PAEDIATRIC PHARMACOKINETICS AND PHARMACODYNAMICS OF AZATHIOPRINE

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The objective of the research is to find the best strategy to optimize the dosage of the immunosuppressive agent azathioprine (AZA), given to paediatric patients affected by Inflammatory Bowel Disease (Crohn disease and ulcerative colitis). It will consider individual phenotype and genotype characteristics that could influence drug's pharmacodynamics and pharmacokinetics, with particular attention to the enzymes involved in the metabolism of the drug.

Inflammatory Bowel Diseases (IBD) are a group of illnesses that affect the intestine, characterised by a chronic inflammation of the intestinal lining. The precise cause of this inflammation is not known, even if it is clear that genetic, infective, immunologic and emotional factors are involved in the beginning and in the development of the disease.

Because there is no cure for IBD, the goal of the medical treatment is to suppress the inflammatory response. Several groups of drugs are used to treat IBD. They are

- Aminosalicylates: sulfasalazine and oral formulations of mesalazine. These medications typically are used to treat mild to moderate symptoms.
- Corticosteroids: prednisone and methylprednisolone are given orally and rectally. They are used to treat moderate to severely active disease. These drugs have significant short- and long-term side effects and are not used as a maintenance medication.
- Antibiotics: metronidazole, ciprofloxacin.
- Immunomodifiers: AZA, 6-mercaptopurine (6-MP), methotrexate, infliximab.

AZA is used as a support or alternative to steroids therapy, in particular:

- to reduce the severity of the illness
- to maintain remission
- to prevent relapse
- to reduce steroids dosage.

AZA is the first choice in subjects resistant to corticosteroids. Despite proven clinical efficacy, not all patients respond favourably; at least 30% of patients with steroid refractory disease fail to respond to the standard dosages used in most published studies. The wide dose range of AZA used in clinical practice today would suggest that a safe and established therapeutic dose has not yet been defined.
AZA is methyl-nitroimidazolyl-6-mercaptopurine, a pro-drug believed to act *in vivo* by the conversion to an active form. The first step in the bio-transformation of AZA involves conjugation with sulphide groups and formation of 6-MP, the active metabolite of the drug. The cytotoxic activity of 6-MP is due to the conversion of the drug to monophosphate nucleotides: this reaction is catalyzed by hypoxanthine phosphoribosiltransferase (HPRT). These thionucleothides (6-TGNs) cause cytotoxicity by interference with purines biosynthesis and metabolism. Some experimental results suggest that AZA has an immunosupressive activity apart from its antimitotic effect. It is possible that the nitroimidazole group of the molecule is responsible for this biological activity, but more studies are needed to prove this hypothesis.
The major adverse effect of AZA is bone marrow suppression with leucopoenia; other important side effects include increased susceptibility to infections, hepatotoxicity, alopecia, pancreatitis and an increased risk of neoplasia.

AZA and 6-MP are rapidly absorbed after oral administration and undergo extensive hepatic first pass metabolism.
The main metabolic pathways involved are:

- oxidation to thiouric acid by xanthine oxidase (XO)
- thiol methylation catalyzed by thiopurine methyltransferase (TPMT).

There is a little inter-individual or interethnic variation in the activity of XO in the healthy population.

On the other hand, TPMT activity is inherited as an autosomal codominant trait, exhibiting genetic polymorphism in all the populations studied to date. Genetic polymorphism in the TPMT gene is such that 90% of Caucasians have high TPMT activity, 10% have intermediate activity and 1 in 300 individual has low activity. The genetic basis and molecular mechanism for inherited differences in TPMT activity have been elucidated. To date, it is known that a wild type allele (TPMT*1) and different mutant alleles with reduced activity exists. Three are the most frequent mutations of this gene: TPMT*2 (G238C transversion), TPMT*3A (G460A and A719G transitions), TPMT*3C (A719G transition). Recent studies have elucidated that alterations in the promoter region of the TMPT gene can modulate its expression and consequently TPMT activity. These alterations consist in a variable number of tandem repeats (VNTR) in the promoter region; in vitro studies have shown an inverse correlation between the number of repeated elements and the TPMT activity.
Mutations of TPMT gene have an important role in the determination of the therapeutic effect and toxicity in patients treated with thiopurine drugs, since they are often associated with lower enzyme activity. Several studies have shown that TPMT deficient patients need a drastic dose reduction of thiopurines (90%) to avoid toxicity with maintenance of the therapeutic effect. On the contrary, the treatment of these patients with standard doses causes a severe and potentially fatal neutropoenia. The risk of toxicity is lower in heterozygous and wild type individuals. Hence the variability in TPMT activity could be related to the success of the therapy but more studies are needed.

The project of the research is to examine, in a three years work, the possible existence of a correlation between therapy outcome and the biological parameters listed below:

- TPMT enzymatic activity in erythrocytes, measured by high performance liquid chromatography (HPLC)
- levels of the toxic metabolites (6-TGNs) in the peripheral blood, measured by HPLC techniques
- genetic characteristics, in particular identification of the most frequent ORF mutations by polymerase chain reaction (PCR) techniques (restriction fragment length polymorphisms analysis) and gene promoter analysis, by means of the determination of the length of the VNTRs present in the 5'-untraslated region
- the levels of nitroimidazole derivatives and thiouric acid in the blood and urine, measured by HPLC.

The final aim is to predict efficacy and toxicity of AZA in paediatric patients and to individually adapt the dose of the drug.
Moreover, the data obtained from TPMT gene analysis will be the initial step for the development of an alternative and innovative method for the study of this gene, using specific immobilized probes, for a rapid screening analysis.
