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Effect of Cyp2c19*2 Polymorphism on the Pharmacokinetics of Gliclazide in Healthy Myanmar Volunteers

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This study aimed to determine the effect of CYP2C19*2 polymorphism on the pharmacokinetics of gliclazide in healthy Myanmar volunteers. In the phase I study, a total of 150 subjects, of either sex of different ethnicities, were randomly selected for determination of CYP2C19 genotype. Among them, 53.3% were normal/wild type, 40% were heterozygous and 6.67% were homozygous for CYP2C19*2 genotype. In the phase II study, one tablet of Reclide containing 80 mg of gliclazide was given to each volunteer and blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10 and 24 hr after drug administration. Plasma gliclazide concentrations were determined by validated HPLC method and the pharmacokinetic parameters were compared among three different genotypes. All the elimination parameters of gliclazide (C_{max}, T_{max}, $T_{1/2el}$, K_{el}, clearance/F) were significantly lower in those with polymorphism but no significant differences were observed in the absorption parameters ($T_{1/2ab}$ and K_{ab}). The results of this study suggested that CYP2C19*2 polymorphism affect the metabolism of gliclazide but not its absorption.

Key words: Cyp2c19*2, Polymorphism, Pharmacokinetics of gliclazide

INTRODUCTION

Pharmacogenetics is the study of the linkage between an individual's genotype and its ability to metabolize a foreign compound. Cytochrome P-450(CYP) enzyme system is one of the most important drug metabolizing enzyme systems in humans. It is composed of families and subfamilies of enzymes which are responsible for metabolism of drugs, steroid and cholesterol. Of the CYP450 content in human liver, CYP3A4 is the most abundant (~28%), followed by CYP2C family (18%), CYP1A2 (~12%), CYP2E1 (7%), CYP2A6 (4%), CYP2D6 (1.5%) and CYP2B6 (0.2%).[1]

Of these, CYP3A4 metabolizes more than half of the currently prescribed drugs (51%) followed by CYP2D6 (24%) and the CYP2C subfamily (~20%).[1] CYP2C9 and CYP2C19 are responsible for metabolizing over 20% of clinical therapeutic drugs. There are altogether 21 variant alleles of CYP2C19 that have been described to date. Normal/wild type genotype is CYP2C19*1 and the two most common variants are CYP2C19*2 and *3, both of which result in the total absence of enzyme activity. They account for approximately 87% of all poor metabolisers (PMs) in Caucasians and 100% of all PMs in Orientals. The variant alleles of CYP2C19 (except*17) explain almost all PMs of CYP2C19. CYP2C19*17 is the one and only allele that results in ultra-rapid metaboliser status. The rest of the variant alleles account for poor metaboliser status.[2]

Genotyping of CYP2C19 has clinical importance particularly for drugs which are mainly eliminated by CYP2C19 especially for those with narrow therapeutic index. Of all the CYP enzymes, Asians have the

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highest incidence of CYP2C19 polymorphism. Substrates of CYP2C19 include tricyclic antidepressants (e.g amitriptyline), antiepileptics (e.g phenytoin), proton pump inhibitors (e.g omeprazole), β blockers (e.g propanolol) and NSAIDs (e.g indomethacin, diclofenac), oral hypoglycemic (e.g gliclazide), etc. Impaired metabolism of these drugs due to polyorphism of CYP2C19 can lead to serious clinical consequences.

