POSSIBILITIES OF PSYCHOPHARMACOTHERAPY ADJUSTED TO PATIENTS' DRUG-METABOLIZING CAPACITY

Ph.D. Thesis

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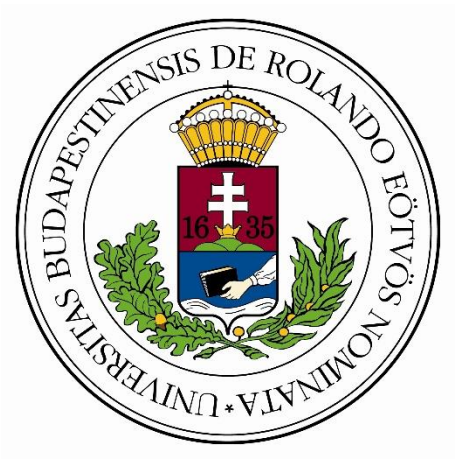

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1 Introduction

The present work investigated the possibilities for prevention of side effects of antiepileptic and antipsychotic therapy, and for optimal dosing strategy of selected psychopharmacons. The application of an appropriate drug therapy for psychiatric patients is a great challenge for psychiatrists. It is partly due to the lack of objective diagnostic tools, whereas the most drugs in psychiatry take effect in weeks. Although international guidelines recommend psychiatric use of therapeutic drug monitoring, in Hungary, there is no possibility for routine monitoring of blood levels of psychopharmacons.

Most of the undesired side-effects of drugs are caused by differences or changes in drug metabolism. Adverse events can damage the function of a healthy liver (and other organs), or can adversely affect the quality of life of patients (weight gain, gait and balance disturbances, muscle stiffness, somnolence, dizziness). The drug-metabolizing capacity of the liver primarily depends on the levels and activities of cytochrome P450 (CYP) enzymes, which can potently influence the efficacy or toxicity of a drug. The current CYP activities are genetically determined which can be modulated by internal and external factors. Patients' drug-metabolizing capacity can be estimated by a complex diagnostic system (CYPtest™) that determines the current expression of CYP enzymes (CYP-phenotyping) in leukocytes and clinically relevant mutations in CYP genes. Combining CYP-genotyping and CYP-phenotyping tools, we identified those CYP polymorphisms which result in reduced or non-functional enzymes, and may lead to the lack of pharmacological effect and/or to development of side effects. Patients' CYP-status can predict potential poor metabolism that may require tailored medication (dosage optimization or withdrawal and replacement to an alternative drug). Personalized drug therapy can increase the therapeutic efficacy, can reduce the unnecessary drug consumption and the risk of potential drug toxicity, and can contribute to the recovery of patients.

2 Aims

The main goal of the present work was the rationalization of psychopharmacotherapy by reducing the unnecessary drug consumption and the risk of potential drug toxicity by the estimation of drug-metabolizing capacity of patients suffering from schizophrenia, bipolar disorder and epilepsy.

The first step was to clarify the fate of psychopharmacons (clonazepam, clozapine, valproate) in human body, 1. whether CYP enzymes are able to metabolize the particular drug or it remains unchanged, 2. which CYP enzymes are responsible for the metabolism of these drugs, 3. and if they are metabolized, toxic or active metabolites are formed. The primary role of CYP3A4 enzyme in clonazepam biotransformation has been verified by *in vitro* and *in vivo* investigations. The role of several CYP enzymes (CYP1A2, CYP2C19, CYP2D6 and CYP3A4) in clozapine metabolism has been assumed, however the data were contradictory. CYP2C9 is the major CYP enzyme of valproate metabolism, whereas CYP2A6 and CYP2B6 play only a minor role. The CYP2C9, CYP2C19, CYP2D6 and CYP3A5 are highly polymorphic enzymes, and different allelic variants lead to different catalytic activities. Furthermore, the drug-metabolizing capacity can be influenced by other non-genetic factors, thus the phenotype cannot always be predicted from genotype. The expression rate of a wild-type gene can highly influence a patient's drug-metabolizing capacity.

We were looking for the answers to the following questions:

1. Which isoenzymes are responsible for the metabolism of clonazepam, clozapine and valproic acid psychopharmacons?
2. Can the genetic polymorphism and/or the expression of CYP enzymes influence the blood levels of the drugs? Is there any association between the blood levels of the parent drugs or their metabolites and the drug-metabolizing capacity of patients?
3. Could the patients' CYP-status predict the dose requirements for the therapeutic concentrations?
4. Are there any benefits of the CYPtest™ guided therapy against the conventional therapy?

