PART III

DISCUSSION
CHAPTER 14

SUMMARY AND GENERAL DISCUSSION
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Over fifty years ago, Gertrude Elion and George Hitchings discovered thiopurines for treatment of cancer (childhood leukaemia). They would be surprised to know that thiopurines nowadays are most extensively used for treating inflammatory mediated diseases, including IBD. There is persisting and even growing interest in the use of thiopurines including thioguanine, reflected by the ongoing publication of pharmacokinetic and -dynamic studies as well as clinical studies. It has become evident that individual thiopurine metabolism for a large part depends on pharmacogenetic properties. Although the research described in the first part of this thesis does not directly encompass genetics, indirectly it does by focusing on the phenotypes of enzymes involved in thiopurine metabolism. It provides understanding the variation in individual metabolism. Understanding individual metabolism is key from a pharmacological point of view, as it may guide drug dosing. It may also help to identify individuals who may or may not respond to the intended therapy and it may predict and explain adverse reactions.

As hepatotoxicity is a frequently observed but poorly understood adverse event upon the use of thiopurines in IBD, the second part of the thesis focuses on gaining insight in potential mechanisms involved. The use of thioguanine was already associated with histopathological irregularities of the liver. We questioned reproducibility of such histopathological findings and carried out an inter-observer agreement study in collaboration with pathologists. Prevalence data on histopathological irregularities of the liver were obtained, focusing on vascular abnormalities during the use of thioguanine and their clinical relevance. Moreover, change in histopathology over time during thioguanine treatment was studied.

PART I: OPPORTUNITIES AND THREATS OF THIOPURINE METABOLISM AND DRUG MONITORING

Chapter 2 provides an extensive overview of the pharmacology of thiopurines, including pharmacogenetic aspects, presumed mechanisms of action and the role of therapeutic drug monitoring. In addition, it discusses the role of thiopurines in specific clinical situations. As a result of their complex metabolism, thiopurines have a narrow therapeutic window and frequently cause adverse events. Thiopurine failure in the treatment of IBD has previously been related with formation of MMP at the cost of TGN. The concomitant use of allopurinol, a known inhibitor of xanthine oxidase (XO), in those patients with a preferential MMP metabolism leads to an impressive decrease of MMP production and subsequent enhanced TGN production, contributing to a better tolerability and effectiveness of azathioprine and mercaptopurine. Moreover, this combination therapy does prevent many other adverse reactions that may occur upon thiopurine monotherapy. Although MMP's are believed to be the result of enzymatic conversions driven by TPMT, there is a poor association between the activity of TPMT and MMP concentration, suggesting that there are other important mechanisms for preferential MMP production. In line with these findings, we could not observe a change in TPMT activity upon co-administration of allopurinol to mercaptopurine. Indeed, we observed a decreased activity of xanthine oxidase. Interestingly, we also observed an increase in the activity of HGPRT that catalyses the production of TGN (Chapter 3). Contrarily, in a group of IBD patients who
received tioguanine, a decrease of HGPRT activity was observed (Chapter 4). The activities of xanthine oxidase and TPMT did not markedly change in this study. A theoretical explanation for the decrease in HGPRT activity with the use of tioguanine is that thiopurines in general inhibit HGPRT. In patients with preferential MMP production HGPRT activity may be further inhibited (possibly by inhibition of the novo purine synthesis), but may be enhanced by the concomitant use of allopurinol. This, of course, needs further study.

From a pharmacological perspective it would be preferable to always use tioguanine instead of azathioprine or mercaptopurine. The metabolism of the latter two is rather complex with much more enzymatically driven steps to the formation of the pharmacologically active TGN and with production of presumably undesirable, potentially toxic products. Tioguanine only needs one enzymatic conversion, catalysed by HGPRT, to TGN. In the sixties, tioguanine was introduced for the treatment of IBD. It was, however, soon abandoned as its use was associated with particular neurological symptoms and liver toxicity6. Rediscovery of tioguanine for treating IBD took place in the year 2001. Results were promising with good response rates in patients intolerant of or refractory to conventional thiopurines7. Most of the studies that report on the use of tioguanine in IBD include Crohn’s disease patients. Therefore we conducted a retrospective cohort study of ulcerative colitis patients who were treated with tioguanine after failing conventional thiopurine therapies (Chapter 5). We found that tioguanine was well-tolerated; after two years of treatment 80% (36/45) of patients still successfully continued their treatment. Endoscopic remission was observed in 40% during follow-up as compared to 10% at baseline. Toxicity was mild with no patients with symptoms of portal hypertension, although two patients developed mild liver test abnormalities and thrombocytopenia as a result of which these patients ceased tioguanine treatment. Our study underlines that tioguanine still may be considered as a rescue therapy in IBD treatment.

