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Original Research Article

CYP2D6 Phenotyping with Dextromethorphan and Comparison with Risperidone Plasma Level

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Abstract: Risperidone (R) is a new generation atypical antipsychotic used for schizophrenia-treatment. Today, most effective method for CYP2D6 enzyme phenotyping is dextromethorphan metabolism. The aim of this study is to compare risperidone plasma level with dextromethorphan metabolite ratio in Turkish psychiatric patients receiving risperidone monotherapy and to investigate whether 9-hydroxyrisperidone ratio could be a phenotype marker for CYP2D6 enzyme. The study included 41 patients receiving R monotherapy in Üsküdar University NP Istanbul Hospital between March 2011 and February 2012. On the 7th day of risperidone,risperidone plasma sample and CYP2D6 enzyme phenotyping was worked. Phenotyping was performed in the urine according to dextromethorphan metabolite ratio. If that ratio is less than 0.003 ultra-rapid metabolizer, 0.003-0.3 and intermediate metabolizer/ normal metabolizer; more than 0.3 phenotyping was calculated as poor metabolizer. Plasma Risperidone / 9-OH risperidone ratio is ≥ 1: poor metabolizer (PM); less than 0.1 ulta rapid metabolizer (UM), values between 0.2-0.9 were evaluated as intermediate- or normal metabolizer(IM/NM). In 92.9% (n:39) of the cases, phenotype was consistent with risperidone blood levels. In 4.8% (n:2) CYP2D6 enzyme phenotype was not consistent with risperidone /9-hydroxyrisperidone. The strong relationship between dextromethorphan metabolite ratio in urine and risperidone hydroxylation. As monitoring of the plasma levels of risperidone is more practical, Therapeutic Drug Monitoring(TDM), as a phenotype marker in predicting CYP2D6 enzyme phenotype on patients using risperidone, can be used as an important data in monitoring the treatment.
Keywords: Risperidone, dextromethorphan, CYP2D6, Therapeutic Drug Monitoring, Phenotype marker.

INTRODUCTION

Risperidone (R) is a new generation atypical antipsychotic used for schizophrenia-treatment. It is metabolized in the liver by the enzyme CYP2D6 to the major metabolite, 9-hydroxyrisperidone (9-OHR) which has a similar pharmacological profile [1]. While it has a half-life of 3 hours, its metabolite 9-OHR has 23 hours[2]. Additionally, in R's 9-hydroxylation, CYP3A4 enzymes are effective at catalyzing[3,4]. 9-OHR is pharmacodynamically similar to R, and clinical effect of R equals to the sum of R and OHR plasma levels[5]. Therapeutic range is the sum of the activities of R and its metabolite 9-OHR and is informed as 20-60 ng/ml[2].

In a CYP2D6 genotyping study on Turkish psychiatric patients by Herken *et al*., poor metabolizer frequency has been reported as 1.45%, and ultra-rapid metabolizer 10.29 %[6]. The sum of the frequency of poor and rapid metabolizers in terms of CYP2D6 activity is about 12.7%. Hence, 12.7% of Turkish populationis at risk for R treatment. In poor metabolizers, side effects of drugs occur more, while the drug ineffectiveness arises in ultra-rapid metabolizers. In evaluation of CYP2D6 enzyme activity, genotyping, phenotyping by prop medication and TDM are methods that are used. Although genotyping for CYP2D6 enzyme is a very important data in terms of drug metabolism, it, alone, is not sufficient in terms of showing enzyme activity. Genotyping is recommended, if a substrate that has quite different metabolism is being used, if the drugs a have narrow therapeutic range (if it has the toxic effects due to genetic differences in metabolism), if the drug or metabolite has an unusual plasma concentration and if genetic factors thought to be responsible, if there is a chronic disorder that needs a lifelong treatment[7]. Additionally, as the enzyme activity can be also affected by environmental factors in addition to genotyping factors, the most effective method for

current enzyme activity of the individual is phenotyping.

In a phenotyping study performed with dextromethorphan metabolites ratio, Gaedigk et.al reported in 60% of cases polymorphism commonly seen in CYP2D6 and in 40% of the cases rare polymorphisms[7]. Hence, estimating phenotype through genotyping has been insufficient in explaining phenotype. Nowadays, phenotypic research indicating this enzyme's activity is increasing.