3 Methods

3.1 *In vitro* CYP enzyme mapping

In vitro CYP enzyme mapping of clonazepam and clozapine was performed in human liver microsomes. CYP inhibition studies were carried out in the presence of various CYP-selective inhibitors: α-naphtoflavone for CYP1A2, sulfaphenazole for CYP2C9, ticlopidine for CYP2C19, quinidine for CYP2D6 and ketoconazole for CYP3A4. The incubation mixture contained human liver microsomes, psychopharmacons (clonazepam and clozapine) at an approximate K_m concentration, inhibitors and NADPH-generating system in 0,1 M Tris-HCl buffer. The parent drugs and their metabolites were detected by HPLC-MS/MS.

3.2 Clinical studies

The investigations were approved by the Hungarian Committee of Science and Ethics.

The blood samples taken from psychiatric patients were from the Department of Psychiatry and Psychotherapy, Semmelweis University, whereas the samples taken from epileptic children were from the 2nd Department of Pediatrics, Semmelweis University and from Heim Pál Children's Hospital. The patients' demographic data as well as the details of antipsychotic and anticonvulsant therapy were recorded.

3.3 Determination of CYP- and NAT2-status

According to our previous work, strong correlation exists between the enzyme activities of CYP1A2, CYP2C9, CYP2C19 and CYP3A4 in the liver and CYP expression in leukocytes. Therefore, hepatic CYP activities in patients with homozygous wild genotype can be estimated from CYP mRNA levels in leukocytes isolated from peripheral blood (CYPtest™).

The patients' CYP-status (CYP-genotype and CYP mRNA expression) were determined from leukocytes. The *CYP2C9*, *CYP2C19*, *CYP2D6* és *CYP3A5* genotypes were determined by assaying the most common allelic variants in Caucasian population using real-time PCR with TaqMan probes.
NAT2 acetylation phenotype was inferred from the 4-SNP panel distinguishing the slow acetylator alleles from the rapid acetylator alleles with TaqMan probes.

All SNPs were in Hardy-Weinberg equilibrium and the genotype frequencies for the CYP and NAT2 genes were not deviated from the expectations.

For assaying CYP1A2, CYP2C9 and CYP3A4 expression, RNA was isolated from leukocytes, reverse transcribed into single-stranded cDNA, and then real-time PCR was performed with TaqMan probes. The quantity of target RNA relative to that of the housekeeping gene glyceraldehyde 3-phosphate-dehidrogenase (GAPDH) was determined. GAPDH expression is constant in all cells and independent of experimental conditions; therefore, its expression was set to 1 and CYP mRNA levels were normalized by GAPDH expression.

3.4 Determination of blood concentrations

The blood samples were taken before the patients were administered the morning dose. The steady-state concentrations of clonazepam, 7-aminoclonazepam, clozapine, norclozapine, and clozapine N-oxide were determined by LC-MS/MS. The steady-state serum concentration of valproic acid was determined by the fluorescence polarization immunoassay method at the laboratory of Heim Pál Children's Hospital.

Normalized blood levels were calculated by dividing the concentration values by the corresponding 24-h dose on a mg/kg bodyweight basis and expressed as [(ng/ml)/(mg/kg)].

3.5 Statistical analysis

The statistical significance of demographic data, CYP expressions and genotypes as covariates was analyzed by ANOVA using linear model of covariate effects with constant terms.

The lower and upper limits for the optimal dose were estimated from the lower and upper limits of the optimal clonazepam and clozapine plasma concentration range. Matlab R2009b was used to perform the analysis and calculate the optimal dosing. Between-group differences were calculated by the use of Kruskal–Wallis ANOVA followed by Mann-Whitney comparison test (clonazepam, clozapine) or Dunn's multiple comparisons test (valproate). $P<0.05$ was considered statistically significant.

4 Results

During the *in vitro* inhibition studies, we confirmed the role of CYP3A4/5 in the formation of 7-aminoclonazepam. Clozapine is extensively metabolized forming two major metabolites, clozapine N-oxid and norclozapine. CYP3A4/5 catalyze N-

oxidation of clozapine, whereas N-demethylation was performed by mainly CYP3A4/5 with minor contribution of CYP1A2 and CYP2D6.

In the clinical studies, we examined the clinical importance of CYP enzymes responsible for the metabolism of the psychopharmacons.

