Summary

Thiopurines and inflammatory bowel disease; pharmacology and toxicity

This thesis provides novel pharmacologic and toxicologic insights in the complex metabolism of thiopurine therapy in inflammatory bowel disease patients. Azathioprine (AZA) and 6-mercaptopurine (6-MP) are considered as classical thiopurines due to longterm historical use as immunosuppressive maintenance drugs. Unfortunately, up to one third of patients is unable to benefit from thiopurine therapy due to the development of adverse events (e.g. myelotoxicity or hepatotoxicity) or therapeutic failure. Several metabolites of AZA/6-MP have been held responsible for the development of adverse events. Myelotoxicity has been associated with elevated levels of the pharmacologically active 6-thioguananinenucleotides (6-TGN) and hepatotoxicity may in part be explained by grossly elevated 6-methylmercaptopurine (6-MMP) levels. A proposed strategy to avoid AZA or 6-MP-induced toxicity is the administration of another thiopurine 6-thioguanine (6-TG), which is metabolized in a single step into 6-TGN. Due to this relative simple metabolism, the number of (potentially) toxic metabolites is reduced. However, the use of (comparatively) high dosages of 6-TG has been associated with induction of nodular regenerative hyperplasia (NRH) of the liver.

Chapter 1

In recent years, the complex pharmacology, metabolism, mechanism of action and toxicity profile of thiopurines have been elucidated to a larger extent in IBD patients. The first chapter provides an -in depth- review on thiopurine therapy, particularly focusing on pharmacologic and toxicologic aspects. The use of therapeutic drug monitoring and prior-to-treat determination of the activity of the pivotal enzyme thiopurine methyltransferase (TPMT) are discussed. More prominently, the role of 6-TG in inducing NRH is described.

Chapter 2

The second chapter deals with the role of 6-TGN in inducing myelotoxicity during 6-TG therapy. High 6-TGN levels during AZA/6-MP therapy (>450 pmol/8x10^8 red blood cells (RBC) according to the method described by Lennard) have been associated with leukopenia. The commonly used dosages in literature of 6-TG up till now normally generated much higher 6-TGN levels but, contra-intuitively, 6-TG therapy has not been associated with an increased risk of myelotoxicity. We prospectively assessed the role of 6-TGN concentrations in developing myelotoxicity during 6-TG treatment in 25 patients and observed no increased risk (4%). Moreover, high 6-TGN levels above 450 pmol/8x10^8 RBC did not have any effect on haemoglobin concentrations, peripheral leucocyte or platelet counts.
Chapter 3

6-Thioguaninenucleotides can be subdivided into three different phosphorylated forms: 6-thioguanine-monophosphate (6-TGMP), 6-thioguanine-diphosphate (6-TGDP) and 6-thioguanine-triphosphate (6-TGTP). The molecular mechanism of immunosuppression by AZA/6-MP is amongst others due to the specific end-metabolite 6-TGTP that binds and inhibits the function of the small GTPase Rac1 in activated T-lymphocytes leading to apoptosis. Metabolic data concerning the generation of the specific phosphorylated 6-TGN during 6-TG treatment in patients with IBD are lacking. In this chapter, we determined standard 6-TGN levels and TPMT activity combined with 6-TGMP, 6-TGDP and 6-TGTP levels in 6-TG using Crohn’s disease patients. High inter-individual variance in all metabolite measurements was observed, not explained by individual TPMT activity or dosage. Standard 6-TGN levels correlated well with the 6-TGTP level, therefore total 6-TGN level monitoring may suffice for clinical practice.

Chapter 4

The fourth chapter describes the influence of 5-aminosalicylates (5-ASA) on AZA/6-MP metabolism. We observed a dose-dependent increase in 6-TGN levels during 5-ASA co-administration. Two grams of 5-ASA daily for four weeks led to a 40% increase in 6-TGN levels compared to a 70% increase after 4 grams per day. The different 5-ASA dosages did not influence 6-MMP levels. This indicates that the methylating enzyme TPMT is not influenced by 5-ASA or its metabolites in vivo. The combination of these two drugs seems to lead to an increased risk of developing myelotoxicity as two patients (7.7%) developed a temporary leukopenia. Patients, that are refractory or unresponsive to standard AZA/6-MP therapy, may benefit from 5-ASA co-administration as higher 6-TGN levels have been associated with a better responsive to thiopurine therapy.

Chapter 5

The use of AZA during pregnancy is believed to be relatively safe, particularly taking into account the potential risks for mother and fetus when the underlying disease becomes active due to withdrawal of AZA therapy. However, it is unknown whether, to what extent, and to which metabolites the unborn child is exposed during maternal use of AZA. Chapter five describes three patients who were treated with stable-dosed AZA throughout all trimesters of their pregnancies. The thiopurine metabolites 6-TGN and 6-MMP were measured in erythrocytes of mother and infant directly after delivery. The 6-TGN concentration was only slightly lower in the erythrocytes of the infant than the mother. No 6-MMP could be detected in the infant. Therefore, the placenta forms a relative barrier to AZA and its metabolites. We believe that intrauterine exposure to 6-TGN may be minimized by careful therapeutic drug monitoring of the mother during pregnancy.
Chapter 6
This chapter describes the incidence of adverse events after one year of 6-TG therapy in 95 AZA and/or 6-MP intolerant IBD patients with a 6-TG dosage leading to 6-TGN levels similar to the currently advised dosages of AZA or 6-MP (approximately 20 mg daily or 0.3-0.4 mg/kg daily leading to a mean 6-TGN level of 540 picomoles/8 x 10^8 RBC according to the method described by Lennard). The majority of patients (79%) tolerated this 6-TG regime well. Reasons for discontinuation were gastro-intestinal complaints (31%), general malaise (15%) and hepatotoxicity (15%). An abdominal ultrasonography was performed in a sub-group of 51 patients after at least 1-year 6-TG use to screen for potential hepatotoxicity. Signs of portal hypertension (splenomegaly) were observed in only one patient.

