CHAPTER 1

INTRODUCTION AND OUTLINE OF THIS THESIS
INTRODUCTION

Inflammatory bowel diseases (IBD), comprising Crohn’s disease, ulcerative colitis and IBD-unclassified, are chronic recurrent inflammatory disorders of the (large) intestine with a heterogeneous, but often disabling disease presentation. The prevalence of IBD is increasing worldwide and medical therapies are introduced earlier to improve health-related quality of life and postpone surgical interventions\(^1\). This has led to the more extensive use of immunosuppressive therapies including biologicals and thiopurines. While the use of tioguanine was soon abandoned due to toxicity concerns\(^3\), over the years efficacy data on the use of azathioprine and mercaptopurine for the treatment of IBD has grown\(^4,5\). However, due to side effects and ineffectiveness, related to unprofitable metabolism, thiopurine therapy often fails; up to 50% of patients discontinue treatment within 2 years\(^6\). Increasing the knowledge on thiopurine metabolism may provide opportunities to further explore the therapeutic usefulness of thiopurines, which both from a patient’s perspective and a pharmacoeconomic perspective is essential.

THIOPURINES

In the 1950’s Gertrude Elion (1918-1999) worked as an assistant to George Hitchings (1905-1998), both employees of Burroughs-Wellcome, focusing on the advent of drugs that inhibited cell growth without doing harm to the host cells. They discovered that purine bases are essential components of nucleic acids and vital for cell growth\(^7\). Subsequently, they designed the purine antagonists diaminopurine and tioguanine that blocked the synthesis of original nucleic acids and inhibited cell growth\(^8\). For the first time a successful treatment of leukaemia became available. Some years later Elion and colleagues also developed mercaptopurine, azathioprine, allopurinol, trimethoprim and acyclovir. Based on their research the important HIV drug azidothymidine was discovered. For their achievements in drug discovery Elion and Hitchings received the distinguished Nobel Prize, awarded in 1988. Although the thiopurines tioguanine and mercaptopurine were originally developed and registered for treating leukaemia, and azathioprine for preventing organ rejection in transplantation medicine, since 1962 these drugs have also been used for treating inflammatory mediated diseases including IBD\(^9\)-\(^13\).

It soon became evident that these thiopurines have a small therapeutic window and that their use is not without risk. Both efficacy and toxicity of these drugs are dependent on the extent to which pharmacologically active and toxic metabolites are produced. On the one hand azathioprine is converted, via the formation of mercaptopurine and other intermediates, into the pharmacologically active (but also cytotoxic) 6-thioguanine nucleotides (6-TGN). These 6-TGN are associated with therapeutic response\(^12,13\). On the other hand, 6-methylmercaptopurine ribonucleotides (6-MMP) are formed and related to the occurrence of adverse events including hepatotoxicity (figure 1)\(^14\).

Around the year 1990, Lennard and colleagues showed that thiopurine metabolism was under genetic influence. Thiopurine S-methyl transferase (TPMT) was found to be responsible for
the degradation of thiopurines into 6-MMP’s and a low TPMT activity, which increases 6-TGN concentrations, was shown to increase the risk at myelosuppression\textsuperscript{15}. Genetic polymorphisms of the TPMT gene are hold responsible for the variable enzyme activity; at the time of writing of this thesis 38 variant TPMT alleles had been identified\textsuperscript{16}. Besides TPMT there are multiple other enzymes involved in purine metabolism, most of which have not been studied extensively. Pharmacogenetics is believed not to be the only determinant of thiopurine pharmacokinetics. Bioavailability of orally administered thiopurines also depends on diet as was illustrated by a lower bioavailability of mercaptopurine when administered with milk\textsuperscript{17}. In addition, drug interactions may influence drug availability conceivably by altering the phenotype of enzymes involved. These interactions may be unfavourable but may also provide opportunities to optimise treatment, as was already shown for allopurinol\textsuperscript{18}. Altogether, this complex metabolism of thiopurines makes therapeutic drug monitoring – defined as a clinical speciality aimed at improving patient care by individually adjusting the dose of drugs for which clinical experience or clinical trials have shown it improved outcome in the general or special population- very challenging.

Tioguanine only needs one enzymatic conversion toward the formation of 6-TGN, which theoretically is advantageous\textsuperscript{19,20}. Therefore the drug was reintroduced for IBD treatment around the year 2000. Some small non controlled studies including chiefly Crohn’s disease patients intolerant of azathioprine or mercaptopurine showed that tioguanine was well tolerated by the majority of patients. In addition, its effectiveness was promising\textsuperscript{21-23}. Further use of tioguanine was largely abandoned when reports of its potential hepatotoxicity were published\textsuperscript{24}.