4.1 Thesis

1.1. The patients' *CYP3A5* genotype seemed to have no effect on clonazepam plasma concentrations, whereas CYP3A4 expression significantly influenced the steady-state levels of clonazepam. The normalized concentrations of clonazepam were significantly lower in the patients displaying normal CYP3A4 mRNA levels than in low CYP3A4 expressers. The plasma concentrations of 7-aminoclonazepam were found to be associated with the patients' CYP3A4 expression and NAT2 acetylator phenotype. High 7-amino-clonazepam/clonazepam concentration ratio was observed in patients with normal CYP3A4 expression and slow N-acetylation, which refers to the accumulation of the 7-amino metabolite.

1.2. The *CYP2C19* and *CYP2D6* genotypes were not likely to have a primary role in clozapine clearance. The majority of the patients expressed CYP1A2 at a relatively low level. In contrast to the findings published in the literature, we did not find any correlation between the clozapine concentrations and CYP1A2 expression. Furthermore, the patients displaying normal CYP3A4 expression showed significantly lower blood levels than the patients with low expression. *CYP3A5*1* carriers with low CYP3A4 expressions displayed similar clozapine concentrations to the patients with normal CYP3A4 expression. Strong association was observed between the metabolite/clozapine ratios and CYP3A4 mRNA levels, which confirmed the primary role of CYP3A4 in clozapine metabolism.

1.3. Valproate serum concentrations in pediatric patients were influenced by the patients' CYP2C9-status determined not only by the genetic variability of CYP2C9, but also by CYP2C9 expression. The normal expressers with two wild type alleles presented significantly lower valproate serum levels than the low CYP2C9 expresser patients. Nevertheless, the presence of one mutated *CYP2C9* allele resulted in high valproate serum levels independently from CYP2C9 expression.

In conclusion, the blood levels of the psychopharmacons (clonazepam, clozapine, valproate) and their metabolites were influenced by the current status of the metabolizing enzymes.

2. We found strong association between drug levels and patients' CYP-status which were found to be applicable for prediction of optimal dosing. The patients expressing CYP3A4 mRNA at low levels required significantly lower dose of clonazepam and clozapine for the optimal plasma level than normal CYP3A4 expressers. The conventional dosing approach (30-40 mg/kg) in pediatric patients' valproate therapy was appropriate for normal CYP2C9 expresser patients with *CYP2C9*1/*1* genotype. Non-valproate therapy was proposed for children with two mutated *CYP2C9* alleles. The low expressers and the patients with one polymorphic *CYP2C9* allele required significantly lower dose (<20 mg/kg) of valproate for the optimal serum level.
3. Finally, we investigated the potential clinical benefit of CYP2C9-status guided valproate therapy over the symptom-driven therapy in pediatric patients. CYP2C9-status controlled valproate therapy could prevent patients from misdosing, resulting in fewer patients out of the therapeutic range of serum valproate concentrations. Valproate-associated serious adverse effects (hyperammonemia and increased levels of serum alkaline phosphatase) were observed less frequently in the CYPtest group than in the control group, whereas the prevalence of mild side effects, including weight gain and somnolence, was similar in the control and CYPtest groups. Our major findings indicated that CYP-status guided treatment could prevent patients from misdosing or from the manifestation of toxic symptoms.

5 Conclusions

Our present work clearly demonstrated that the blood levels of the psychopharmacons (clonazepam, clozapine, valproate) were associated with the expression of the drug-metabolizing enzymes. These findings can facilitate the improvement of personalized medication.

The patients' CYP3A4 expression was found to be the major determinant of clonazepam plasma concentration normalized by the dose and bodyweight, whereas the plasma concentrations of 7-aminoclonazepam were found to be associated with the patients' CYP3A4 expression and NAT2 acetylator phenotype. Prospective assaying of patients' CYP3A4 expression can identify poor metabolizers and can contribute to the avoidance of misdosing-induced side effects in patients. The knowledge of patients'

CYP3A4 and NAT2-status may improve the efficiency of clonazepam therapy and can minimize the risk of side effects and withdrawal symptoms.

The patients' clozapine-metabolizing capacity can be a useful information regarding dose requirement for therapeutic blood concentration. Although the present work involved a limited number of patients and further investigation enrolling larger cohort is required, it clearly demonstrated that CYP3A-status could be the major determinant of normalized clozapine concentration and dose requirement, in particular, that the patients expressed CYP1A2 at a relatively low level. For achieving the therapeutic concentration, twice as high dose was necessary for the normal/high CYP3A4 expressers and *CYP3A5*1* carriers than for low CYP3A4 expressers. The routine dosing regimen appears to be appropriate for the patients with low CYP3A4 mRNA levels. High proportion of the patients were CYP3A4 normal expressers, thus their clozapine blood levels were below the therapeutic concentrations.

