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Research Article

STEADY-STATE PHARMACOKINETICS OF METFORMIN IN OBESE PATIENTS WITH TYPE 2 DIABETES MELLITUS: A PRELIMINARY STUDY

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ABSTRACT

Objective: This study aimed to determine the metformin plasma steady-state concentration (PSSC) either trough and peak level in Type 2 diabetes mellitus patients with obesity and the impact of *SLC22A1* gene organic cation transporter 1 (OCT) rs628031 A>G on PSSC of metformin.

Methods: Validated reversed-phase high-performance liquid chromatography method with ultraviolet detector was used to determine the metformin PSSC, as well as genotype variation was performed using the restriction fragment length polymorphisms-polymerase chain reaction method.

Results: A total of 13 patients were recruited from five Primary Health Centers in Yogyakarta Province of Indonesia. The results showed that the means of their trough and peak PSSC were 0.285±0.192 and 1.175±0.814 μg/ml, respectively. Only 10 patients (77%) had peak PSSC within the plasma therapeutic level (PTL) of metformin, and 14-fold variability was observed for the peak PSSC. None of the patients achieved the PTL of metformin with regard to their trough PSSC. The PSSC of metformin was independent of the OCT1 genotype in rs628031 (A>G) 408M/V *SLC22A1*.

Conclusion: This study found a huge variability in the trough concentration of metformin (>100-fold) and 14-fold for the peak PSSC, and no impact of a variant of rs628013 SLC22A1 OCT1 on metformin PSSC was revealed.

Keywords: Metformin, Steady-state pharmacokinetics, Obesity, Type 2 diabetes mellitus.

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INTRODUCTION

Metformin is the chosen oral antidiabetics to treat Type 2 diabetes mellitus (T2DM) patients with obesity [1]. In addition to its ability in reducing hemoglobin A1c (HbA1c) level up to 1-2% and in improving insulin sensitivity, metformin has been known to be able to reduce body weight slightly. Those positive effects, along with lower incidence of hypoglycemia, have placed metformin in the first line of T2DM treatment, particularly, in patients with obesity [2,3]. However, the current dosing of metformin mostly refers to the drug-dosing data resulted from clinical studies on average-weight patients, and hence it is less clear to obtain metformin dosing-guidelines for the obese [4]. In addition, the results of Garber *et al*. demonstrated that the effectiveness of metformin as antidiabetic was correlated with its doses [5].

Metformin is available in the plasma largely as hydrophilic cation making it an unmetabolized drug with low plasma protein-binding so that its clearance is mostly affected only by cardiac output and renal function [6,7]. Therefore, the pharmacokinetic parameters of metformin could be changed in obese individuals, especially for the renal clearance because of increased glomerular filtration and tubular secretion [7,8]. Despite its wide therapeutic range, which is 0.75-5.00 μg/ml [9,10], it is suggested that the pharmacokinetic variability in an individualizing dosage of metformin should also be considered to gain optimal effectiveness without lactic acidosis [7]. In a clinical setting, the usual pharmacokinetic parameter to adjust dosage regimen in multiple drug administration is the drug steady-state level.

Several studies of plasma steady-state concentration (PSSC) of metformin have been conducted. A study of metformin trough PSSC in patients with unknown body mass index (BMI) was conducted on varying renal functions and doses [11]. Meanwhile, two studies investigated the low function of transporters, which is responsible for influx-efflux metformin in hepatocyte, and its influence on trough concentration of metformin [12,13]. However, studies on the peak steady-state level of metformin that could be used to estimate the maximum doses of metformin and its variability that could reflect the reduced-function of transporters mainly in the distribution process of metformin have never been conducted.

One of the transporters mediating the intestinal uptake of metformin [14] as well as the hepatic uptake of metformin across the sinusoidal membrane primarily is organic cation transporters 1 (OCT) [15]. Considerable works have been carried out with the OCT1 and its impact on both of pharmacokinetics as well as the response of metformin. Studies of genetic variants in specific population may illuminate important polymorphisms on metformin therapy [15]. Up to the present, investigation of OCT1 polymorphisms in Asian T2DM patients has been conducted in limited study. Results of Umamaheswaran *et al*. found a high frequency of the single nucleotide polymorphism (SNP) rs628031 OCT1 in Indian Population [16]. This variant could play an important role in pharmacokinetics and therapeutic response of metformin.

