Genes polymorphism that determine effectiveness of anticancer and antimicrobial agents

Polymorphism of the genes of the second phase of biotransformation - conjugation, for example **N-acetyltransferase**, determines the activity of the corresponding reactions in the liver. Arylamine-N-acetyltransferases (NATs) are a unique group of enzymes that play an important role in drug detoxification (isoniazid, sulfonamides, etc.) and activation of carcinogens. There are two isoenzymes (NAT1 and NAT2) encoded by polymorphic genes in human. Significant variation in drug acetylation is associated with NAT2 gene polymorphisms. The NAT2 enzyme is synthesized mainly in the liver, but is detected in small amounts in other tissues: in the skin, lungs, and kidneys. NAT2 gene polymorphism determines extensive, intermediate and slow types of acetylation. On the one hand, the genotype of the NAT2 *slow acetylator* can be an important determinant of the development and clinical course of a number of diseases (diabetes mellitus, bladder cancer, etc.). On the other hand, the NAT2 polymorphism is important for the rate of inactivation and, accordingly, the concentration of various drugs, including isoniazid, co-trimoxazole, novocainamide, etc..

At the end of the 1960s, it was established that the concentration of isoniazid in the blood after the administration of the same dose largely depends on the rate of its acetylation in the liver, according to which patients were divided into "extensive", "intermediate" and "slow acetylators". To determine the phenotype, the patient was administered a standard dose of the sulfonamide drug and the amount of acetylated sulfonamide derivative in the urine was determined after a certain period of time. Today, individuals are divided into "slow" and "fast" inactivators (acetylators) according to the amount of active isoniazid excreted in the urine relative to the dose taken. The first category includes patients in whom up to 10% of isoniazid is excreted in the urine per day, and the second - more than 10% per day. At the same time, the half-life of isoniazid in the blood plasma with rapid acetylation is 0.5–1.6 hours, with slow acetylation it is 2–4 hours.

In the early 2000s, the daily dose of isoniazid was 5-15 mg/kg of body weight per day (600-900 mg on average), which allowed to ignore the acetylation genotype, since this dose ensured the achievement of a therapeutic concentration of isoniazid regardless of the *NAT2* genotype. But after the introduction of the DOTS (directly observed treatment short-course) treatment strategy in 2006, the dose of isoniazid was reduced to 4-6 mg/kg per day (300-400 mg on average). The dynamics of the concentration of isoniazid during the day shows significant differences in the achievement of the therapeutic concentration of isoniazid according to the *NAT2* genotype, which indicates the importance of the *NAT2* polymorphism for maintaining the therapeutic concentration of isoniazid in the blood during TB treatment. For example, in "fast acetylators" the concentration

of isoniazid remains above the recommended minimum therapeutic concentration up to 13 hours after the introduction of the drug, in "slow acetylators" - up to 18 hours after the introduction of a standard dose. Research conducted in the Odesa region in 2014 showed that around 46% of the population are "rapid acetylators" according to the NAT-2*5,*6 genotype, while among tuberculosis patients - 38%.