The usefulness of drug monitoring of thiopurines by means of measurement of TGN and MMP has frequently been debated. There may be several reasons for the poor sensitivity, specificity, positive predictive value and negative predictive value of measuring TGN to predict clinical response. In response to an uncontrolled study performed in Spain8, which failed to observe an association between TGN concentrations and therapy effectiveness, we bring up arguments why we think this happened (Chapter 6). First of all, stability of TGN is limited, hence prompt handling and analyses is important. Furthermore, IBD is characterized by a relapsing and remitting natural course which hampers assessment of therapy effectiveness and consequently drug monitoring. We conclude that we think measurement of TGN and MMP may still be of interest, in particular when treatment fails due to either side effects or non-response.

In Chapter 7 we describe a case of an IBD patient who had been treated with azathioprine for several years and developed leukocytopenia. Often leucocytopenia during thiopurine treatment is a result of the drug treatment. Associations with low TPMT activity and consequently high TGN concentrations are well-known. However, not every episode of leucocytopenia is due to thiopurine therapy. In this case an infection with Parvovirus B19 was found to be responsible. The important message being that thiopurine treatment is not always the culprit if leucocytopenia occurs and, thus, need not always be permanently discarded as treatment for IBD.
PART II: EXPLORING HEPATOTOXICITY OF THIOPURINES

Approximately 15% of IBD patients develop liver test abnormalities upon the use of thiopurines \(^9,10\). This hepatotoxicity might be an allergic reaction but most of the time it is idiosyncratic and dose-dependent. Thiopurine associated hepatotoxicity can be divided into short-term and long-term toxicity, the latter being vascular liver toxicity such as nodular regenerative hyperplasia (NRH)/sinusoidal obstruction syndrome (SOS). Mechanisms behind this thiopurine associated hepatotoxicity are not well known. There are some clues with respect to the aetiology of this longer term vascular toxicity of the liver. A Notch1 knockout mouse-model showed spontaneous development of NRH and sinusoidal dilatation in the absence of venous obstruction \(^11,12\). Other animal and in vitro studies provide evidence for the mechanism behind the short term thiopurine associated hepatotoxicity. It was shown that reduced glutathione was depleted upon azathioprine induced liver injury and that supplementation of N-acetylcysteine (NAC), as a precursor of cysteine and glutathione, protected against this, presumably oxidative stress initiated, liver injury \(^13,14\). We therefore carried out a clinical trial in which we studied the role of oxidative stress and supplementation of NAC in IBD patient who developed thiopurine associated hepatotoxicity (Chapter 8). In this study we could not find a significant hepatoprotective effect of the use of NAC in these patients reflected by an absence of effect on liver tests. As opposed to these negative results we observed that the supplementation of NAC did cause a reduction of oxidative stress as reflected by a decrease of plasma myeloperoxidase levels. In addition, upon thiopurine reintroduction we observed that the amount of F2-isoprostanes were increased, underlining the hypothesis that the use of thiopurines give rise to increased oxidative stress. It would be of interest, also from a clinical point of view, to redo this study with allopurinol instead of NAC.

In Chapter 9 we showed that IBD patients who developed MMP associated hepatotoxicity did not develop this hepatotoxicity upon the (short term) administration of tioguanine. This implicates causality, the mechanism of which remains speculative. These results strengthen the optional use of tioguanine as an alternative for co-administration of allopurinol in these preferential MMP producers.

Chapter 10 provides an overview of the association between tioguanine and SOS. It is believed that SOS results from sinusoidal endothelial injury, resulting in the loss of sinusoidal wall integrity \(^15\). In addition, cytotoxic CD8+ lymphocytes seem to be involved \(^16\). Specific morphological abnormalities such as NRH frequently accompany SOS \(^17\). In this review, we discuss potential mechanisms behind the association between SOS and tioguanine. A mouse-model nicely illustrates that there is a dose-dependent effect.