Among the substrates of CYP2C19, gliclazide is of particular interest because it is frequently prescribed drug in current clinical practice since the incidence of diabetes mellitus is increasing nowadays. Unlike other substrates of CYP2C19, gliclazide has to be given daily for long period since diabetes is a chronic metabolic disorder. It is relatively free of side effects in normal subjects but may lead to serious consequences in subjects with CYP2C19 polymorphism.[3]

Genetic polymorphism of CYP2C19 was examined in three South-east Asian populations. The study was conducted in 772 Thai, 127 Burmese and 131 Karen. Genotype analysis revealed that allelic frequency of CYP2C19*1, *2 and *3 in Thai were 0.68, 0.29 and 0.03, respectively and in Burmese were 0.66, 0.30 and 0.04, respectively. And Karen were 0.71, 0.28 and 0.01, respectively. The prevalence of PMs estimated from data among these three ethnic populations were 9.2%, 11% and 8.4%, respectively.[4]

CYP2C19 polymorphism and pharmacokinetics of gliclazide modified release (MR)

Zhang and co-workers investigated the influence of CYP2C9 and CYP2C19 genetic polymorphism on the pharmacokinetics of gliclazide MR in healthy Chinese subjects with both single and multiple dose studies. In the single dose study, $AUC_{0-\infty}$ of gliclazide was significantly increased by 3.4 fold ($p<0.01$) in CYP2C19 PM subjects compared to CYP2C19*1 homozygotes (normal/wild type). The half-life (T½el) was prolonged from 15.1 to 44.5 hrs ($p<0.01$). Similar differences were found in the multiple dose study. AUC and C_{max} of gliclazide were 4.5 fold and 2.9 fold increased ($p<0.01$) in CYP2C19 PM subjects, respectively, compared with CYP2C19*1 homozygotes, and T½ was also increased from 13.5 to 24.6 hrs ($p<0.01$).[5]

When extensive metabolisers (EMs) of CYP2C19 were compared with PMs of CYP2C19, AUC and C_{max} were significantly higher ($p=0.000$) and oral clearance was significantly lower ($p=0.000$) in CYP2C19 PMs. The half-life of gliclazide in CYP2C19 PMs was also prolonged but the difference was not significant ($p=0.052$).

In that study, $AUC_{0-\infty}$ ratio between CYP2C19 PMs and CYP2C219 EMs is 5-12, suggesting that CYP2C19 PMs have a considerably increased exposure to gliclazide in Chinese Han subjects.[6] The main objective of this study was to find out the frequency of CYCP2C19*2 polymorphism in Myanmar and to study its effect on pharmacokinetics of gliclazide MR.

MATERIALS AND METHODS

This study was a cross-sectional, analytical study. The method for phase I study (genotyping of CYP2C19 polymorphism) was developed and established in Renal Immunology Laboratory, Institute of Medical and Veterinary Sciences (IMVS), Royal Adelaide Hospital, Adelaide, Australia and actual genotyping of Myanmar volunteers was carried out in the Pathology Department, National Health Laboratory (Yangon), Myanmar. Phase II study i.e., pharmacokinetic assay was carried out in the Common Laboratory of University of Medicine 1 (Yangon).

One hundred and fifty healthy Myanmar volunteers, ages between 25 to 50 years, of either sex and different ethnicities who gave consent to participate in this study were chosen for the phase I study.

*Phase I: Identification of CYP2C19*2 polymorphism*

After obtaining informed consent, 10 ml of venous blood was taken from each subject for genotyping. DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Mini kit. The extracted DNA were dissolved in sterile distilled water and stored at -20ºC until PCR analysis. PCR amplification of CYP2C19 was done in an Eppendorf PCR system (Mastercycler Gradient) using the specific forward and reverse primer 5-'ATTACAACCAGAGCT TGGC-3' and 5'TATCACTTTCCATA AAA GCAAG-3'. The amplification conditions were as followed: initial denaturation at 95ºC for 2 minutes, then, 40 cycles of denaturation at 95ºC for 30 seconds, annealing at 54ºC for 30 seconds, extension at 72ºC for 30 seconds.

Final extension at 72ºC for 5 minutes was performed.[7] PCR products were then digested with restriction enzyme Sma1 at 25ºC overnight.[8] Then, digested PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide. In the CYP2C19*1 (wild-type) allele, the restriction enzymes Sma1 spliced the 169 bp DNA fragments into 120 bp and 49 bp fragments.