Alteration of pharmacokinetic parameters in obese patients, as well as reduced function of metformin transporters potentially, influence the obtained metformin PSSC, and further dose adjustment is required in case the intended PSCC and glycemic control are not reached. Considering the limited studies on PSSC of metformin in obese patients in Asian population as well as the existence of variant transporters of metformin that could affect both metformin pharmacokinetics and pharmacodynamics, this preliminary study aimed to determine the

PSSC as well as the impact of rs628031 408M/V *SLC22A1* OCT1 on metformin PSSC in Indonesian T2DM patients with obesity.

METHODS

T2DM patients with obesity, indicated by the BMI of ≥30 kg/m^2, were recruited from five Primary Health Centres during January-November 2015 in Yogyakarta province, Indonesia. Patients aged 35-60 years old, receiving a therapy of generic metformin at a dose of 500 mg every 12 hrs daily for at least 2 weeks as either a monotherapy or in combination with other antidiabetics, and willing to participate in the study were included. Patients with creatinine serum >1.00 mg/dL, history of chronic liver diseases, medication having potentially pharmacokinetic interaction with metformin such as cimetidine, furosemide, nifedipine [17], and systemic steroid treatment because of its glycemic uncontrolled effect [18] and patients not adhering to metformin therapy were excluded. Ethics approval for this research has been granted by the Faculty of Medicine – University of Gadjah Mada Ethics Committee. Written informed consent was obtained from all subjects.

Steady state of metformin measurement

To measure the trough PSSC of metformin, blood sample was taken immediately before administrating the next dose (pre-dose). Subsequently, the blood sample taken 3.5-4 hrs after metformin administration was used to measure the peak PSSC (post-dose). Determination of metformin plasma concentrations was carried out using validated reversed-phase high-performance liquid chromatography assay with a Sunfire® C-18 column, 4.6 × 150 mm × 5 µm from waters, and SM7 injector with ultraviolet (UV) detector at 233 nm of wavelength. The mobile phase used a mixture of 45% acetonitrile and 55% buffer phosphate (6 mM KH_2PO_4, 6 mM sodium lauryl sulfate). All of the parameters for bioanalytical method have fulfilled the Guidance for Bioanalytical Method Validation from FDA. The linearity of the standard curve was (r) 0.9999, and the accuracy value (% difference) was <15%. Meanwhile, the value of precision (CV) was <15%. The obtained selectivity value (CV) was <15%, while the CV of recovery ranged 1.22-1.89% (unpublished observations). The obtained PSSCs were used to calculate metformin elimination rate by following one-compartment pharmacokinetic model [19]. The equation for elimination rate is [20]:

$$K_{el} = \frac{\ln C_{max,ss} - \ln C_{trough,ss}}{\tau}$$

Genotyping

Genomic DNA was isolated from whole blood, and genotyping for (rs rs628031 A→G) variations was performed using the restriction fragment length polymorphism-polymerase chain reaction (PCR) method. For PCR amplification, the following primers were used: Forward 5'-TTTCTTCAGTCTCTGACTCATGCC-3' and reverse 5'-AAAAAACTTTGTAGACAAAGGTAGCACC-3'. A thermo Scientific® 5020 PCR was used for the amplification with PCR conditions as follows: Initial denaturation at 94°C for 5 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 63°C for 45 seconds, primer extension for 1 minutes at 72°C, and final extension at 72°C for 10 minutes. The PCR products were digested with *MscI*. The digestion of 397 bp amplicon of rs628031 GG genotype resulted in 397 bp fragments, and the AA genotype remained 210 and 187 bp, whereas the heterozygous genotype was 187, 210, 397 bp fragments. The accuracy and reliability of all the genotyping methods have been confirmed by direct DNA sequencing in the previous study [16].

Control glycemic assay

For patients receiving metformin as a single therapy for 6 weeks, the parameters of glycemic responses were also assessed by measuring fasting blood glucose (FBG) and glycated albumin (GA) level. The GA was determined using ELISA reader ADVIA®, and UV/VIS spectrophotometry was utilized for the FBG. The intended FBG was defined as <100 mg/dl while the GA was <16% [21].

