Pharmacogenetics of Valproic Acid as Unmodified Risk Factor of Adverse Drug Reactions

The purpose of the research is the assessment of the role of CYP2C9 gene polymorphisms of the isoenzyme 2C9 of cytochrome P450 of the liver as unmodified risk factor of adverse drug events development in case of intake of an average therapeutic doses of PA drugs at patients with epilepsy and epileptic syndromes.

The studies was carried out within the limits of complex theme of scientific project “Epidemiological, clinical and genetics aspects of diseases of central, peripheral and autonomic neural systems and preventive health care” in 2010-2011. Sampling included 41 cases (patients with epilepsy and epileptic syndromes). Patients were from 1 up to 60 years of age, the median was 23 years.

All the carriers of mutant polymorphous allelic variants CYP2C9*2 and CYP2C9*3, both homozygous and heterozygous carriers and also in case of their combination (for example, genotype CYP2C9*2/CYP2C9*3) had 100% occurrence of treatment-emergent adverse events in case of standard usage of VPA drug dosage in accordance with the Pharmacopeia (20-30 mg/kg/per day for children, 20 mg/kg/per day for adults) even during the daily dose titration (10-15 mg/kg/per day for children, 5-10 mg/kg/per day for adults). The homozygous carrier of the gene CYP2C9 of the minor allelic variant of the isoenzyme 2C9 of cytochrome P450 had the most severe adverse drug reactions in the form of epileptic seizures aggravation, cognitive and behavioral disorders in case of standard usage of VPA drug dosage (kg/body weight per daily).

Keywords: Epilepsy, antiepileptic drugs, valproic acid, pharmacogenetics, adverse drug reactions.

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Introduction

Epilepsy is a widespread socially important chronic disease of brain which is treated for many years; 30% of the patients with epilepsy should take antiepileptic drugs (AEDs) for the life term. Main aim of antiepileptic therapy is full epileptic seizures control and, in case of their absence, minimization of adverse drug reactions and their patient’s life quality negative influence. But the occurrence of adverse drug reactions still remains rather high and is estimated at 7-25%, according to different authors (Guekht et al., 2005; Vlasov et al., 2003; Zenkov, 2000; Chid, 1997). Only in the USA annually 2 million adverse drug reactions are registered and more then 100 thousand patients die due to this cause. Adverse drug reactions economic damage increased from 76.6 billion dollars in 1997 up to 177.4 billion dollars in 2001 (Ingelman-Sundberg, 2004). At the same time, drug therapy effectiveness leaves much to be desired: up to 40% of patients with different disorders do not respond the drug therapy Silber (2001).

In case of severe complications there is a necessity of the drug cancellation (even if it controls the epileptic seizures effectively) and the way of therapy changing. Treatment-emergent adverse events may influence negatively the patient’s social and family adaptation sometimes to a greater extent then the disease itself. None of the well-known AEDs which are used nowadays in practice lacks side effects. The number of AEDs side effects is great and is connected with the pharmacokinetics and the pharmacogenetics of the drugs themselves.
Client-centered approach in case of epilepsy drug therapy includes assessment of the factors which influence the inter- and individual variability, in particular pharmacokinetic parameters changes which are due to the patient’s physiologic and pathologic characteristics (Ingelman-Sundberg, 2001). Pharmacokinetic variability may be assessed by measurement of the drug serum concentration. Therapeutic Drug Monitoring (TDM) in epileptology is a measurement and clinical usage of AEDs concentrations in body fluids, usually in serum or blood plasma. TDM is used because of considerable interindividual variability of pharmacological parameters in cases of: nonlinear pharmacokinetics, for example due to phenytoin administration; little difference between average therapeutic and toxic doses (the danger of side effects and toxic signs); specific patients (pregnant and nursing women, aged persons, sucking children) whose pharmacologic parameters (and therefore little difference between average therapeutic and toxic doses of AEDs) differ greatly from the usual average number; renal disorders, lever function abnormality, gastrointestinal tract dysfunction which influence the AEDs pharmacologic parameters; polytherapy with account of several AEDs interaction for the purpose of anti-epileptic therapy effectiveness optimization and/or toxic effect detection or subject to low level of AED in blood and control of the patient’s compliance to their intake and dosage.

In case of routine methods of AEDs monitoring its general level in blood plasma is detected. It was found out that with young and healthy people monitoring of AED general level gives rather exact information but with aged sick persons there were some problems (Shnayder et al., 2008).

AED level stabilization in blood plasma depends on the drug type and for major part of them sums fivefold value of the half lifetime. Therapeutic level is such a level under which the drug concentration in blood plasma is enough to gain curative effect but the concentration is not enough to entail side clinical aspects or treatment-emergent adverse events. Although AED level stabilization in patient’s blood plasma depends to a greater extent on individual sensitivity characteristics and body metabolism of the patient, there are certain pharmacokinetic and pharmacodynamic parameters for every AED determining approximately their therapeutic possibilities which should be taken into account while drug administration. To assess these data biochemical detection of drug level in patient’s blood plasma is used.