Therefore, only two fragments (120 bp and 49 bp) were seen after the digestion.[8] In the case of heterozygous polymorphism of CYP2C19*2 (*1/*2) carrying one normal allele and one mutant allele, there were altogether three fragments (120 bp, 49 bp and 169 bp) after enzyme digestion as a result of incomplete or partial digestion. But, in those with homozygous polymerphism of CYP2C19*2 (*2/*2), carrying two mutant alleles, since there was no restriction site in both alleles, the DNA fragments were totally undigested and only one undigested fragment (169 bp) was seen.[8]

Phase II: Pharmacokinetic analysis of gliclazide MR

Among the subjects from phase I, up to 15 each of normal/wild type genotypes (*1/*1) and heterozygous genotypes (*1/*2) and all 10 of homozygous genotypes (*2/*2) who met the inclusion criteria were included in the phase II (kinetic) study. All eligible subjects underwent routine history taking, physical examination and thorough clinical examination. No medication was used for at least 2 weeks before phase II study and alcohol was forbidden within 72 hours prior to drug administration.

All the volunteers were asked to undergo fasting since 10:00 pm onward the night before. On the day of the experiment, before drug administration, a control/blank venous blood sample was collected from each volunteer using sterile 5 ml syringe. Then, each subject received one tablet of Reclide containing 80 mg of gliclazide with 250 ml of drinking water in a fasted state in the morning. The subjects could have their breakfast 15 minutes after the drug administration.

Sample collection and storage

Five milliliters of serial blood samples were taken at 1, 2, 3, 4, 5, 6, 8, 10 and 24 hours after administration of gliclazide using disposable syringes and sera were separated within 3 hours of venepuncture. Serum samples were stored frozen at -20°C until time of assay. Plasma gliclazide concentrationsin the collected samples were analyzed by validated HPLC method.[9] The separation was performed on an analytical HPLC C_{18} (5 μm particle size) column using glipizide as internal standard.

The wave-length was set at 229 nm. The mobile phase was a mixture of acetonitrile and water (49:51 v/v) titrated at pH 2.6 by using phosphate buffer, and the mobile phase was adjusted with a flow rate of 1 ml min^{-1}. The average retention time of gliclazide and glipizide were 7.9 minutes and 4.4 minutes, respectively.

Data analysis

The allelic frequencies were calculated and the results of this study were compared with published results of the previous studies in

Asians as well as Westerners. Confidence intervals were set at 95% for all observed allelic frequencies. The correlation between genotypes and pharmacokinetic parameters was analyzed by method of analysis of variance (ANOVA) followed by post-hoc analysis using Dunnett 2 sided Test. 'p' Value of less than 0.05 was taken as the minimum level of statistical significance.

Ethical Consideration

This study was approved by Ethical Committee of the Academic Board of Post-graduate Studies, University of Medicine 1 (Yangon).

RESULTS

Phase I: Identification of CYP2C19 polymorphism

Among 150 healthy Myanmar volunteers, 80(53.33%) were homozygous for CYP2-C19*1 (normal/wild type) (*1/*1) and 60(40%) were heterozygous for CYP2-C19*2(*1/*2) and only 10 subjects (6.67%) were homozygous for CYP2C19*2 geno-type (*2/*2) (Fig. 1).

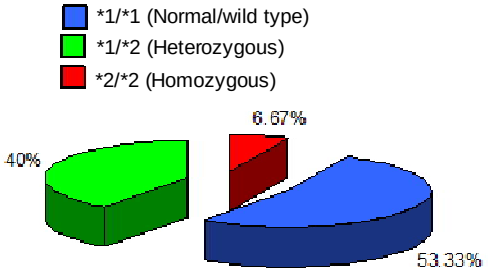


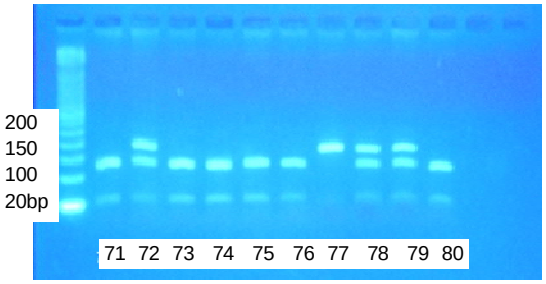
Fig. 1. CYP2C19 polymorphism among healthy Myanmar volunteers

