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REVIEW

Individually administered or co-prescribed thiopurines and mesalamines for inflammatory bowel disease

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Abstract

Data from both basic research and clinical experience continue to suggest that mesalamines and thiopurines are effective and efficient for the maintenance of remission of inflammatory bowel diseases. Several decades following the formalization of their indications, attention on these two drugs has been fostered by recent achievements. Demonstration of the ability of mesalamine to activate a colonocyte differentiation factor has shed light on its chemopreventive effects on colorectal cancer; in addition to their anti-proliferative efficacy, thiopurines have been shown to be specific regulators of apoptosis. The two drugs are often co-administered in clinical practice. Recent advancements have shown that mesalamines exert a positive synergism in this context, insofar as they can inhibit side-methylation of thiopurines and hasten the function of the main immunosuppressive pathways. Considering that up to 40% of patients cannot tolerate thiopurines, such renovated targets have stimulated efforts to improve compliance by research on the toxicity mechanisms. The definition of genetic polymorphisms in the enzymes of thiopurine metabolism, and the uncovering of synergistic drug interactions, such as that with allopurinol, are just two of the results of such efforts. Interaction between basic research and clinical practice has continued to inform indications and refine the prescriptions of mesalamines and thiopurines; these have not been restrained (they have been implemented in some cases) by the advent of the novel biological molecules with anti-cytokine activity.

Key words: Inflammatory bowel disease; Mesalamine; Thiopurines; Azathioprine; Remission; Drug toxicity

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INTRODUCTION

Maintenance of remission of inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD) is a crucial target for at least two reasons: unchecked bowel inflammation behaves as an independent factor in colon cancer development[1]; and the inflammatory pathways do mutate in relapsing inflammatory bouts, thus favoring development of drug unresponsiveness and activation of apoptosis-resistant non-professional immunocytes[2]. Two classes of drugs form the traditional arsenal for IBD remission maintenance: mesalamines and thiopurines, and the remainder of this review emphasizes that they are far from being set aside. Mesalamines and thiopurines share a few facts in common: they have both been studied in the second half of the last century for indications that were eventually changed; they were studied by two female scientists, one of them was awarded the Nobel prize; they are both effective and cheap; and they both continue to remain under the limelight as novel pharmacological actions are being uncovered beyond those already known. Mesalamines and thiopurines are

often co-prescribed, and among other topics, we shall review the pros and cons of this association.

MESALAMINES

Brief history

The ancestor of mesalamine is a compound named salazopyrin (SASP) made by a sulfamidic moiety linked by an azo bond to 5-aminosalycilic acid (5-ASA). Such combined anti-bacterial and anti-inflammatory actions came to the attention of the Swedish rheumatologist Nanna Svartz, who believed that the joint lesions in her patients may have been caused by latent infections and inflammation. In the early 1940s, while giving SASP to her rheumatic patients, to take advantage of its double actions, she noticed an improvement in IBD in co-morbid subjects. Her observations were reported in 1942[3] and were to be confirmed in a controlled fashion 20 years later[4]. The abandonment of the infectious theory in rheumatoid arthritis and the acknowledgement that the significant allergic toxicity of SASP was mainly caused by its sulfa moiety led to the development of 5-ASAs compounds in the following decades, which are known as mesalazine in Europe and as mesalamine in the US.

Pharmacology, metabolism, and mechanism of action of 5-ASA

Upon ingestion, 5-ASA is partially oxidized in the stomach, absorbed in the proximal gut, and acetylated in the liver. Although it is not as efficiently absorbed in the terminal gut, the efficiency of the above described processes causes a significant portion of 5-ASA to be transferred to the bloodstream, thus posing the need for an efficient carrier to effect drug delivery to distal areas of disease. Diverse ways to address the need for the distal delivery of 5-ASA have been pursued in the last decades: use of a pH-dependent carrier that releases the active drug distal to the ileo-cecal valve, an ethyl-cellulose capsule to release 5-ASA evenly in the digestive lumen, or, the synthesis of dimers in which the azo bond is supposed to be broken by the colonic flora[5]. *In vitro*, 5-ASA has been shown to share several pharmacological properties with non-steroidal anti-inflammatory compounds, including: inhibition of nuclear factor (NF)-κB-dependent inflammatory pathways[6]; limitation of the oxidative stress in epithelial cells[7,8]; increase in the cellular heat-shock protein response[9]; inhibition of leukotriene production[10]; and modulation of prostaglandin metabolism[11].