Clinical picture (character, epileptic seizures severity and frequency) is the main criteria of epilepsy treatment effectiveness and, if the treatment is successful and there are no adverse drug reactions, there is no need as a rule to determine the drug concentration by means of biochemical methods.

In case of drug intoxication besides standardized measurement, a free AEDs portion non-related with blood protein should be determined. Usually three and more blood samples should be taken daily to assess daily fluctuations of AED level and to identify its peaks of concentration which exceed the therapeutic reference area and enter toxic area. Repeated blood samples may be helpful in some case of therapy ineffectiveness (AEDs resistance), as assessment of balance of the time of epileptic seizures development and the time of the least AED blood concentrations may help to correct drug intake schedule and their dosage.

Therapeutic drug monitoring should be carried out if:

- the treatment is ineffective,
- the patient’s condition changed or drug intake schedule was changed,
- signs of intoxication appeared,
- the drug was administered or cancelled in case of polytherapy or combined treatment (including non-AEDs), if pharmacological interaction is possible.

AEDs (free drugs and protein-bound drugs) general level measurement is informative only if the drug does not bind to blood proteins or if the balance of free fractions and bound fractions is stable. Meanwhile carbamazepine and especially phenytoin and valproic acid (VPA) to a greater extent bind to proteins (Shnayder et al., 2010; Vojtenkov and Borisova, 2005; Vlasov et al., 2003; Aksenova et al., 1997). So any changes in the ability to
bind protein may influence the free fraction number leading to certain physiological effect. AEDs may both aggravate metabolism of other drugs reducing their effectiveness and lower leading to dose-dependent toxicity. Thus, team approach is essential in case of all AEDs intake. Drugs binding to proteins greatly may promote AED chasing leading to free fraction increasing and lowering of general AED level in blood plasma and thus increasing the epileptic seizures frequency and the risk of epileptic status development is more possible (Mashkovsky, 2006).

Therefore, assessment of epilepsy treatment effectiveness should be carried out in a complex way: taking into account epileptic seizures control and improvement of patient’s life quality, the results of treatment-emergent adverse events monitoring and lowering the risk of their development. If it is necessary the AED general level and AED free level in blood is determined, CBC and biochemical blood assay and electroencephalography (EEG) is carried out (Zenkov, 2000).

However, the phenotyping of patients with epilepsy has some disadvantages:

- to carry out a test a single AED marker intake is essential in this case treatment-emergent adverse events may appear;
- invasiveness (multiple blood sampling is essential) and patients discomfort (difficulties of outpatient usage);
- the necessity to determine AED concentration and/or its metabolite in blood serum at several temporary points;
- tests assess the activity of biotransformation enzymes which can be determined not only by patient’s genetic characteristics but by the combined used drugs (inhibitors, inductors), age and sex, daily biorhythm, kind of food, bad habits (smoking, alcohol drinking etc)
- tests are difficult to use for studying large populations to assess ethnic AEDs responsitivity.

Pharmacogenetics is a rapidly growing field of interest encompassing genetic variation in genes encoding drug transporters, drug-metabolizing enzymes and drug targets, as well as genes related to the action of drugs. The interest is based on the fact that only 30-60% of common drug therapy is successful and that adverse drug reactions cause 7% of all hospital admissions, 4% withdrawal of new medical entities, and cost society an amount equal to the cost of drug treatment per se (Ingelman-Sundberg, 2004). It should be noted that pharmacogenetic tests have no such disadvantages as they are based on detection of allelic multiform variants of genes of biotransformation systems and AEDs transporters which determine the pharmacologic response, i.e. patient genotyping itself which is the base of developing rapidly during the last 5 years personalized medicine (Kukes, 2004; Silber, 2001).

Pharmacogenetic tests advantages:

- genetic tests do not require to intake AEDs, i.e. it is possible to predict their pharmacological response before the beginning of the intake (there is a possibility of high risk of treatment-emergent adverse events groups stratification);
- a single blood sample or other biological material is needed (for example, buccal smear) at any time once in patient’s life (economic effectiveness);
- genetic tests are based on the method of polymerase chain reaction usage (PCR) and do not require determination at several temporary points;
- molecular genetic testing results are unchangeable during the whole life of a patient and gives an opportunity to create a so-called Pharmacogenetic Passport of a patient with epilepsy;
- tests assess only genetic part which influence the AED pharmacologic response;
- genetic tests are not expensive and do not require equipment for carrying out TDM;
- using methods of personalized medicine of the tests we can carry out large population studies and it’s of great importance from the point of epileptic characteristics of AEDs metabolism.
The genotype of an individual is essentially invariable and remains unaffected by the treatment itself. Several examples exist where subjects carrying certain alleles do not benefit from drug therapy due to ultrarapid metabolism caused by multiple genes or by induction of gene expression or, alternatively, suffer from adverse effects of the drug treatment due to the presence of defective alleles. However, it will take time before this will be a reality within the clinic.

