

PHARMACOGENETIC ASPECTS WHICH INFLUENCE THE PHARMACOKINETIC PROPERTIES OF ATYPICAL ANTIPSYCHOTICS – PRELIMINARY STUDY

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Abstract

Patients under medical treatment show significant differences in treatment response. Among the reasons of this variability, the genetic factors play an important role. Pharmacogenetics is a new science, at the crossroads between pharmacology and genetics, which studies how genes polymorphism influences inter-patient variability in drug response, in terms of efficacy and side effects profile. The existence of a large number of schizophrenic patients showing resistance to antipsychotic treatment requires the development of such methods that could be able to predict the individual responsiveness to antipsychotics treatment. The ability to predict treatment response based on gene variation aims to optimize drug therapy by prescribing the most effective drug in the right dose and with the lowest risk of side effects. This paper highlights the current knowledge about the clinical utility of determining the genetic factors that may affect the metabolism of atypical antipsychotics. The paper does not want to present an exhaustive list of all pharmacogenetic studies in the field, but is focused on the most studied examples of DNA sequence variations in genes that encode cytochrome P450 enzymes, in relation to treatment response. Pharmacokinetic studies were identified by means of combinations of the keywords in the Pub Med database.

Keywords: antipsychotics, pharmacogenetics, polymorphisms, cytochrome P450.

Introduction

Atypical antipsychotics are a class of drugs that have as main indications schizophrenia and bipolar disorder. Since clozapine, the prototype of this class, which was introduced in medical practice in 1989, various novel compounds have entered the market: risperidone, olanzapine, quetiapine, ziprasidone, aripiprazole, paliperidone, asenapine, iloperidone. Compared to classical antipsychotics, they have a number of benefits: reduced risk of extrapyramidal syndrome, superior efficacy for improving negative symptoms, treatment of refractory cases and little effect on prolactin secretion. These advantages are

considered to be due to the dual antagonism of the dopaminergic and serotonergic system. The atypical antipsychotics have of course their own side effects: weight gain, predominantly for clozapine and olanzapine, hyperlipidemia and hyperglycemia. Over time it was observed that for the same antipsychotic given in the same dosage there is a wide variation from patient to patient regarding length of the onset of action, intensity of pharmacological action and severity of side effects. Thus, the current research directions are oriented towards understanding the interindividual variability of treatment response in order to customize and optimize drug choice.

With the completion of the Human Genome Project, researchers became interested in the genetic differences between humans and identification of those genes that have an impact on health status.

The fact that patients respond differently to antipsychotic treatment is largely due to the different genetic imprinting existing between one another. Psychiatric pharmacogenetics aims to identify the genetic inheritance of a patient and how this influences drug treatment outcomes.

Most pharmacogenetic research conducted in the field, uses as strategy the candidate gene approach, that explores the association between an allelic variation of candidate gene and the characteristic of interest (such as treatment response). This requires knowledge of the pharmacology of antipsychotics in order to select those genes that encode proteins that the drug interacts with in the course of the pharmacokinetic process (cytochrome-P450 enzymes, plasma binding protein, transport protein, etc.) or of the pharmacodynamic process (receptors, enzymes, etc.). Subsequently, the molecular genetic techniques can establish the existence of genetic polymorphisms (variations) in these genes of interest which might be responsible for phenotypic differences. This means that a certain gene in the form of an allele determines a certain type of treatment response, while another allele of the same gene, may generate an altered response.

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The discovery of pharmacogenetic markers that influence antipsychotic pharmacodynamics, with strong predictive value for clinicians, is difficult because the mechanism of drugs action involves multiple proteins and second messengers, and each of them can be subject to genetic variability.

By now, the greatest progress made in antipsychotic therapy individualization based on molecular diagnosis, is represented by cytochrome P450 genotyping. Since most antipsychotics are metabolized by cytochrome P450 enzymes, the existence of genetic variation affecting the enzymatic activity will influence the plasma concentration of antipsychotics, and therefore the efficacy and tolerability of medication.

Material and method

Prior to inclusion in this study, informed consent was obtained from each subject. A special attention was given to protect the privacy of the subjects. This study respected the guidelines which regalements the utilization of patients data and the use of patients DNA samples in research use.

A lot of 50 patients were selected for molecular genetic investigations. Inclusion on the study lot was made based on several criteria. Were selected patients who undergo treatment with aripiprazol and risperidone.