Soon after the reintroduction of tioguanine for the treatment of IBD it became clear that its use was associated with hepatotoxicity characterized by liver test abnormalities and portal hypertension as evidenced by thrombocytopenia and increased hepatic venous pressure gradient \(^18,19\). Histopathological analyses, which was selectively carried out, showed NRH in a substantial number of patients probably reflecting SOS \(^18,20\). It is very likely that in these studies tioguanine was overdosed. However, NRH was also reported in a post-mortem
series and a series of healthy potential liver donors, suggesting that NRH might not always be of clinical relevance\textsuperscript{21,22}. Then the question rose to what extent the histopathological evaluation of the pathologists were consistent. An observer agreement study was carried-out (Chapter 11). Pathologist evaluated liver biopsy specimen that were previously diagnosed as NRH. Both intra- and inter-observer agreement were poor. Then a consensus meeting took place which resulted in an adaption of the definition of NRH. Finally, this new definition was validated by two pathologist. Observer agreement got slightly better, but was still far from good. This might be due to some limitations in the design of the study, but also emphasizes the difficulty of the diagnosis. The conclusion of this study was that NRH is a clinicopathologic diagnosis, meaning that a histopathological diagnosis of NRH is not meaningful unless it coincides with clinical symptoms or signs. It underlines the importance of providing clinical information to the pathologist.

Although the further use of tioguanine was abandoned in the United States after the worrisome reports of Dubinsky and collaborators\textsuperscript{18}, in Europe some investigator kept interest in the drug and believed that it could be used safely provided that it was lower dosed. In 2005 a working party of European specialists plotted guidelines for the monitoring of tioguanine treatment in IBD\textsuperscript{23}. One of these recommendations was obtaining liver biopsy specimen irrespective of clinical symptoms. This led to our next study described in Chapter 12. As part of safety monitoring 115 liver biopsy specimen were obtained from 115 IBD patients that were being treated with tioguanine for a median duration of 20 months (range 4-64). In 7 out of 115 NRH was diagnosed. In line with the conclusions from chapter 11, a histopathological diagnosis of NRH is not necessarily of clinical importance as only approximately half of patients had liver test abnormalities, thrombocytopenia and/or splenomegaly. Moreover, there were no episodes of variceal bleeding or (severe) ascites. Yet, besides nodularity also phlebosclerosis could be associated with liver test abnormalities. These results are in line with the concept of endothelial cell injury which occurs in SOS and may give rise to not only NRH but also other histopathological alterations as seen with non-cirrhotic portal hypertension\textsuperscript{24}. Finally, in Chapter 13 we describe those patient that tolerated tioguanine and underwent sequential liver biopsies, mostly part of toxicity screening. In this study we observed that while continuing tioguanine treatment histopathology generally did not change except for a non-statistically significant increase of phlebosclerosis.

**FUTURE PERSPECTIVES**

We have entered interesting times when we look at treating IBD. On the one hand there is ongoing drug development resulting in the availability of multiple effective, but often expensive drugs. On the other hand, evolving the further use of common drugs or drug rediscovery (finding new indications for existing drugs) may safe costs by preventing or postponing the use of more expensive treatments. A major drawback in this regard is that there is little interest from pharmaceutical industries to sponsor research on out of patent-drugs. This means that community money is needed to fuel these further developments.
Of course the future is not foreseeable, but presumably personalised medicine will have a central role in drug treatments including treatments of IBD, just like it has in oncology. With respect to thiopurine treatment, there is a further need for pharmacogenetic studies which identify variant alleles that alter the activities of the enzymes involved in thiopurine metabolism. Of equal importance is the further study on pharmacodynamic effects of thiopurines. In this regard Rac1, a small signalling GTPase that is important for cell to cell signalling and anti-apoptosis of activated T cells, has been identified as a target of thiopurine treatment. As current therapeutic drug monitoring of thiopurines by the measurement of thiopurine nucleotides in red blood cells has many limitations, discovering new mechanisms of action provides opportunities for development of new drug monitoring techniques. It is interesting to realize that just recently, over fifty years after the discovery of thiopurines, we learn to understand pharmacology and toxicity of thiopurines. There are, however, still much questions left unanswered.
REFERENCES


