Glibenclamide Therapy in Type 2 Diabetes

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ABSTRACT: Glibenclamide is a second generation sulfonylurea used in the treatment of type 2 diabetes. The study included 57 type 2 diabetic patients of either sex, aged above 21 years, who were under treatment with glibenclamide. Blood samples were collected after 2 hours of drug intake and the next day, early morning before taking the drug. Serum glucose levels and glycosylated hemoglobin levels were estimated. The effect of glibenclamide treatment on long-term glycemic control was studied. There was no correlation between peak serum drug concentrations and post prandial glucose level as well as between trough serum drug and fasting glucose concentrations as could be observed from the coefficient of correlation (R²) values of 0.0319 and 0.0013, respectively. There is no correlation between peak serum drug concentration and HbA1c% as can be observed from the R² value of 0.0101. The insignificant correlation between the parameters indicates that upon chronic therapy with glibenclamide desensitization and receptor down regulation occurs leading to loss of glycemic control. It was observed that the increased dose of glibenclamide also did not improve glycemic control.

KEYWORDS: Glibenclamide, blood glucose, glycosylated hemoglobin and type-2 diabetes.

Introduction

Diabetes mellitus, a chronic disease, is one of the main threats to human health in the 21st century. Type 2 diabetes arises as a result of β-cell failure combined with concomitant insulin resistance (DeFronzo et al., 1992). Modern treatment for diabetes aims at the overall management of the disease by monitoring various parameters which is called as diabetes care. Glibenclamide is a second generation sulfonylurea used in the treatment of noninsulin dependent diabetes (NIDDM). It is reported that there is a need for the management of glibenclamide therapy in type 2 diabetic patients who are at risk of developing one or more adverse reactions due to over utilization of the drug (Yap WS 1998). It has beneficial effects of lowering the serum glucose levels. It is able to maintain a more prolonged increase in serum insulin levels by inhibiting the degradation of insulin in the vascular endothelial cells of the liver. The inhibition contributes to the blood glucose lowering effect of glibenclamide (Mulder, 1991 and Prosser, 1985). It has beneficiary effects on serum lipids (Singh, 1992 and Wainscot, 1988).

Metabolic control in NIDDM patients failing to respond to therapy with maximum dose glibenclamide or glipizide is not improved by switching to the alternate second generation sulphonylurea (Martin, 2003; Peters et al., 1996 and Simcic, 1991). The most important advantage regarding the monitoring of serum glibenclamide levels is the cost factor. Since there is equivalent glycemic control with glibenclamide when compared with other second generation drugs, economic considerations regarding choice of therapy may be appropriate. There is a need for modifying prescribing behavior for the treatment of NIDDM in countries like India, and the use of glibenclamide when compared with other second generation drugs was proved to be the most effective approach (Geetasharma, 2003). Therefore, keeping in view the need and advantages for monitoring serum glibenclamide levels, the present work was aimed at monitoring the serum glibenclamide levels in type 2 diabetic patients.

Materials and Methods

Materials

Glibenclamide tablets (Dianil® 2.5) were purchased from Aventis Pharmaceuticals Limited, Mumbai, India. Glibenclamide pure substance was a kind gift from Cadila
Pharmaceutical Limited, Ahmedabad, India. Diethyl amine from S.D. Fine Chemicals, Mumbai, India. Acetonitrile (HPLC grade) and diethyl ether from E. Merck Limited, Mumbai, India. Glucose oxidase-peroxidase (GOD-POD) kit and HbA1c (Glycosylated hemoglobin) kit from Excel Diagnostics Pvt. Limited, Hyderabad, India were purchased.

Study design
The patients participated in this study were from Mahatma Gandhi Memorial Hospital, Warangal, (A.P) India. The study included 57 type-2 diabetic patients of either sex who were above 21 years, under treatment with glibenclamide only were observed in this study after subjecting to a thorough physical examination and standard laboratory tests. All the patient were briefed about the study and a written informed consent was obtained from them. The study protocol was approved by institutional ethics committee. The study was conducted as per good clinical practice guidelines, in accordance with the Declaration of Helsinki. Study drug (2.5 mg/day) was taken in the morning with 100 ml of water just after voiding. Blood samples were collected after 2 hours of drug intake (representing peak levels of drug) and at early morning before taking drug in the next day i.e. fasting serum samples (representing trough levels of drug). Blood samples were centrifuged at 3000 rpm for 15 min and serum samples were stored at -80°C until analysis.

