

Any Polymorphisms of CYP2C9 Affects the Biochemical Profile of Diabetic Patients Receiving Glibenclamide

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Abstract

Introduction: Glibenclamide, a hypoglycemic agent, is a member of the sulfonylurea family. It is metabolized by the hepatic P450 CYP2C9. Despite thorough pharmacokinetic characterization of glibenclamide, the evidence for a pharmacogenetic influence on the pharmacodynamics of this medication is scarce. The current study aims to evaluate a possible association between different CYP2C9 alleles and the pharmacodynamic effect of glibenclamide, including hypoglycemic events and required daily glibenclamide dosage.

Methods: The patients included were aged ≥ 18 years, diagnosed with type II diabetes mellitus, and treated with metformin and glibenclamide for at least 3 months. Exclusion criteria were treatment with other hypoglycemic agents or with medications that affect CYP2C9 function. Patients underwent a medical interview, including detailed anti-glycemic treatment dosage and duration. Blood was drawn for CYP2C9 allele genotyping, glycosylated hemoglobin A1C, chemistry and a complete blood count. Patients were re-interviewed 1 month after inclusion for hypoglycemic event incidence. The patient's genotype was classified as wild-type (WT) for *1/*1 alleles, or CYP2C9 polymorphism for any other alleles.

Results: Fifty eight patients were recruited. Characteristics were similar in the two groups. Forty patients had WT genotype (69.0%), twelve patients had *1/*2 genotype (20.7%), five (8.6%) had *1/*3 genotype and one (1.7%) had *2/*3 genotype. Patients with CYP2C9 polymorphism had similar A1C levels (7.5 ± 0.99 vs. 7.6 ± 1.3 , NS) but suffered more hypoglycemic episodes (≥ 2 episodes in 3 months; 5% vs. 22.2%, $p=0.04$) under similar glibenclamide dosage (6.5 ± 4.2 vs. 5.3 ± 3.9 , NS).

Conclusion: Our study suggests that diabetic patients with any CYP2C9 polymorphism have higher tendency to develop hypoglycemia when treated with glibenclamide, compared to those with wild type alleles. Further studies are needed to evaluate the clinical benefit from genotyping CYP2C9 before treatment initiation with glibenclamide or other drugs metabolized by CYP2C9 enzyme.

Study points

- Most of the sulfonylurea drug family members are metabolized by the hepatic P450 CYP2C9 enzyme to their inactive form.
- Polymorphism in CYP2C9 may encode a lower activity CYP2C9 enzyme, with different alleles encoding enzymes with varied activity degree.
- Our study shows that any polymorphism in CYP2C9 gene expose patients treated with glibenclamide to higher risk of hypoglycemia compared with the wild type alleles.

Keywords: Diabetes mellitus; Glibenclamide; CYP2C9 gene; Diabetic patients

Introduction

Diabetes mellitus (DM) is a growing global health burden that affects more than 150 million patients worldwide, with increasing prevalence [1,2]. Diabetes under treatment might lead to its complications, such as end stage kidney disease and retinopathy [3]. However, tight glycemic control might lead to hypoglycemia and to its consequences [4,5].

The effect of non-insulin glucose lowering drugs is characterized by interindividual differences in efficacy [6]. This variation is influenced by factors such as weight, physical activity, diet and genetic factors [7]. The genetic influence on interindividual drug response is studied by pharmacogenetics [8]. This is an emerging field in which genetic polymorphisms are studied in order to help clinicians to individualize drug therapy. The information gathered from pharmacogenetics might be used in the future for the establishment of personalized medicine.

Current guidelines for management of glycemic control in DM indicate metformin as first line therapy, with other medications as

additive second line agents, among them sulfonylurea [9]. Sulfonylurea is the most widely used hypoglycemic drug group [10,11]. Most of the sulfonylurea family members are metabolized by the hepatic P450 2C9 (CYP2C9) enzyme. CYP2C9 has more than thirty known alleles, marked as *1 to *35. CYP2C9*1 is the wild-type (WT) allele, CYP2C9*2 (Ile359Leu) and CYP2C9*3 (Arg144Cys) are single nucleotide

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polymorphisms (SNPs) and their prevalence among caucasians is 23% and 13%, respectively. Both alleles encode reduced activity enzymes compared to the WT allele. Other alleles are of small clinical importance [4].

