

Figure 1: Serum glucose level for test and reference products

Discussion:

The importance of our study are derived from thefrom medicines quality monitoring survey done in 2008 in Sudan pointed the fact that

one of the authorized Glibenclamide products was among the top three products that the doctors complained about its therapeutic outcomes[4].

WHO guidance on bioavailability and bioequivalence stated the following *"Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives AND if their bioavailability after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same"*[5].Our study design benefited from the experience and guidance of other bodies such as FDA[6].Most of the published studies in this area adopt sample size between 12 – 26 subjects (depends on the variability of drug under study) and this number showed sufficient and statistically significant evidence[7].

The chemical contents of each medicine usually play important role in medicines ineffectiveness problems. Because it indicates that the desired quantity of the medicine is available or not as patient need it; and any interruption in this relationship could lead to failure of therapeutic process[12].

As indicated before, based 2008 quality monitoring survey and according to the results obtained from both data collection process and laboratory analysis, it was clear there are other reasons contributed to ineffectiveness problems associated with specific Glibenclamide product in the market. Currently there are 16 different formulations authorized and available in Sudan and from different sources[3]. On the other hand very limited information about their bioequivalence or interchangeability was known. Due to lack of information about the interchangeability of these products and lack of programs to inform and educate the patients, all of these highlighted the concerns about the effectiveness of these products in managing the disease in more than (50,000) estimated cases of diabetes mellitus (type II) in Sudan[13].

As stated by Meredith PA[14]: *"For economic reasons, the use of generic substitution is increasingly being supported by health authorities, ..., many developing countries do not have the resources or expertise to carry out appropriate quality control resulting in widespread distribution of substandard drugs, ..., a number of reports, largely anecdotal, of treatment failure or increased adverse events after switching brands have cast some doubts upon whether bioequivalence testing is sufficient in all cases.* On the other hand, Tschabitscher D and his colleagues urged[15]: "*Since the introduction of generic drugs to the pharmaceutical market a sometimes emotional debate exists whether they are well-investigated and of high quality. There is some uncertainty about [whether evidence of bioequivalence is enough to guarantee efficacy and safety of generic drugs]....., the importance of bioequivalence studies is increasing also due to the large growth of the production and consumption of generic products....., the registration of generic products does not demand complicated and expensive clinical study contrary to original product. The comparison of the original and the generic product via bioequivalence study is suggested as sufficient".*

After reviewing the results of each product under testing it was noticed that the main difference between the two products, beside other minor differences, was its formulation characteristics. The local product was a non-micronized formulation unlike the reference product which was formulated using a micronized powder. This was significant finding and has major impact on the registration requirements of this drug (and other similar drugs) in the country. According to the results obtained from the survey, from chemical laboratory analysis and form this study; it becomes clear that the risks associated with poor formulation of Glibenclamide products is the major cause behind the complaints reported about this drug. These observations and feedback about the specific locally manufactured product support the complaints received about the problem. This was supported by the study taken by Coppack and other co-workers in

which the results showed the clear differences in pharmacokinetic characteristics of the different types of Glibenclamide formulation[16]. This also supports the hypothesis behind the insufficiency of chemical analysis alone to verify the quality as it was shown in this bioequivalence study. Although, measuring the quality of pharmaceutical products was changed markedly in the last decade, nevertheless, chemical content of pharmaceutical products alone is not the only suitable indicator to measure the quality of some generics. As evidences showed, other important indicators that should be considered when we evaluate the quality of these drugs. The combined interpretation of quality control and quality assurance results with other data from clinical trials and feedback about therapeutic outcomes becomes highly important. This is particularly significant in order to include or exclude any potential risk factors that contribute to treatment failure or poor clinical outcomes.

Conclusions:

This study provided necessary information about the assessment of medicines quality in order to inform the decision makers about reviewing the registration decisions of Glibenclamide products that are available in the market. The study highlighted the impact of poor drug formulation on quality and effectiveness especially for medicines that are chronically used.

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