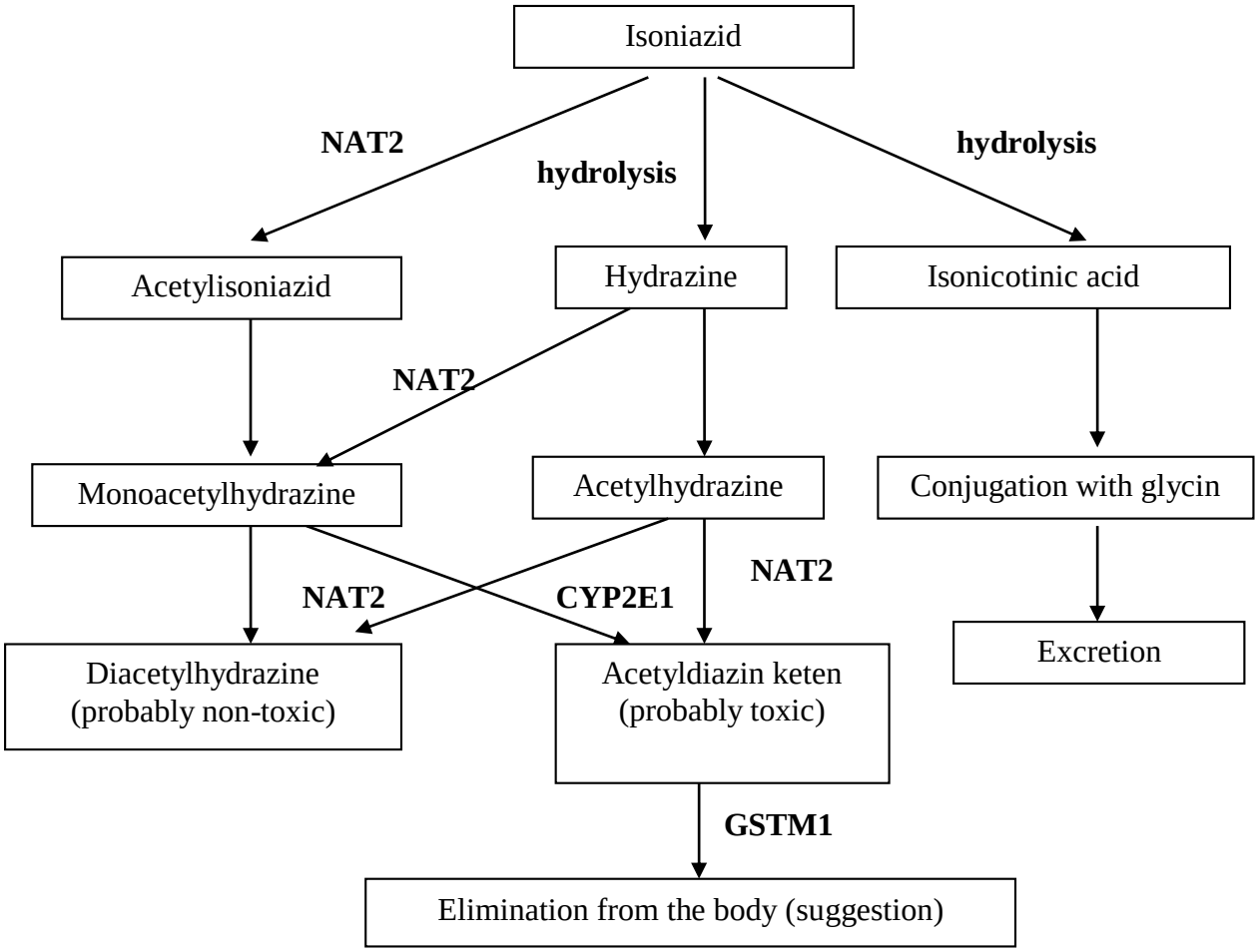

Fig. 1. Hypothetical metabolic cycle of isoniazid (згідно Geetha Ramachandran, Soumya Swaminathan, 2012).

At the same time, "*slow acetylators*" have an increased risk of developing hepatotoxicity (increased bilirubin content, activity of cytolysis markers - alanine aminotransferase and aspartate aminotransferase, etc.). To understand the dependence of isoniazid toxicity depending on the genotype/phenotype of acetylation, it is necessary to refer to the isoniazid biotransformation scheme (fig. 1).

Cytochrome P-4502E1 (CYP2E1 gene) participates in cell damage through the formation of reactive oxidation products and participates in the metabolism of acetone, benzene, benzopyrene, carbon tetrachloride and other compounds. As a result, hydrogen peroxide and free

radical peroxide and hydroxyl are formed, which causes damage to organs and, above all, to the liver. A change in the amount or activity of an enzyme leads to a change in the risk of damage to the body. The enzyme is also involved in the elimination of N-nitrosamines from tobacco smoke - carcinogens that also cause breast cancer.

Isoinazide and its metabolites can increase the activity of CYP2E1 in "*rapid metabolizers*". On the other hand, in patients with the genotype of "*intermediate*" and "*slow metabolizers*", isoniazid can inhibit CYP2E1 activity. According to fig. 1 CYP2E1 is involved in the formation of the toxic metabolite of isoniazid, and possibly other hepatotoxins. Therefore, the presence of the *CYP2E1*DD* genotype ("*rapid metabolizers*") is associated in tuberculosis patients with a higher risk of developing hepatotoxicity than with the presence of variant genotypes - **CD* ("*intermediate metabolizers*"), **CC* ("*slow metabolizers*"). Research conducted in the Odesa region in 2014 showed that according to the *CYP2E1* genotype 82% of the population are "*rapid metabolizers*", among tuberculosis patients - 89%.

