Thiopurines' Metabolites and Drug Toxicity

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Thiopurines (comprising azathioprine (AZA), 6-mercaptopurine (6-MP), and 6-thioguanine) have been used for over 5 decades in the treatment of a myriad of disorders, including acute lymphoblastic leukemia (ALL), inflammatory bowel disease (IBD), auto-immune hepatitis (AIH), and also in the prophylaxis of rejection in organ transplant recipients. Many questions remain unanswered regarding therapeutic drug monitoring (TDM) utility with thiopurines. We performed a systematic review with inclusion of studies evaluating the relationship between thiopurines' metabolites and drug toxicity. Meta-analysis of mean difference (MD), correlations and odds ratio (OR) was performed. We identified 21,240 records, 72 of which were eligible for meta-analysis. Levels of 6-thioguanine nucleotides (6-TGN) were higher in patients with leukopenia (MD 127.06 pmol/8 × 10^8 RBC) and gastrointestinal intolerance (MD 201.46 pmol/8 × 10^8 RBC), and lower in patients with hepatotoxicity (MD −40.6 pmol × 10^8 RBC). We established a significant correlation between 6-TGN and leukocytes (r = −0.21), neutrophils (r = −0.24) and alanine aminotransferase levels (r = −0.24). OR for leukopenia in patients with elevated 6-TGN was 4.63 (95%CI 2.24;9.57). An optimal cut-off of 135 pmol/8 × 10^8 RBC for leukopenia was calculated (sensitivity 75.4%; specificity 46.4%). 6-methylmercaptopurine ribonucleotides (6-MMPR) were significantly associated with hepatotoxicity (MD 3241.2 pmol/8 × 10^8 RBC; OR 4.28; 95%CI 3.20; 5.71). Levels of 6-MMPR measured in the first 8 weeks of treatment were associated with leukopenia. We conclude that TDM could be used to prevent thiopurines' toxicity. As optimal metabolites level may vary according to indication, physicians may adapt posology to decrease toxicity without compromising efficacy.

Keywords: thiopurines ; therapeutic drug monitoring ; adverse events

1. Introduction

Thiopurines (comprising azathioprine (AZA), 6-mercaptopurine (6-MP), and 6-thioguanine) have been used for over 5 decades in the treatment of a myriad of disorders, including acute lymphoblastic leukemia (ALL), inflammatory bowel disease (IBD), auto-immune hepatitis (AIH), and also in the prophylaxis of rejection in organ transplant recipients [1].

As prodrugs, thiopurines have a complex metabolism which leads to the formation of 6-thioguanine nucleotides (6-TGN). Regarding conventional thiopurines, other pathways compete with the production of the active metabolite 6-TGN, leading to the formation of 6-methylmercaptopurine (6-MMP) and 6-MMP ribonucleotides (6-MMPR). These metabolites can be determined by different methods, such as the Lennard [2] and Dervieux–Boulieu assays [3], that perform the measurement in red blood cells (RBC), with concentrations expressed as pmol/8 × 10^8 RBC.

2. Thiopurine's toxicity - an overview

Thiopurines present toxicity at distinct levels: myelosuppression, hepatotoxicity, pancreatitis and gastrointestinal intolerance, among others. Toxicity is an important cause of treatment cessation; in IBD, about 15% of patients discontinue thiopurines due to adverse events [4] [5]. The toxicity of thiopurines can be divided into dose-dependent and idiosyncratic. Due to the distinct metabolisms, the safety profiles of thiopurines may differ. The most worrisome adverse event of 6-thioguanine is liver nodular regenerative hyperplasia (NRH), which still detracts some physicians from its use [6].

The balance between efficacy and toxicity can be achieved with tailored dosing and monitoring, using a weight-based regimen. However, the dose of thiopurines does not correlate with the levels of metabolites [7]. The level of metabolites, specifically 6-TGN, has been associated with improved clinical outcomes in ALL, renal transplantation, and IBD [8] [9] [10] [11]. An optimal therapeutic range of ~230 to 400 pmol/8 × 10^8 RBC is often cited for patients with IBD and other disorders [12]. Values of 6-TGN of 450 pmol/8 × 10^8 RBC and of 6-MMPR of 5700 pmol/8 × 10^8 RBC were reported as thresholds for myelotoxicity and hepatotoxicity, respectively [13] [14]. However, the benefit of therapeutic drug monitoring (TDM) for thiopurines is still uncertain [15]. Based on the risk of myelosuppression, Food and Drug Administration (FDA) and the Clinical Pharmacogenetics Implementation Consortium recommend genotyping or phenotyping for thiopurine S-

methyltransferase (TPMT) deficiency prior to starting thiopurines [16] [17]. American Gastroenterology Association and proceedings of the first Thiopurine Task Force meeting [6] [15] state that the benefit for routine TPMT testing is still uncertain for most patients, and some real-life studies support this statement [18].