HPLC instrumentation
Shimadzu high performance liquid chromatography unit equipped with the LC-8A Solvent delivery module, SPD-10AVP UV-Visible spectrophotometer detector, Class CR-10 Data Processor, Rheodyne (with 20 μl capacity loop) Injection Port and Wakosil II C-18 Column (stainless steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5μm diameter, 100 Å pore diameter) were used for analysis of samples. Mobile phase consisting of acetonitrile and phosphate buffer adjusted to pH 7.4 with diethyl amine (65:35v/v) at 1ml/min flow rate, UV-detection set at 230 nm and sensitivity of 0.001 a.u.f.s was used for the analysis. Serum glucose was estimated using glucose-oxidase peroxidase method (Trinder, 1969) at 510nm and serum HbA1c was estimated using ion exchange resin method (Trivelli, 1971 and Bunn, 1981) at 415 nm using Photoelectric Colorimeter (Systronics).

Calibration curve of glibenclamide in human serum
Glibenclamide in serum samples was estimated by reversed-phase high performance liquid chromatography (Nieder et al, 2005). To 100 μl of serum, 20 μl of different concentrations of (25-800 ng/ml) of glibenclamide was added followed by 6 ml of diethyl ether, and vortexed for 4 min and centrifuged for 5 minutes at 3000 rpm. The supernatant was transferred into another centrifuge tube and evaporated to dryness. The residue was then reconstituted with 50 μl of mobile phase. From this 20 μl of this solution was injected onto the HPLC column. The samples were extracted as described and peak areas obtained at different concentrations of the drug were plotted against the concentration of the drug. The slope of the plot determined by the method of least square regression analysis ($r^2 = 0.9991$) was used to calculate the glibenclamide concentration in the unknown sample. The retention time of glibenclamide was 8.5 min.

Statistical analysis
All variables are expressed as mean ± SD. All analyses were performed using INSTAT 1.12 (Graph-Pad Software, Inc., San Diego, CA).

Results
Peak serum drug concentration is the concentration of drug in the serum collected 2 hr after drug intake. Trough serum drug concentration is the concentration of the drug in the serum collected early morning when no drug was taken but the serum retains small amounts of drug taken previous day. These were separated and plotted individually. The correlation between peak drug and postprandial serum glucose concentrations was shown in fig.1 and the correlation between trough drug concentration and fasting serum glucose concentrations was shown in fig 2. The correlation between peak serum drug concentration and HbA1c % was shown in fig 3. There was no correlation existed between peak serum drug concentrations and postprandial glucose level as well as between trough serum drug and fasting glucose concentrations as can be observed from the coefficient of correlation ($R^2$) values of 0.0319 and 0.0013, respectively. There is no correlation between peak serum drug concentration and HbA1c% as can be observed from the $R^2$ value of 0.0101. Of the 57 patients, number of patients with peak serum drug concentration and postprandial glucose level were 51, that of trough serum drug concentrations and fasting serum glucose level were 22 and HbA1c values were determined in 8 patients.
Fig. 1 Correlation between peak serum drug concentration and postprandial serum glucose levels. 
\( y = 0.0241x + 219.43, R^2 = 0.0319 \)

Fig. 2 Correlation between trough serum drug concentration and fasting serum glucose levels. 
\( y = 0.0142x + 186.75, R^2 = 0.0013 \)
Discussion

The main objective of present study was to monitor type 2 diabetes by finding a linear relationship between glibenclamide serum concentration and serum glucose levels and HbA1c levels. This objective was in support of an earlier report which emphasized the need for careful dosage titration of glibenclamide to achieve a desired therapeutic response in type 2 diabetes. They found a significant difference in pharmacokinetics and pharmacodynamics existing between single and steady state conditions (Jaber, 1994). In the present study we did not find any correlation between two parameters indicating the patients were not showing a linear relationship with glibenclamide therapy.