Glutathione-S-transferases (GST) are a group of enzymes involved in the detoxification of carcinogens, toxic substances and drugs. Glutathione-S-transferase ensures the conjugation of glutathione with the substrate. This contributes to the removal of xenobiotics from the body. In total, GST enzymes are encoded by at least five different DNA regions, of which *GSTM1* and *GSTT1* are associated with hepatotoxicity. Research conducted in the Odesa region in 2014 showed that around 50% and 81% of the population are "rapidt metabolizers" according to the GSTM1 and GSTT1 genotype, respectively (Ostapchuk K.V. etc., 2015). GST enzymes play an important role in the metabolism of isoniazid. Glutathione binds free radicals intracellularly by conjugation with toxic metabolites of isoniazid. A decrease in GST activity under conditions of the del GSTM1, del GSTT1 ("*slow metabolizers*") genotype can increase the risk of liver damage, including the development of chronic hepatitis C. The negative influence of the del GSTM1, del GSTT1 ("*slow metabolizers*") genotype has been established in patients with tuberculosis on the processes of detoxification and accumulation of metabolites in the body, which causes the development of toxic and allergic reactions. In patients with pulmonary tuberculosis, genotypes *del GSTM1*, *del GSTT1* ("*slow metabolizers*") are associated with disorders of the excretory function of the kidneys under the influence of anti-tuberculosis drugs. In 100% of patients with a deletion of the *GSTT1* gene, a violation of the excretory function of the kidneys is recorded at the pre-hospital stage of treatment (Bazhora Yu.I. etc., 2011).

Magnesium superoxide dismutase. It is known that products of lipid peroxidation (LPO) have a hepatotoxic effect. The enzyme magnesium superoxide dismutase reduces the level of lipid products in the mitochondria. The appearance of mutated allele C of the magnesium superoxide dismutase (SOD) gene is characterized by a higher risk of hepatotoxicity from the use of

antituberculosis drugs. This is probably due to the appearance of the amino acid valine, which increases the formation of toxic hydrogen peroxide, which in turn leads to hepatotoxicity.

Rifampicin, which is an important first-line antituberculosis drug, is a substrate of organic anion transporter peptides (OATP) and P-glycoprotein. Organic anion transport peptides play an important role in the transport and disrtibution of medicinal substances in the human body. It is known that the genotype of organic anion transporting peptides *SLCO1B1 463CA* is characterized by a lower content of rifampicin in the blood than with the *SLCO1B1 463CC* genotype. Patients with intermediate and slow activity genotype *SLCO1B1 rs4149032* differ in reduced bioavailability of rifampicin by 20% and 28% compared to individuals with high activity genotype. Therefore, in the presence of the genotype of moderate and slow activity of *SLCO1B1*, it is necessary to increase the dose of rifampicin.

Sulfonamide drugs (**co-trimoxazole, sulfasalazine**) are metabolized in the liver with the participation of the enzyme (NAT2). Carriers of the "*slow metabolizers*" genotype are likely to increase the concentration of drugs and, accordingly, increase their toxicity.

Antiretroviral drug - **abacavir** - in the presence of the *HLA-B*57:01* allele, the risk of allergic reactions increases, therefore, in this case, abacavir should not be used. The administration of another antiretroviral drug, efavirenz, in "*slow metabolizers*" is accompanied by an increase in the concentration in the blood and an increase in the risk of side effects (prolongation of the QT interval).

The enzyme **thiopurine S-methyltransferase (TRMT)** catalyzes the S-methylation reaction. This is the main way of metabolism of cytostatics (**mercaptopurine, azathiopurine and thioguanine**). Although the low efficiency of TRMT is inherited by an autosomal recessive type, increased sensitivity to thiopurines is noted not only in homozygotes ("*slow metabolizers*"), but also in heterozygotes ("*intermediate metabolizers*"). Eight different alleles are known, encoding an enzyme with low activity, which leads to a violation of the metabolism of anticancer drugs. In the presence of such alleles, it is necessary to reduce the standard dose of cytostatics by 2-4 times, since toxicity (immunosuppression) increases significantly. The prevalence of homozygotes for all allelic variants of the *TPMT* gene among residents of Europe and Africa is 4-5%. Safe doses of mercaptopurine for patients homozygotes for the mutant allele are 10-15 times lower than average therapeutic doses, among heterozygotes - 2-4 times. To ensure the safety of chemotherapy with mercaptopurine (acute lymphoblastic leukemia, lymphomas), it is necessary to carry out phenotyping (TPMT activity in erythrocytes) or genotyping for mutant variants of the *TPMT* gene. In clinics in Europe and the USA, one of these typing procedures is mandatory before starting