Statistical analysis

The results were expressed as mean±standard deviation. The peak PSSC of metformin was not normally distributed, thus the Mann–Whitney U-test was used to assess the difference mean among the groups, and the Spearman test was for the numerical correlation. As the nature of the study was slightly explorative, no sample size calculation was performed, but the size of the study population was estimated from a previous pharmacokinetic study of antibiotics in obese patients [22] as well as genetic variations in specific patients [13].

RESULTS

Thirteen T2DM patients were included in this study, in which all patients were female, and the patients' characteristics are presented in Table 1. Regarding the BMI, most patients were within the category of moderate obesity with age under 50-year-old. Most of the patients had estimated glomerular filtration rate (GFR) ≥100 ml/min. The proportion of patients diagnosed as having T2DM for more or <5 years was quite similar. However, more patients used metformin twice daily as oral antidiabetics for more than 6 weeks before participating in this study, and so did patients with variant heterozygote Met/Val in *SLC22A1*.

In addition, metformin users had higher (1.98-fold) peak PSSC of metformin than patients with a combination therapy ($p<0.05$). It was not significantly different for the other characteristic groups in either trough or peak level of metformin.

Fig. 1 displays the achieved trough and peak PSSC of metformin for each patient participating in this study. The average sampling time for the determination of trough PSSC in this study was 12.18±0.24 hrs after previous metformin administration, and the obtained PSSC ranged between 0.003 and 0.586 µg/ml. Meanwhile, the average sampling time to determine the peak PSSC was 3.83±0.09 hrs after the next administration of metformin, and the peak PSSC ranged 0.240-3.359 µg/ml. From the obtained PSSC, an elimination rate of metformin was calculated accordingly, and it was found that the average elimination rate of metformin among the 13 patients was 0.15±0.11/hr.

With regard to the trough PSSC, it was found that no patients reached the metformin plasma therapeutic level (PTL), and only 10 patients (77%) whose peak PSSC reached metformin PTL were identified. However, the obtained PSSC in this study can be further used to estimate the adequate dose of metformin. By assuming that metformin followed the first order kinetics, the pharmacokinetic parameters of metformin such as half time, volume of distribution, and renal clearance were unchanged in patients. Hence, the recommended PTL of metformin with respect to the avoidance of lactic acidosis, which is PSSC >5 µg/mL, and the maximum

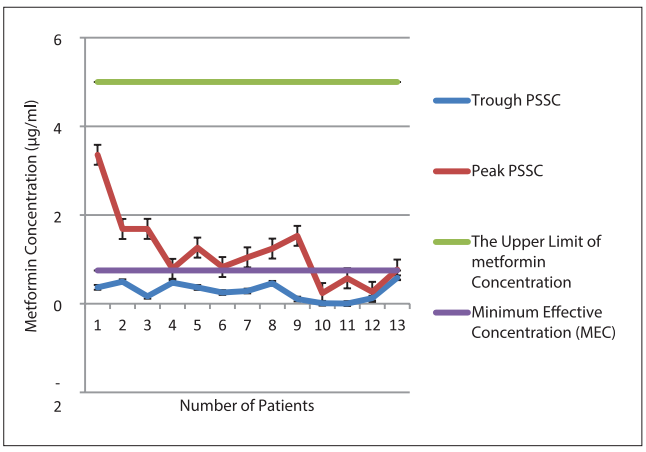

Fig. 1: Observed metformin plasma steady state concentration among 13 patients included in the study compared to the upper limit and minimum effective concentration of metformin

dose of metformin for T2DM patients with obesity can be calculated accordingly. Given that the average peak PSSC obtained in this study was 1.175±0.814 μg/mL, the estimated maximum dose of metformin for T2DM patients with obesity was 2513.83 mg ∞ 2500 mg daily. Unfortunately, the minimum dose could not be calculated because of the large variability (>100-fold) of the trough PSSC in this study [11]. As listed in Table 1, only 6 patients received metformin as a single therapy. For these 6 patients, an assessment of glycemic control was performed by using two parameters: The change of FBG and GA. It can be shown in Table 2 that no patient achieved the intended FBG, and only 2 patients had GA value within the defined range. In addition, due to the small number of cases in glycemic control assay, no formal analysis was conducted.

DISCUSSION

To conduct monitoring and evaluation of administered drug therapy is very important to ensure that the defined therapeutic responses have been achieved [23]. The most important aspect leading to the need of monitoring and evaluation for patients receiving metformin therapy is the variability of metformin pharmacokinetics and the risk of lactic acidosis in those patients with metformin plasma concentration >5 μg/mL even in patients with normal renal function [24].