An evaluation form was established and applied for all the patients who undergo treatment with the above mentioned drugs. It were collected dates about the age at which the individual first experienced a diagnosis or symptoms of schizophrenia. The severity of manifestations was established based on the PANSS score. Different grades were used in order to correlate the severity of each symptom (1 = absent, 2 = minimal, 3 = mild, 4 = moderate, 5 = moderate severe, 6 =severe, 7 = extreme). For each patient from the study lot, PANSS score before initiation of the treatment was available. In order to evaluate the therapeutic effect, PANSS assessments at 1, 3 and 6 month after initiation of anti-psychotic treatment was done.

The patients selected for study were divided in 2 sub-lots based on the PANSS score evolution. Those who presented clinical improvement of the manifestation, based on the PANSS score were included in sub-lot 1 which served as a reference lot. The second lot included patients that did not exhibit the expected evolution in clinical manifestations.

For each patient 2 mL of venous blood was collected using a heparinized blood collection tube. The DNA extraction was done by using the commercial DNA extraction kit (Qiagen Mini DNA Extraction kit). In this study, the CYP2D6 alleles, CYP2D6*3, CYP2D6*4, CYP2D6*5, CYP2D6*6 were selected for detection based on the frequencies of those polymorphism in Caucasian population.

For the CYP2D6 SNPs genotyping, we choose the TaqMan® Pre-Developed Assay for Allelic Discrimination Kit (Applied Biosystems). Amplification and analysis of patients' samples are run on a 7500 Real-time PCR sistem (Applied Biosystems).

The first stage of the study had the purpose of selecting the cohort of patients under treatment with risperidone and aripiprazole, followed by identification of sublots based on the clinical evolution after the therapy, selection of the polymorphisms that will be evaluated, DNA samples preparation as well as the optimization of the protocol for SNPs identification. The second part of the study includes SNPs evaluation and statistical analysis of the results. Statistical analysis has the purpose of evaluating the existence of a positive or negative correlation between the presence of a specific genotype and the PANSS score evolution after treatment institution.

Discutions

Cytochrome P450 iso-enzymes are found primarily in the endoplasmic reticulum of hepatocytes, but are also present in the gut and brain. They are involved in phase I reactions of the hepatic biotransformation of antipsychotics, and are responsible for the modification of functional groups by oxidation reactions that increase hydrophilicity of molecules, in order to eliminate them from the body. Each CYP enzyme is the product of a particular gene. The occurrence of genetic mutations in the gene, will produce different allelic variants of that gene. Allelic variants will encode CYP enzyme variants with different degrees of activity. When genetic mutation occurs with a frequency greater than 1% in the population is called a genetic polymorphism. The most polymorphic izoforms are CYP2D6, CYP3A4, CYP2C19 (1). Following the characterization of patients' genetic profile in relation to polymorphic forms of these enzymes, genotype-phenotype correlations can be established. For example, based on allelic variants of P450 CYP2D6 gene, four phenotypes have been identified:

- "ultrarapid metabolisers" are patients with allelic variants encoding highly functional enzymes
- "intermediate metabolizers" and "poor metabolisers" are patients with allelic variants coding for enzymes with deficient or dysfunctional activity
- "extensive metabolizers", are patients with wild-type allelic variants, with the highest frequency in the population, which have normal enzymatic activity.

Atypical antipsychotics have primary and secondary pathways of biotransformation. These should be well known in order to properly assess the clinical relevance of polymorphisms (Table 2) (3). For a poor metaboliser CYP2D6, prescribing an antipsychotic such as aripiprazole, iloperidone, paliperidone or risperidone, which are extensively metabolized by this particular isoenzyme, should be avoided. If there is no therapeutic alternative available, it is recommended to reduce their doses.

Until recently, the way in which the optimal dose of antipsychotic could be determined, with clinical efficacy and minimal risk of adverse effects, was by regular determination of drugs plasma levels, but this required repeated blood sampling. Conducting a therapy using pharmacogenetic testing has the advantage of anticipation of plasma levels, before antipsychotic administration, so that the initiation of therapy should be judiciously done. For

example, Hendset et al. recommends for CYP2D6 poor metabolisers patients, a 30-40% reduction of the maximum aripiprazole daily dose, so that they reach the same steady

state concentration of aripiprazole and dehydroaripiprazole (the active metabolite) as extensive metabolisers (4).