Extensive research of the pharmacogenetic effect on sulfonylurea pharmacodynamics have shown reduced dosage requirements for glycemic control and increased hypoglycemic event incidence among non-WT allele carriers. However, these results were based only on sulfonylureas other than glibenclamide [12,13]. Despite thorough pharmacokinetic characterization of glibenclamide, the evidence for a pharmacogenetic influence on the pharmacodynamics of this medication is scarce [14].

The current study aimed to evaluate a possible association between CYP2C9 polymorphism and the response to glibenclamide. The effects of glibenclamide might be decreased in patients with ultrarapid CYP2C9 activity, while patients with slow CYP2C9 metabolism might have a higher incidence of hypoglycemia or decreased daily dosage requirements.

Patients and Methods

We conducted an observational prospective study, including patients admitted to Assaf Harofe Medical Center during the study period. The patients were interviewed, underwent physical examination and phlebotomy at inclusion, and then were followed prospectively for three months. After study was ended patients were compared according to their CYP2C9 genotype.

The patients included were ≥ 18 years, with type II DM, treated with metformin and glibenclamide for at least 3 months, with no other hypoglycemic drug. Patients on medications that might affect the CYP2C9 function, including chlorpromazine, valproic acid, sulfamethoxazole, metronidazole, antimycotic drugs were excluded from the study.

Study protocol

All patients were interviewed and questioned about past medical history, date of DM diagnosis, DM complications (ischemic heart disease, peripheral artery disease, cerebrovascular disease, retinopathy, nephropathy and neuropathy), medical treatment, and duration of treatment with glibenclamide and metformin and drug dosage. Physical examination was performed including vital signs, waist circumference, weight and height. Each patient underwent phlebotomy once, at study entrance. Blood samples were drawn for CYP2C9 allele genotyping, glycosylated hemoglobin A1c, fasting insulin and blood chemistry creatinine, urea and glucose and a complete blood count. Three months after inclusion, patients were interviewed for hypoglycemic events. Hypoglycemia was considered when glucose was <70 mg% on self measurement.

Beta cell function was assessed with the homeostatic model assessment for beta cell function (HOMA-B) using the equation $20 \times \text{fasting insulin } (\mu\text{U/ml}) / \text{fasting glucose } (\text{mmol/ml}) - 3.5$ [15]. Study protocol was approved by the institutional Helsinki Committee.

Laboratory methods

Peripheral blood was drawn and collected into Vacuette[®] Serum Tubes with gel for insulin testing and Vacuette[®] EDTA coated tubes for glycosylated hemoglobin testing and DNA extraction. Samples were kept at 4°C (glycosylated hemoglobin, molecular typing) or frozen at -20°C (insulin). Serum insulin concentrations were measured using a chemiluminescent immunoassay [16]. Glycosylated hemoglobin was measured in whole blood samples using the COBAS INTEGRA[®] 800 [17].

DNA was extracted from whole blood samples using the MagNA Pure Compact Instrument [18]. Genotyping for CYP2C9 alleles was conducted with the Light Mix[®] Kit human CYP2C9*2 and CYP2C9*3 and the Roche Diagnostics 'Light Cycler' Fast Start DNA Master HybProbe[®] in the Light Cycler[®] 1.x/2.0/480 instrument [19].

A 374bp fragment and 180bp fragment of the human CYP2C9 genome were amplified with specific primers. Human CYP2C9*2 was detected with a Simple Probe (detected in channel 530), human CYP2C9*3 with probes labeled with Light Cycler Red 640 (detected in channel 640). Primers and probes were provided by Light Mix kit human CYP2C9*2 and CYP2C9*3 of TIB MOLBIOL Light Mix[®] Kit (TibMolbiol, Berlin, Germany). The genotypes were identified by running a melting curve with specific melting points of 58.5°C for the WT and 55.5°C for the mutant for human CYP2C9*2 in channel 530 and 48.3°C for WT and 58.3°C for the mutant for human CYP2C9*3 in channel 640. Lab technicians were blinded to the patients.