Since 2010 on the base of Neurological Centre of Epileptology, Neurogenetics and Brain Research of the University Clinic (hereafter Centre) jointly with the Scientific Laboratory of Medical Genetics and the Central Scientific and Research Laboratory (CSRL) of the Krasnoyarsk State Medical University, we for the first time in our region introduced in everyday practice of neurologist and epileptologist molecular genetic study of polymorphism of isoenzyme 2C9 of cytochrome P450 of liver, taking part in VPA drugs metabolism, which have liver way of metabolism.

The cytochrome P450s (CYP) are responsible for about 75% of phase I dependent drug metabolism and for the metabolism of a huge amount of dietary constituents and endogenous chemicals. The human has 59 active genes, and 6 of those encode important drug metabolising enzymes. About 40% of cytochrome P450 dependent drug metabolism is catalysed by polymorphic enzymes and such drug P450 interactions are frequently seen in adverse drug reaction reports (Ingelman-Sundberg, 2004).

Gene CYP2C9 of isoenzyme 2C9 which reduces VPA metabolism has the most clinical importance in predicting adverse drug reactions. Meanwhile the polymorphisms CYP2C9*2 (R144C, c.430 C>T) and CYP2C9*3 (I359L, c.1075 A>C) at chromosome 10q24.1-24.3 are the most well-studied. As polymorphous allelic variants CYP2C9*2 and CYP2C9*3 are bound to rather slow VPA metabolism, their carriage with patients with epilepsy leads to the reducing of speed of VPA drugs biotransformation and increasing its concentration in the blood plasma due to the activity reduction of isoenzyme 2C9 of cytochrome P450. It increases the risk of treatment-emergent adverse events induced by VPA genetic polymorphisms CYP2C9*2 and CYP2C9*3 increase treatment-emergent adverse events development both with heterozygous and with (especially) homozygous carriages.

Taking into account forgoing and modern principles of personalized medicine the choice of VPA drugs should be personalized in every case dependent not only on character of epileptic seizures and clinical form of epilepsy but also on personalized genetically dependent characteristics of their metabolism system. During last decades in our country and abroad scientific researchers have been carrying out to study the problem but the question of their introduction in everyday clinical work of neurologists and epileptologists is still unsolved that was the reason of our choice of the study, the aim of the investigation.

The purpose of the research is assessment of the role of genetic polymorphisms CYP2C9 of isoenzyme 2C9 of cytochrome P450 of liver as non-modifying risk factor of treatment-emergent adverse events in case of average therapeutic doses of VPA drugs at patients with epilepsy intake.

**Materials and methods**

The research is executed within the limits of complex theme of federal scientific project “Epidemiological, clinical and genetics aspects of diseases of central, peripheral and autonomic neural systems and preventive health care”, the state registration number 0120.0807480. The research was approved at the meeting of the Local Ethical Committee of Krasnoyarsk State Medical University named after Prof. V.F. Vojno-Yasenetesky (record №23/2010 of 02.04.2010). The research was encouraged by the members of Council of experts in the Russian Branch of International League Against Epilepsy (ILAE) in December 2010.
The research mentioned above was conducted on the base of the Interdepartmental Laboratory of Medical Genetics and the CSRL of the Krasnoyarsk State Medical University. We studied database of the Register of epilepsies of the Neurological Center of Epileptology of the University Clinic in 2010-2011.

Methods: analysis of doses of VPA preparations, TDM of VPA level in serum, study of readings in biochemical blood analyses (level of aspartate aminotransferase - AsAT, alanine aminotransferase - AlAT, bilirubin, amylase, complete albumen), illustrating functional activity of liver; video-EEG-monitoring; pharmacogenetic testing of polymorphisms of gene CYP2C9 (chromosome10q24.1-24.3) of isoenzyme 2C9: wild-type allele variant CYP2C9*1 without mutation, mutant-type allele variants (CYP2C9*2 - single nucleotide replacement of cytosine by thymine in the position 430; CYP2C9*3 - single nucleotide replacement of adenine by cytosine in position 1075).

Blood sampling, picking out of DNA and molecular-genetic studies were performed after a patient had given a documentary confirmation to be followed up and for filling in a patient’s case record which is composed in accordance with the aim and tasks of the research. On questioning about the presence of adverse drug reactions at the moment of blood sampling or in the past history we paid attention not only to subjective (complaints) and objective (results of examinations made by neurologist and physician of the Neurological Center of Epileptology, Neurogenetic and Brain Research) symptomatology but data of anamnesis. We performed blood sampling from the ulnar vein in aseptic laboratory condition at the Department of Medical Genetics and Clinical Neurophysiology of the Post-Diploma Institute.

