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Formulations Dependent Variability in the Pharmacokinetics: A Case Study with Metformin

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Abstract: A retrospective analysis was carried out to compare the bioavailability of variety of preparations of metformin using the data of four bioequivalence studies of metfomin formulations. Four separate bioequivalence studies have been investigated where a single dose of the specific formulation was administered to 12 healthy, male Asian Indian volunteers. Three studies (Studies 1, 2 and 3) were done after an overnight fast whereas one study (No. 4) was conducted in fed condition. The present findings illustrate that the three formulations used in studies 1 and 2 showed comparable plasma concentration-time profiles and four formulations used in studies 3 and 4 demonstrated comparable maximum plasma concentration (C_{max}), area under the curve from 0 h to time t (AUC_{0-t}) and area under the curve from 0 h to time ∞ ($AUC_{0-\infty}$). The results infer that the same dose of drug gives different plasma concentration-time profiles of the drug if different marketed formulations are being used. Hence metformin may have variable results, including adverse drug reaction or therapeutic failure, as it has formulation dependent pharmacokinetics.

Key words: Metformin · formulation dependent pharmacokinetics

INTRODUCTION

The biguanide metformin (dimethylbiguanide) is an oral antihyperglycaemic agent widely used in the management of non-insulin-dependent diabetes mellitus (NIDDM or type 2 diabetes). Moreover, Gin *et al*. [1] found reduction of insulin requirements in insulin-dependent (type I) diabetic patients given metformin. Metformin reduces blood glucose levels, predominantly by improving hepatic and peripheral tissue sensitivity to insulin without affecting the secretion of this hormone [2]. Its efficacy in reducing hyperglycemia in type 2 diabetes mellitus is similar to that of sulfonylureas, thiazolidinediones and insulin [3]. Also metformin seems to exert its effect rapidly [4].

Lactic acidosis is the most commonly observed adverse effect of biguanide [5, 6]. However, because of difference in chemical structure and pharmacokinetic profile between the various biguanide, this serious adverse reaction is much rare with metformin than with phenformin and buformin [7-10]. Gastrointestinal absorption of metformin is incomplete, 20 to 30% of an oral dose being recovered in the faeces [11]. The rate of oral absorption of metformin is slower than that of plasma elimination [2]. The slow and incomplete absorption in combination with a rapid elimination makes the rationale for sustained release (SR) preparations of a drug less obvious. Consequently, several SR formulations of metformin have been developed.

Considerable renewal of interest in this drug has been observed in recent years. It has been used extensively in Europe without significant adverse effects and was approved for use in the United States in 1995. As the regulatory authorities have made it mandatory to conduct a bioequivalence study for generic substitutions, many bioequivalence studies of metformin have been conducted. In this manuscript, a retrospective analysis was carried out to compare the bioavailability of controlled release metfomin formulations of four bioequivalence studies performed at our centre.

MATERIALS AND METHODS

Four separate bioequivalence studies have been reported in this paper, where a single dose (500 mg) of the specific formulation was administered to 12 healthy, male Asian Indian volunteers. Three studies (Studies 1, 2 and 3) were done after an overnight fast whereas one study (No. 4) was conducted in fed condition. All the formulations used were controlled release formulations. The demographic profiles of all the volunteers are given in Table 1. All the volunteers conformed to the inclusion

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Table 1: Demographic profile of the volunteers participated in the bioequivalence studies

Study No. (N)	Frame		Age (years)	Height (cm)	Weight (kg)
1 (12)	Medium	Mean	25.91	168.95	57.09
		SD	4.87	7.73	6.69
2 (12)	Medium	Mean	24.92	169.92	60.54
		SD	4.21	5.53	10.05
3 (12)	Medium	Mean	22.89	166.78	59.78
		SD	4.62	4.24	5.93
4 (12)	Medium	Mean	26.83	171.58	63.50
		SD	4.78	6.46	9.41

N - Number of volunteers participated in the study
SD - Standard deviation

and exclusion criteria. Volunteers who were habitual users of tobacco, alcohol or other potentially enzyme inducing drugs, those who had participated either in another bioequivalence study or donated blood in the 3 month period prior to the study, as well as those with abnormal liver enzyme function, were excluded. In addition, all the volunteers underwent a physical examination, urine analysis and blood chemistry determinations. The volunteers were requested to refrain from all medication for 7 days prior to each study and until the study was completed. Alcohol was not permitted 24 h prior to and during each treatment period.

