Myelotoxicity and hepatotoxicity during azathioprine therapy

N.K.H. de Boer*, C.J.J. Mulder, A.A. van Bodegraven

Department of Gastroenterology and Hepatology, VU University Medical Centre, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)20-444 06 11, fax: +31 (0)20-444 05 54, e-mail: KHN.deBoer@vumc.nl

ABSTRACT

Azathioprine is a frequently used immunosuppressant for managing inflammatory bowel disease (IBD). In recent years the hepatotoxic profile of thiopurines has been recognised. We report the case of a 40-year-old man with Crohn’s disease treated with azathioprine. After taking azathioprine (2.2 mg/kg daily) for two years, his liver function tests were found to be elevated. Moreover, a myelodepression was established as platelet and leucocytes counts were lowered. The 6-thioguaninenucleotide level was 738 picomoles/8 x 10^8 per red blood cell, which is well above the proposed upper limit of efficacy and associated with an increased risk of developing a myelodepression. Genotyping of the enzyme thiopurine methyltransferase revealed no mutant alleles. The ultrasonography and CT scan showed signs of portal hypertension (spleen 17 cm and widened splenic vein). A liver biopsy was performed and an incomplete septal liver cirrhosis was found. An upper endoscopy revealed oesophageal varices (grade 2 to 3). Autoimmune and viral liver diseases were ruled out by laboratory parameters. After cessation of therapy, all laboratory parameters normalised. Therefore, azathioprine is believed to be the causative factor for inducing the liver cirrhosis. Continuous monitoring of patients taking thiopurines is mandatory. The role of 6-thioguaninenucleotide levels in inducing myelotoxicity and hepatotoxicity is discussed.

KEYWORDS
Azathioprine, Crohn’s disease, hepatotoxicity, liver cirrhosis, myelotoxicity, 6-thioguaninenucleotides

INTRODUCTION

Azathioprine (AZA) and 6-mercaptopurine (6-MP) have gained a prominent place as an immunosuppressive maintenance therapy for Crohn’s disease (CD). However, their therapeutic role has been under discussion because of toxicity. Adverse effects may occur in 15 to 30% of patients. AZA-induced hepatotoxicity is believed to be a rare adverse event. Hepatitis is considered to be an idiosyncratic reaction to AZA. Nodular regenerative hyperplasia (NRH), veno-occlusive disease, peliosis hepatis, fibrosis and sinusoidal dilatation are regarded as signs of dose-dependent hepatotoxicity. During the complex metabolisation process of AZA and 6-MP, multiple metabolites are generated. The pharmacologically active metabolites are the 6-thioguaninenucleotides (6-TGN), which are believed to induce apoptosis of activated T lymphocytes, hence leading to suppression of the overactive immune defence mechanisms. The proposed range of 6-TGN is 235 to 450 picomoles/8 x 10^8 per red blood cell (RBC). High 6-TGN levels (>450 picomoles/8 x 10^8 RBC) have been associated with an increased risk of developing a myelodepression and high levels of the methylated product of 6-MP (6-methylmercaptopurine (6-MMP)) with hepatotoxicity. Here we describe a patient who developed histological liver abnormalities combined with a myelodepression most likely caused by the use of AZA.

CASE REPORT

In 2000 the diagnosis of CD of the ileum and jejunum with perianal fistulas was established in a 35-year-old man.
A steroid pulse therapy was administered to induce remission. However, in December 2000 a surgical ileocaecal resection was performed due to intractable symptoms caused by a stenosis of the terminal ileum. Postoperative therapy with calcium, vitamin D, H\textsubscript{2}-receptor antagonist, vitamin B\textsubscript{12} and AZA was initiated. AZA was given in doses of 200 mg a day (2.2 mg per kg). The disease remained quiescent and regular laboratory controls were normal (\textit{table 1}). In December 2003 abnormal laboratory parameters were found as liver function tests were elevated. Leucocyte and platelet counts were found to be below the reference limits (\textit{table 1}). No physical complaints were mentioned by the patient. As AZA was believed to be the causative factor, an abdominal ultrasound was performed, and the level of 6-TGN/6-MMP and the status of the enzyme thiopurine methyltransferase (TPMT) were determined. The examination by abdominal ultrasound revealed an enlarged spleen (17 cm) and a distended diameter of the splenic vein (1.9 cm).

The hepatic parenchyma showed no abnormalities. The 6-TGN was found to be 738 picomoles/8 x 10\textsuperscript{8} RBC.\textsuperscript{12} Determination of the 6-MMP level was not carried out due to technical failure. TPMT genotyping revealed no mutant alleles (TPMT*1/*1). Autoimmune and viral liver diseases were ruled out by laboratory parameters. Alcohol use was repeatedly denied. Subsequently, a CT scan was performed but no novel insights were obtained. A fine needle liver biopsy was performed in May 2004 and the specimen was stained with haematoxylin and eosin, (silver)reticulin and trichrome. An incomplete septal cirrhosis was found microscopically without signs of primary sclerosing cholangitis, peliosis hepatis, veno-occlusive disease or NRH. Oesophageal varices (grade 2 to 3) were found by upper gastrointestinal endoscopy. Treatment with a \beta-receptor antagonist (propranolol 10 mg twice daily) was initiated as primary prophylaxis for oesophageal bleeding. All laboratory tests normalised after cessation of AZA (\textit{table 1}). The \alpha-fetoprotein level was not increased. The disease has remained in remission to date. Abdominal ultrasounds and upper gastrointestinal endoscopies are performed at regular intervals.

