Pheno- and Genotyping the Prescription of Drugs Metabolized by CYP2D6

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 8, pp. 184-185, August, 2002
Original article submitted June 20, 2002

Mutant alleles *CYP2D6*3* and *CYP2D6*4* were detected by PCR in 33 individuals. All examinees were given 50 mg methoprolol (orally). Plasma concentrations of the drug and its major metabolite was determined by high performance liquid chromatography and arterial pressure and heart rate were monitored. The rate of methoprolol metabolism was significantly lowered in subjects with *CYP2D6*4* mutation. This determined blood pressure decrease and bradycardia in these subjects after drug intake.

Key Words: *CYP2D6; metabolism; methoprolol*

Cytochrome P-450 subfamily CYPIID includes 2D6 isoenzyme. This isoenzyme metabolizes about 20% drugs, including neuroleptics, antidepressants, and β-adrenoblockers. Marker substrates for evaluation of CYP2D6 activity are debrisoquine, dextromethorphan, and spartein [2-4]. Of all the known isoenzymes, CYP2D6 is characterized by the highest gene polymorphism. The hypotensive effect of debrisoquine is more pronounced in subjects with slow metabolism of this drug (slow metabolizers); metabolism of some other drugs (phenacetine, amitryptiline, procainamide, and propaphenone) is also decelerated in these subjects, which modulates their side effects. For example, more pronounced β-adrenoblocking effect of antiarrhythmic drug propaphenone is observed in slow CYP2D6 metabolizers [1]. Genetic studies showed that slow CYP2D6 metabolizers carry mutant alleles of *CYP2D6* gene. These mutations determine the absence of CYP2D6 synthesis, or synthesis of a defective protein with reduced activity, or synthesis of inactive enzyme [6]. In Europe 5-10% population are slow metabolizers. In order to prevent side effects and intoxication, slow CYP2D6 metabolizers should be prescribed CYP2D6 substrate drugs in lower doses [5].

We investigated the relationship between *CYP2D6* gene polymorphism, plasma concentration of methoprolol, and its pharmacological effects.

MATERIALS AND METHODS

Mutant *CYP2D6*3* (2549A→deletion) and *CYP2D*4* (1846G-A) alleles were detected by PCR in 33 patients with arterial hypertension without gastrointestinal or hepatorenal diseases. This stage of the study was carried out in collaboration with the State Institute of Genetics (Moscow).

The examinees were given 50 mg methoprolol (orally) and plasma concentrations of the drug and its main metabolite were measured. Blood pressure (BP) and heart rate (HR) were also monitored.

Plasma concentrations of methoprolol and its metabolite were measured before and 1, 1.5, 2, 3, and 5 h after intake by high-performance liquid chromatography on a Shimadzu chromatograph equipped with a fluorometric detector at λ_{ex}=277 nm and λ_{em}=305 nm. The components were separated on an μ-Bondapak Phenyl column (3.9×300 mm, Waters) using $^{1}/_{15}$ M sodium dihydrophosphate as the mobile phase (pH was adjusted to 3.5 with o-phosphoric acid and aceto-

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0007-4888/02/1342-0159$27.00

nitrile at a 85:15 ratio. Methoprolol and its metabolite were isolated from the plasma by an ether:chloroform mixture (4:1) after addition of 1 M NaOH to the plasma.

The intensity of methoprolol metabolism was evaluated by the ratio of methoprolol/metabolite concentrations. The results were statistically processed using Systat 5 software. The significance of differences was assessed using Student's *t* test at 95% significance.

RESULTS

Twenty-one of 33 examinees carried none of studied mutations, *i. e.* were wild type (*CYP2D6*1/CYP2D6*1*) homozygotes, 9 were *CYP2D6*1/CYP2D6*4* heterozygotes, and 3 had complete replacement of G for A in the studied locus (*CYP2D6*4/CYP2D6*4*). No *CYP2D6*3* mutations were detected in the studied group. Anthropometric data (body length and weight, age) were similar in the groups of homo- and heterozygotic examinees.

All subjects with complete substitution of G for A (*CYP2D6*4/CYP2D6*4*) were slow metabolizers. The mean peak concentration of methoprolol in the plasma of these subjects was significantly higher than in other groups (Fig. 1), and the mean maximum concentration of methoprolol metabolite was significantly lower (Table 1). No significant differences in the peak concentrations of methoprolol and its metabolite in the *CYP2D6*1/CYP2D6*1* and *CYP2D6*1/CYP2D6*4* groups were detected.

Six-hour monitoring of BP and HR after methoprolol intake showed pronounced bradycardia (HR below 52 bpm) with a drop of BP below 110/70 mm Hg in all examinees with *CYP2D6*4/CYP2D6*4*, but not with *CYP2D6*1/CYP2D6*1* or *CYP2D6*1/CYP2D6*4*.

Hence, all examinees with *CYP2D6*4/CYP2D6*4* were slow methoprolol metabolizers, which manifested in more pronounced hypotensive effect of the drug with subsequent development of side reactions (bradycardia). Correction of the doses improved clinical efficiency of methoprolol and reduced the incidence of side effects.

This clinical case demonstrates the importance of *CYP2D6* gene phenotyping and genotyping for most prevalent mutation in order to choose the optimal dose of β-adrenoblocker methoprolol.

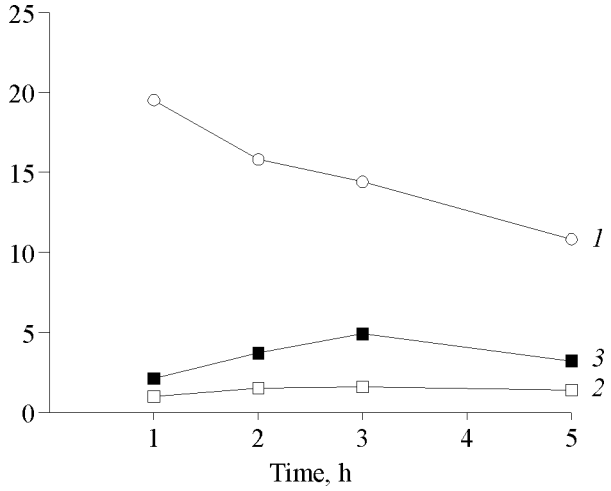

Fig. 1. Mean ratios of plasma methoprolol/metabolite concentration in *CYP2D6*4/CYP2D6*4* (*1*, *n*=3) and *CYP2D6*1/CYP2D6*1* (*2*, *n*=21) homozygotes and *CYP2D6*1/CYP2D6*4* (*3*, *n*=9) heterozygotes after administration of 50 mg methoprolol.

TABLE 1. Mean Maximum Concentrations of Methoprolol and Its Metabolite in the Plasma after Oral Intake of Methoprolol (50 mg)

Genotype	Maximum concentration, ng/ml	
	methoprolol	metabolite
*CYP2D6*1/CYP2D6*1* (*n*=21)	45.1	16.3
*CYP2D6*1/CYP2D6*4* (*n*=9)	52.1	14.5
*CYP2D6*4/CYP2D6*4* (*n*=3)	109.4	5.6

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