All the volunteers provided written informed consent. The studies were conducted according to the principles outlined in the Declaration of Helsinki. The protocols were approved by the Institutional Ethics Committee.

Experimental design:

Studies 1, 2 and 3: The studies were conducted according to a single-dose, two period, two way cross over design with 12 volunteers in each of the treatment groups and a wash out period of 1 week between the two phases of the study. Twelve volunteers were administered a single, 500 mg controlled release tablet of metformin (either test or reference) with 240 mL of water in each phase. Five milliliter blood samples were collected at 0 h and then 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 18 and 24 h post dose. No drink was allowed for 2 h prior to dosing and 2 h after dosing. A light standardized meal was provided 4 and 12 h postdose.

Study 4: The study was conducted in the same way as studies 1, 2 and 3 but in fed condition. Five milliliter blood samples were collected at 0 h and then 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36 and 48 h post dose. A heavy breakfast was permitted approximately 2 h before dose administration. A standardized meal was provided 4 and 12 h post dose.

Analytical method: Blood sample analysis was performed using an isocratic system consisting of a pump (L-7110, Merck Hitachi), UV Detector (L-7400 Merck Hitachi), auto sampler (L-7200 Merck Hitachi) and column oven (L-7350 Merck Hitachi).

The analytical column used was Nucleosil C_{18} (5 µ, 250 X 4.6 mm, Merck Germany). The guard column was Flexit Jour C_{18}e (4 X 4 mm). The mobile phase was a mixture of buffer and acetonitrile by 46:54% v/v. The buffer was 0.1 M Potassium Dihydrogen Phosphate and pH adjusted to 3.5 using o-phosphoric acid (85%). This solution was filtered by 45 µ membrane filter and mixed with methanol. The flow rate was 1 mL min^{-1} and the eluents monitored at 236 nm. The column was maintained at a temperature of 40±0.1°C. Samples were carried out by precipitating plasma followed by evaporation of supernatant. The evaporated samples were reconstituted with mobile phase and 80 µL was injected on HPLC. The range of detection of the drug was 30 to 5000 ng mL^{-1}.

The system was connected with the help of D-7000 interface to multi HSM software in a computer system for data collection and processing.

Pharmacokinetic analysis: The maximum plasma concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were directly determined from the plasma concentration versus time curves. The area under the curve from 0 h to t (AUC_{0-t}) was calculated using the linear trapezoidal rule. The area under the curve from 0 h to infinity ($AUC_{0-\infty}$) was calculated by summing the area from 0 to t (AUC_{0-t}) and t to infinity ($AUC_{t-\infty}$), where $AUC_{t-\infty} = C_t/kel$, with 'C_t' defined as the last measured plasma concentration at time t and 'kel' the slope of the terminal portion of the ln plasma concentration versus time curve, obtained by linear regression.

Statistical analysis: ANOVA was performed to test the similarity of the demographic profiles of volunteers participated in the various studies. ANOVA followed by Tukey's multiple comparison was applied to test the statistical significance between C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of various formulations used in different studies.

RESULTS

The mean plasma concentration-time profiles of test and reference formulations of metformin following administration of single oral 500 mg dose to 12 healthy male volunteers are shown in Fig. 1. Table 2 gives the summary of the pharmacokinetic parameters, time of the study and the formulation number for all the four studies. The 90% confidence intervals meet the criterion of bioequivalence for all the studies individually, implying that there was no statistically significant difference

Table 2: A comparative study of pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ (mean ± SD) using various formulations of metformin

Study No. (N*)	Period of the study	Condition	Dosage**	Formulation No.	C_{max} (ng mL^{-1})	T_{max} (h)	AUC_{0-t} (ng.h mL^{-1})	$AUC_{0-\infty}$ (ng.h mL^{-1})
1 (12)	Oct'2001	Fasting	Test (SR 500 mg)	1	450.93±64.23	3.42±1.00	3270.30±1037.55	4990.57±2476.58
			Ref (XR 500 mg)	2	460.26±71.58	3.21±0.66	2981.35±1107.99	4423.91±2229.64
2 (12)	Aug'2001	Fasting	Test (SR 500 mg)	3	456.29±142.77	3.58±1.22	3375.67±1232.45	4134.58±1902.85
			Ref (XR 500 mg)	4	483.14±129.90	3.67±1.07	3194.39±1509.39	4679.99±2441.82
3 (12)	Feb'2002	Fasting	Test (ER 500 mg)	5	769.04±177.75	3.63±0.71	5320.08±1130.43	5721.89±1189.50
			Ref (ER 500 mg)	6	773.68±207.87	3.38±1.00	5119.18±2052.98	5841.85±2266.63
4 (12)	Jan'2002	Fed	Test A (SR 500 mg)	7	766.87±129.10	3.42±0.85	5412.80±1436.71	6205.21±2179.31
			Test B (SR 500 mg)	8	765.44±194.94	4.75±0.62	5699.53±1109.44	6091.70±1178.95