Chapter 7
Nodular regenerative hyperplasia of the liver has been associated with thiopurine use in IBD patients. However, data on the prevalence of this histological abnormality in non-thiopurine using IBD patients are lacking. In chapter seven, we pathohistologically assessed 85 liver biopsy specimens, obtained from non-thiopurine using IBD patients during surgery, with special attention for NRH. In 6% of the liver specimens NRH was detected. Correlation was observed with the age at biopsy. These findings indicate that IBD itself may be considered as a risk factor for developing NRH. Moreover, laboratory liver tests were found to be unspecific and insensitive to predict pathohistological abnormalities. The association between thiopurine use and NRH may be weaker as reported in recent literature, as there is a relatively high background prevalence.

Chapter 8
In this chapter, we describe the incidence of NRH in a unique series of IBD patients using adapted-dose 6-TG (approximately 20 mg per day with a corresponding 6-TGN level of 442 pmol/10^8 RBC according to the method described by Lennard) as a maintenance therapy (mean period of 23 consecutive months). We observed no case of NRH in this cohort. We state that the induction of NRH during 6-TG therapy is a 6-TG dose (or 6-TGN level) dependent phenomenon.

Chapter 9
We proposed that the induction of histological liver abnormalities, in particular NRH, during 6-TG therapy may well be dose or 6-TGN level dependent. Chapter nine provides a detailed histological assessment of 28 liver biopsies, that were obtained from AZA/6-MP intolerant, AZA/6-MP refractory or AZA/6-MP naive patients using adapted-dose 6-TG therapy for at least 30 consecutive months. In 26 patient (93%) no signs of NRH were detected, in two additional patients NRH could not be excluded due to inconclusive pathological findings. We observed no cases of hepatotoxicity of myelotoxicity by laboratory parameters.
monitoring. The mean 6-TG dosage, 6-TGN level, duration of use and cumulative dosage were 19.5 mg, 564 pmol/8×10^8 RBC, 38 months and 22491 mg, respectively. The use of adapted-dose 6-TG maintenance therapy in IBD patients is not likely to be associated with induction of NRH. We state that the induction of NRH is a 6-TG dose or 6-TGN level dependent feature.

**Chapter 10**

In this chapter, we describe an IBD patient using AZA (2.2 mg/kg daily) for 2 years who developed a pancytopenia and an incomplete septal liver cirrhosis with portal hypertension. The 6-thioguaninenucleotide level was 738 picomoles/8×10^8 per red blood cell, which is well above the proposed upper limit of efficacy. After cessation of therapy, all laboratory parameters normalised. We discuss the role of 6-TGN in inducing myelotoxicity and hepatotoxicity. More prominently, we stress the need for close monitoring of patients taking thiopurines by routine laboratory parameters and therapeutic drug level controls.

**Chapter 11**

The proposal to use 6-thioguanine (6-TG) as an alternative thiopurine in patients with IBD has been discarded due to reports about possible (hepato)toxicity. During meetings arranged in Vienna and Prague in 2004, European experts applying 6-TG further on in IBD patients presented data on safety and efficacy of 6-TG. After thorough assessment of its risk-benefit ratio, the group consented that 6-TG may still be considered as a rescue drug in stringently defined indications in IBD. In chapter eleven, we present the proposed guidelines for 6-TG use. As a potential indication for administering 6-TG, we delineated the requirement for maintenance therapy as well as intolerance and/or resistance to aminosalicylates, AZA, 6-MP, methotrexate and infliximab. The standard 6-TG dosage should not exceed 25 mg daily. Routine laboratory controls are mandatory in short intervals. Liver biopsies should be performed after 6–12 months, three years and then three-yearly accompanied by gastroduodenoscopy, to monitor for potential hepatotoxicity. Treatment with 6-TG must be discontinued in case of overt or histologically proven hepatotoxicity.

**Conclusions**

This thesis provides novel insights in the complex pharmacology and toxicity of thiopurines. If AZA or 6-MP therapy clinically fails due to inadequate metabolite levels, than co-administration of 5-ASA compounds seems a promising alternative. In case therapy with AZA or 6-MP fails due to adverse events, the use of low-dose 6-TG (not exceeding 25 mg daily) may be considered. The induction of NRH due to thiopurine therapy (especially 6-TG) is likely to be a dose (subsidiary 6-TGN level) dependent effect. High 6-TGN levels during 6-TG therapy should be considered as a risk factor for developing NRH. Levels of 6-TGN above the proposed upper normal limit (450 picomoles/8×10^8) during AZA or 6-MP therapy are not indicative for (developing) myelotoxicity during 6-TG therapy.
The association between thiopurine use and NRH may be weaker as reported in recent literature, as there is a relatively high background prevalence (6%) in non-thiopurine using IBD patients. Physicians should be aware that the metabolites 6-TGN easily cross the placenta during pregnancy and that the unborn child may be exposed to high (potentially toxic) levels in case the mother has elevated 6-TGN levels.