treatment. According to the recommendations of the FDA, in the presence of the genotype of "*slow metabolizers*" (*TPMT* and/or *NUDT15 gene*), it is necessary to replace azathioprine with another drug; in the presence of "*intermediate metabolizers*" it is necessary to reduce the dose of the drug. While using mercaptopurine or thioguanine in the carriers of "*slow metabolizers*" genotype (*TPMT* and/or *NUDT15* gene), it is necessary to reduce the dose of the drug, since patients can tolerate only 10% of the usual dose or less; in "*intermediate metabolizers*" - it is necessary to significantly reduce the dose according to tolerance.

Dihydropyrimidine dehydrogenase (DPDH, DPYD). The physiological function of the DPDH enzyme is to restore uracil and thymidine. In addition, DPDH is the main enzyme that metabolizes fluorouracil. Fluorouracil is widely used as part of combined chemotherapy for cancer of the breast, ovaries, esophagus, stomach, colon and rectum, liver, bladder, prostate, tumors of the head, neck, salivary glands, adrenal glands, and pancreas. Since the mid-1980s, there have been reports of severe complications arising from the use of fluorouracil in individuals with low DPDH activity. Low activity of DPDH is inherited according to the autosomal recessive type (that is, in both "*slow*" and "*intermediate metabolizers*"). In patients with low DPDH activity, a longer half-life of fluorouracil is noted - up to 160 minutes, with normal - 8-22 minutes. There is a clear relationship: the lower the activity of DPDH is associated with more severe side effects (neurotoxicity, cardiotoxicity) of fluorouracil and even fatal consequences. Genetic studies have revealed a number of mutations in the DPYD gene, which encodes this protein, which are responsible for reduced activity of this enzyme and increased sensitivity to fluorouracil. Therefore, today it is recommended to introduce DPYD genotyping into genetic practice, and when the genotype of "*intermediate/slow metabolizers*" is detected, it is probably better to replace the drug with another one.

UDP-glucuronyltransferase (UGT). Glucuronidation is the most important reaction of the II phase of drug metabolism. UDF is added to medicines due to catalysis with the help of UDF-glucuronyltransferase enzymes, which include two families and more than 20 isozymes. They catalyze a large number of drugs (morphine, chloramphenicol, paracetamol, etc.), their metabolites, hormones, carcinogens. The physiological function of UGT is glucuronidation of endogenous compounds (for example, bilirubin). Medicines from the following groups undergo glucuronidation: phenols (propofol, paracetamol); alcohol (chloramphenicol, codeine, oxazepam); aliphatic amines (lamotrigine, amitriptyline); carboxylic acids (phenylbutazone, etc.); carboxylic acids (naproxen, ketoprofen). A hereditary disorder of bilirubin glucuronidation is observed in Gilbert's syndrome. Mutations in the *UGT1* gene lead to the synthesis of UGT with 25-30% less activity compared to the norm, therefore patients with Gilbert's syndrome have a decrease in the clearance of tolbutamide, paracetamol, and rifampicin. Other genetic polymorphisms of genes

encoding different UGT isoforms affect the pharmacokinetics and pharmacodynamics of lorazepam, morphine, carvedilol, and other drugs. *UGT1A1* gene polymorphism testing is approved in the United States for correction of irinotecan therapy.

When the anticancer drug **irinotecan** is administered to patients with the *UGT1A1*28/*28* genotype ("*slow metabolizers*"), there is an increase in the concentration of active metabolites in the blood and an increase in the risk of side effects due to profound neutropenia. In this case, the issue of reducing the initial dose with a subsequent gradual increase according to individual tolerance is considered.

The introduction of the immunosuppressant **tacrolimus** in "intermediate" and "extensive metabolizers" according to the *CYP3A5* genotype leads to a decrease in the concentration of the drug in the blood and a decrease in the effectiveness of the drug. Therefore, it is recommended to measure the concentration in the blood and adjust the dose accordingly.

The anticancer and antiestrogenic drug **tamoxifen** is metabolized with the participation of the CYP2D6 enzyme. In the presence of the "*intermediate*" or "*slow metabolizers*" genotype, a decrease in the concentration of the active metabolite in the blood is observed, but the clinical significance of this phenomenon remains unclear.