Phase II: Pharmacokinetic analysis of gliclazide MR

As seen in Table 1, $AUC_{(0-24)}$, $AUC_{(0-\infty)}$ and C_{max} of those with polymorphic alleles were significantly higher than those with normal/wild type genotypes. T_{max} was also significantly prolonged in those with poly-morphic alleles. When plasma gliclazide concentrations at specified times among three different genotypes were compared, plasma gliclazide concentrations obtained in those with normal (*1/*1) genotypes were significantly lower than those of hetero-zygous and homozygous genotypes starting from 4th hour after drug administration upto the time of last collection i.e., 24th hr.

Table 1. Comparison of pharmacokinetic parameters among three different genotypes

Parameters (Mean±SD)	Genotypes			ANOVA (p value)		
	*1/*1	*1/*2	*2/*2	*1/*1 *vs.* *1/*2	*1/*1 *vs.* *2/*2	*1/*2 *vs.* *2/*2
C_{max}(µg/dL)	4.27 ±0.68	5.32 ±0.88	6.79 ±1.51	0.014	0.000	0.002
T_{max}(hr)	2.93 ±0.26	4.47 ±0.64	5 ±0.82	0.000	0.000	0.056
$AUC_{(0-24)}$	44.34 ±9.09	65.12 ±16.37	87.34 ±20.20	0.000	0.000	0.002
$AUC_{(0-\alpha)}$	53.99 ±10.88	91.21 ±22.68	125.51 ±2.92	0.000	0.000	0.001
$T_{1/2\ ab}$(hr)	0.82 ±0.27	0.94 ±0.28	0.89 ±0.26	0.352	0.687	0.883
$K_{ab}(hr^{-1})$	0.94 ±0.32	0.81 ±0.28	0.85 ±0.32	0.407	0.690	0.925
$T_{1/2el}$(hr)	8.71 ±0.59	11.77 ±1.07	12.19 ±1.08	0.000	0.000	0.404
$K_{el}(hr^{-1})$	0.079 ±0.01	0.059 ±0.01	0.057 ±0.01	0.000	0.000	0.494
$CL/F(Lhr^{-1}kg^{-1})$	0.025 ±0.01	0.015 ±0.00	0.010 ±0.00	0.000	0.000	0.020
Vd/F(L/Kg)	0.31 ±0.06	0.25 ±0.04	0.19 ±0.04	0.004	0.000	0.005



77 = Homozygous (2*/2*)
72, 78, 79 = Heterozygous (1*/2*)
71, 73, 74, 75, 76, 80 = Normal genotype (1*/1*)

Fig. 2. Electrophoretic patterns of CYP2C19 polymorphism evaluated by PCR-RFLP based assay

Pharmacokinetic parameters of gliclazide MR among three different genotypes

In this study, the peak plasma concentration C_{max} of normal (*1/*1) genotypes was

significantly lower than those of heterozygous (*1/*2) and homozygous (*2/*2) (p=0.014 and 0.000, respectively). T_{max} of those with normal genotypes was significantly shorter than those with heterozygous and homozygous genotypes (p=0.000). However, there was no statistically significant difference between T_{max} of heterozygous and homozygous. $AUC_{(0-\infty)}$ of gliclazide obtained in those with normal genotypes were significantly lower than those of heterozygous and homozygous (p=0.000). $AUC_{(0-\infty)}$ of homozygous was also significantly larger than that of heterozygous (p=0.001) (Table 1 & Fig. 4).