To pick out DNA we applied reagents from the set “DNA-sorb B” made in the Public entity Central Research Institute of Epidemiology of the Ministry of Health of Russia (Moscow).

DNA was picked out according to the following scheme. As a pattern 100 microliters of blood was taken in a patient with epilepsy. Then 300 microliters of lysis solution were added to this pattern; 25 microliters of reconstituted sorbent universal were added in every vial. After that all this was mixed with the help of centrifuge “Fugue-Vortex Micro-Spin FV-2400” and left for 2 minutes in the rack. Then mixed again and left for 5 minutes in the upright position for precipitation of DNA on the sorbent. Then we precipitated the sorbent in the vials with the help of centrifuge “Mini Spin plus” (Eppendorf) at the speed of rotation of 5000 turns per a minute for 30 seconds. Over sedimentary liquid was deleted from the vials with medical suction device “OM-1” (RF) using a separate tip to prevent contamination of the pattern. In every vial we added 300 microliters of “Solution No.1” for cleaning and mixed with the centrifuge-vortex “Mini Spin plus” (Eppendorf). Then again we precipitated the sorbent by centrifugation at the speed of 5000 turns per a minute for 30 seconds. Again, we deleted the over sedimentary liquid with the suction device “OM-1” using separate tips to prevent contamination of the pattern. Then we added 500 microliters of “Solution No. 2” in every sample for cleaning, mixed with the centrifuge-vortex “Mini Spin plus” (Eppendorf) for 30 seconds at the speed of rotation of 10000 turns per a minute. After this manipulation the sedimentary liquid was deleted with the suction device “OM-1” again using separate tips for every sample. The procedure of cleaning was repeated once more.

Then we placed vials with the samples into the thermostat “Thermo 24-15” (Biokom) in the upright position at 65 °C for 10 minutes to dry the sorbent. After that we added 50 microliters of TE-buffer in the vials to elute DNA, mixed with the centrifuge-vortex “Mini Spin plus” (Eppendorf), then placed them into the thermostat “Thermo 24-15” (Biokom) in the upright position at 65 °C for 5 minutes shaking them from time to time in the centrifuge “Mini Spin plus” (Eppendorf).

At the final stage of picking out of DNA the vials underwent 1 minute centrifugation at the speed of rotation of 12000 turns per a minute. Over sedimentary liquid of 40 microliters was removed with the help of suction device “OM-1” with separate tips and the over sedimentary liquid containing refined DNA was replaced into the clean vial.
As a result of described above process samples of patients’ DNA were obtained which at a later date were kept in the freezer at -20°C.

Genetic typing was conducted by the method based on the polymerase chain reaction (PCR). A method of analysis of PCR using oligonucleotide samples marked by fluorescent agents (TaqMan technology) was applied. Genotypes were determined depending on the presence or lacking of the product of amplification using two DNA probes (to 2 ways of the examining polymorphism CYP2C9), each of them containing fluorescent mark and suppressors of fluorescence. Presence of this or that polymorphisms (CYP2C9*2 or CYP2C9*3) was determined by the presence of fluorescence in the amplified mixture. Negative control was included into every experiment where DNA matrix for the PCR was substituted for distilled water (dH2O). The PCR was conducted in the amplifier “Rotor-gene 6000” (Corbet Life Science, Australia).

We learned 41 clinical cases (patients with epilepsy and epileptic syndromes at the age of 1-60 years old, mean age - 23 years old).

Criteria of inclusion into the study:
- children and adults suffering from epilepsy and epileptic syndromes who addressed neurologist-epileptologists (or those patients who were followed up) for a consultation in the Neurological Center of Epileptology, Neurogenetics and Brain Research of the University Clinic of the Krasnoyarsk State Medical University named after Prof. V.F. Vojno-Yasenetsky;
- patient’s age - from 1 till 60 years old;
- more than 3 months of treatment with VPA drugs;
- a daily dose of VPA drugs (registered in the RF) didn’t exceed the recommended average therapeutical dosage at 20-30 mg/kg body weight per a day for younger children, 10-20 mg/kg body weight per a day but not more than 1800 mg per a day for adults;
- presence of clinical symptoms of VPA-induced adverse drug reactions at the moment of questioning, a consultation of an expert in epilepsy (neurologists-epileptologists) or epilepsy in the past history;
- the informative agreement of a patient (or parents of a child) for molecular-genetic test of gene CYP2C9.

Criteria of exclusion:
- patients over 60 years old (patients with slow VPA metabolism depending on the age were excluded);
- burdened history (present diseases of liver or the same in the past history);
- Gilbert syndrome;
- average daily doses of VPA drugs more than 30 mg/kg body weight per a day for children and more than 1800 mg per a day for adults;
- refusal of a patient (or parents of a child) to perform molecular-genetic test of polymorphism in gene CYP2C9;
- unwillingness of a patient (or parents of a sick child) to collaborate with an expert in epilepsy and to be constantly followed up.

