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Effects of CYP3A5, CYP2C19, and CYP2B6 on the clinical efficacy and adverse outcomes of sibutramine therapy: A crucial role for the CYP2B6*6 allele

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ABSTRACT

Background: Various cytochrome P450 isoforms modulate sibutramine activity and influence sibutramine plasma levels and pharmacokinetics. However, there are no available data to demonstrate the association of these polymorphisms with the clinical outcomes of sibutramine administration.
Methods: This study was a sub-investigation of a 12-week, double-blind, placebo-controlled trial examining the additive effect of orlistat on sibutramine. The final analysis was restricted to 101 women who had fulfilled the protocol. We evaluated the effects of genetic polymorphisms of *CYP3A5*, *CYP2C19* and *CYP2B6* on the % weight loss and the occurrence of adverse events.
Results: The change of pulse rate from baseline value was affected by both *CYP2B6* and *CYP3A5* genetic polymorphisms ($P < .01$ for *CYP3A5* and $P = .01$ for *CYP2B6*). Both *CYP2B6* and *CYP3A5* showed gene–gene interactions ($P < .01$). After adjusting for significant variables in the backward stepwise regression model, the change of pulse rate and time-dependent weight reduction were significant only among the *CYP2B6* genotypes ($P = .027$ and $P < .01$, respectively).
Conclusion: The *CYP2B6*6* allele influences the extent of weight reduction and pulse rate changes in patients undergoing sibutramine treatment.

1. Introduction

Sibutramine induces weight loss by selectively inhibiting the neuronal reuptake of serotonin and noradrenalin within the hypothalamus [1]. The amount of weight lost in response to sibutramine treatment is greatly influenced by genetic, metabolic, nutritional and psychobehavioral factors [2]. Appropriate patient–drug matching could provide benefits for the individual in terms of increasing the chance of successful weight loss and minimizing the risk of discouraging failures in attempts to lose weight.

Sibutramine itself is a prodrug and is rapidly absorbed from the gastrointestinal tract following oral administration; it undergoes extensive first-pass metabolism in the liver to form active metabolites [3]. The M1 (mono-desmethylsibutramine) and M2 (di-desmethylsibutramine) metabolites of sibutramine are pharmacologically active with more potent reuptake inhibition than sibutramine [4], and plasma M1 and M2 concentrations appear to be linearly related to weight loss [5].

The manufacturer's information claims that cytochrome P450 CYP3A4 is involved in the metabolism of sibutramine, but recent microsomal studies have revealed that other CYP enzymes including CYP2B6, CYP3A5, and CYP2C19, are also involved [6]. The genes expressing these enzymes are highly polymorphic, and their polymorphisms modulate the blood levels of substrates of each enzyme and their pharmacokinetic characteristics [7]. Additionally, two pharmacokinetic (PK) studies were recently conducted to examine the relationship between these CYP polymorphisms and the PK parameters of sibutramine and its metabolites [8,9].

Assuming that weight loss is directly related to the levels of active metabolites and that pharmacogenetically polymorphic drug-metabolizing enzymes involved in the disposition of sibutramine affect the pharmacokinetics of sibutramine and its active metabolites [10], polymorphisms of these genes might influence the clinical effects and adverse reactions of sibutramine. However, there is no available data to confirm this hypothesis. Therefore, we investigated the effects of candidate polymorphisms related to sibutramine metabolism on clinical efficacy and adverse events of sibutramine treatment.

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2. Materials and methods

2.1. Participants

This study was a sub-investigation of a 12-week prospective, double-blind, placebo-controlled trial (NCT01184560) for the additive effect of orlistat on sibutramine treatment. Inclusion criteria were patients over 18 and under 50 years, patients naïve to both antiobesity drugs, and a body mass index (BMI) of ≥27 kg/m^2. Exclusion criteria were endocrine obesity, hypercortisonism, thyroid dysfunction, uncontrolled hypertension, known history of diabetes mellitus defined by past history and/or diabetic therapy, pregnancy and lactation. Subjects were enrolled under the condition of proof of using a safe and medically accepted contraceptive method. The analysis presented here was restricted to 101 women who fulfilled the protocol in terms of eligibility, interventions, and outcome assessment. The study was approved by the Institutional Review Board of Gachon University Gil Hospital, Incheon, Korea. All procedures were performed in accordance with the recommendations of the Declaration of Helsinki with all subjects giving their informed consent for participation and genotyping.

2.2. Study design and interventions

Eligible participants were assigned to the sibutramine alone group or the sibutramine-orlistat group in a 1:1 randomized, parallel group design: One group received sibutramine 10 mg/d p.o. and orlistat placebo 120 mg three times daily p.o., and the other group received sibutramine 10 mg/d p.o. and orlistat 120 mg three times daily p.o. Participants were followed up every 4 weeks for assessment of primary outcome and occurrence of adverse effects. Adverse effects were recorded via interview during each visit, and participants were encouraged to describe any unusual changes, complaints or symptoms. Participants who, at any point after baseline assessment, failed to attend the clinical appointments or suffered from serious adverse events were excluded. Serious adverse events were defined as those which required hospitalization, were life threatening or resulted in a persistent or significant disability or death.