Other than the specific characteristics of obesity, the limited number of patients receiving metformin as a single therapy was the substantial reason for getting limited research subjects. Although several approaches have been conducted to obtain male patients for this study, no male patients were willing to participate; as a result, all recruited patients were female. Therefore, this study cannot rule out significant gender differences in the metformin PSSC of obese patients.

Based on the obtained PSSC in this study, the elimination rate was estimated at 0.15±0.11/h, and it was higher than the elimination rate stated in literature, that is 0.112/h [9], probably indicating that metformin is excreted more effectively in obese individuals owing to increased GFR and tubular excretion than in patients with normal body weight.

One important finding of this study is that there were no patients reaching the PTL with regard to the trough PSSC. This means that the administered dose of metformin was inadequate to achieve the intended therapeutic level, which potentially leads to the failure of obtaining defined therapeutic responses [23]. Considering that the half-life of metformin is approximately 5 hrs in T2DM patients with normal renal function [25], administering conventional dosage form of metformin every 12 hrs (2.4-fold) resulted in trough PSSC [20] that is under the minimum effective concentration of metformin. Researches on pharmacokinetics and pharmacodynamics modeling exhibit a correlation between metformin plasma levels and glycemic responses [26]. In addition, doses were a dependent of the glycemic response of metformin [5].

Furthermore, this research found that only 10 patients (77%) had peak PSSC within the PTL while none of the patients had metformin levels >5 mg/mL indicating low risk of lactic acidosis. Although the plasma level of metformin can be used to estimate the occurrence of lactic acidosis, other parameters such as symptoms of nausea and fatigue as well as abnormalities in lactate levels (plasma lactate levels >4 mmol/L and/or pH <7.37) should also be confirmed [27]. PSCC <5 mg/mL was observed by Duong *et al*. in dosing sumulations for patients with ClCr 30 ml/min receiving metformin 1000 mg daily [10]. Commonly, the risk of lactic acidosis due to metformin administration increases when the

Table 1: Characteristics of patients

Characteristics	Number (%)	Trough PSSC±SD (μg/ml)	p	Peak PSSC±SD (μg/ml)	p
Age (years)					
<50	8 (61.5)	0.2935±0.1611	0.850	1.1291±0.9822	0.464
≥50	5 (38.5)	0.2714±0.2553		1.2496±0.5361	
BMI (kg/m^2)					
30-35	10 (76.9)	0.2739±0.1732	0.721	1.0912±0.4795	0.612
≥35	3 (23.1)	0.3220±0.2904		1.4563±1.6689	
eGFR (ml/min/1.73 m^2)					
70-100	4 (30.8)	0.2235±0.1841	0.466	1.2930±0.6879	0.280
≥100	9 (69.2)	0.3123±0.2001		1.1232±0.8978	
Duration of T2DM (years)					
<5	7 (53.8)	0.2520±0.1816	0.528	1.1566±1.0248	0.475
≥5	6 (46.2)	0.3235±0.2142		1.1975±0.5729	
Duration of metformin (weeks)[a]					
2-<6	6 (46.2)	0.2498±0.2035	0.565	1.3250±1.1191	0.886
≥6	7 (53.8)	0.3151±0.1929		1.0473±0.4893	
Metformin regimen					
Single	6 (46.2)	0.3543±0.1275	0.245	1.6020±0.9466	0.046*
In combination with other antidiabetics	7 (53.8)	0.2256±0.2270		0.8099±0.4899	
Genotype[b]					
GG	3 (25.0)	0.2433±0.2361	0.862	1.3693±0.9226	0.166
AG	9 (75.0)	0.2654±0.1707		0.7290±0.1366	

*p<0.05, [a]At dose of 500 mg every 12 hrs, [b]1 patient with wild-type AA. PSSC: Plasma steady-state concentration, T2DM: Type 2 diabetes mellitus, eGFR: Estimated glomerular filtration rate, BMI: Body mass index

Table 2: Glycemic control responses among 6 patients after receiving a single therapy of metformin 500 mg every 12 hrs

S.No.	Genotype	Baseline of FBG (mg/dL)	FBG after 6 weeks (mg/dL)	Baseline of GA (%)	GA after 6 weeks (%)
1	AG	108	105	15.96	15.45
2	AG	135	127	17.89	18.39
3	GG	152	131	16.59	15.53
4	AG	119	173	17.36	19.92
5	GG	175	268	24.37	22.1
6	AG	199	189	22.27	23.21

Spearman test: Between baseline FBG and GA (p=0.072, r=0.771); between FBG and GA after metformin administered for 6 weeks (p<0.05, r=0.886). FBG: Fasting blood glucose, GA: Glycated albumin

tissue suffers from hypoxia as in the case of myocardial infarction, acute left heart failure or septicemia as well as renal or liver failure [27,28].