In accordance with the aim and tasks of the present research 2 groups for study were formed: Group 1 (control) - homozygous carriers of wild allelic polymorphic variant of gene CYP2C9 of isoenzyme 2C9 of cytochrome P450 (genotype CYP2C9*1/CYP2C9*1) - 26 persons (63%); Group 2 (comparable) - heterozygous and homozygous carriers of mutant (minor and major) allelic polymorphic variants of gene CYP2C9 (CYP2C9*2 and CYP2C9*3) of isoenzyme 2C9 of cytochrome P450 (CYP2C9*1/CYP2C9*2; CYP2C9*1/CYP2C9*3; CYP2C9*2/CYP2C9*3; CYP2C9*2/CYP2C9*2; CYP2C9*3/CYP2C9*3) - 15 persons (47%). Difference in age in both groups of patients (control and comparable) was insignificant ($p > 0.05$).
Statistic processing of the results of the research was performed with the use of methods of biomedical statistics which were recommended for data handling of dissertation by The Higher Certification Commission of the Ministry of Education and Science of the RF, with the help of set of application programmes Statistica v. 7.0 (StatSoft Inc. USA). Differences in characteristics were considered to be significant when \( p < 0.05 \).

Results of research

The division of patients in sampling according to the dosage form of VPA drugs taken was the following: 30/41 (73\%) of patients received Depakine-chrono in the dose of 300 mg/day up to 2000 mg/day; 8/41 (20\%) - Depakine-chronosphere in the dose of 150 mg/day up to 1250 mg/day; 3/41 (7\%) - Convulex-retard in the dose of 750 mg/day up to 1750 mg/day. Average daily dose of VPA drugs in sampling was 800 ± 55.8 [95% CI: 450-900] mg/day. Thus VPA drugs were prescribed in average therapeutic doses, 20-30 mg/kg/day for children, 20 mg/kg/day for adults (according to the Guidelines of International League Against Epilepsy accepted in RF). It allowed us to exclude the risk of development of adverse drug reactions initially by means of overdose of VPA drugs.

The data of our molecular genetic testing are presented in Table 1.

### Table 1. Frequency of polymorphic allele variants of gene CYP2C9 of isoenzyme 2C9 of cytochrome P450 (N = 41)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Clinical cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>CYP2C9<em>1/CYP2C9</em>1</td>
<td>26</td>
</tr>
<tr>
<td>CYP2C9<em>1/CYP2C9</em>2</td>
<td>7</td>
</tr>
<tr>
<td>CYP2C9<em>1/CYP2C9</em>3</td>
<td>6</td>
</tr>
<tr>
<td>CYP2C9<em>2/CYP2C9</em>3</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C9<em>2/CYP2C9</em>2</td>
<td>1</td>
</tr>
</tbody>
</table>

We had shown that adverse drug reactions revealed during questioning or check-up (clinical aspects are the following: body weight gain, aggravation of epileptic seizures, increased fatigability, depression, aggression, defective memory, hepatopathy, splenomegaly, diffuse alopecia, menstrual disorders, bleeding disorder and so on; laboratory disorders: increasing level of SGPT, SGOT, increasing level of bilirubin, thrombocytopenia) in 50\% of cases were associated with homozygous or heterozygous carriage of mutant polymorphisms of gene CYP2C9 (Table 2). However, in all carriers of mutant polymorphic allelic variants (CYP2C9*2 and CYP2C9*3) both in homozygous and heterozygous conditions and also in case of their combination (for example CYP2C9*2/CYP2C9*3) adverse drug reactions were registered in 100\% of cases along with standard approach to the dosing of VPA drugs according to the guidelines of Pharmacopeia and International League Against Epilepsy (20-30 mg/kg/day for children, 20 mg/kg/day for adults) even in the period of titration of the daily dose (10-15 mg/kg/day for children, 5-10 mg/kg/day for adults).

The most severe adverse drug reactions were registered in patient, who was a homozygous carrier of minor allelic polymorphic variant of gene CYP2C9, in the form of aggravation of epileptic seizures, cognitive and behavioral disorders given standard dosing (per kg of body mass daily) of VPA drugs.

Clinical case 1. Patient Yu., 7 years old, is observed in Neurologic Centre of Epileptology, Neurogenetics and Brain Research of the University Clinic since August 2010. He complains of moderate simple and complex focal psychomotor epileptic seizures during the day time (up to 3-4 times daily), lasting for some seconds, and night seizures, which
appear during the sleep (up to 2-3 times nightly), lasting for some seconds without confused mental state after the seizure; and there are myoclonic seizures in left extremities, disinhibition, hyperactivity, weakness of right extremities, difficulties in learning educational material.

