Journal of Crohn's and Colitis (2013) 7, 812–819

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The effect of allopurinol and low-dose thiopurine combination therapy on the activity of three pivotal thiopurine metabolizing enzymes: Results from a prospective pharmacological study

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Received 10 October 2012; received in revised form 4 December 2012; accepted 10 December 2012

KEYWORDS
Azathioprine;
Allopurinol;
Inflammatory bowel disease;
Thiopurine methyl transferase;
Hypoxanthine guanine phosphoribosyl transferase

Abstract

Introduction: Thiopurine therapy is often discontinued in inflammatory bowel disease (IBD) patients. The xanthine oxidase (XO) inhibitor allopurinol has previously shown to enhance thiopurine efficacy and to prevent adverse reactions, the mechanism of this beneficial interaction is not completely clarified. The aim of this study is to observe possible effects of allopurinol and low-dose thiopurine combination therapy on the activity of three pivotal thiopurine metabolizing enzymes.
Methods: A prospective study of IBD patients failing thiopurine therapy due to a skewed thiopurine metabolism was performed. Patients were treated with allopurinol and azathioprine or mercaptopurine. Xanthine oxidase, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and thiopurine S-methyl transferase (TPMT) activities, and thiopurine metabolites concentrations were measured during thiopurine monotherapy, and after 4 and 12 weeks of combination therapy.
Results: Of fifteen IBD patients, XO activity decreased from 0.18 (IQR 0.08–0.3) during thiopurine monotherapy to 0.14 (IQR 0.06–0.2) and 0.11 (IQR 0.06–0.2; $p=0.008$) mU/hour/ml at 4 and 12 weeks, respectively. HGPRT activity increased from 150 (IQR 114–176) to 180 (IQR 135–213) and 204 nmol/(h×mg protein) (IQR 173–213; $p=0.013$). TPMT activity seemed not to be affected. 6-Thioguanine nucleotide concentrations increased from 138 (IQR 119–188) to 235 (223–304) and to 265 pmol/8×10^8 (IQR 188–344), whereas 6-methyl mercaptopurine ribonucleotides concentrations decreased from 13230 (IQR 7130–17420) to 690 (IQR 378–1325) and 540 (IQR 240–790) pmol/8×10^8 at 4 and 12 weeks of combination therapy (both $p<0.001$).

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1873-9946/$ - see front matter
http://dx.doi.org/10.1016/j.crohns.2012.12.006

Conclusion: Allopurinol and thiopurine combination-therapy seems to increase HGPRT and decrease XO activity in IBD patients, which at least in part may explain the observed changes in thiopurine metabolite concentrations.

1. Introduction

The immunomodulating pro-drugs azathioprine (AZA) and mercaptopurine (MP) are commonly used in the treatment of inflammatory bowel disease (IBD), as these conventional thiopurines are recommended in most IBD-guidelines as first line immunosuppressive maintenance treatment. Nevertheless, in daily practice up to half of IBD patients discontinue this therapy within 2 years. Treatment withdrawal is mostly due to the development of adverse drug reactions or therapy resistance, allegedly related to an aberrant metabolism, which seems theoretically disadvantageous.[1,2]

Several enzymes, of which the activities are partly pharmacogenetically determined, are crucial in the complex metabolism of thiopurines.[3] Among these enzymes are: hypoxanthine-guanine phosphoribosyl transferase (HGPRT), xanthine oxidase (XO) and thiopurine S-methyl transferase (TPMT). Following conversion of AZA into 6MP, the first step in metabolism of 6MP to the pharmacologically active 6-thioguanine nucleotides (6-TGN) is driven by HGPRT. However, 6MP can also be oxidized by xanthine oxidase (XO) into inactive 6-thiouric acid (6TUA) or methylated by thiopurine S-methyl transferase (TPMT) either directly into 6-methyl mercaptopurine or as a nucleotide into 6-methyl mercaptopurine ribonucleotides. These methylated products together have been named 6-MMPR (Fig. 1).[4] Patients with a skewed metabolism produce high red blood cell (RBC) 6-MMPR concentrations at the cost of 6-TGN formation. High 6-MMPR concentrations are associated with toxicity, in particular hepatotoxicity but even with myelotoxicity, whereas 6-TGN concentrations above a certain cut-off (>235 mmol/8×10^8 RBC) are associated with therapeutic efficacy.[5–8] However, prospective studies have not be able to demonstrate that 6-TGN-guided dosing is superior to