Indications

The indications for mesalamines do differ between UC and CD. At least one study[12] has claimed that SASP is superior to placebo in the treatment of active left-sided CD. On the contrary, the results are mixed as to the indication for remission maintenance; a recent review[13] has recommended that prescription of mesalamines for the maintenance of CD be avoided. Regarding the management of UC, there are different data. In one large study[14], mesalamine doses ranging between 1 and 4 g/d have been shown to induce remission in 30% of patients, as compared with 12% remission achieved by placebo. A recent Cochrane[15] analysis has shown that all of the FDA-approved formulations can offer a 30% therapeutic gain over placebo.

Table 1 Adverse effects of mesalamine and thiopurines in a cohort of IBD patients[18]

Event	Number	Percentage (%)
Mesalamine (n = 44)		
Pulmonary dysfunction	3	6.8
Pancreatitis	1	2.2
Hemolytic anemia	1	2.2
Intolerance to local drug vehicle	1	2.2
Platelet reduction	1	2.2
Diarrhea	1	2.2
Total	8	17.8
Thiopurines (n = 57)		
Leukopenia	10	17.5
Hepatic damage	5	8.77
Infection	4	7.0
Pancreatitis	4	7.0
Idiosyncracy	2	3.50
Nausea	1	1.75
Malignancy	1	1.75
Total	27	47

Toxicity

Although they are prescribed worldwide, SASP/mesalamines can exert occasionally complex toxicity that targets the skin, kidneys, pancreas, liver and cardiovascular system. As a result of the sulfa moiety, SASP can target the skin more often with manifestations ranging from rashes to major Stevens-Johnson lesions. In contrast, the phenacetin-like structure confers on mesalamine the ability to effect necrosis of the renal papilla, thus explaining the concern for clinically meaningful renal toxicity. In an English survey[16], the frequency of 5-ASA-related interstitial nephritis was 11.1 cases per million prescriptions, with the figure being 7.5 for pancreatitis. The lung manifestations linked with mesalamine deserve particular attention[17], being probably based on allergic mechanisms shown to occasionally cause a range of lung damage from eosinophilic pneumonitis to bronchiolitis obliterans. In our series[18], an unexpected percentage of 6.8% patients out of 44 that received mesalamine showed respiratory distress or pleuro-pneumonitis (Table 1).

THIOPURINES

History

Belonging to the class of fraudulent nucleotides, the thiopurines are expected to exert a prevalent anti-proliferative and immunosuppressive effect by interfering with DNA replication and causing strand breakage. The thiopurines received a lot of attention in the 1950s and 60s from Gertrud Elion and George Hitchings, who aimed at exploiting their anti-proliferative actions for the treatment of pediatric malignancy. Some of the shrewd

perceptions of Elion, including the attempt to hasten the antineoplastic effects by adding the synergic drug allopurinol (see below), were already contained in her manuscripts of the 1960s[19], and such work led to her award of the Nobel prize in 1988[20]. In the following years, the oncological indications for thiopurines were challenged by the release of more potent antineoplastic drugs; However, an anecdotal claim at the beginning of the 1960s[21] opened the era of their use for IBD, an era that it still far from being concluded.