**Table 2. Frequency of adverse drug reactions of valproic acid at patients with different variants of genotypes CYP2C9**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency of the genotype No. (%)</th>
<th>Frequency of adverse drug reactions at the genotype Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9<em>1/CYP2C9</em>1</td>
<td>26 (63%)</td>
<td>13/26 50</td>
</tr>
<tr>
<td>CYP2C9<em>1/CYP2C9</em>2</td>
<td>7 (17%)</td>
<td>7/7 100</td>
</tr>
<tr>
<td>CYP2C9<em>1/CYP2C9</em>3</td>
<td>6 (15%)</td>
<td>6/6 100</td>
</tr>
<tr>
<td>CYP2C9<em>2/CYP2C9</em>3</td>
<td>1 (2.5%)</td>
<td>1/1 100</td>
</tr>
<tr>
<td>CYP2C9<em>2/CYP2C9</em>2</td>
<td>1 (2.5%)</td>
<td>1/1 100</td>
</tr>
</tbody>
</table>

In June 2003 at the age of 3 months the boy got a severe closed craniocerebral injury, underwent an operation (the resection of epidual hematoma on the left frontal lobe) in City Hospital №20 named after I. Berzon in Krasnoyarsk. At the age of one there was a single episode of atypical febrile seizures, AEDs were not received. At the age of 3-4 complex focal psychomotor and myoclonic seizures appeared, atonic seizures with fallings and repeated injuries and fractures of bones, as well as secondary generalized tonic-clonic seizures, the frequency of which was about 10 times daily. Since 2008 the child was prescribed VPA drugs by children's neurologist from the City Pediatric Polyclinic in Krasnoyarsk, it was Depakine–chrono with gradual titration of the dose (according to the instruction to AED approved by Pharmacological Committee of the Ministry of Public Health of RF 31 January 2002, protocol №2). During the last year the child took Depakine-chrono in the dose of 1250 mg/day (500 mg in the morning, 750 mg in the evening). The study of VPA level in serum was not conducted till the first visit to our centre. Given the beginning of conducted antiepileptic therapy the condition of the patient slightly improved - secondary generalized tonic-clonic seizures were cut off, at first focal seizures appeared less frequently but during the last year they became more frequent, the reason is vague. The mother of the boy notes the development of adverse drug reactions in the form of body weight gain of the child more than 10 kg for 1.5 year, growth of cognitive and behavioral disorders. In 2009 the child underwent reconstructive operation (plastic reconstruction of postoperative skull defect). The EEG of 2009 shows “instable focal slowing-down of cortical rhythmic activity in frontotemporal region on the left as well as photosensitive epileptic paroxysms during rhythmical photostimulation in high-frequency band”. Computer tomography of the brain (2009): focal glial-cystic-atrophic changes of brain substance of frontoparietal localization in the region of past brain injury.

Objectively: the child is hyperactive, disinhibited, the concentration is unstable and quickly weakening; slight delay in speech development and moderate cognitive disorders are noted. The boy is calmer without parents, fulfills diagnostic tests with minor mistakes, anxiety level is moderately increased. Asymmetry of cerebral and facial skull in frontotemporal region on the left, “hollow” left eye socket (slight enophthalmos), neurolymph “pad” in the region of posttrepanation achiloplasty of skull defect on the left. Slight alternating squint, right smoothed nasolabial fold, deviation of the tip of the tongue to the left by protrusion out of mouth cavity. No bulbar disorders are noted. There is central hemiparesis syndrome with mild locomotor function disorders.
After the check-up of the patient in our centre the following things were revealed. Video-EEG monitoring (2010): focal epileptic activity of high intensity in the frontal region of the right hemisphere with the phenomena of secondary bilateral synchronization according to three-dimensional localization of “Brain Loc” origin correlating with topography of posttraumatic glial-cystic-atrophic changes of the brain.

Molecular genetic testing (2010): mutant polymorphic allelic variant of gene CYP2C9 (genotype CYP2C9*2/CYP2C9*2, single nucleotide replacement of cytosine by thymine in the position 430 on 10q24.1-24.3; T/T), being a predictor of high risk of decreasing VPA metabolism (“superslow metabolizer”) and VPA accumulations in the patient’s organism and development of adverse drug reactions are revealed.

Therapeutic drug monitoring of VPA in serum against Depakine-chrono intake in the dose of 1250 mg/day or 31.25 mg/kg/day (17.08.2010): VPA cumulation with achievement of toxic level - 142 ug/ml is revealed (reference values are 50 - 100 ug/ml).

Therefore the data of molecular genetic testing (pharmacogenetics) and TDM correlated with clinical adverse drug reactions in child, including aggravation of epileptic seizures, behavioral and cognitive disorders in the form of aggression and anxiety syndrome, obesity. As far as after the first consultation in our centre we have explained the mother of the child the meaning of molecular genetic testing and TDM for detection of reasons of adverse drug reactions developed in boy, the parents of the boy, having received the foregoing results of the study 2 days before the planned repeated consultation of neurologist-epileptologist cancelled Depakine-chrono intake on their own (in the manner of self-treatment). During the second consultation the mother of the child noted that on the second day after cancellation of Depakine-chrono intake behavioral disorders in child decreased, daytime epileptic seizures disappeared but one nighttime complex focal epileptic seizure was fixed, the duration of this seizure was about 3-4 minutes longer than it was before. Neurologist-epileptologist of our centre conducted an explanatory talk with the child's parents about the danger of self-treatment and sharp reduction of AED dose or its sudden withdrawal. Stepped reduction of Depakine-chrono dose under VPA level control in serum in dynamics is recommended.

