The role of isoniazid pharmacogenetics in the individualized treatment of tuberculosis

Rolul farmacogeneticii izoniazidei în tratamentul individualizat al tuberculozei

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<td>Tuberculosis (TB) is a major health problem, a disease that ranks first regarding mortality associated with an infectious agent and for which sustained efforts are made to reduce the global incidence up to disappearance. The treatment is for a long period, while the presence of adverse effects, especially hepatotoxicity (HT), and the noncompliance overshadow the success of therapy. Isoniazid (HIN) has a strong effect on TB management and, along with rifampicin and pyrazinamide, is most likely to induce HT. HIN metabolism by N-acetyltransferase, cytochrome P450E1, glutathione S transferase, liable to genetic polymorphisms, are the main ways to induce HT and HIN resistance. Determining the genetic profile of major enzymes involved in the metabolism of anti-TB drugs provides patient information that allows the choice of the appropriate treatment and an optimal dose for the therapeutic success. The clinical implementation of genetic testing increases the treatment efficiency and is a huge step in eradicating tuberculosis. Keywords: tuberculosis, isoniazid, CYP2E1, NAT2, hepatotoxicity</td>
<td>Tuberculosa (TB) reprezintă o problemă majoră de sănătate, ocupând primul loc în privința mortalității printre bolile asociate unui agent infecțios și pentru care se fac eforturi susținute de scăderea până la dispariția incidenței mondiale. Tratamentul tuberculozei este de lungă durată, prezintă efectelor adverse, în special hepatotoxicitatea (HT), și a necomplianței umbrind succesul terapiei. Isoniazida (HIN) are un puternic efect în managementul TB, alături de rifampicină și pirazinamidă fiind cele mai predispuse să inducă HT. Metabolizarea HIN, cu ajutorul N-acetyltransferazei, citocromului P450E1 și a glutatinei S transferazei, predispune la polimorfisme genetice, fiind principalele căi de apariție a HT și a rezistenței la HIN. Determinarea profilului genetic al principalelor enzime implicate în metabolizarea medicamentelor antituberculoase aduce informații pentru pacient, permitând alegerea tratamentului adecvat și a unei doze optime pentru succes terapeutic. Implementarea clinică a testării genetice crește eficiența tratamentului și reprezintă un pas ușor în eradicarea tuberculozei. Cuvinte-cheie: tuberculoză, isoniazidă, CYP2E1, NAT2, hepatotoxicitate</td>
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Introduction

Tuberculosis (TB) represents an important challenge worldwide, being the first cause of death from a single infectious agent, an infectious disease for which the World Health Organization (WHO) makes huge effort to have an end TB strategy. In order to reduce the TB incidence and mortality, including the reduction of global TB incidence by 80% by 2030 and by 90% by 2035, and of the global TB mortality by 90% by 2030 and by 95% by 2035, there are management and guide strategies to achieve these targets, at global and national levels, for preventing, diagnosing and treating TB(1). In 2017, 10 million people developed TB, which caused 1.3 million deaths. Even though Romania is not in the first 30 countries with a high TB incidence reported by WHO, our country is situated now on the first place in the European Union and on the fourth place in Europe(2). Only the correct long-term treatment leads to the cure of the disease, and pharmacogenetics brings to light important individual aspects regarding the therapeutic response, adverse effects and the toxicity of the administered drugs.

Drugs used to treat TB are antibiotics, which are ranked according to the level of the attack line. The first-line therapeutic agents are isoniazid (H or HIN), rifampicin (R), pyrazinamide (Z), ethambutol (E), rifabutin, and rifampentine, with a regimen of 2HRZE/4HR for a 6-month treatment of non mono- or multidrug-resistant tuberculosis (MDR-TB)(3,4). For the determination of resistance of Mycobacterium tuberculosis (MTB), the bacteriological examinations that can be used include sputum smear microscopy, culture and drug susceptibility testing, as well as molecular techniques such as Xpert® MTB/RIF assay and rapid line probe assay (LPA) for resistance to rifampicin and isoniazid, and to fluoroquinolones and injectable anti-TB drugs (referred to as a second-line LPA)(5). Xpert® MTB/RIF is the most common method for diagnosis and for rifampicin drug-susceptibility testing, but it lacks the ability to test for isoniazid resistance, and the patients with isoniazid resistance are at higher risk of developing additional drug resistance(6).

Isoniazid, introduced 66 years ago in therapy, quickly reaches all body fluids and tissues by achieving similar intracellular and extracellular levels, inhibiting the synthesis of mycolic acids. But the antituberculosis drugs also induce liver injury. H, R and Z may induce hepatic damage, especially isoniazid.

Particularities of isoniazid metabolism

The pro-drug H enters the cytoplasm of MTB through simple passive diffusion and kills only actively dividing bacteria and no stationary phase or mycobacteria growing under anaerobic conditions. The inactivation is done in the liver by acetylation, hydrolysis and again acetylation with...
the vital role of N-acetyltransferase (NAT) (Figure 1 and Figure 2), and also cytochrome P450 2E1. Noncompliance associated with TB treatment leads to resistance. The rate of H-resistant TB cases is increasing globally and represents a negative outcome of TB treatment and the possibility of acquiring additional drug resistance\(^5\). Also, InhA promoter mutations lead to low level H resistance, while mutations in katG lead to high level resistance\(^5\). The mutation in katG gene is the major cause for H resistance, followed by inhA, ahpC, kasA, ndh, iniABC and fadE\(^5\). The nat gene, a cluster gene, coding for NAT, is unique, as it is the sole gene involved in the metabolism of H in MTB prior to katG activation\(^8\).