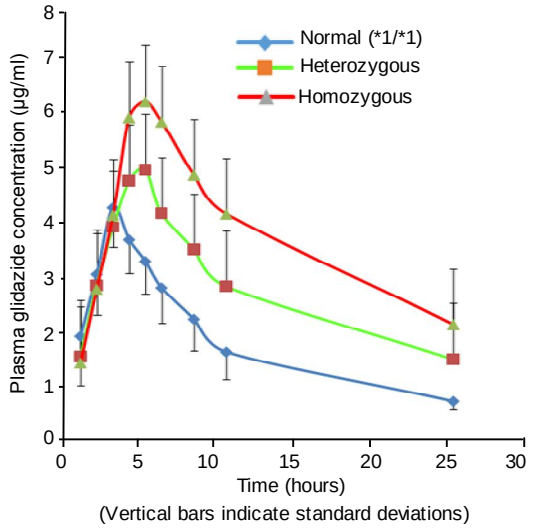


Fig. 3. Mean plasma gliclazide concentration versus time graph after single oral administration of 1 tablet of Reclide (gliclazide MR) 80 mg

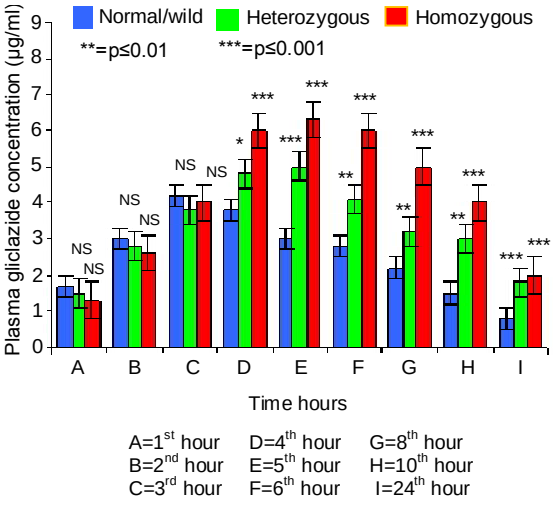


Fig. 4. Comparison of plasma gliclazide concentrations (µg/ml) of normal/wild type genotypes at specified times with heterozygous and homozygous genotypes

There was no statistically significant difference in absorption half-life ($T_{1/2ab}$) and absorption rate constant (K_{ab}) of gliclazide among three different genotypes. However, elimination rate constant (K_{el}) and elimination half-life ($T_{1/2el}$) of gliclazide in normal genotypes were significantly lower and shorter than those with heterozygous and homozygous genotypes with p values of 0.000. But, $T_{1/2el}$ of gliclazide in those with heterozygous and homozygous genotypes were not statistically different (p=0.404). The clearance/F ($L.hr^{-1}.kg^{-1}$) of gliclazide in those with normal genotypes was significantly higher than those with heterozygous and homozygous genotypes (p=0.000) (Table 1 & Fig. 4).

DISCUSSION

*CYP2C19*2 polymorphism in Myanmar*

The findings of this study were in agreement with one study.[4] In this study, the frequency of CYP2C19 *1/*1, *1/*2 and *1/*3 in Myanmar populations were 44.1%, 39.4% and 9.4% and those of Thai were 44.5%, 42.6% and 6.7%, respectively. However, the frequency of CYP2C19 *1/*1 in the present study was higher than these findings.

It may be due to the fact that, in the present study, those who were neither *1/*2 nor *2/*2 were regarded as *1/*1 (normal) genotypes. But in fact, there are other polymorphisms apart from*1/*2 and *2/*2 (*3 to *17). So, the discrepancy of *1/*1 between the two studies might be due to these other polymorphisms of CYP2C19. It is especially noteworthy for Myanmar since 40% of Myanmar populations are heterozygous and 6.7% are homozygous for CYP2C19*2 polymorphism which can significantly lower the activity of the enzyme. Therefore, it is clinically important

to take into consideration of CP2C19*2 polymorphism before administration of its substrate drugs.