On the basis of clinical, laboratory and instrumental research methods the clinical diagnosis of the patient was specified: Symptomatic (posttraumatic) frontal epilepsy with simple and complex focal psychomotor seizures both with and without automatisms, myoclonic seizures of moderate frequency in right extremities, rare atonic seizures, secondary generalized myoclonic-tonic-clonic seizures in anamnesis, incomplete clinical-electroencephalographic compensation against VPA monotherapy (Depakine-chrono 1250 mg/day); homozygous carrier of minor polymorphic allele variant CYP2C9*2 (T/T) gene of isoenzyme 2C9 of cytochrome P450 (“superslow metabolizer” of VPA), high risk of VPA-induced adverse drug reactions. Adverse drug reactions are the following: VPA cumulation in serum at average therapeutic doses of Depakine-chrono, toxic syndrome, aggravation of epileptic seizures, behavioral disorders, VPA induced obesity of the 3 rd severity level.

We reduced the dose of Depakine-chrono from 1250 to 600 mg/day under control of VPA level in serum. L-carnitine (medication “Elcar”, RF) was recommended additionally for reduction of VPA-induced complications also. This medication is approved for use in pediatric neurological practice in RF. During the first months of dispensary observation the condition of the patient improved, behavioral disorders reduced considerably, frequency of complex focal seizures reduced twice, secondary generalized tonic-clonic seizures were not registered. During the repeated check-ups the child actively comes in contact with neurologist-epileptologist of the centre, anxiety syndrome and aggression are cut off, the child shows interest to the world around, toys and books.

In spite of the rarity of minor polymorphic allelic variant of gene CYP2C9, clinical meaningfulness of its early detection before the prescription of VPA drugs is extremely high, because it allows to prevention of severe adverse drug reactions and deterioration of life quality of patients.
In general the correlation between homozygous and heterozygous carriage of mutant alleles of gene CYP2C9 with frequency of adverse drug reactions development given VPA drugs intake was high ($p < 0.001; r = 0.55$) (Figure 1). However, in other 50% of clinical events the development of adverse drug reactions by VPA drugs intake was revealed in homozygous carriers of wild-type genetic polymorphism (genotype CYP2C9*1/CYP2C9*1). It induced us to the second stage of the present research within the bounds of which we analyzed fluctuations of VPA level in serum of homozygous carriers of wild-type allele of gene CYP2C9 and both heterozygous and homozygous carriers of mutant allele of the same gene. As a result of statistical analysis we have shown the complete correlated dependence of high degree between carriage of mutant alleles (the most high is in case of homozygous carriage) and VPA level in serum in the form of tendency to VPA cumulation up to subtoxic or toxic levels, in spite of antiepileptic medications intake in average therapeutic doses according to pharmacopoeia (Table 3).
Conclusion

The cumulation of VPA in the organism of patients with epilepsy (homozygous and heterozygous carriers of major and minor mutant polymorphic allele variants of gene CYP2C9 of cytochrome P450) could be explained by retardation of metabolism VPA in the liver. VPA is exposed to glucuronization, C-oxidation, CYP-catalyzed desaturation and hydroxylation (2-hydroxylation, 1-hydroxylation) in the liver. Although CYP-catalyzed VPA metabolism is quantitatively insignificant concerning another ways of metabolism, it is interesting due to toxic effect because of formation of unsaturated fatty acids which are intermediate products of VPA metabolism. Specifically for metabolite 4-ene-VPA there are hepatotoxic and nephrotoxic effects shown. Less than 15-20% of VPA dose is usually metabolized by other oxidative mechanisms. Less than 3% of a dose is excreted in an unchanged way with the urine. That is why the aggravation of seizures (their quickening and exacerbation) in patients under observation is explained by appearance of intermediate VPA metabolites that are unusual for homozygous carriers of wild-type allele of gene CYP2C9 (Shnayder et al., 2008; Ingelman-Sundberg, 2001).

The underestimation of this pathogenetical factor by neurologists leads to a misinterpretation: they considered the continuation or exacerbation of seizures on the stage of a VPA drugs dose titration not as an overdose but as a low, insufficient dosage. Thus patients got higher daily dose of VPA drugs that conduced to acute aggravation of patient’s condition and false attribution of the given epilepsy form to a drug-resistant one. And the VPA drugs were considered as “ineffective”, that was not true.