It was reported that there is a difficulty to relate serum glibenclamide levels with the biological response in disease state, multiple sites of sulfonylurea action and inter-patient variability (Pearson, 1985). This study collaborates with our present observations which were based on short term treatment with glibenclamide. Since our study included patients on chronic therapy, failure to find a linear relationship should be attributed to the pathological and other inexplicable conditions of the patients during long term treatment. There are also reports indicating slow elimination of glibenclamide in NIDDM patients. The long half life (15.0 ± 607 hrs) adds support to use of a once-daily dosage administration (Jonsson, 1994). It was reported the reasons for failure of glibenclamide therapy on long term treatment. Type 2 patients who have hyperglycemia lose acute incremental insulin responses to glucose. In these patients, exposure of pancreatic beta cells to sustained levels of sulfonylureas induces a reversible state of refractoriness to acute stimulation with sulfonylureas (Karam, 1986).

Glibenclamide effect on insulin release was relatively higher than proinsulin, which was more pronounced in patients on low glibenclamide dose. This was because of either impaired beta cells in those receiving low doses or due to reduced sulfonylurea sensitivity in those on higher dosages (down regulation) (Jonsson, 2001). More evidence support comes from a study in which no relation found between glibenclamide dose and concentration of glibenclamide in serum and steady state plasma levels of glibenclamide vary extensively irrespective of dose (Sartor, 1980). Saturation of membrane binding process at higher concentrations could explain the lack of effect of higher sulfonylurea dosages. Experiments with glibenclamide suggest the insulinotropic effect reaches a plateau at plasma concentrations of 100 to 200 nmol / L, corresponding to the dose of 10 mg / day (Stenman, 1993). This suggests that increasing glibenclamide concentration doesn’t improve glycemic control and hyperglycemia continues to persist. In fact, it was found that increasing dose led to impairment of glycemic control rather than improvement. This supports our present study since the number of patients who could achieve euglycemia with glibenclamide therapy was very few and many patients had persistent hyperglycemia despite treatment with glibenclamide for many years.
The maximal glibenclamide plasma concentrations were significantly higher in the euglycemic than in hyperglycemic state (448 Vs 228 mg/L) and these peak concentrations were attained faster in euglycemia than in hyperglycemia (3.7 Vs 5.0 hrs) (Hoffman, 1994). This suggests the absorption of glibenclamide was less in hyperglycemic state. This could be one of the reasons to explain the failure of drug therapy in the present study as most of the patients were hyperglycemic. There was poor management of the disease as can be seen from the insignificant correlation between serum glibenclamide levels and serum glucose and HbA1c levels in the present study. The number of people who have achieved euglycemia were very few and non compliance of the patients regarding drug intake, which may be due to poor economic conditions prevailing among the patients. There is evidence to support this. In one study there was a positive relationship between dose of glibenclamide and HbA1c indicating disease progression, patient compliance, underlying stressful condition, or a combination of these things (Linda et al., 1997). However, this was a retrospective study and it had its own limitations.

The overall well being of the patients improved and continued to be so far longer periods. The aim of study was to evaluate the effect of a 5 day teaching program for diabetic patients on their quality of life 1 and 2 years afterwards. Results from this study demonstrate that structured patient education improves patients well being after teaching programs (Tankova et al., 2004). Educational interventions which included a face to face interaction, cognitive reframing teaching method and exercise content were more likely to improve glycemic control (Ellis, 2004). However the glycemic control achieved in most of these studies is modest and needs refinement. Support to this statement comes from a metaanalysis study in which educational and behavioural intervention led to only moderate improvement in glycemic control (Gary, 2003). The present study was inadequate to support the above study and a more comprehensive work is needed in this direction which will greatly benefit the patients achieve good self management of the disease.

Conclusions

The present study concludes that with chronic glibenclamide therapy, glycemic control will be lost due to desensitization and receptor down regulation of β cells. Patient non compliance is an important factor for loss of glycemic control. It is also concluded that the glycemic control is not improved by increasing the dose. The present investigation suggests that intensive patient education programmes on a continuous basis would greatly improve glycemic control and help in the overall well being of the patients.

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References

Karam JH. Selective unresponsiveness of pancreatic beta cells to acute sulfonylurea stimulation during sulfonylurea therapy in NIDDM. Diabetes. 35(12): 1314-1320 (1986).