Phase II: Pharmacokinetic analysis of gliclazide MR

Plasma drug concentration at any time is a factor of absorption and elimination and is directly proportional to absorption and inversely to elimination. It is also directly proportional to the dose of the drug administered. If the dose is the same, as in this study, differences in the C_{max} might be due to the differences in the rate of absorption or elimination between the subjects.[11] In the drugs which follow first order kinetics, rate of absorption decreases with time until it becomes equal to the rate of elimination (i.e., T_{max}) which gradually increases with time.[11]

But, if the elimination rate is slowed, as with polymorphic alleles, then it takes longer for absorption to become equal to elimination and hence T_{max} is delayed. Therefore, there will be more time for absorption leading to higher C_{max} and larger AUC. The observed differences in plasma drug concentrations, C_{max}, T_{max} and AUC between polymorphic and normal/wild type genotypes could be either due to absorption or elimination since amount of drug in the body is a function of these two parameters. But, according to the comparison of the results, there were no significant differences in all the absorption parameters (K_{ab}, $T_{1/2ab}$) among three different genotypes. Therefore, it could be concluded that CYP2C19*2 polymorphism did not influence the absorption kinetics of gliclazide.

Therefore, the observed differences should only be due to the differences in elimination. At the same time, highly significant differences were noted in all the elimination parameters ($T_{1/2el}$, K_{el}, CL) among three different genotypes suggesting that CYP2C19*2 polymorphism influences the elimination of gliclazide. Again, elimination is mainly influenced by age, liver and renal function. Gliclazide is extensively metabolized into inactive metabolites which were excreted from the kidney and less than 1% of administered gliclazide is excreted unchanged.[10] In this study, all the subjects had to undergo renal and liver function test before the study and those with abnormal renal or liver function were excluded.

Therefore, the observed differences in the elimination parameters could not be due to differences in renal function. Previously, CYP2C9 was assumed to be responsible for the metabolism of gliclazide. A study[5] incidentally found pharmacokinetic profile of gliclazide modified release (MR) was in good relation with CYP2C19, not with CYP2C9.[5] Those findings were further supported by another once study[6] which also found that CYP2C9 was not associated with any change in the disposition of gliclazide but CYP2C19 polymorphism appeared to exert the dominant influence on the pharmacokinetics of gliclazide in healthy Chinese Han subjects, and might also affect the observed pharmacodynamics of the drug as a result.[6]

Therefore, differences in the pharmacokinetic parameters of gliclazide in those with polymorphism in this study should be due to the differences in the activity of CYP2C19. Although plasma gliclazide concentration in normal genotypes fell below the threshold concentration for hypoglycemic activity (i.e., 1.5 μg/ml)[10] after 10^{th} hr, that of heterozygous and homozygous genotypes were still above the threshold concentration even after 24^{th} hr. This could be dangerous since gliclazide is usually administered as twice daily regimen, especially in homozygous genotypes whose plasma gliclazide levels were much high above the threshold concentration even at 24^{th} hr. Furthermore, poor metabolisers also had longer $T_{1/2el}$ and would take longer for their plasma gliclazide concentration to fall below the threshold, making it even more dangerous. According to this study, about one third of Myanmar population has at least one polymorphic allele, rendering one in three chance of administering gliclazide to poor metabolisers.

Conclusion

In the present study, incidence of CYP2C19*2 polymorphism in healthy Myanmar population was found to be higher than expected, accounting for a significant portion of Myanmar population. This study also indicated that CYP2C19 played an important role in the metabolism of gliclazide MR providing a plausible explanation for interindividual variations of the pharmacokinetics of gliclazide MR.

This was clinically important since there was significant accumulation of gliclazide MR in poor metabolisers which could lead to serious adverse effects with usual twice daily regimen. Pharmacokinetic changes of gliclazide MR due to CYP2C19*2 polymorphism in this study can also be applied to other substrate drugs of CYP2C19. The findings of this study highlight the clinical significance of CYP2C19*2 polymorphism regarding administration of its substrates drugs.

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