Metabolism and mechanism of action

6-mercaptopurine (6-MP), derived in the liver after non-enzymatic, glutathione-dependent, elaboration of the pro-drug azathioprine (AZA) opens the thiopurine metabolic cascade, which leads to the final synthesis of the immunosuppressive 6-thioguanine metabolites (6-TGNs). While AZA is mostly prescribed in Europe, 6-MP is preferred in the US. At the initial pathway, two enzymatic systems compete for the common substrate 6-MP: thiopurine methyltransferase (TPMT) catalyzes the formation of the methylated non-immunosuppressive compounds of methyl-mercaptopurine (MMP); and hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) leads to the synthesis of 6-thio-inosine-monophosphate. This metabolite may either enter a phosphorylation loop regulated by a pyrophosphatase (ITPase), or it may undergo elaboration by a dehydrogenase to form 6-TGN, which finally exerts their specific DNA strand breakage effect by incorporation into the DNA replication pathways (Figure 1). A few typical features of the toxicity of thiopurines can be accounted for by at least three points of genetic/biochemical variability, as contained in the described metabolic pathway. Also, such points have recently become the target for finely tuned interventions that are aimed at modulating the patterns of the metabolic cascade, and at restraining the clinical meaningfulness of the relevant thiopurine toxicity[22]. The three points include: the genetic polymorphism of TPMT; the possibility to inhibit the enzyme xanthine oxidase by allopurinol (Elion's initial idea); and the ITPase polymorphisms. The following paragraph on toxicity gives more insight into this matter.

Indications

The immunomodulatory action of thiopurines is characterized by a delayed onset that may take 3 mo. This feature, caused by some fine aspects of their immunomodulatory mechanisms as described below, has traditionally made the thiopurines specific remission maintenance drugs. A pivotal controlled study in 1980 has shown that 6-MP is superior to placebo for fistula closure and steroid sparing in CD[23]. Drug withdrawal experiments have shown a significant trend to relapse, with > 50% of the withdrawl patients relapsing at the third year of follow-up[24-26]. The efficacy of AZA in the maintenance of UC has been suggested by a similar withdrawal experiment conducted in 1992: out of 79 patients in remission on AZA and randomized to either continue or withdraw treatment, 36% of the former relapsed during follow-up, but as many as 59% of the latter relapsed[27]. Several multicenter and monocentric studies, including one from our group[28], have confirmed the efficacy of thiopurines in the maintenance of remission and avoidance of colectomy for UC. In particular, we were able to show that AZA is effective in the maintenance of fastidious forms of UC that are initially responsive to cyclosporin[29].

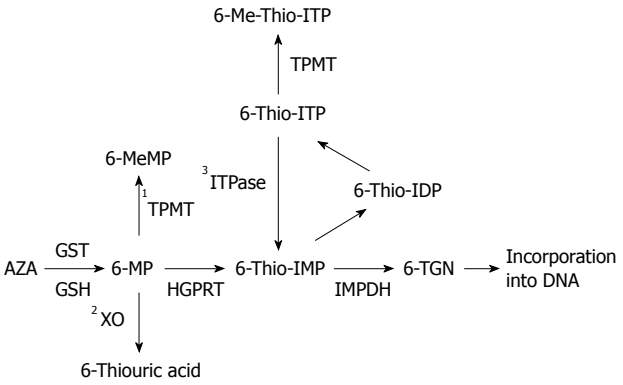

Figure 1 Thiopurine metabolism. For a caption to this figure please refer to metabolism and toxicity paragraphs and ref. 22. [1]TPMT is polymorphic and can be inhibited by mesalamine; [2]Xanthine oxidase can be inhibited by allopurinol; [3]ITPase is polymorphic.