3. Meta-analysis - main results

In our recently published meta-analysis [19], we identified a relationship between thiopurines´ metabolites and several adverse events: i) 6-TGN were associated with leukopenia, neutropenia and gastrointestinal intolerance, and inversely associated with liver toxicity; and ii) 6-MMPR were associated with liver toxicity and early leukopenia.

As myelosuppression has long been linked to 6-TGN, we calculated an optimal 6-TGN threshold (135 pmol/8 × 10^8) for the occurrence of leukopenia. Although this cut-off is below the therapeutic levels for monotherapy with thiopurines in IBD, it is above the optimal cut-off for the levels demanded in combination therapy. This constitutes an additional argument for using lower doses of thiopurines when the drug is combined with infliximab. Regarding neutrophils and platelets, correlation with 6-TGN was only significant when the analysis was restricted to conventional thiopurines. Scientific evidence indicates that 6-TGN levels derived from 6-thioguanine have a different impact than those resulting from conventional thiopurines. Indeed, low doses of 6-thioguanine can lead to high 6-TGN levels without evidence of myelosuppression [20]. Some explanations can be pointed out for this fact. As most methods do not measure 6-TGN directly, but reduce it to thioguanine, the ingested 6-thioguanine is indistinguishable from 6-TGN, resulting in false high levels of 6-TGN if the drug is ingested close to the assay [21]. In addition, 6-MMPR are not produced with 6-thioguanine. Our results evidenced a possible association of early 6-MMPR assessment (i.e., in the first 8 weeks of treatment) with leukopenia. In previous studies, these metabolites were shown to be cytotoxic and to inhibit purine de novo synthesis, contributing to the antiproliferative properties of these drugs, responsible for both therapeutic and myelotoxic effects [22].

We have also confirmed a positive association of hepatoxicity with 6-MMPR, and a negative association with 6-TGN. In patients who metabolize thiopurines preferentially through the methylation pathway, generating high levels of 6-MMPR (known as “shunters”), dose escalation will not always improve clinical outcomes. This explains thiopurines’ inefficacy despite optimal weight-based dosage [23]. Some strategies can be used to improve the metabolite profile in these patients: i) dose-splitting regimen [24]; ii) addition of allopurinol [1] [24]; or iii) use of 6-thioguanine instead of a conventional thiopurine [20].

Gastrointestinal intolerance to thiopurines is one of the most frequent adverse events with thiopurines treatment, causing many patients to abandon treatment [25]. Some authors postulated that this adverse event could be related to the nitro–imidazole compound released in AZA metabolism to form 6-MP [26]. In this way, 6-MP could be an adequate alternative to AZA treatment in patients experiencing gastrointestinal intolerance, as was demonstrated in some studies [26] [27]. However, we have found that 6-TGN levels were associated with the occurrence of gastrointestinal intolerance. Accordingly, in one of the studies included in the analysis, switch of AZA to 6-MP was only tolerated in a small proportion of patients [28].

4. Implications for patient care

As many thiopurine-associated adverse events are related to the level of metabolites, physicians should take this information into account for dose selection, to achieve the best compromise between efficacy and toxicity. The importance of establishing a clear relationship between metabolite levels and toxicity may also be of value in patients receiving concomitant medications with similar toxicity profiles. In these cases, metabolites’ measurement will help to determine the culprit. The same applies to disorders in which the clinical presentation resembles drug toxicity, as in the case of AIH flares. If the context enables TPMT and nudix hydrolase−15 (NUDT15) screening before starting the treatment to inform on eligibility or drug dosage, subsequent adjustments can be guided by the measurement of metabolites, in a tiered approach [24]. However, at this point, these strategies should be used as adjuncts in clinical practice and cannot yet replace blood and clinical monitoring for early detection of toxicity. A more personalized medicine should overcome the traditional weight-based dosing of thiopurines and rely more on TDM. Still, higher quality studies are needed to confirm this strategy.

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