Statistical analysis

Sample size of 21 patients is required for finding difference in daily glibenclamide dose of 6 mg, with standard deviation of 4 mg, estimating CYP2C9 WT prevalence of 58% in the Israeli population [20], with statistical power of 0.8 and p value <0.05 .

A comparison between patients with the WT CYP2C9 genotype and patients with CYP2C9 genotype polymorphisms was performed.

Continuous variables were described using mean \pm standard deviation unless mentioned otherwise (including age, disease duration, drug dosage, body mass index, waist circumference and plasma levels of hemoglobin, leukocytes, platelets, creatinin, low-density lipoprotein, high-density lipoprotein, total cholesterol, triglycerides and HOMA-B). Categorical variables were described using frequency distributions and are presented as frequency (including gender, ancestry and genotype). The t-test for independent samples was used to compare continuous variables between subjects with the WT genotype or polymorphism. The chi-square test was used to assess associations between genotype and other categorical variables.

Statistical analysis was conducted using SPSS software version 13 (SPSS Inc., Chicago, IL, USA). Study protocol was approved by Assaf Harofe Medical Centre Helsinki Committee, and all study participants signed written informed consent.

Results

A total of 58 patients were included, among them 34 women (58.6%). Mean age of the patients was 60.3 ± 10.0 years and mean disease duration was 8.7 ± 6.1 years. In terms of cardiovascular risk factors, the rates of hypertension, dyslipidemia and tobacco smoking among study participants were 55.2%, 74.1% and 22.4%, respectively, and the diabetes complication rates were 19.0%, 3.4% and 10.3%, for neuropathy, nephropathy and retinopathy, respectively. Patient characteristics compared according to genotype are depicted in Table 1.

Forty patients had WT genotype (69.0%), twelve patients had *1/*2 genotype (20.7%), five (8.6%) had *1/*3 genotype and one (1.7%) had *2/*3 genotype. Patients with CYP2C9 polymorphism had similar A1c levels (7.5 ± 0.99 vs. 7.6 ± 1.3 , NS) but suffered more hypoglycemic episodes during the 3 months follow up period, despite similar glibenclamide dosage (>2 hypoglycemic episodes, 5% vs. 22.2%, $p=0.04$; with glibenclamide dose 6.5 ± 4.2 vs. 5.3 ± 3.9 mg (NS) in the WT and CYP2C9 polymorphism groups, respectively). HOMA-B value was similar among the two groups (63.68 ± 76.88 vs. 44.58 ± 17.36 , $p=0.28$). Glycemic control parameters are detailed in Table 2.

	WT CYP2C9 (n=40)	CYP2C9 polymorphism (n=18)	p value
Age (years)	59.8 ± 10.9	60.6 ± 8.1	NS
Female gender n (%)	14 (35.0)	9 (50)	NS
DM duration (years)	9.1 ± 6.7	7.7 ± 4.9	NS
Cardiovascular risk factors			
Dyslipidemia n (%)	27(69.2)	15(83.3)	NS
Hypertension n (%)	20(51.3)	11(61.1)	NS
Tobacco smoking n (%)	8(20.5)	5(27.8)	NS
Diabetes complications			
Ischemic heart disease n (%)	11 (28.2)	1 (5.6)	0.05
Retinopathy n (%)	6 (15.4)	0 (0)	0.08
Nephropathy n (%)	1 (2.6)	1 (5.6)	NS
Neuropathy n (%)	8 (20.5)	3 (16.7)	NS
Physical examination parameters			
Weight (kg)	84 ± 16	82 ± 15	NS
Height (cm)	167 ± 9	166 ± 10	NS
BMI (kg/m ²)	29.5 ± 6.3	29.9 ± 2.4	NS
Waist circumference (cm)	105 ± 14	101 ± 7	NS
Systolic blood pressure (mmHg)	133 ± 19	139 ± 22	NS
Diastolic blood pressure (mmHg)	80 ± 11	80 ± 8	NS
Heart rate (/min)	76 ± 10	78 ± 8	NS

Table 1: Patient characteristics according to CYP2C9 genotype. Body mass index (BMI) - Calculated by dividing body weight (kg) by squared height (meters). WT: Wild-type; CYP2C9 polymorphism included *1/*2, *1/*3 and *2/*3 alleles.

