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Thiopurines are frequently used in the treatment of patients suffering from (auto)immune diseases. Since their introduction in the mid fifties of the 20th century the way these compounds exert their therapeutic action has been the subject of many clinical and laboratory studies. In chapter 1 the reader is introduced into this topic. After a brief introduction on purines and pyrimidines and the importance of synthetic analogues of these compounds, the metabolism of purines is described. As is outlined purine metabolism can be divided into 3 main parts: purine de novo synthesis (PDNS), interconversion and the degradation-salvage pathway. The complexity of these pathways reflects the importance of these compounds in life, by tight regulation mechanisms the intracellular concentrations of purines are kept in balance. Modern molecular genetic methods, generating vast amounts of genetic information, have opened the field of pharmacogenetics and it was shown that several enzymes involved in purine metabolism are essential in the handling of synthetic purine analogues, used as therapeutics in a variety of diseases. Thiopurines are metabolized by the enzymes from the purine pathways and it is apparent that alterations in the activity of these enzymes by genetic polymorphisms may influence the efficacy of thiopurine therapy. This thesis focuses on two enzymes involved: thiopurine-S-methyltransferase (TPMT) and inosine triphosphatase (ITPase). The effects of alterations in the expression of these enzymes are discussed in relation to thiopurine metabolism. The therapeutic targets of thiopurines and drug/drug interactions influencing thiopurine efficacy are briefly mentioned.

The selectivity of TPMT to thiopurine compounds is still puzzling. This topic is addressed in chapter 2. Experiments using human recombinant TPMT (hrTPMT) and (methyl-14C)-S-adenosylmethionine (SAM) confirmed the transfer of the methyl group from SAM to the different substrates. In human erythrocyte lysates this was seen with 6-MP and 6-TG, but not with 6-TIMP. In contrast, using MOLT-3 cells, we were able to confirm the formation of 6-Methyl-TIMP from 6-MP and 6-thioguanosine (6-TG). The thionucleotide profile in MOLT-3 lysates after incubation with 6-methyl-MP showed no formation of 6-Methyl-TIMP. Further investigations are warranted to elucidate the way thiopurine compounds are metabolized in human cells.

Chapter 3 describes the development and application of a method to determine thiopurine metabolites in body fluids, using ultra performance liquid chromatography – tandem mass spectrometry. Using stable isotope labeled internal standards (methylated) thiopurines and metabolites could be detected in urine and plasma in concentrations down to 50 nanomolar. Intra assay variations were within acceptable limits for the compounds measured. Instability of 6-MP and 6-TG, most probably due to oxidation, made it nearly impossible to establish inter assay variation for these compounds. For methylated thiopurines inter assay variations ranged from 4-7% in plasma. In urine the low concentration range intra assay variations were >15%. The method was further validated by analyzing samples from patients on thiopurine
therapy. In urine and plasma 6-MP and 6-thiouric acid (6-TU) were readily detectable. The other compounds were only found in trace amounts, below the limit of quantification (LOQ) of the method.

The relevance of measuring TPMT before starting thiopurine therapy is emphasized by the clinical history of the case presented in chapter 4. The patient was treated for refractory Ulcerative Colitis (UC). Within 5 weeks after starting Azathioprine (AZA), a prodrug of 6-MP, the patient developed severe leucopenia and anemia. After admission to the hospital an adverse drug reaction caused by AZA was suspected and AZA was stopped immediately. TMPT activity was measured in erythrocytes and was found decreased. Molecular analysis revealed a *1/*3C TPMT genotype, associated with a decreased TMPT activity. After 4 weeks the patient recovered and treatment was continued with mesalazine.

A second enzyme involved in (thio)purine metabolism is inosine triphosphatase (ITPase). In chapter 5 the method for the measurement of ITPase activity in erythrocytes is described, using liquid chromatography with UV-detection. The method was optimized for measuring ITPase activity in dried blood spots (DBS). Although the method was applicable for the measurement in DBS and decreased activities could easily be detected, the stability of the enzyme was poor. It was therefore concluded that ITPase activity measurement in DBS is not reliable. Therefore the use of fresh erythrocyte lysates for the measurement of ITPase activity is required.

The debate on ITPA polymorphisms and ADR during thiopurine therapy is still ongoing. In chapter 6, the focus is on the enzyme ITPase and its kinetic properties. Genotype associated reference values for ITPase in erythrocytes were established. The specificity of ITPase for the substrates ITP and thio-ITP was determined, both for the wild type genotype as for the activity lowering polymorphisms. Surprisingly the binding of the substrates was comparable for the genotypes tested, however, the velocity of pyrophosphohydrolysis was greatly decreased when the c.94C>A polymorphism was present. The efficiency of ITPase for both substrates was found to be comparable under the assay conditions. From these results it appears that other (epigenetic) factors may be responsible for the occurrence of ADR during thiopurine therapy in the presence of ITPA polymorphisms.

Chapter 7 describes a pilot study in which the association between ITPA polymorphisms and clinical outcome in patients with pulmonary Langerhans' cell histiocytosis is evaluated. Interestingly, patients with a with an ITPase activity lowering polymorphism were found to have a more unfavorable outcome compared to the patients with a wild type genotype. The cause of this phenomenon is unclear: possibly the house keeping function of ITPA is compromised in patients with a decreased ITPase activity.

Finally, the major findings of this thesis are discussed in chapter 8 and the suggestions for future research are given. Although the knowledge on TPMT and ITPase is expanding continually, still many questions have to be answered. To answer some of these questions future research needs to follow two lines: one will be focused on the
biological function of ITPase in health and disease. The other line will focus on the handling of thiopurines by the organism, especially the way thiopurines are absorbed by the intestinal tract and how inflammation is influencing this process.