* N-Number of volunteers participated in the study

** Dosage form was tablet for all the studies. Ref-Reference, SR-sustained release, ER-extended release, XR-extended release

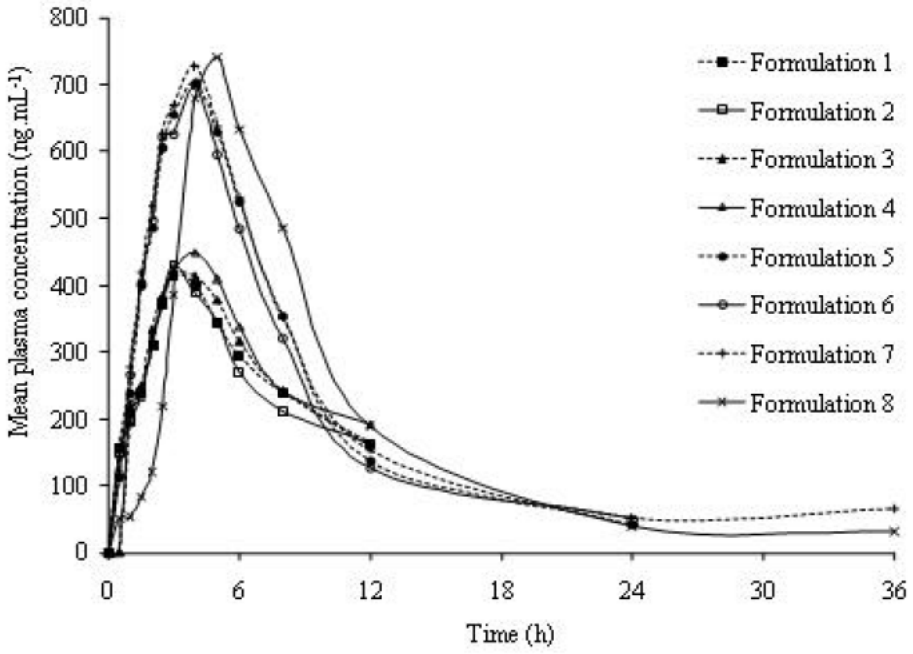

Fig. 1: Mean (n=12) plasma concentration-time profiles of metformin in healthy Indian male subjects after oral administration of 500 mg tablet

between the test and reference formulations in any of the studies for the parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.

The mean age (SD) and mean weight (SD) of the volunteers participated in various studies were 25.91 (4.87) years and 57.09 (6.69) kg for study 1, 24.92 (4.21) years and 60.54 (10.05) kg for study 2, 22.89 (4.62) years and 59.78 (5.93) kg for study 3 and 26.83 (4.78) years and 63.50 (9.41) kg for study 4 (Table 1). No statistically significant difference was observed in age ($p>0.15$) and weight ($p>0.20$) of volunteers in various studies. This justifies the comparison of pharmacokinetic parameters of metformin in various studies.

On comparing the parameters C_{max} and AUC_{0-t}, it was concluded that there was statistically significant difference in the parameters C_{max} and AUC_{0-t} of various formulations used in different studies ($p<0.0001$). Reference formulations used in studies 1 and 2 were the same (Formulations 2 and 4) and multiple comparison showed that the mean pharmacokinetic parameters were also similar in both the studies for this reference formulation ($p>0.05$, Table 2). This implies that pharmacokinetics of metformin was not time dependent. Also these results infer that inter individual variability was not very high for metformin. Consequently, it supports the comparison of pharmacokinetic parameters of metformin using different sets of volunteers at different times.

