ANOVA: A valuable tool in clinical trials

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Abstract

Statistics encompass a wide range of endeavors and applications. Statistics is utilized by varied strata of science from pharmaceutical science to health science in making vital decisions. Before a new drug can be marketed, the regulatory authority requires that it should be subjected to clinical trials. The data from this trials must be analyzed to determine whether the drug is not only effective, but safe. The safety and efficacy of a novel formulation of a drug is often checked by comparing its effects with a pre-existing formulation of the same drug. Various statistical methods are employed for this purpose out of which Analysis of Variance (ANOVA) can be considered the most efficient. In the present study, we compare a novel formulation of Glimepiride, with a formulation that is already available in the market by ANOVA. Glimepiride (2.0 mg) is a sulphonyl urea agent that stimulates insulin release from pancreatic cells and is administered once daily to patients with type 2 diabetes. The study was designed to compare the bioavailability of a new preparation of Glimepiride (2.0 mg) with commercially available Glypiride (2.0 mg) tablet in eleven healthy Indian male subjects following a single dose administration. Blood samples were collected through an indwelling cannula placed in a forearm vein. The pre-dose blood samples were collected before dosing and post-dose samples up to 16 hours. The subjects were randomized to receive formulations test and reference in each period in order to eliminate any bias factors. In each period, total of sixteen blood samples were collected. The plasma concentrations of both the reference and the test drug were obtained, analyzed and the values are interpreted with the use of ANOVA. We found the values of pharmacokinetic parameters Cmax, AUC0-t, and $ACU_{0-\infty}$ with the help of Measures of Central Tendency. Based on the ANOVA results, we found that the test formulation had a slightly higher performance compared with the reference and also demonstrated greater inter-individual variability among the groups. And from the 90% confidence interval we found that, the two formulations were inequivalent.

INTRODUCTION

ANOVA is the most powerful statistical tool. It is a general method of analyzing data from designed experiments, whose objective is to compare two or more group means. The t test is a special case of ANOVA in which only two means are compared. By designed experiments, we mean experiments with a particular structure. Well-designed experiments are usually optimal with respect to meeting study objectives. The statistical analysis depends on the design, and the discussion of ANOVA therefore includes common statistical designs used in clinical trials. Glimepiride (2.0 mg) is a sulphonyl urea agent that stimulates insulin release from pancreatic cells and may act via extra-pancreatic mechanisms. It is administered once daily to patients with type 2 (non-insulin dependent) diabetes mellitus in which glycemia is not controlled by diet and exercise alone, and may be combined with insulin in patients with secondary sulphonylurea. The greatest blood glucose lowering effects of Glimepiride occur in the first hour after the dose. Glimepiride has fewer and less severe side effects on cardiovascular variables than glibenclamide. Pharmacokinetics is mainly unaltered in elderly patients or those with renal disease. Present study was carried out to examine the relative bioequivalence of Glimepiride (2.0 mg) tablet with Glypiride (2.0 mg) tablet.

MATERIALS AND METHODS

Name	Meaning
AUC_{0-t}	Area under the plasma concentration versus time curve from the first time point (t=0) to the time point of the last measured concentration (t_{last})
C_{max}	Maximum plasma concentration
hr	Hours(s)

Name	Meaning
N	Number of subjects
SD	Standard division
T_{max}	Time of maximum plasma concentration
SEM	Standard error of mean

This study was designed to compare the bioequivalence of an oral film coated tablet of Glimepiride (2.0 mg) with Glypiride (2.0 mg) tablet. Each film coated reference and test tablets contained 2.0 mg of drug. Eleven (11) normal, healthy, male volunteers of age 20 - 40 years were included in the study. All the volunteers underwent a thorough physical experimentation, urinalysis and routine blood tests. They were instructed to refrain from all medications seven day prior to the study, and until the study was completed. Alcohol was not permitted 24 h prior and during the study. The study was conducted according to the principles outlined in the declaration of Helsinki. The study protocol was approved by the institutional ethics committee overseeing clinical studies in human. Volunteers gave a written consent before initiation of the study.

STUDY DESIGN:

A double blind, randomized crossover study was conducted. The clinical investigators as well as the volunteers were unaware of the allocation of the treatment. Allocation of treatments to volunteers was done using random numbers generated by computer. On the day of study, an intravenous cannula was inserted by the consulting physician at least one hour prior to the dosing time. The volunteers were administered one of the two treatments (after an overnight fast) with 150 ml of water. Five (5.0) ml blood samples was collected at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 14.0 and 16.0 h post dose. Blood was collected into heparinised disposable syringes and transferred into clean glass through the indwelling venous cannula. The intravenous cannula was kept in patient up to 24 hours with 0.5 ml of saline and 0.2 ml of 1000 IU/ml heparin. Blood was transferred immediately to plasma separation room and plasma was separated by centrifugation, divided into two aliquots and stored in plastic vials at -20°C, till assayed for the drug Glimepiride by LC/MS. Standardized food was given after 4 hours and 12 hours of dosing. MS Office Excel sheet was used for the calculations.