Toxicity

Owing to the high degree of genetic polymorphism that affects a few of the key enzymes in their metabolic pathway, thiopurines tend to exert varied toxicity. A recent relevant review[30] includes the following: leukopenia 1.3%-12.6%; infection 0.3%-7.4%; liver damage 0%-4.2%; pancreatic damage 0%-4%; gastric intolerance 1.3%-6%; idiosyncratic reactions 0%-3.9%; and drug withdrawal 5.7%-22%. These figures have been upgraded in a recent review, which estimates that up to 40% of the subjects assigned to thiopurine treatment cannot benefit from these drugs because of the adverse effects[31], and do not differ from our monocentric experience[18] in which, of 57 IBD patients receiving thiopurines, 25 (43%) experienced adverse effects including leukopenia (17.5%), hepatic damage (8.7%), and infection (7.0%) (Table 1). Attempts at understanding and preventing leukopenia and hepatic and pancreatic events have brought about a significant increase in our basic knowledge. Examination of the thiopurine pathway suggests that leukopenia may chiefly be caused by dysfunction of the side methylator enzyme TPMT, which allows HGPRT to form excess levels of the bone-marrow-toxic 6-TGNs. Indeed, studies of TPMT activity have found it to be uneven, and the gene that encodes for TPMT has been shown to have a degree of polymorphism, as follows. Determination of erythrocyte TPMT activity has led to identification of three subsets of subjects: 90% show high activity; 10% show intermediate activity; and 0.3% are almost devoid of TPMT. The latter (1:300 in the Caucasian population) have a significant risk of developing fatal leukopenia if exposed to thiopurines. TPMT is encoded for by a highly polymorphic 27-kb long gene that is localized to chromosome 6p22.3. Seventeen

variant alleles have so far been described: three of these (TPMT*2, TPMT*3A, TPMT*3C) can cause the synthesis of an unstable protein, caused by an altered tertiary structure, and have been causally linked with 80%-95% of all cases of null TPMT activity. Such information in the last two decades has fostered the search for a screening test and has led to the standardization and release of commercial kits that are allegedly able to identify homozygous subjects at risk for developing fatal leukopenia. Such TPMT studies have provided the paradigm of the neonatal pharmacogenomic studies and have shown the extent of the clinical impact of these studies. If a hypoactive TPMT can cause leukopenia, recent studies[32] have suggested that hyperactivity of the enzyme may cause accumulation of an excess of methylated by-products and liver damage that may affect up to 10% of the so-called hypermethylator subjects. This problem has been addressed[31] by the co-prescription of allopurinol. By inhibiting xanthine oxidase and the production of the inactive thiouric acid, a 30% lower AZA dose can be administered, thus reducing the substrate for the hyperactive TPMT. Idiosyncratic pancreatitis has been claimed to respond to this strategy. In addition, idiosyncratic response to thiopurine, as shown by fever and flu-like syndromes, has been shown to be avoided by allopurinol. In these cases, probably the accumulation of ill-defined methylated toxins in the area of a polymorphic ITPase is the culprit. Although promising, research in the area of ITPase polymorphisms is still in its infancy.

CO-PRESCRIBED MESALAMINES AND THIOPURINES

Frequency in clinical practice

Data from a recent survey conducted by us and other four Italian centers has revealed a frequency of co-prescription of 71%.

Evidence of synergism between the two drugs

This comes essentially from withdrawal data in clinical practice. In two independent reports, others and ourselves have shown that patients on AZA and mesalamine in remission from their UC may relapse severely and progress to eventual colectomy if mesalamine is withdrawn; a consistent fall in the blood concentration of 6-TGNs is found in these circumstances[33,34]. In addition, the above cited review has shown that regular mesalamine therapy behaves as the only independent factor of continuous remission before AZA withdrawal.