For the first time, in 1993, phenotypic investigation performed through deprisguin metabolic way has reported a similarity to R metabolism[1]. In a study investigating the relationship between CYP2D6 enzyme activity and R hydroxylation, deprisguinprob was used and it was reported that phenotyping performed according to deprisguin metabolite and R/9-OHR rates are related and R plasma level might reflect enzyme activity[8]. Today, most effective method for CYP2D6 enzyme phenotyping is dextromethorphan metabolism. According to this, 4 types of phenotyping namely intermediate metabolizer (IM), normal metabolizer (NM), ultra rapid metabolizer (UM), and poor metabolizer (PM) was determined. In 1999, Huang *et al*. has investigated the relationship between dextromethorphan (DEX) phenotyping and R plasma level in 6 cases for the first time, and 7-times decrease in the clearance of R in PM phenotype has been reported[9].

On the other hand, TDM will provide information on pharmacokinetics by showing the drug plasma concentration (10,11). A strong correlation between CYP2D6 enzyme activity and R hydroxylationhas been reported. According to this, risperidone / paliperidone ratio was adopted as CYP2D6 index. If risperidone/ paliperidone ratio is 01-02 normal range>1: PM or CYP2D6 inhibition has been reported[12,13]. In another study,in UM cases with risperidone/ paliperidone less than or equal to 0.1, genotyping was performed and the ratio for UM was evaluated ashighly sensitive but nonspecific[12].

AIMS

The aim of this study is to compare R plasma level with DEX metabolite ratio in Turkish psychiatric patients receiving R monotherapy, and to investigate whether R/9-OHR ratio could be a phenotype marker for CYP2D6 enzyme.

MATERIAL AND METHODS

Medical files of 84 patients in Uskudar University Health Practice and Research Centers between March 2011 and February 2012 and who received risperidone treatment and Phenotyping was performed using the metabolic ratio (MR) were examined in details retrospectively. In the research center hospital, drug plasma level monitoring on a weekly basis for the drug dose adjustment of all inpatients is a routine of clinical practice. Patients with hepatic or renal failure, patients who were receiving additional drugs that might affect CYP2D6 enzyme activity were excluded. The study was performed with 41 patients receiving R monotherapy. On the 7th day of R treatment, R plasma sample and CYP2D6 enzyme phenotyping was worked.

Blood sampling

With the object to measure plasma levels of R plasma level, 5cc venous blood samples were taken into EDTA Vacutainer tubes 12 hours after the last dose. The blood samples were processed immediately by centrifugation. Patient's samples were stored at – 70 °C immediately after centrifuging. Plasma samples were withdrawn from freezer maintained at −70 °C and allowed to thaw at room temperature for 30–45 min High Performance Liquid chromatography with Tandem Mass Spectrometry (HPLC/MS) analysis administered Blood samples are taken at trough concentration under laboratory blood sample drawing procedures.

Measurement of plasma drug level

In order to measure R and 9-OHR plasma levels, 5cc blood sample was taken from the patients on the 7th day, 12 hours after the last dose. R and 9- OHR levels of the blood samples were measured with Agilent 6410 triple quadrupole mass spectrometer [Agilent Tech., Switzerland]. R and 9OHn in the plasma samples were separated by gradient elution using C8 columnand volume determinations were performed with Quadrupole Mass Spectrometer (Agilent 6410 triple quadrupole mass spectrometer) by using electrospray positive ionization technique in multiple-reaction monitoring (MRM) mode. Blood samples are taken at trough concentration under laboratory blood sample drawing procedures. If Risperidone / 9-OH risperidoneratio is (R/9-OHR)≥ 1: poor metabolizer (PM); less than 0.1 ultra-rapid metabolizer (UM), values between 0.2-0.9 were evaluated as intermediate- or normal metabolizer (IM/NM) [14].

Phenotyping

CYP2D6 enzyme activity after a single oral dose of DEX is calculated by dextrophan (DOR)metabolite ratio[15,16]. Phenotyping was applied on the 7th day of R treatment and after 12-hour diet without caffeine. After a single oral dose of DEX 30 mg, 8-hour urine was collected. Phenotyping was performed in the urine according to [DEX] / [DOR] ratio. If that ratio is less than 0.003 UM, 0.003-0.3 and IM/NM; more than 0.3 phenotyping was calculated as PM[13,15,17,18].

Statistical Analysis

Data were given as arithmetic means with the corresponding standard deviation (SD).

RESULTS

Phenotyping of 41 cases using R was examined while monitoring their drug blood levels. The mean age of patients is 33.80. All patients were male. The average R dose is 3,76 mg. In the study, there was no significant relationship between CYP2D6 enzyme activities and disorder duration and mental disorder family-history of the sample group. Inphenotyping examination, 58.5% IM/NM, 29.5% UM, and 12.2% PM. According to R/9-OHR ratio, 53.1% IM/NM, 29.3% UM, and 17.1% PM. Table 1 summarizes CYP2D6 enzyme phenotyping results and distribution ratio of R/OHR.