	WT CYP2C9 (n=40)	CYP2C9 polymorphism (n=18)	p value
Glibenclamide			
Dosage (mg/d)	6.5 ± 4.2	5.3 ± 3.9	NS
Duration (years)	2.6 ± 4.3	2.4 ± 3.5	NS
Metformin			
Dosage (mg/d)	1870 ± 561	170 586	NS
Duration (years)	3.1 ± 3.2	2.7 ± 3.1	NS
A1c			
Mean (%)	7.5 ± 0.99	7.6 ± 1.3	NS
<6.5% n (%)	6 (15.8)	3 (15.7)	NS
<7% n (%)	12 (31.6)	7 (38.9)	NS
<8% n (%)	28 (73.7)	14 (77.8)	NS
Fasting plasma glucose (mg%)	140.9 ± 50.0	146.0 ± 31.9	NS
Hypoglycemia events			
Mean (n)	0.2 ± 0.6	0.6 ± 1.1	0.1
≥ 2 events n (%)	2 (5)	4 (22.2)	0.04

Table 2: Glycemic control and hypoglycemia data according to CYP2C9 genotype. WT: Wild-type; CYP2C9 polymorphism included *1/*2, *1/*3 and *2/*3 alleles.

Discussion

In this study we investigated the effect of CYP2C9 genotype variation on the glibenclamide dosage needed for glycemic control and on the prevalence of hypoglycemic events. Patients with CYP2C9 polymorphism, including patients with alleles *1/*2, *1/*3 and *2/*3, suffered more hypoglycemic episodes compared with those harboring WT alleles despite similar glibenclamide dosage used, and similar glycemic control achieved according to fasting plasma glucose and A1c levels.

The prevalence of *2 alleles (27.1%) and *3 alleles (13.5%) in our study is similar to that found in former studies, mainly in European and Eastern-Asia populations [17-19], and to the known prevalence of

these alleles in the Israeli population [19,20]. The prevalence found in Israel for the WT allele was 58.4%, compared with 26.3% and 18.4% for the *2 and *3 alleles, respectively [20].

Traditionally, the CYP2C9 genetic variants *2/*3 and *3/*3 are considered poor metabolizers compared to the WT genotype. This classification is based on an increased area under the glibenclamide plasma level curve as demonstrated in pharmacokinetic studies [17,21]. Nevertheless, pharmacokinetic changes such as elevated sulfonylurea half life have also been demonstrated in patients considered as intermediate metabolizers [22]. Furthermore, studies on other medications found decreased CYP2C9 activity in patients with the *1/*2 genotype [23,24]. Based on these metabolic activity differences we compared patients with the WT genotype with other genetic polymorphisms of CYP2C9.

In our study, patients with any polymorphism of CYP2C9 suffered from higher risk for hypoglycemia, with no effect of glibenclamide dose, and in relatively low doses. This tendency is clinically important, especially in the population with highest use rates of this drug, such as pregnant women with diabetes during pregnancy (either gestational or overt), as there is no other approved oral antiglycemic therapy [25]. Such clinical utility was already shown for patients using the anticoagulant warfarin, another substrate of CYP2C9 [20].

The limitations of this study include the small sample size. The heterogeneity of diabetic patients further limits conclusions based on the results of this study. Nevertheless, we did show higher prevalence of hypoglycemia among CYP2C9 Polymorphism carriers.

Our study proved that diabetic patients with any CYP2C9 polymorphism have higher tendency to develop hypoglycemia when treated with glibenclamide, compared to those with wild type alleles. Further studies are needed to evaluate the clinical benefit from genotyping CYP2C9 before treatment initiation with glibenclamide or other drugs metabolized by CYP2C9 enzyme.

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