Genotype	Phenotype	Consequences at usual recommended dosage of active drugs	Frequency in the Caucasian population (%) (2).
Gene duplication in absence of inactive or low activity allele	ultrarapid metabolisers	- subtherapeutic plasma levels - therapeutic ineffectiveness	2
Two alleles (wild-type) with normal activity	extensive metabolizers	- therapeutic plasma levels - clinical efficacy	80
Two low-activity allele <i>or</i> carriers of one active allele and one inactive <i>or</i> one low-activity allele and one inactive	intermediate metabolizers	- increased plasma concentrations - adverse effects, toxicity - biotransformation in another unfavorable pathway - reduced prodrug activation	10
Two inactive alleles	poor metabolisers	- increased plasma concentrations - adverse effects, toxicity - biotransformation by another unfavorable pathway - lack of prodrug activation	8

Active alleles * 1, * 2, * 33, * 35

Low activity alleles * 9, * 10, * 17, * 29, * 36, * 41

Inactive alleles * 3 - * 8 * 11 - * 16, * 19 - * 21, * 38, * 40, * 42

Table 1. CYP2D6 genotype-phenotype relationship

Not always pharmacogenetic studies have led to recommendations for clinical practice, because many of them have failed to achieve significant results.

In 1995, Arraz et al. undertook one of first studies on correlation between the CYP2D6 genotype and response to antipsychotic treatment. The study included 130 Caucasian patients that had been undergoing treatment with clozapine. They observed that poor metabolisers and ultrarapid ones were equally distributed in the 2 groups of patients - responsive and non responsive to treatment -, failing to establish a correlation between CYP2D6 genotype and drug response (5). This may however be due to the fact that CYP2D6 is a minor metabolic pathway for clozapine, the

major being CYP1A2. It goes without saying that the poor metaboliser phenotype in a minor metabolic pathway will have a lower impact on treatment response as compared with the poor metaboliser phenotype in a major metabolic pathway. Also, one pharmacogenetic study of Melkerson et al. failed to establish an association between the poor and intermediate CYP2D6 metaboliser phenotype and plasma levels of clozapine and its metabolite N-desmetilclozapina. Instead, CYP1A2 poor metabolisers (major route of metabolism of clozapine) have higher plasma concentrations and a higher risk of hyperlipidemia and increased insulin resistance (6).

Atypical antipsychotic	CYP2D6	CYP3A4	CYP1A2
Aripiprazole	+++	+	
Clozapine	+	+	+++
Iloperidone	+++	+	
Olanzapine	+		+++
Paliperidona	+++	+	
Quetiapine		+++	
Risperidone	+++	+	
Ziprasidone		+	

Table 2. CYP450 enzymes responsible for metabolism of atypical antipsychotics.

Once genotyping of cytochrome P450 became possible, Dutch Pharmacogenetics Working Group of Royal Dutch Pharmacist Association, based on current research evidence, recommends testing of P450 genotype in order to individualize therapeutic doses of:

- aripiprazole: reduction of the maximum dose to 10 mg / day (67% maximum recommended daily dose) for CYP2D6 poor metabolisers;
- risperidone: in poor, intermediate or ultrarapid CYP2D6 metabolisers, administration of an alternative drug is recommended (eg, quetiapine, olanzapine, clozapine) or careful monitoring of side effects and adjustment of dose based on clinical response. Information from pharmacogenetic studies is insufficient to determine the exact optimal dose (7).

In the list of drugs that have included pharmacogenetic information in their labels, Food and Drug Administration (FDA) included aripiprazole, clozapine, iloperidone, risperidone. The biomarker that is referred to is CYP2D6. In CYP2D6 poor metabolizer patients, for aripiprazole and iloperidone, a 50% reduction of the initial dose is recommended, with subsequent dosage adjustment based on the clinical response. Also, reducing the dose of clozapine is recommended, without specifying the exact percentage (8).

Another advantage of pharmacogenetic testing, prior to administration of an antipsychotic, is the possibility to protect the patient from drug-drug interactions that can have serious consequences. The cytochrome P450 enzyme system can be disrupted by factors such as cigarette smoke, alcohol but also drugs. There are drugs (e.g. antiepileptics, antiretrovirals etc.) with enzyme induction effect, which increase the enzymatic activity and accelerate the hepatic biotransformation of the co-administered drug (this case, an atypical antipsychotic), reducing its plasma concentration. This induction leads to diminishing or annulment of the therapeutic effect of the atypical antipsychotic drug. Other drugs that have enzyme inhibition effect (e.g. macrolides), leads to a decrease in rate of hepatic biotransformation of

the atypical antipsychotic, causing increased plasma half-life, with the risk of accumulation and occurrence of toxic effects from overdose. (9) This types of drug interaction can turn an extensive metabolizer into a rapid one (by enzymatic induction) or into an intermediate/poor one (by enzymatic inhibition).