The elimination of valproate in pediatric patients was influenced by the patients' CYP2C9-status. The knowledge of *CYP2C9* genotype has some benefit in valproate therapy, but the individuals may become transient poor metabolizers as an effect of non-genetic variations. For example, cytokine release following seizures has a reducing effect on CYP2C9 expression. Due to CYP2C9 phenoconversion, the majority of epileptic children expressed CYP2C9 at low level, thus low expressers required reduced valproate dose for the therapeutic serum concentration. CYP2C9-status guided valproate treatment could prevent patients from misdosing and from exaggerated serum concentrations of valproate, resulting in the manifestation of toxic symptoms.

6 Publications

6.1 Related publications

T Budi, **K Toth**, A Nagy, Z Szever, A Kiss, M Temesvari, E Hafra, M Garami, A Tapodi and K Monostory (2015). "Clinical significance of CYP2C9-status guided valproic acid therapy in children." *Epilepsia* **56**(6): 849-855.

K Toth, T Budi, A Kiss, M Temesvari, E Hafra, A Nagy, Z Szever and K Monostory (2015). "Phenoconversion of CYP2C9 in epilepsy limits the predictive value of CYP2C9 genotype in optimizing valproate therapy." *Personalized Medicine* **12**(3): 199-207.

K Toth, G Csukly, D Sirok, A Belic, A Kiss, E Hafra, M Deri, A Menus, I Bitter and K Monostory (2016). "Optimization of Clonazepam Therapy Adjusted to Patient's CYP3A Status and NAT2 Genotype." *International Journal of Neuropsychopharmacology* **19**(12): pyw083.

K Monostory, T Budi, **K Toth**, A Nagy, Z Szever, A Kiss, M Temesvari, E Hafra, A Tapodi and M Garami (2016). "In response: Commentary on clinical significance of CYP2C9-status-guided valproic acid therapy in children." *Epilepsia* **57**(8): 1339-1340.

K Toth, G Csukly, D Sirok, A Belic, A Kiss, E Hafra, M Deri, A Menus, I Bitter and K Monostory (2017). "Potential role of patients' CYP3A-status in clozapine pharmacokinetics." *International Journal of Neuropsychopharmacology* **20**: pyx019.

6.2 Other publications

A Belic, **K Toth**, R Vrzal, M Temesvari, P Porrogi, E Orban, D Rozman, Z Dvorak and K Monostory (2013). "Dehydroepiandrosterone post-transcriptionally modifies CYP1A2 induction involving androgen receptor." *Chemico-Biological Interactions* **203**(3): 597-603.

K Monostory, **K Toth**, A Kiss, E Hafra, N Csikany, J Paulik, E Sarvary and L Kobori (2015). "Personalizing initial calcineurin inhibitor dosing by adjusting to donor CYP3A-status in liver transplant patients." *British Journal of Clinical Pharmacology* **80**(6): 1429-1437.

AF Kiss, **K Toth**, C Juhasz, M Temesvari, J Paulik, G Hirka and K Monostory (2016). "Is CYP2D6 phenotype predictable from CYP2D6 genotype?" *Microchemical Journal*, 10.1016/j.microc.2016.10.018.

K Toth, D Sirok, A Kiss, A Mayer, M Patfalusi, G Hirka and K Monostory (2016). "Utility of in vitro clearance in primary hepatocyte model for prediction of in vivo hepatic clearance of psychopharmacons." *Microchemical Journal*, 10.1016/j.microc.2016.10.028.

6.3 International conference abstracts

K Toth, A Belic, M Temesvari, P Szabo, G Csukly, J Bulucz, D Filipovits, I Bitter and K Monostory "Estimation of serum concentrations of clozapine by CYP3A4 expression (preliminary results)" (Molecular Medicine and Biotechnology, Ljubljana, Slovenia, June 27-29, 2012)

K Toth, M Temesvari, F Kiss, P Szabo, G Csukly, I Bitter and K Monostory "The role of CYP3A enzymes in clonazepam metabolism " (FEBS3+ Meeting "*From molecules to life and back"*, Opatija, Croatia, June 13-16, 2012)

K Toth, D Sirok, G Csukly, J Bulucz, I Bitter, P Szabo, B Magda and K Monostory "Clinical relevance of patients' CYP3A-status in clozapine therapy" (FEBS3+ Meeting "*Molecules of Life*", Portorož, Slovenia, September 16-19, 2015)

K Toth, D Sirok, A Belic, G Csukly, I Bitter and K Monostory "Contribution of CYP3A enzymes and NAT2 alleles to clonazepam metabolism" (XV Italian-Hungarian Symposium on Spectrochemistry "*Pharmacological Research and Analytical Approaches*", Pisa, Italy, June 12–16, 2016)