To standardize for potential differences in the behavioral aspects of weight reduction therapy, all participants were given a standard behavioral weight management text, the "LEARN" Manual [11]. Participants met for 15 min with a master's or doctoral level physician at the start of the study and at weeks 4, 8, and 12. The physicians followed a structured study session outline and documented the content of their visits to ensure adherence to the behavior therapy protocol.

The main outcome of this study was the percentage of weight loss (% weight loss), not the absolute value, because previous reviews have indicated that a higher initial weight is strongly related to higher absolute losses during treatment [12].

2.3. Anthropometric measurements

Height and weight were measured using an automatic digital stadiometer (InBody BSM330, Biospace, Co., Seoul, Korea) while subjects wore a lightweight gown and stood bare foot. BMI was calculated as body weight in kilograms divided by height squared in meters. Blood pressure (BP) and pulse rate (PR) were measured using the automatic clinical blood pressure monitor (TM-2655, A&D Co., Tokyo, Japan) while the subjects were seated comfortably after a rest period of at least 10 min. The participants were instructed to refrain from drinking coffee, smoking cigarettes, or engaging in strenuous exercise for 30 min prior to the taking of the measurements.

2.4. Genotyping

For genetic analysis, a 2-ml EDTA blood sample was drawn from each subject and stored below −70 °C until DNA extraction. DNA was extracted using the 96 Genomics Blood Kit (NeclecGen, Korea), according to the manufacturer's protocol. PCR was performed in a volume of 50 μl containing Taq Polymerase (Genotech, Korea) and 5–100 ng of genomic DNA. The amplification protocol consisted of an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 45 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. All polymorphisms were confirmed by directed sequencing using the BigDye Terminator Cycle Sequencing Kit v3.1 and 3730XL DNA Analyzer (ABI, Applied Biosystems, USA) [13]. *CYP3A5*3* (6986A>G, rs776746), *CYP2B6*4* (18053A>G, rs2279343), *CYP2B6*6* (15631G>T and 18053A>G, rs3745274), *CYP2C19*2* (681G>A, rs4244285) and *CYP2C19*3* (636G>A, rs4986893) sequences were retrieved from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP). We also screened for *CYP3A4*18* (878T>C, rs28371759) and *CYP2C19*17* (−806C>T, rs12248560), but did not find any subjects with these alleles. The participants were divided into three groups according to their *CYP2C19* genotype: homozygous extensive metabolizers (EMs; *CYP2C19*1/*1*), intermediate metabolizers (IMs; *CYP2C19*1/*2* and *CYP2C19*1/*3*), and poor metabolizers (PMs; *CYP2C19*2/*2*, *CYP2C19*2/*3*, and *CYP2C19*3/*3*) [9]. Subjects were also grouped by allele pair of *CYP2B6* according to the number of **6* alleles in order to investigate the effect.

2.5. Statistical analysis

Data are expressed as number, mean ± standard deviation (SD) or regression coefficient (β). Genotype frequencies were evaluated for Hardy–Weinberg equilibrium using a χ^2 test. Frequency differences were assessed by the Fisher's exact test, and comparisons of continuous variables were carried out using one-way analysis of variance. Literature review shows that maximum weight loss by an antiobesity drug is achieved after 6–8 months [14,15], so the intervention duration in this study may be too short to demonstrate a significant difference. We therefore used a repeated analysis of variance (ANOVA) model [16] with adjustment for significant variables in a backward stepwise regression model to demonstrate a time-dependent influence of genotypes on weight reduction. The analysis of covariance (ANCOVA) model was used to estimate the differences of % weight loss or pulse rate changes between genotype groups, after adjusting for potential confounders that are significant in the backward stepwise regression model. The data were analyzed using the statistical program STATA SE 9 (STATA Corporation, Texas). Differences were considered statistically significant at $P < 0.05$.

Table 1
Genotype and allele frequencies of *CYP3A5*, *CYP2C19*, and *CYP2B6* genetic polymorphisms in 101 Korean women and comparison with previously published data.

Gene	Genotypes	Number	Frequency	Allele	Frequency	Korean[a] (%)	*P*
			Present (%)		Present (%)		
CYP3A5	**1/*1*	7	6.9	**1*	21.8	17.4	.70
	**1/*3*	30	29.7	**3*	78.2	82.6	
	**3/*3*	64	63.4				
CYP2C19	**1/*1*	40	39.6	**1*	62.9	56.5	.74
	**1/*2*	35	34.7	**2*	28.7	34.8	
	**1/*3*	12	11.9	**3*	8.4	8.7	
	**2/*2*	9	8.9				
	**2/*3*	5	5.0				
CYP2B6	**1/*1*	51	50.5	**1*	71.8	68.5	.37
	**1/*4*	16	15.8	**4*	9.4	9.3	
	**1/*6*	27	26.7	**6*	18.8	16.4	
	**4/*4*	1	1.0				
	**4/*6*	1	1.0				
	**6/*6*	5	5.0				

P values were calculated by the χ^2 test.
[a] Data obtained from Kim et al. [9].