Further, multiple comparison revealed that formulations 1, 2, 3 and 4 were not statistically significantly different from each other with respect to C_{max} and AUC_{0-t}. Also there was no statistically significant difference in formulations 5, 6, 7 and 8 with respect to the parameters C_{max} and AUC_{0-t}. The present results show that the time to reach maximum concentration (T_{max}) was comparable for formulations 1, 2, 3, 4, 5, 6 and 7 (Table 2).

DISCUSSION

There were no statistically significant difference ($p>0.05$) in C_{max} and AUC_{0-t} for formulations 1, 2, 3 and 4, whereas these parameters were statistically significantly different ($p<0.05$) from those of formulations 5 and 6 (study 3). The C_{max} and AUC_{0-t} for formulations 5 and 6 were approximately 1.5 times higher than the C_{max} and AUC_{0-t} for formulations 1, 2, 3 and 4 (Table 2).

If controlled release formulation of metformin is administered with food, the AUC is increased and at the same time the C_{max} is unaffected [12, 13]. Study 4 was conducted in fed conditions but, C_{max} of study 4 (Formulations 7 and 8) was more than that of studies 1 and 2 (Formulations 1, 2, 3 and 4). This finding was supported by the multiple comparison test inferring statistically significant difference in the parameters C_{max} ($p<0.05$) between the formulations of studies 1 and 2 and study 4. This difference in C_{max} should be due to difference in formulation as food does not affect the parameter C_{max} [12]. In contrast, though food affects the parameter AUC [12], the pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ in study 4 (Formulations 7 and 8) conducted in fed condition were comparable with that of study 3 (Formulations 5 and 6) ($p>0.05$) performed in fasting condition.

In summary, this connotes that the same amount of drug gives different plasma concentration-time profiles of the drug if different SR formulations are being used. Consequently, swapping from one formulation to another may lead to changed plasma concentration time profile of metformin in an individual leading to lower exposure and no therapeutic benefit or leading to higher exposure to the drug may be toxicity. Moreover, the maintenance of plasma glucose levels in diabetes patients is very important, hence glycemic control should be closely monitored and dosage adjustments should be made accordingly. This task becomes more complicated if metformin plasma concentrations are formulation dependent. For example, if a patient is using one of the formulations used in studies 1 and 2 and if he/she switches over to formulations used in studies 3 and 4 assuming the same effect of the formulation because of the same dose and similar type of (SR) formulation, he/she may get higher plasma concentration and toxic effects. Similarly if it is other way round, he/she may get lower plasma concentration and the drug may not be effective at all. As consumer has the habit of switching from one brand to another especially if it is cheaper, it may be very hazardous with metformin treated patient. A US Congressional Budget Office estimate is that in 1994 alone consumers saved US$8-10 billion on prescription drugs by buying generic drugs instead of their branded counterparts [14]. Hence it is the responsibility of the physician to observe the patients who switch over from one formulation of metformin to another formulation of metformin as metformin has formulation dependent pharmacokinetics; it may have variable results, including adverse drug reaction or therapeutic failure.

Metformin is a very old drug, one of the reasons for formulation dependent pharmacokinetics may be that there was no regulatory requirement to show bioequivalence 35-40 years back. Once a new drug formulation had been shown safe and effective, other formulations of the drug were used to market based on dissolution criterion. As metformin absorption is limited to the upper part of the intestine [2], controlled release oral formulations of metformin should release the drug during transit from stomach to jejunum completely. If this is not the case, the formulations may give diverse *in vivo* profiles even if their dissolution profiles are alike.

Additionally, introduction of new formulation (generic) involves pharmacokinetic comparison with the innovator's formulation. In many developing countries, the comparison is made with any existing formulation and not necessarily with innovator's formulation. As 20% difference from the reference formulation in pharmacokinetic parameters is permissible for the new (to be marketed) formulation, the new formulation (if it is second or subsequent entry in the market) may have 40% or more difference in pharmacokinetic parameters in comparison to the innovator's formulation. This is another reason why we have several formulations of the same drug in the market with variable PK behavior, as shown here; hence the drug regulatory authority should consider pharmacokinetic comparison of the new formulation with only the innovator's formulation. This will in our opinion cut down some of the formulation dependent variability seen here.

This retrospective study suggests marked formulation-dependent pharmacokinetics of metformin, which may have important clinical implications. Moreover, this analysis also warns us that similar findings may be there for other comparatively older drugs.

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