In 1999, Markowity et al. noted that the association of ciprofloxacin with olanzapine causes the doubling of plasma concentrations of olanzapine, effect due to CYP1A2 enzyme inhibitory activity of ciprofloxacin (10). In 2006, the case of a 70 years old patient was presented, that underwent chronic treatment with azathioprine, vasartan and olanzapine, to whose therapeutic regimen ciprofloxacin (800 mg / day) and furosemide were introduced. After 3 days, the patient experienced a prolongation of 610 ms of the QT interval, which gradually returned to normal after replacing ciprofloxacin with a cephalosporin. This iatrogenic prolongation of the QT interval may be caused by ciprofloxacin administration itself or may be due to the cumulative effect of ciprofloxacin and olanzapine. Ciprofloxacin has a CYP1A2 enzyme inhibition effect on olanzapine, which causes accumulation of unmetabolised olanzapine, with the risk of adverse effects, including prolongation of QT interval (11). There is no information about the CYP1A2 phenotype of the patient. As a rule, the effects of enzymatic inhibition interaction are more severe if the patient is a poor metabolizer.

Aripiprazole is metabolized via CYP2D6 and CYP3A4. Thus, if initial aripiprazole doses in patients how are CYP2D6 poor metabolizers have to be decreased by 50%, they will be reduced to 25% if the patient is concomitantly under treatment with a potent CYP3A4 enzyme inhibitor (e.g. ketoconazole) (12).

Most studies on pharmacokinetic aspects of pharmacogenetics of atypical antipsychotics have focused on the influence of cytochrome P450 genotype on plasma concentrations of drug and its active metabolite, which were correlated with treatment response.

Studies attempting direct correlation between cit. P450 polymorphisms and clinical efficacy of the treatment are fewer and most of them have negative results. After failure of Arranz et al. to establish such a correlation, Riedel and his collaborators assessed the influence of CYP2D6 genotype on the plasma concentrations of risperidone and 9-HO-risperidone (active metabolite) and the relationship with clinical efficacy. Efficacy was defined as 30% reduction of the PANSS score (positive and negative syndrome scale) after 6 weeks of treatment. Although CYP2D6 *4 allele carriers, (associated with reduction of enzyme activity) showed an increase in plasma levels of risperidone and its active metabolite, the polymorphism could not be correlated with efficacy of treatment defined by the PANSS score (13). In 2008, Thomas P. published a study which also fails to associate the efficacy of olanzapine treatment with CYP2D6*4, CYP1A2*1C, CYP1A2*1F polymorphisms (14). Although cit. P450 phenotype influences plasma drug concentration, more studies are needed to assess consequences from the clinical point of view.

Promising progress is made in CYP genotyping to assess propensity to obesity induced by atypical antipsychotics. Ellingrot et al. have established a relationship between CYP2D6 polymorphisms and weight gain for 11 patients that were under olanzapine treatment. Patients with genotypes *1/*3 and *4, associated with the status of poor metabolizers, had a higher increase of body mass index as compared to carriers of the wild type allelic variant (*1/*1) (15). These results are supported by the study of Lane et al., conducted over 123 ethnic Chinese schizophrenic patients. The presence of CYP2D6 188-C/T polymorphism (CYP3D6 *10 - reduced enzyme activity) was associated with a greater increase in weight after 6

weeks of monotherapy with risperidone, as compared to the homozygotes wild-type (16).

Recently AmpliChip CYP450 (Hoffmann-LaRoche, 2003), the first pharmacogenetic test was approved by the U.S.A. and the European Union allowing identification of CYP2D6 and CYP2C19 gene polymorphisms in order to assess the patients' metabolizer status. Although molecular diagnosis technology has advanced, genotyping for P450 cytochrome is not usually integrated into clinical practice. The physicians often resort to pharmacogenetic testing only when pharmacokinetic parameters are so much modified that there are problems in clinical practice.