Mechanisms underlying the synergy

As illustrated above (Figure 1), the purine metabolic pathway unfolds primarily following a process by which the pro-drug AZA is finally converted to the metabolites of 6-TGN. The immunosuppressive power of this biochemical machinery depends on the accumulation of the latter compounds, insofar as they are able to decrease the number of dividing lymphocytes by DNA strand breakage. The immunosuppressive steps of the pathway may be influenced by diversion of the metabolites towards two side-streams at the beginning of the process: one, catalyzed by TPMT, leads to the production of MMP, and the other, catalyzed by xanthine oxidase, produces thiouric acid, both of which are devoid of immunosuppressive activity. Of the two, TPMT has resulted in the most protean system, being under the influence of both genetic polymorphism and drugs. 5-ASA compounds have shown strong affinity for TPMT[35], with a significant inhibitory activity that results in increased feeding of metabolites towards the main axis, which results in a boost to the immunosuppressive power of the pathway. Mesalazine, sulphasalazine and olsalazine have all been shown to influence TPMT activity, with the effects emerging at both the biochemical and clinical levels, as detailed below.

Results from clinical reports

An increase of the effectiveness of AZA in relation to administration of mesalamine, and a concomitant increase in 6-TGN concentration, with attendant leukopenia, have been described in several studies[36-39]. Two other studies, on the contrary, have failed to find an advantage from co-prescription, whereas increased toxicity that has hastened the need to discontinue AZA has been emphasized[40,41]. The overall available evidence speaks in favor of co-prescribing AZA and 5-ASAs. Together with allopurinol, the mesalamines seem to offer an effective strategy to optimize AZA administration in hypermethylator patients. Whether this readily translates into improved clinical outcomes remains debatable. A recent systematic review[42], although not providing a definitive answer, has concluded that co-administration of thiopurines and mesalamines can lead to a decreased risk for colorectal cancer in long-standing disease.

REAPPRAISAL OF THE INDICATIONS FOR MESALAMINES: CHEMOPREVENTION

Background

Both CD and UC are known as pre-cancerous lesions, with the risk for transformation becoming significant within 8 years of the diagnosis of UC, and attaining 7%-14% at 25 years[43]. Two orders of evidence achieved in the last decades have focused attention on mesalamine as a chemopreventive agent against UC-dependent colorectal cancer. On one hand, a pivotal paper[1] has shown that unchecked inflammation acts independently in the promotion of cancer through dysplasia; and on the other hand, modern technology has provided evidence that mesalamine can exert a specific anti-neoplastic action thanks to its ability to interfere with both prostaglandins and nuclear transcription factors for the pro-inflammatory cytokines. The next question was whether mesalamine can protect against colon cancer *in vivo*. A literature search has retrieved at least three retrospective studies of correlation offering relevant answers: a 3-mo course of SASP significantly reduced

the cancer risk in a population of 3000 patients with colitis; in two subsets of colitis patients, of whom, only one received therapeutic doses of 5-ASA, the eventual percentage of cancer development was 3% *vs* 31%, respectively; and in the final study of 102 patients, the drug-induced risk reduction in cancer was 75%-90%[44].

Mechanisms

Peroxisome proliferator activated receptor gamma (PPAR-γ). PPAR-γ is a nuclear receptor that belongs to a family of at least 50 members that are involved with an array of biological functions. Once located to the nucleus and heterodimerized with retinoid X receptor alpha, PPAR-γ begins to regulate four gene classes. Such a gene complex is known to direct four major biological functions: metabolism, proliferation, signal transduction, and cell motility. PPAR-γ is maximally expressed in the gut, with a gradient increasing from the proximal to the distal bowel. A local negative gradient of expression has been shown in the colon, with the lowest expression levels found in the distal colon; microscopically, the expression is high among the proliferating cells of basal crypts and progressively fades away from bottom to top, to almost indistinguishable levels in cells that detach from the crypt apex and fall free in the lumen. This clearly depicts PPAR-γ as a potent differentiation factor that exerts its pivotal role in an environment where a dramatic proliferative drift completely renews the epithelium every 3 d.