\section*{DISCUSSION}

The metabolism of thiopurines has partly been elucidated in recent years. Several metabolites have been held responsible for induction of adverse events. The enzyme TPMT plays a key role in the bioavailability of 6-TGN. High TPMT activity may lead to elevated levels of 6-MMP, which have been associated with hepatotoxicity (>5700 pmol/8 x 10\textsuperscript{8} RBC) and refractoriness on therapy.\textsuperscript{9} A diminished TPMT activity will likely result in shunting 6-MP away from 6-MMP towards overproduction of 6-TGN. Levels of 6-TGN >450 pmol/8 x 10\textsuperscript{8} red blood cells (RBC) induced by AZA or 6-MP therapy have been associated with an increased risk of developing myelotoxicity (e.g. leucopenia or thrombocytopenia).\textsuperscript{3} Despite shortage of conclusive evidence, 6-TGN levels between 235 and 450 pmol/8 x 10\textsuperscript{8} RBC are currently considered to corre-

\begin{table}
\centering
\caption{Laboratory parameters}
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Test} & \textbf{December 2000} & \textbf{December 2003} & \textbf{March 2005} & \textbf{Normal values} \\
\hline
Aspartate aminotransferase (U/l) & 12 & 57 & 25 & <40 \\
Alanine aminotransferase (U/l) & 19 & 33 & 26 & <45 \\
Gamma-glutamyltransferase (U/l) & 15 & 85 & 26 & 10-50 \\
Alkaline phosphatase (U/l) & 86 & 141 & 97 & 40-120 \\
Bilirubin (unconjugated) (\textmu mol/l) & 10 & 35 (0.28) & 16 (0.23) & <20 \\
Haemoglobin (mmol/l) & 9.5 & 7.7 & 9.5 & 8.7-11 \\
Leucocytes (x 10\textsuperscript{9}/l) & 17.2 & 2.1 & 8.1 & 3-10 \\
Platelets (x 10\textsuperscript{9}/l) & 303 & 64 & 161 & 150-400 \\
\hline
\end{tabular}
\end{table}

\textit{De Boer, et al. Myelotoxicity and hepatotoxicity during azathioprine therapy.}
spond with an increased likelihood of optimal therapeutic response. The 6-TGN levels during AZA (2 to 2.5 mg/kg) and 6-MP (1 to 1.5 mg/kg) therapy vary between individuals but in most cases they are found to be between 200 and 500 pmol/8 x 10^9 RBC. These observations may explain the leucopenia and thrombocytopenia of our patient as the 6-TGN level was 738 picomoles/8 x 10^9 RBC, which is well above the proposed upper limit of efficacy. However, the diminished platelet count may also be explained by the splenomegaly due to the portal hypertension. The relatively high 6-TGN level is not explained by the TPMT status as no mutant alleles were found by genotyping. Additionally, the medication used by our patient is not expected to influence the metabolism of AZA. The possibility of an AZA overdose can not be ruled as the 6-MMP level was not available. An adequate clarification for the elevated 6-TGN is not available.

In recent years the hepatotoxic potential of thiopurines, in particular 6-thioguanine (6-TG), has been discussed in literature. The use of 6-TG in IBD patients has currently been abandoned due to its presumed hepatotoxic profile, as it has been associated with the induction of NRH. The higher occurrence of histological liver abnormalities during 6-TG treatment in comparison with AZA or 6-mercaptopurine (6-MP) may be explained by the significantly higher levels of 6-TGN reached by 6-TG. In the past, it was demonstrated that AZA may lead to veno-occlusive disease due to dose-dependent toxicity to murine sinusoidal endothelial cells and hepatocytes. Additional different pharmacokinetic characteristics or a different first-pass effect may play a role as well. Interestingly, there is a clear predominance of hepatic lesions in male patients which raises the question of a genetic predisposition.

The hypothesis that high 6-TGN levels are hepatotoxic may provide an explanation why our patient developed an incomplete septal cirrhosis. Moreover, this concept may provide a clue why hepatotoxicity is reported to be relatively rare during AZA and 6-MP therapy as the majority of IBD patients will have much lower 6-TGN levels during AZA or 6-MP treatment compared with our patient. However, an underestimation of histological liver abnormalities caused by AZA or 6-MP therapy may be expected, since the current practice is to stop the drug without performing a liver biopsy when finding elevated liver function tests during AZA or 6-MP treatment. Furthermore, not all histological liver abnormalities lead to derangement in liver function tests.

Our case illustrates the potential toxicity of AZA and stresses the need for continuous close monitoring of patients taking thiopurines in general. Routinely performed laboratory controls including full white blood counts and liver function tests seem mandatory.

## REFERENCES