The study that we conducted allowed elaborating the Algorithm of starting therapy of epilepsy with VPA with the aim of the prevention of adverse drug reactions in high risk groups including:

- choice of VPA starting dosage,
- time-frames of TDM of VPA on the stage of dose titration,
- definition of a therapeutic dose of VPA in long-term intake,
- volume and time-frames of laboratory and clinical diagnostics.

We have proposed the Algorithm of VPA dosage.

In case of homozygous carrying of wild-type polymorphic allele variant of gene CYP2C9 (genotype CYP2C9*1/CYP2C9*1) the VPA dose titration is standard. Therapeutic drug monitoring of VPA is performed in 1 month from the setting of therapeutic dose, after that - once in 3 months.

In case of adverse drug reactions development and/or VPA accumulation in blood serum in homozygous carriers of wild-type allele variant of gene CYP2C9 (genotype CYP2C9*1/CYP2C9*1) it is recommended to perform an additional molecular-genetic testing in order to reveal mutations of other isoenzymes of cytochrome P450 that take part in VPA metabolism (for example, genes CYP1A1, CYP2D6, CYP2E1, CYP1A2).

The starting VPA dose in case of heterozygous carrying of mutant polymorphic allele variants of gene CYP2C9 (genotypes CYP2C9*1/CYP2C9*2 or CYP2C9*1/CYP2C9*3) must be lower than the recommended one to 25-30%, and rates of dose titration slower with regular dynamic TDM-control of VPA rate in blood serum on titration stage - not less than once in 2 weeks. Average VPA therapeutic dose must be lower than the recommended one up to 20-30% depending on the patient’s age.

In homozygous carrying of major (genotype CYP2C9*3/CYP2C9*3) or minor (genotype CYP2C9*2/CYP2C9*2) polymorphic allele variants of gene CYP2C9 of isoenzyme 2C9, and also in their combination (genotype CYP2C9*2/CYP2C9*3), the starting VPA dose ought to be lowered up to 50% from the recommended one; the titration rates are twice lowered with a dynamic regular VPA rate control in blood serum not less than once a week during the first month, after that - not less than once in 1-2 months.

Thus, even on the first consultation of patients with epilepsy or epileptic symptoms, when the type of seizures or/and the clinic form of epilepsy requires the antiepileptic therapy
administration with VPA drugs, the neurologist-epileptologist can mark out 3 possible risk
groups of adverse drug reactions, considering the results of pharmacogenetic research of
polymorphic allele variants of gene CYP2C9 of isoenzyme 2C9 of cytochrome P450.
The Stratification of the development of adverse drug reactions risk groups during VPA
drugs intake:
- low risk group («widespread metabolizers») - homozygous carriers of wild-type allele of
gene CYP2C9 (genotype CYP2C9*1/CYP2C9*1);
- average risk group («slow metabolizers») - heterozygous carriers of mutant allele
variants of gene CYP2C9 (genotypes CYP2C9*1/CYP2C9*2 or
CYP2C9*1/CYP2C9*3);
- high risk group («super-slow metabolizers») - homozygous carriers of minor or major
polymorphic allele variants of gene CYP2C9 (genotypes CYP2C9*2/CYP2C9*2 or
CYP2C9*3/CYP2C9*3) or their heterozygous combination (genotype
CYP2C9*2/CYP2C9*3).
The Algorithm and Stratification of the development of adverse drug reactions risk groups
during VPA drugs intake were elaborated form the point of view of personalized medicine
(pharmacogenetics). They were introduced into the daily practice of neurologists-
epileptologists of the Neurological Centre of Epileptology, Neurogenetics and Brain
Research of the University Clinic and into the work of doctors and laboratory assistants of
the CSRL of the Krasnoyarsk State Medical University named after Professor V.F. Vojno-
Yasenetsky (we were given the implementation statements dated from 28 March 2010).
This technique has been presented to discussion during the International Conference on
Clinical Pharmacology (the breakup group «Clinical pharmacogenetics») in Kazan, RF in
October, 2010, and it has also been presented at the International exhibition “Yenisey-
Medica 2011” (Krasnoyarsk, RF).

Summary

Certain polymorphic genes of cytochrome P450 can be used as markers for optimization
of the drug therapy of epilepsy. The personalized approach to VPA drugs dosing in
treatment of children and adults suffering from epilepsy is clinically grounded.
It is likely that predictive genotyping is of benefit in 10-20% of drug treatment and
thereby allows for prevention of causalities as a cause of adverse drug reactions and thus
improves the health for a significant fraction of the patients. In 15-40% of the cases, the
penetration of genetic polymorphism is of less importance because of the polygenic
influence on the outcome of drug treatment and in 50% of the cases, pharmacogenetics
would be without influence because of other more important physiological and
environmental factors (Ingelman-Sundberg M., 2001).
The personalized approach to VPA drugs therapy with consideration of pharmacogenetic
traits of the metabolism is effective medically, socially and economically. Its approach can
be recommended for a broad introduction into neurologist’s practice.

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