At present, most research on the metabolism of drugs already in therapy or on new drugs study the reactions catalysed by the group of enzymes cytochrome P450 (CYP), the major biotransformation, which has more than 1,000 isoenzymes, of which five (CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2) metabolize 90% of all drugs. From 73% by drugs that can be metabolized, type CYP3A isoenzyme metabolizes 46% of drugs, type CYP2C9 isoenzyme metabolizes 16% of drugs, type CYP2C19+CYP2D6 isoenzymes metabolize 12% of drugs, type CYP1A1 isoenzyme metabolizes 9% of drugs, and type CYP2B6+CYP2E1 isoenzymes metabolize 2% of drug\(^9\). Drug metabolizing enzyme (DME) activity has been divided into two categories, phase I and phase II. Phase I is characterized by oxidative metabolism, resulting in pharmacological inactivation or activation, facilitated elimination and/or addition of reactive groups for subsequent phase II conjugation. Of interest are phase I DMEs with CYP enzyme and phase II DMEs with glutathione S-transferases (GST), NAT\(^10\). The CYP2E subfamily has only been described in mammals and there is only one gene in the CYP2E locus in humans. CYP2E1 is constitutively expressed in the liver and its expression is regulated by different cytokines; thus the level of CYP2E1 is decreased by IL-1, IL-6 and TNF-\(\alpha\) and II-4 induces the expression of CYP2E1\(^11\). Very low CYP2E1 expression is found in brain, kidney and lung\(^12\). There is an increased risk of drug-induced hepatotoxicity by CYP2E1 in individuals carrying the C1/C1 genotype of the CYP2E1 RsaI/PstI polymorphism\(^13\).

In humans, there are two NAT genes (NAT1 and NAT2) which are located on the short arm of chromosome 8. NAT activity has a dominant autosomal transmission. 53 alleles of NAT2 and 27 alleles of NAT1 have been identified\(^14,15\). “Wild type” alleles are considered NAT2*4 and NAT1*4, which exhibit normal enzyme activity. Allele variants present one or more nucleotides, leading to decreased expression, decreased activity or enzymatic instability. Both NAT1 and NAT2 alleles possess a particular combination of SNPs associated with the slow acetylator phenotype\(^16,17\). NAT2*4 allele is the most common NAT2 allele, conferring the rapid acetylator phenotype, and is associated with increased metabolism and clearance of H with the polymorphic NAT2 alleles of the main allelic groups *5, *6, *7 and *14 encode for slow acetylator enzyme variants that may compromise the drug-metabolizing ability of individuals\(^18\). The slow acetylator status of NAT2, associated with the NAT2*6A/6A diplotype, is a risk factor for the induction
of isoniazid-induced high hepatotoxicity. The NAT2*6A genotype is correlated with increased risk of hepatotoxicity, and the NAT2*4 genotype with moderate hepatotoxicity. This makes NAT2*6A a new biomarker useful in the prognosis of hepatotoxicity induced by this drug(25-27).

The slow acetylators are homozygous (ss), while the intermediates are heterozygous (Fs) and fast homozygous (FF). So, we distinguish between slow release individuals and individuals with rapid inactivation of isoniazid or intermediate. Poor acetylators are more likely to develop hepatotoxicity, especially if they are treated with rifampicin concomitantly. Besides the fact that isoniazid will be acetylated slowly, rifampicin is an inducer of metabolic enzymes, this association making it very likely to activate some metabolic pathways that will lead to the formation of toxic compounds. It should be added that in the absence of concomitant rifampicin, the risk of hepatotoxicity remains increased at slow activity. Slow acetylation will not only affect the first stage starting from isoniazid, but the second acetylation for diacetylhydrazine is also slowed down, as the concentration of unchanged isoniazid is higher because it will compete with the hydroxylamines resulting from the oxidation of acetylhydrazine, both of which are substrate for N-acetyl transferase. Therefore, toxic compounds will accumulate. Another pathway to inactivate and eliminate hydroxylamines is GST and the polymorphisms of the GSTM1 and GSTT1 genes are incriminated in liver injury. The association of NAT2, GST and CYP2E1 polymorphisms in line contribute to liver injury(24).

NAT2 enzyme is associated with classical acetylation polymorphism, while NAT1 controls distinct polymorphism with respect to para-aminosalicylic acid, para-aminobenzoic acid, sulfamethoxazole, and arylamine carcinogenic substances. NAT2 is responsible for the metabolism of many compounds, including sulfamides (sulfadiazine, sulfadoxine, sulfamerazine, sulfamethazine and sulfapyridine), various other drugs (aminoglycosides, dapsone, endralazine, hydralazine, isoniazide, prazidilol, procainamide), clonazepam and arylamine carcinogenic substances.