Although this research was unable to estimate the metformin dose based on a pharmacokinetics-pharmacodynamics profile due to the limited number of patients, the findings can be considered for a maximum dose recommendation of metformin in T2DM patients with obesity. The maximum twice daily dosing of metformin in T2DM patients with obesity based on the observed peak PSSC in this research was 2500 mg daily, and this is almost similar to the recommended maximum dose of metformin for adult patients with average body weight stated in the literature (2550 mg/day) [18].

In addition, the unachieved PTL in regard to the trough PSSC of metformin in this research indicated that the administered daily dose of 1000 mg metformin was inadequate, and this was further proved by a decrease of FBG only in 4 patients and 3 patients for the GA after metformin administration for 6 weeks. In a study involving 45 obese patients with T2DM, a significant reduction of FBG was observed after treated with metformin 500 mg twice daily for 1 year [29]. Yasser *et al*. found 18.3% reduction of HbA1c in 10 patients with newly diagnosed of T2DM after receiving 1000 mg metformin [30]. The difference on the duration of metformin administration, the baseline of glycemic index, as well as involved patients with normal renal function could be the reasons of this present results. In addition, since GA reflects a shorter glycemic control resulted from shorter life span of albumin (17 days) compared to erythrocyte [31], make GA is an intermediate-term glycation index. A study including 18 patients with T2DM showed a GA reduction up to 8.1% after being administered with metformin and monitored every 4 weeks for 24 weeks [32]. Because of limited sample size, no statistical analysis for the glycemic control was performed in this study.

A higher peak PSSC of metformin instead of trough level in variant homozygote GG group was found in this study (p=0.166). OCT1 (gene SLC22A1) is expressed on the basolateral membrane and cytoplasm of the enterocytes and predominantly found in hepatocytes [14]. Since the variant homozygote have greater impact to protein function, thereby OCT1 reduced-function may contribute to a decrease in influx of metformin to hepatocytes and result in higher peak PSSC of metformin than in heterozygote variant group in this study.

Meanwhile, OCT1 is also expressed on the apical and subapical domain side of both the proximal and the distal tubules in the kidney [33], so it plays an important role in the renal clearance of metformin. In the present study, there was no impact on metformin PSSC of the frequent OCT1 SNP rs628031 (408M/V), which was contrary to the result of Shikata *et al*. that demonstrated lower expression of mRNA in OCT1 in the hepatocyte of human liver with 408M/V variant in *SLC22A1* [34]. Since OCT1 is the main transporter responsible in the influx process of metformin into hepatocyte, the existence of variance leads to a reduced function of the transporter. Consequently, metformin in a level higher than that in wild-type patients was observed. The smaller size of sample and number of observation could explain the insignificant difference in these findings.

This present study also revealed a quite large number of frequency of SNP rs628031 OCT1 in Indonesian T2DM patients, so further studies about the impact of this variant on gastrointestinal side effect and risk of hypoglycemia events as already reported in several studies [35,36] should be conducted for Indonesian population. As this research also found a variability of metformin peak PSSC, as well as a significantly higher level in metformin users, a pharmacogenetic and detailed pharmacokinetic study on monotherapy compared to combination therapy with sulfonylurea, are required to improve the therapeutic management of T2DM patients receiving metformin.

CONCLUSION

This study found a huge variability in the trough concentration of metformin (>100-fold) and 14-fold for the peak PSSC. The PSSC of metformin was independent of the OCT1 genotype in rs628031 408M/V *SLC22A1*. This study indicated that the maximum doses of metformin were relatively similar to what is listed in the literature; also, the prescription of metformin with twice daily dose of 500 mg was inadequate for T2DM patients with obesity. However, due to the small size of samples, further studies are needed to draw firm conclusions.

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