STATISTICAL METHODOLOGY:

The plasma pharmacokinetic parameters that have been estimated include, the observed maximum plasma concentration Cmax, the time to reach Cmax, (Tmax) and the area under the plasma concentration-time curve from 0 hour to last measurable concentration (AUC0-t) and 0 hour to infinity (ACU_{0-}). The maximum plasma concentration (Cmax) and the time to reach maximum concentration (Tmax) were directly determined from the plasma concentration versus time curves. The area under the curve from 0 h to t (AUC0-t) was calculated by the linear trapezoidal rule. The area under the curve from 0 h to infinity ($ACU_{0-\infty}$) was estimated by summing the area from 0 to (AUC0-t) and t to infinity (ACU_{0-}), where $ACU_{0-\infty} = C_t/k_{el}$, with '$C_t$' defined as the last measured plasma concentration at time t, and 'k_{el}' the slope of the terminal portion of the plasma concentration versus time curve, obtained by linear regression. Logarithmic transformation was done before data analysis for Cmax, AUC0-t, and $ACU_{0-\infty}$. Analysis of variance (ANOVA) was used to assess effects. Intra-subject variability in terms of the overall percentage coefficient of variation (%CV), were evaluated from the ANOVA results for ln-transformed data. For the pharmacokinetic parameters Cmax, AUC0-t and AUC0- , 90% confidence intervals for the ratios of test and reference product averages were calculated using the ANOVA of the ln-transformed data. Consistent with the two one sided test for bioequivalence, 90% confidence interval for the ratio of both the products averages were calculated by first calculating the 90% confidence interval for the differences in the averages of the ln-transformed data and then taking the antilogarithms of the obtained confidence limits. Bioequivalence was evaluated using average bioequivalence approach; this is based on the ratio of average ln-transformed responses. The 90% confidence interval for ratio of average ln-transformed Cmax, AUC0-t of Glimepiride was the basis for concluding the equivalence of test and reference. The 90% CI should hence lie within the bioequivalence limit (80.00-125.00).

RESULTS AND DISCUSSION

The mean of maximum plasma Glimepiride concentrations (Cmax, ng/ml) mean time to reach Cmax,(Tmax, hr) for the test and reference formulations were 95.07 ng/ml (range: 49.17 - 189.31), 73.80 ng/ml (range: 35.65 - 144.01) and 3.59 hr (range: 2.50 - 4.00), 3.64 hr (range: 2.50 - 4.00) respectively. The mean of area under the curve from 0 to last measurable time point 't' (AUC0-t ng.hr/ml) for test and reference formulations were 603.72 ng.hr/ml (range: 234.36 -1644.97), and 458.49 ng.hr/ml (range: 212.17 - 1140.17) respectively. The mean of area under the curve from 0 to infinity (ACU_{0-} ng.hr/ml) for test and reference formulation were 715.78 ng.hr/ml (range: 250.61 - 2127.06) and 593.25 ng.hr/ml (range: 252.87 - 1564.36) respectively. The mean of elimination rate constant (k_{el} hr^{-1}) and mean of time required for the plasma Glimepiride concentration to decrease by one half (t1/2 hr) for test and reference formulations were 0.17 hr-1 (range: 0.08 - 0.36), and 0.11 (range: 0.06 - 0.18) respectively. The p-values obtained from the ANOVA for sequence and period effects were not all greater than 0.05 for Cmax, AUC0-t and AUC0- which indicates that there were statistically significant difference due to sequence, treatment and period. 90% confidence intervals using two one sided t test of the Cmax, AUC0-t and $ACU_{0-\infty}$, were (102.42, 153.08), (107.56, 140.45), and (98.15, 129.46) respectively. It has been observed that $ACU_{0-\infty}$ variability is closer to acceptable range when considered for all 11 volunteers. For the whole group the lower limits of confidence interval for Cmax as well as AUC_{0-t} are not within acceptable range while upper limits for Cmax as well as AUC_{0-t} is slightly higher than the permissible limit of 125. Thus, the 90% CI for all the pharmacokinetic parameters were not within bioequivalence acceptance criteria.

TABLE 1: Individual pharmacokinetic parameters of Glemepiride in plasma, following administration of the test formulations.

	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-t} (ng.h/ml)	$ACU_{0-\infty}$ (ng.h/ml)	T$_{\infty}$
MEAN	95.07	3.59	603.72	715.78	3.
SD	43.58	0.54	399.29	518.72	2.
SEM	13.14	0.16	120.39	156.40	0.
N	11	11	11	11	1

TABLE 2: Individual pharmacokinetic parameters of Glemepiride in plasma, following administration of the reference formulations.

	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-t} (ng.h/ml)	$ACU_{0-\infty}$ (ng.h/ml)	T$_{\infty}$
MEAN	73.80	3.64	458.49	593.25	7.
SD	28.70	0.50	245.46	346.69	3.
SEM	8.65	0.15	74.01	104.53	1.
N	11	11	11	11	1

TABLE 3: ANOVA summary for C_{max} values

TABLE 4: ANOVA summary for AUC_{0-t} values

Source	d.f.	SS	MSS	F	Conclusion
Total	21	1.0298			
Treatment	1	0.0442	0.0442	8.05	NS
Subject	10	0.8769	0.0877	15.98	NS
Period	1	0.0593	0.0593	10.80	NS
Error	9	0.0494	0.0055		

TABLE 5: ANOVA summary for $ACU_{0-\infty}$ values

Source	d.f.	SS	MSS	F
Total	21	1.0878		
Treatment	1	0.0525	0.0525	4.521
Subject	10	0.9673	0.0968	16.350
Period	1	0.0462	0.0462	7.8031
Error	9	0.0533	0.0059	

TABLE 6: 90% confidence interval for 80 - 125% equivalence interval

C_{max}	102.42 – 153.08
AUC_{0-t}	107.56 – 140.45
$ACU_{0-\infty}$	98.15 – 129.46

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