Table 1: CYP phenotyping analysis results and R/OHR distribution

	CYP enzyme phenotyping results (n, %)	Phenotyping according to R/OHR ratio (n, %)	R/OHR ratio (Average±SD)	DEX/MET ratio
IM/NM	24,58.5	22,53.1	0,34±0,2	0.102±0.0
PM	5, 12.2	7, 17.1	3,73±4,7	0.978±1.1
UM	12,29.5	12, 29.3	0,06±0,07	0.001±0.0

In 9 2.9% (n:39) of the cases, phenotype was consistent with risperidone blood levels. In 4.8% (n:2), CYP2D6 enzyme phenotype was not consistent with R/9-OHR ratio.

CONCLUSION

In 92.9% (n:39) of the cases, phenotype was consistent with risperidone blood levels. This result supports the strong relationship between DEX metabolite ratio in urine and R hydroxylation. In literature, any other study investigating phenotyping based on R plasma levels and DEX metabolism on Turkish psychiatric patients was not found. Huang at. al investigated the relationship between DEX phenotyping and R plasma level in 6 cases. They reported 7 times decrease in the clearance of R in PM phenotype[9]. According to our findings, plasma R ratio increased by approximately 12 times when compared to IMand NMin PM Phenotype. The conflicting findings may be attributed to factors such as methodological pharmacokinetics and pharmacodynamics. Before phenotyping, it was considered that acceleration of this enzyme activity was affected byfood/environmental factors, or that CYP3A4 enzyme system might be inhibited by genetic or environmental factors. On the other hand, R and DEX are p glycoprotein (P-gp) substrates, and it was reported that R plasma level increased in some carrier enzyme polymorphisms[19,20] and DEX metabolism slowed down in weak carrier polymorphism[21]. In these two patients, polymorphism that might affect R plasma level in P-gp enzyme level was considered.

In 4.8% (n:2), CYP2D6 enzyme phenotyping results were not consistent with R/9-OHR ratio. In both of these cases, while R plasma level was consistent with PM, the case was defined as IM/NM in phenotyping. Mean age of these both cases are 29 years old. Average R dose is 1.5 mg/day. R+OHR values, respectively, are 4.06+1.75ng/ml and 33.67+9.27 ng/ml. Prolactin levels of these cases are 62ng/ml and 106.21ng/ml. Normal prolactin level is 5-35 ng/ml. CYP2D6/CYP3A4 enzyme system might be inhibited by genetic or environmental factors.

The strong relationship between R/9-OHR ratio and CYP2D6 enzyme activity[22].According to these study findings that in phenotype-examination of CYP2D6 enzyme, 58.5% was found IM/NM, % 29.5 UM, 12.2 % PM. In the CYP2D6 genotyping study performed by Herken *atal*. on Turkish psychiatric patients, poor metabolizer frequency was 1.45% and ultra-rapid metabolizer frequency 10.29%[6]. In our study, phenotypic frequency of the poor metabolizer 13%, and ultra-rapid metabolizer frequency was 29.4%. This result suggested that CYP2D6 phenotypic changes in enzyme activity needs to be more careful for R treatment. In the study performed by Herken *atal.*, phenotype was not examined [6]. In our patient group, there might also be fast or slow rare alleles in the gene encoding the CYP 2D6 enzyme and which have therefore not analyzed in routine tests. Limitation of our study might have been not performing genotype examination.

CYP2D6 enzyme has more than 70 alleles and it is difficult and costly to diagnose all of these alleles. Therefore, in routine tests, alleles that might play a key role are diagnosed[22]. Phenotypic PM ratio is consistent with genotype in homozygote carriers, while in heterozygote carriers; enzyme activity can be increased or decreased. Hence, predicting the correct phenotype through genotyping analysis can be sometimes difficult.

R/OHR ratio gives information about CYP 2D6 enzyme activity. Considering R/OHR ratio in Risperidone–CYP2D6 relationship as phenotype marker, pharmacotherapy would clinically be a more practical application. As defining phenotype reflects combined effects of genetic, environmental and endogenous factors on enzyme activity, it provides practically applicable information. As monitoring of the plasma levels of risperidone is more practical, TDM, as a phenotype marker in predicting CYP2D6 enzyme phenotype on patients using risperidone, can be used as an important data in monitoring the treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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