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Review

Effective long-term solution to therapeutic remission in Inflammatory Bowel Disease: Role of Azathioprine

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ABSTRACT

Azathioprine (AZA) is a well-known immunosuppressant used for many years for its ability to ensure long term disease remission in inflammatory bowel diseases (IBD) at an affordable cost to the public. However, the side effect profile has raised many concerns with numerous investigations into the risk, cause and prevention of these effects. Much of the side effect profile of AZA can be linked to a single nucleotide polymorphism (SNP) in the thiopurine methyltransferase (TPMT) gene which ensures the breakdown and efficacy of AZA. Mutated TPMT alleles result in low or deficient TPMT levels which directly correlate to cytotoxity. This is a review of the role of AZA in the treatment of IBD. Knowing a patient's TPMT status allows the prescribing doctor to make an informed decision about dosage and be more alert to the signs of cytotoxicity. It is essential to include "early warning" SNP testing into common practice to ensure therapeutic efficacy.

1. Introduction

Azathioprine (AZA) is an immunosuppressant drug that was first produced in 1957 and is included in the World Health Organization's (WHO) List of Essential Medicines. AZA is a purine analogue which interrupts the synthesis of purine ribonucleotides guanine and adenine, causing mis-incorporation of bases and preventing deoxyribonucleic acid (DNA) repair mechanisms [1–3]. It has a most notable effect on fast dividing cells such as T- lymphocytes; at low doses AZA works as an anti-inflammatory while at high doses it has immunosuppressant and cytotoxic characteristics [4,5]. The drug class of thiopurines is commonly used to treat dermatological conditions, malignancies, rheumatic diseases, prevention of rejection after organ transplant or for the treatment of inflammatory gastrointestinal disorders such as Inflammatory Bowel Disease (IBD). Thiopurine drugs have a very narrow therapeutic index and can cause life threatening toxicity [6].

2. Mechanism of action

As shown in Fig. 1, AZA is a prodrug which once administered, is converted to 6-mercaptopurine (6-MP). 6-MP undergoes methylation via the key enzyme thiopurine methyltransferase (TPMT) to form an inactive methylated metabolite of 6-mercaptopurine (6-Me-MP) [7]. In the absence of methylation by TPMT, 6-MP is converted into 6-thioguanine (6-TG) by xanthine oxidase, where after hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) converts 6-TG into 6-thioguanine nucleotide (6-TGN) metabolites. 6-TGN is the active metabolite that determines cytotoxicity or efficacy. TPMT competes with xanthine oxidase and HGPRT to determine how much of the 6-MP is catalyzed to 6-TGN [8,9]. TPMT enzyme activity varies greatly in patients due to the presence of polymorphic variation in the TPMT gene [3].

At normal levels of TPMT activity, 6-TGN inhibits intracellular signalling pathways and induces lymphocytic apoptosis. An increase in TPMT enzyme activity above normal results in decreased 6-TGN and hence a decrease in drug efficacy. The decrease or absence of TPMT activity (such as that seen in the TPMT polymorphisms) results in increased levels of 6-TGN which incorporates into the DNA and trigger cytotoxicity [9–11].

3. Pharmacology

Azathioprine can be administered intravenously or orally in both a delayed release oral (DRO) capsule, or tablet form. A study by Van Os *et al* (1996) compared the bioavailability of 50mg AZA when administered in the form of a DRO tablet, oral capsule, rectal hydrophilic foam (HBF), rectal hydrophobic foam (HPF) and intravenously. The oral bioavailability was 41.6%, DRO 9.6%, HBF 5.9% and HPF 1.8%, assuming the intravenous bioavailability was 100% [12]. Oral tablets are considered to be a "local" approach as it delivers a lower

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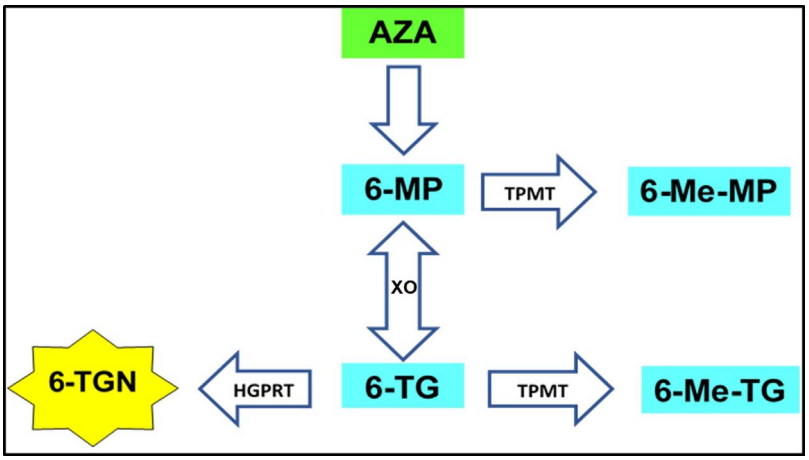

Fig. 1. Mechanism of action of thiopurine drugs and potential pathways of metabolism [8].
AZA (azathioprine), **6-MP** (6-mercaptopurine), **HGPRT** (hypoxanthine guanine phosphoribosyl transferase), **XO** (xanthine oxidase), **TPMT** (thiopurine S-methyltransferase), **6-Me-MP** (6-methyl-mercaptopurine), **6-TG** (thioguanine), **6-TGN** (6-thioguanine nucleotides).

bioavailability and thus less risk of toxicity [13].