Conclusions

We have done a literature overview regarding the role of CYP450 polymorphisms in anti-psychotic treatment response and we believe that our study is justified by the lack of information about the frequency of specific genotypes in the Romanian population and their impact in establishing the best treatment and the correct dose of anti-psychotics.

In any case, even if phenotyping of enzymatic status is useful for predicting plasma concentration of antipsychotics and of active metabolites and to make recommendations on therapy starting and dosage, further future studies are needed so that pharmacokinetic aspects of pharmacogenetics of antipsychotics should find applicability in the exact anticipation of treatment response from a clinical point of view. What is certain is that the future holds an important place to psychopharmacogenetics in therapeutic management and personalized therapy.

References

1. By Julio Licinio (Editor), Ma-Li Wong (Editor) Pharmacogenomics: The Search for Individualized Therapies Publisher: Wiley-VCH | ISBN: 3527303804 | edition 2002 ISBN: 3527303804 Edition: 1 Pub. Date: May 2002 Publisher: Wiley, John & Sons; 390
2. Nadine Cohen (Editor), Pharmacogenomics and Personalized Medicine (Methods in Pharmacology and Toxicology) (Hardcover), 2008; 83
3. Ravyn D, Ravyn V, Lowney R, Nasrallah HA. CYP450 Pharmacogenetic treatment strategies for antipsychotics: A review of the evidence. Schizophr Res. 2013 Sep;149(1-3):1-14.
4. Hendset M, Hermann M, Lunde H, Refsum H, Molden E. Impact of the CYP2D6 genotype on steady-state serum concentrations of aripiprazole and dehydroaripiprazole. Eur J Clin Pharmacol 2007;63:1147-51
5. Arranz MJ, Dawson E, Shaikh S, Sham P, Sharma T, Aitchison K, et al. Cytochrome P4502D6 genotype does not determine response to clozapine. Br J Clin Pharmacol 1995;39:417-20
6. Melkersson KI, Scordo MG, Gunes A, Dahl ML. Impact of CYP1A2 and CYP2D6 polymorphisms on drug metabolism and on insulin and lipid elevations and insulin resistance in clozapine-treated patients. J Clin Psychiatry. 2007 May;68(5):697-704.
7. <http://www.pharmgkb.org/page/dpww>
8. US Food and Drug Administration 2013, Table of Pharmacogenomic Biomarkers in Drug Labels, <http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>, accessed August 10, 2013)
9. Cristescu C., Pharmacy clinique, ed. Eurobit, Timișoara, 2013; 63-69
10. Markowitz JS, DeVane CL. Suspected ciprofloxacin inhibition of olanzapine resulting in increased plasma concentration. J Clin Psychopharmacol 1999;19:289-91.
11. Konstantinos P. Letsas, Antonios Sideris, Stavros P. Kounas, Michalis Efremidis, Panagiotis Korantzopoulos, Fotios Kardaras Drug-induced QT interval prolongation after ciprofloxacin administration in a patient receiving olanzapine International Journal of Cardiology, Volume 109, Issue 2, 10 May 2006, Pages 273-274
12. US Food and Drug Administration, Prospect aprobat Aripiprazole la 30.07.2013, (http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/)

- 021436s037,021713s029,021729s021,021866s0221bl.pdf , accessed August 11, 2013)
13. Riedel M, Schwarz MJ, Strassnig M, Spellmann I, Müller-Arends A, Weber K, Zach J, Müller N, Möller HJ. Risperidone plasma levels, clinical response and side-effects. *Eur Arch Psychiatry Clin Neurosci.* 2005 Aug;255(4):261-8
14. Pramod Thomas, Vibhuti Srivastava, Anuradha Singh, Priya Mathur, Vishwajit L. Nimgaonkar, Bernard Lerer, B.K. Thelma Correlates of response to Olanzapine in a North Indian Schizophrenia sample, *Psychiatry Research*, Volume 161, Issue 3, 15 December 2008, Pages 275-28
15. Ellingrod VL, Miller D, Schultz SK, et al. CYP2D6 polymorphisms and atypical antipsychotic weight gain. *Psychiatr Genet.* 2002;12:55–58.
16. Lane HY, Liu YC, Huang CL, Chang YC, Wu PL, Lu CT, Chang WH. Risperidone related weight gain: genetic and non genetic predictors, *J Clin Psychopharmacol.* 2006 Apr;26(2):128-34
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