These effects favor the candidacy of PPAR-γ to be identified among the effective antineoplastic agents in the colon. The insulin-sensitizing drugs, the glitazones, which bind PPAR-γ show anticancerous activity in animal models. Hemizygous knock-out animals for PPAR-γ show lesser resistance to carcinogenic treatments. At this point, the final crucial question is whether mesalamines can bind PPAR-γ, and two lines of evidence have contributed to the answer: (1) colitic animals that are heterozygous for PPAR-γ respond least to 5-ASA, which implies a role for PPAR-γ in the mediation of the antineoplastic effect of 5-ASA; and (2) 5-ASA can be accommodated into a loop in the structure of PPAR-γ through hydrogen bonding. Taken together, the above indicate that 5-ASA can exert a chemopreventive action against UC-related colorectal cancer, and this action is mediated through anti-inflammatory activity, and depends on its binding to a potent differentiation factor of colonocytes[45,46].

REAPPRAISAL OF THE POTENTIAL OF THIOPURINES: IMMUNOMODULATION

IBD patient caregivers have long become familiar with one of the hallmarks of the action of thiopurines: a latency of effect that may last for 3 mo. Traditionally, this delay has been attributed to the time supposed to be required for the 6-TGN metabolites to saturate myeloid precursors and exert their anti-proliferative effects, a tenet that has recently been challenged on the basis of two lines of evidence[47,48]: (1) the use of an intravenous load of AZA has not significantly reduced the latency; and (2) the process of myeloid cell saturation has been shown to be completed within 2 wk. The results of further studies prompted by these doubts have contributed to uncover that, well beyond their known anti-proliferative capacity, the thiopurines may exert a more finely tuned immunoregulatory action that is largely independent from DNA strand breakage and immune cell death. Focus on this novel aspect of their action is maintained by two recent publications.

Already back in 2003[49], the Neurath group had shown that thiopurines can induce T-cell apoptosis in controls as well as in IBD patients, by replacing GTP as a ligand for the Rac-1 receptor, thus hindering its main function of inducing NF-κB. This process is CD-28-dependent and triggers a mitochondrial pathway to apoptosis. This study was the first to demonstrate that a product of the intermediate thiopurine metabolism (6-thioguanine triphosphate, as generated from the phosphorylation loop described above) cannot simply break the native DNA, but specifically triggers apoptosis directed towards the autoimmune clones at the root of IBD perpetuation. This provides a fine immunological indication for the use of thiopurines, and hints at a disease-modifying role that is still to be studied.

The Ben-Horin group in Israel has recently developed research on this apoptosis process further, and has concentrated on timing and mechanistic issues[50]. Using a double *in vitro* and experimental approach in animals, they have gathered evidence that thiopurines effect a proliferative arrest of T lymphocytes, without any apoptotic effect being obvious until the fifth day post-stimulation. During this latency, these lingering T lymphocytes are still capable of adherence and mediating inflammation; thus, the immunological events lying behind the clinical latency of thiopurines have been uncovered. In their second set of experiments, this time conducted *in vivo*, they have shown that the animals must be exposed for > 1 mo to mercaptopurine before it restricts the memory pool to antigenic re-challenge. These data depict thiopurines as fine immunomodulators that require several weeks to express full-blown activity. Far from being speculative laboratory exercises, these approaches serve (1) to remind the clinician of the underlying reasons for the specific indication for thiopurines, as maintenance drugs; and (2) to remind doctors to reiterate to their patients the need for maximum compliance in order to maximally exploit the drug and benefit from the longest disease-free period.

CONCLUSION

Despite the time elapsed since their initial study, mesalamines and thiopurines continue to remain under investigation as research from basic immunology fosters novel clinical approaches, as shown by a few publications that have appeared even while preparing this review. Mesalamine has recently been proposed as the first candidate drug to be endowed with a disease-modifying role in UC[51]. Another review[52] has raised the question of

the timing of the introduction of AZA for IBD, and has proposed to use it earlier in a top-down strategy in order to best exploit its effects, chiefly the mucosal-healing potential. Finally, mesalamines and thiopurines, together with cyclosporin, are still considered unbeaten in terms of cost-effectiveness, when compared with the most recent drugs made by genetic engineering, and have been awarded the status of "backbone therapy" for IBD[53].

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