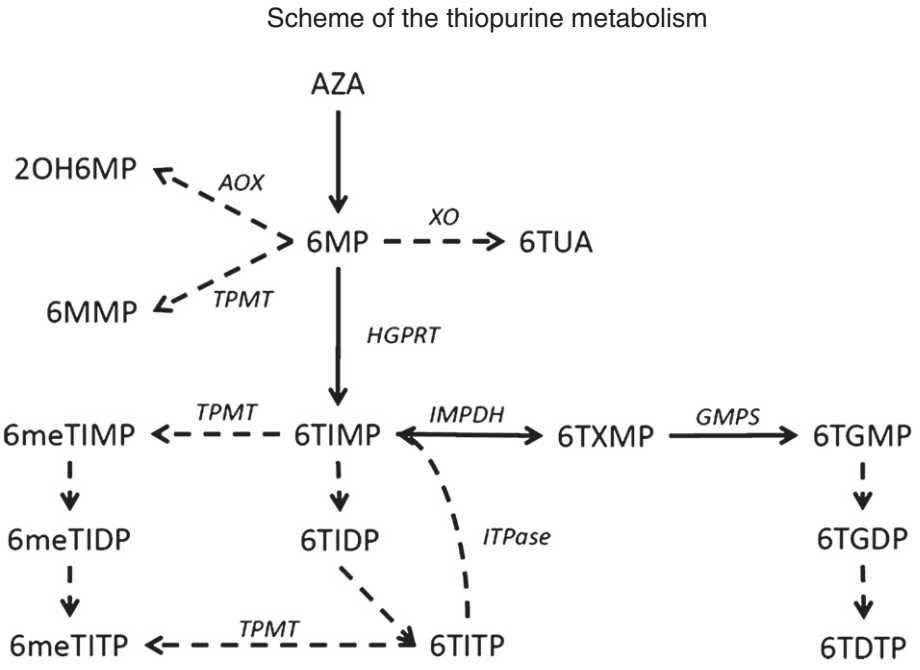

Figure 1 Following conversion of azathioprine (AZA) into 6-mercaptopurine (6MP), the first step in bioactivation of 6MP is mediated by hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and yields 6-thioinosine monophosphate (6TIMP). However, 6MP can also be oxidized by xanthine oxidase (XO) into inactive 6-thiouric acid (6TUA), or methylated by thiopurine S-methyl transferase (TPMT) into 6-methyl mercaptopurine (6MMP). Moreover, 6MP can be inactivated by aldehyde oxidase (AOX), into 2-hydroxy-6-mercaptopurine (2OHMP). 6-Thioinosine monophosphate (6TIMP) can further be metabolized in three different ways. First, 6TIMP is a substrate for TPMT, which results in the formation of 6-methyl mercaptopurine ribonucleotides (6-MMPR). These 6-MMPR include 6-methyl thioinosine monophosphate (6-meTIMP), 6-methyl thioinosine diphosphate (6meTIMP) and 6-methyl thioinosine triphosphate (6meTITP). Second, 6TIMP can be phosphorylated via 6-thioinosine diphosphate (6TIDP) to 6-thioinosine triphosphate (6TITP), which in turn can be converted back to 6TIMP by inosine triphosphate pyrophosphohydrolase (ITPase). Third, inosine-5-monophosphate dehydrogenase (IMPDH) converts 6TIMP into 6-thioxanthosine monophosphate (6TXMP). 6-Thioxanthosine monophosphate (6TXMP), then, is converted by guanosine monophosphate synthetase (GMPS) into 6-thioguanine monophosphate (6TGMP), which in turn is phosphorylated by kinases to 6-thioguanine diphosphate (6TGDP) and 6-thioguanine triphosphate (6TGTP). Together these nucleotides form 6-TGN.