\textbf{Figure 1.} Simplified scheme of thiopurine metabolism illustrating the difference in metabolism between azathioprine (AZA), mercaptopurine (6-MP) and tioguanine (6-TG). XO, xanthine oxidase; 6-TUA, 6-thiouric acid; TMPT, thiopurine S-methyltransferase; HGPR, hypoxanthine-guanine phosphoribosyl transferase; 6-MMP, 6-methylmercaptopurine; 6-TIMP, 6-thioinosine monophosphate; 6-TITP, 6-thioinosine triphosphate; ITPA, inosine triphosphate pyrophosphohydrolase; 6-TGN, 6-thioguanine nucleotides; 6-MTG, 6-methyl thioguanine.
HEPATOTOXICITY

A well known off-target effect of thiopurine therapies includes hepatotoxicity that can be either a dose dependent or an idiosyncratic reaction\textsuperscript{25}. Although an association between 6-MMPs and the occurrence of hepatotoxicity upon azathioprine or mercaptopurine therapy has been reported, the underlying mechanisms remain elusive. It is unknown whether patients with 6-MMP associated hepatotoxicity are prone for hepatotoxicity upon the use of tioguanine. A possible explanation for thiopurine associated hepatotoxicity might be a depletion of the hepatoprotective antioxidant reduced glutathione, due to production of radical oxygen species\textsuperscript{26}. These results imply that the supplementation of (precursors of) glutathione may protect against thiopurine-induced hepatotoxicity. In addition to hepatocyte toxicity, sinusoidal obstruction syndrome has been observed during thiopurine treatment\textsuperscript{27}. This results from toxicity directed to the sinusoidal endothelial cells that may lead to occlusion of the hepatic (micro-) vasculature and may ultimately give rise to portal hypertension\textsuperscript{28}. This type of portal hypertension is coined as non-cirrhotic portal hypertension, characterised by several histopathological irregularities including nodular regenerative hyperplasia (NRH), phlebosclerosis (obliterative portal venopathy), sinusoidal dilatation and pericellular fibrosis\textsuperscript{29}. It is this type of hepatotoxicity that was associated with the use of tioguanine, although it may also be related to IBD itself\textsuperscript{30}. Most of the articles about the use of tioguanine in IBD focus on NRH and do not report other histopathological irregularities. Moreover, it is not always evident whether the definition of NRH as proposed by Wanless is being used, although even this definition has not been validated so far\textsuperscript{31}. Despite the association between tioguanine and liver injury, research groups in Australia and Europe kept interest in tioguanine as a potential rescue drug. A European working group consequently published clinical recommendations on how to monitor safety when prescribing tioguanine\textsuperscript{32}. It was suggested that the dreaded hepatotoxicity is dose dependent, which is further acknowledged by several publications from that time on\textsuperscript{33-35}. Yet, the actual prevalence of histopathological alterations of the liver and its relation with clinical symptoms has not been systematically established in IBD patients treated with tioguanine.

OUTLINE OF THE THESIS

The first part of the thesis focusses on the pharmacology of thiopurines in IBD patients in different clinical situations. Therefore, in Chapter 2 a general overview of thiopurine metabolism including pharmacokinetic and pharmacodynamic aspects is provided. This overview covers the role of therapeutic drug monitoring and drug interactions, but also treatment recommendations. As there is little data on the effect of thiopurines themselves on their metabolism, the effect of tioguanine on the activities of involved enzymes is described in Chapter 3. In several studies the effectivenes and tolerability of tioguanine in the treatment of Crohn’s disease has already been addressed. There is much less experience with tioguanine in the treatment of ulcerative colitis. Therefore, a cohort study on the
tolerability and effectiveness of tioguanine as a maintenance treatment for ulcerative colitis was performed, the results of which are reported in Chapter 4.

In Chapter 5 the very potential interaction between allopurinol and thiopurines is further explored from a pharmacokinetic point of view by measuring the activities of crucial enzymes before and during co-treatment.

Drug monitoring of thiopurines may have its limitations but may also be of additional value, as discussed in Chapter 6 and 7.

The second part of the thesis focusses on the hepatotoxic potential of thiopurines, which often hampers further thiopurine treatment. In Chapter 8 the role of oxidative stress in hepatotoxicity during azathioprine or mercaptopurine treatment was further explored, whereas in Chapter 9 it will be discussed that the particular 6-MMP associated hepatotoxicity does not recur upon tioguanine treatment. This illustrates the possible different origins of thiopurine associated hepatotoxicity.

Chapter 10, being a review, provides an overview of specific tioguanine associated liver injury and reveals evidence from a mouse model that tioguanine associated liver injury is dose-dependent. Histopathological assessment of liver biopsies may be limited by inter observer variability between pathologists. In this respect the intra- and interobserver variability of NRH was studied in Chapter 11.

And since available literature on NRH in IBD patients treated with tioguanine was subjected to major selection bias (most of the time only patients who had clinical signs or symptoms underwent a liver biopsy), a cross-sectional study which reports the prevalence of NRH during tioguanine treatment more accurately, by the structural incorporation of taking liver biopsies as part of drug safety monitoring, is described in Chapter 12. This thesis is finalised with Chapter 13 which reveals the histopathological alterations observed during tioguanine treatment by comparing the findings from 2 consecutive liver biopsies.
INTRODUCTION AND OUTLINE OF THIS THESIS

REFERENCES


16 Website: http://www.imh.liu.se/tpmtalleles.


24 Dubinsky MC, Vasiliauskas EA, Singh H, Abreu MT, Papadakis KA, et al. 6-Thioguanine can