Clinical aspects

In clinic, slow acetylators could benefit from reduced daily H doses at 150 mg once a day, and would maintain high treatment efficacies and simultaneously reduce the exposure to the toxic metabolites hydrazine and acetylhydrazine in the off target organ, the liver. For intermediate and fast acetylators, the H dose administered once a day could be increased up to 600 mg and 900 mg, respectively, without toxicity. Another important aspect is that H doses and administration regimens must be used for immuno-competent and immune deficient individuals of all acetylator phenotypes. Also, studies showed that slow acetylator phenotypes were selectively favored in populations with reduced folate supply, whereas the fast acetylators were neutral or even advantaged in the presence of folate-rich diets and also, diet and herbal medications are suggested as a possible cause for NAT2 genotype-phenotype discordance – for example, carrot, cruciferous vegetable and grapefruit consumption is related to NAT2 activity.

Regarding CYP2E1, the resulting oxidative stress and therefore liver injury are exacerbated by a diet low in carbohydrates and rich in fats, which promote CYP2E1 induction because fatty acids and ketones are substrates of CYP2E1. Also, CYP2E1 is the only P450 isoform that has been shown to be active in the conversion of acetone to acetal, an intermediate in the gluconeogenesis pathway.

Garlic possesses volatile oil that contains active principles such as diallyl sulfide (DAS), which acts as a potential biological antioxidant and a compelling free radical scavenger in suppressing the actions of reactive oxygen species, protecting the biomolecules against oxidative damage, and also is a potential therapeutic or prophylactic agent in liver injury because of its inhibitory actions on CYP2E1-mediated metabolic activation of various chemicals and carcinogens. DAS is capable of inhibiting CYP2E1 activity and with dexamethasone (DEX) it minimizes the steatotic-induction ability of H. Serum ALT and AST levels are also reduced in the pre-treatment with DAS and DEX, maintaining their levels near normal and conferring protection, which is confirmed by histopathological findings. Another possible mechanism to reduce the hepatotoxicity of MTB medication is Sagittaria sagittifolia polysaccharide, which is used as a protective agent against hepatotoxicity induced by the coadministration of isoniazid and rifampicin. There were observed both lower transaminase levels, and the effective reduction of the pathological tissue damage. Another choice is polyherbal formulation or bicyclol.

H is excreted in urine (80%), unmodified, acetylated or conjugated. Less than 10% of the oral INH dose is excreted in the feces. As expected in slow-release individuals, a higher urinary concentration will occur for the unmodified form.

H induces hepatotoxicity in most cases. During treatment, it is strongly recommended to monitor the alanine aminotransferase (ALT) levels for patients who are old, have low body weight/malnutrition, in chronic alcohol users, in those who use concomitant other hepatotoxic medicinal products, have viral hepatitis B or C, with preexisting liver disease/transplantation or only a high level of ALT, for those who have a history of liver failure after treatment with isoniazid, for women during pregnancy or during the first three months postpartum. In addition to these patients, the ALT value should also be monitored in AIDS patients and in those with specific genetic conditions. Some experts recommend even the biochemical monitoring of all patients over 35 years of age. The treatment should be discontinued and, generally, the medical regimen of patients with ALT values three times the normal in the presence of hepatitis or jaundice should be modified. In the absence of these, changes in the treatment regimen should be made when the ALT value exceeds five times the normal value, but weighing the situation. The patients may also have been prescribed Z, which is another risk factor associated with hepatotoxicity in addition to H and R. The
presence of HLA-DQB1*0201 is an independent risk factor for the development of TB liver injury (35).

Twenty-eight days of exposure to first-line anti-TB drugs show alterations in the histopathology of the liver. Liver cells are showing hypertrophy, hyperplasia, and multiple foci of Kupffer cell infiltration of sinusoids. Lobular inflammation is seen. Hemorrhagic lesions are seen throughout the hepatic lobule (36).

Another mechanism for liver injury is that H is capable of directly activating macrophages and could lead to immune-mediated liver toxicity or autoimmunity; also, it can induce a lupus-like syndrome (35).

Slow acetylators seem to be up to 50% in the Caucasian population, 70% in Africa (Ethiopia), high in Asia, with implication in liver injury, therefore the need for NAT2 genetic characterization for the treatment optimization targeting the individual/patient (18, 23, 34, 36).

Conclusion

For tuberculosis patients, genetic testing to determine the acetylator status before starting the treatment would be useful in the risk of isoniazid-induced hepatotoxicity. Pharmacogenetic data, along with phenotypic and genotypic testing, are useful for patients with tuberculous failure which cannot be attributed to therapeutic nonadherence or chemosensitivity. Determining the genetic profile of major enzymes involved in the metabolism of anti-TB drugs provides patient information that allows the choice of the appropriate treatment and an optimal dose for therapeutic success, and the clinical implementation of genetic testing increases treatment efficiency and is a huge step in eradicating TB.

More studies are needed for the clinical pharmacogenetics implementation guidelines for NAT2 and cytochrome P450 2E1 (CYP2E1) genotype and isoniazid therapy.

References