Direct delivery to the colon is ideal as it surpasses first pass metabolism by the liver. However, direct delivery of rectal foam to the colon produces a lower bioavailability due to reduced absorption by the colon mucosa when compared to that of the gastric mucosa [12]. In a recent animal study by Helmy et al. (2017), a new therapeutic strategy was developed by loading AZA into colon-targeted chitosan beads and inserting the beads into an acid-resistant capsule [14].

The half-life of AZA is reported to be 26–80 min, or 3–5 hours if metabolites are included [15,16]. The half-life of TGN in erythrocytes is reported to be 5 days, and months may be needed to reach a steady state [17]. This may explain why a prolonged treatment period is needed before a clinical response occurs.

Therapeutic dosage concentrations range from less than 1 mg/kg bodyweight/day, to 3 mg/kg bodyweight, depending on the severity of the disease and the side effect profile. On average it takes 17 weeks for a therapeutic response to appear in most patients, but it has been suggested that the response time can be sped up by administering a loading dose of 5 mg/kg bodyweight/day to achieve a greater cumulative concentration [3,12,18]. Van Os et al (1996) observed that the patient response time can also be decreased by administering a loading dose of AZA intravenously, thus providing a portion of the cumulative dose more swiftly [12]. On average 47% of orally administered AZA reaches systemic circulation and just more than 80% of AZA is converted to 6-MP, which has a 16% bioavailability [12,13].

Although AZA has been proven to increase miscarriages by 20 fold in mice, conflicting studies and reports have been published with regards to the teratogenic nature of the drug in humans, with some studies [4,19] finding that the drug only causes low birth weight and premature birth while other studies [20,21] claim the drug is definitely teratogenic. It is difficult to relate AZA directly to foetal abnormalities as majority of the females taking the medication are on combination therapy and are advised to stay on treatment during the pregnancy to prevent disease relapse, many studies therefore conclude that any other birth abnormalities are due to underlying disease rather than the medication itself [13,19]. Yet, AZA has been listed as a class D teratogen according to the Food and Drug Administration (FDA), and is not recommended while breastfeeding [12].

AZA has been associated with a variety of side effects ranging from general nausea to myelosuppression, in very rare cases red-cell aplasia or death (in cases of TMPT polymorphism) [22,23]. Most side effects can be separated into two reaction groups, namely dose-independent (DI) and dose-dependent (DD) reactions. Drug Induced reactions tend to be hypersensitive, allergy-like reactions which tend to occur within the first few weeks after initial dosing. Symptoms such as pancreatitis, fever, joint pain, gastrointestinal disturbances and rash are common in DI reactions [17,24,25]. DD reactions tend to appear at later stages of therapy due to metabolite build up and often present as leukopenia, cholestatic jaundice, uncommon bacterial infections, hepatitis, nausea and myelosuppression. DD related side effects will generally disappear once the dosage is decreased, while DI reactions will continue until therapy is discontinued [12,13,17]. Many studies have reported patients developing toxicity long after therapy was initiated, most notably two studies totalling 1135 patients observed the onset of toxicity ranging from immediately after first dose to patients developing toxicity after 11 years of therapy [26–28]. In a more recent study by Björnsson et al (2017), it was noted that nearly three-quarters of patients who had developed thiopurine-induced hepatotoxicity, also developed cholestatic hepatitis within 3 months of starting the dose or increasing the dosage [29].

There are known associations between thiopurine treatment for rheumatoid arthritis or renal transplant and the increased risk of developing a malignancy, however there are differing opinions regarding the relationship when it comes to IBD patients [30,31]. Some studies claim there is no significant association between thiopurine therapy for IBD patients and the risk of malignancies [30], while other studies claim that there is a significant association with an increased risk of malignancies [32,33]. These malignancies include urinary tract cancer in older men, non-melanoma skin cancer (NMSC) in younger patients and lymphoma in the general population [34,35]. However, both study groups agree that there is limited data to establish causality [2].

Kandiel et al (2005) argued that a 4-fold increase in lymphoma is observed in IBD cases, but again whether this is due to the underlying disease, as a result of thiopurines or a combination of the two factors, remains unclear [2,36]. A study by Lewis et al (2000) pointed out that despite the 4-fold increase in risk of lymphoma, AZA is still a vital component of immunomodulatory therapy in IBD management and that the increased risk would have to be greater than 9.8-fold for the benefit of alternative therapies to outweigh the benefit of AZA therapy [17,37]. This statement has been reiterated recently in a study by Clowry et al (2017) on the relationship between thiopurine therapy and the risk of NMSC where, as previously concluded, due to the rising prevalence of IBD in young patients with little other alternative to immunosuppressive therapy, the benefit of this treatment outweighs the risk. Furthermore, it was noted that combination therapy for long periods of time also increased the risk factors [35].

Individuals using AZA are commonly on a combination therapy with a corticosteroid; where the steroid will slowly be weaned off once disease remission status is achieved. A study by de Jong et al (2014) has found that patients who are on a combination therapy with a higher