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feature

A well-tolerated and rapidly acting thiopurine for IBD?

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Thiopurine drugs continue to be a cornerstone of inflammatory bowel disease (IBD) treatment. Thiopurines are economical compared with many newer medical treatments for IBD, other chronic inflammatory diseases and leukaemia, although they are not without their shortcomings. These include a slow-onset therapeutic action and many adverse drug reactions. This feature article surveys published data, unpublished in vitro and in vivo experiments, as well as clinical experience, underpinning a rationale for bringing a novel thiopurine drug formulation to market. This formulation has a rapid action making it suitable for the induction and maintenance treatment of IBD and avoids most thiopurine-associated adverse reactions.

Introduction

For many years, thiopurine drug therapy has been used to treat ulcerative colitis (UC) [1,2] and Crohn's disease [3–5]. Succinctly, thiopurine therapies reduce the need for hospitalisations and surgery, improve quality of life, prevent relapse and decrease the burden of colorectal cancer [6–10]. However, although the conventional thiopurines: azathioprine (AZA) and mercaptopurine (MP), have been a cornerstone of immunosuppressive maintenance therapy in inflammatory bowel disease (IBD), they are not beneficial in 30–50% of IBD patients, mainly owing to the development of adverse events within the first few months of treatment [9,11]. Recently, there has been emphasis on their well-documented association with lymphomas. However, the absolute risk of lymphoma is very low [12], especially when it is considered that patients might accept higher risks of life-threatening complications to gain control of their IBD [13], and the most common thiopurine-associated lymphomas are treatable.

With an estimated 1.5 million IBD patients on them [14], these drugs remain a cornerstone of treatment of IBD but a clinically relevant problem with the conventional thiopurines is their slow onset of action. This is not related to systemic concentrations of the drug. For example, the slow pharmacodynamic action is not avoided by intravenous loading [15]. Rather, the slow pharmacodynamic action relates to the mechanism of drug action. It is pertinent in this regard to understand how conventional thiopurines work. They are pro-drugs that require systemic conversion via the highly conserved purine salvage pathway to the main active nucleotide drug, which is thioguanine nucleotide triphosphate (TGTP) [16,17].

Although excessive conversion to active nucleotide drug can cause unwanted myelosuppression, the toxicity of conventional thiopurines is mainly associated with their catabolic methylated metabolites. The generation of most methylated thiopurine metabolites can be bypassed by another thiopurine pro-drug: thioguanine (TG). TG is converted more directly to TGTP in a pathway that avoids generation of methyl-mercaptopurine or methyl-thioinosine nucleotides. Impressively from a clinical viewpoint, TG is tolerated in 80–90% of those who previously ceased conventional thiopurines because of side-effects, including in the case of pancreatitis [9,18]. There has been a reluctance by gastroenterologists to prescribe TG. This is largely because of concern about hepatic nodular regenerative hyperplasia (NRH). However, the development of NRH is explained by high concentrations of TGTP in the portal circulation (*vide infra*) and is rare and clinically not significant where IBD patients are prescribed either low-dose daily TG (20 mg O.D. for adults) or split daily dosing of TG (several doses of $\leq$20 mg

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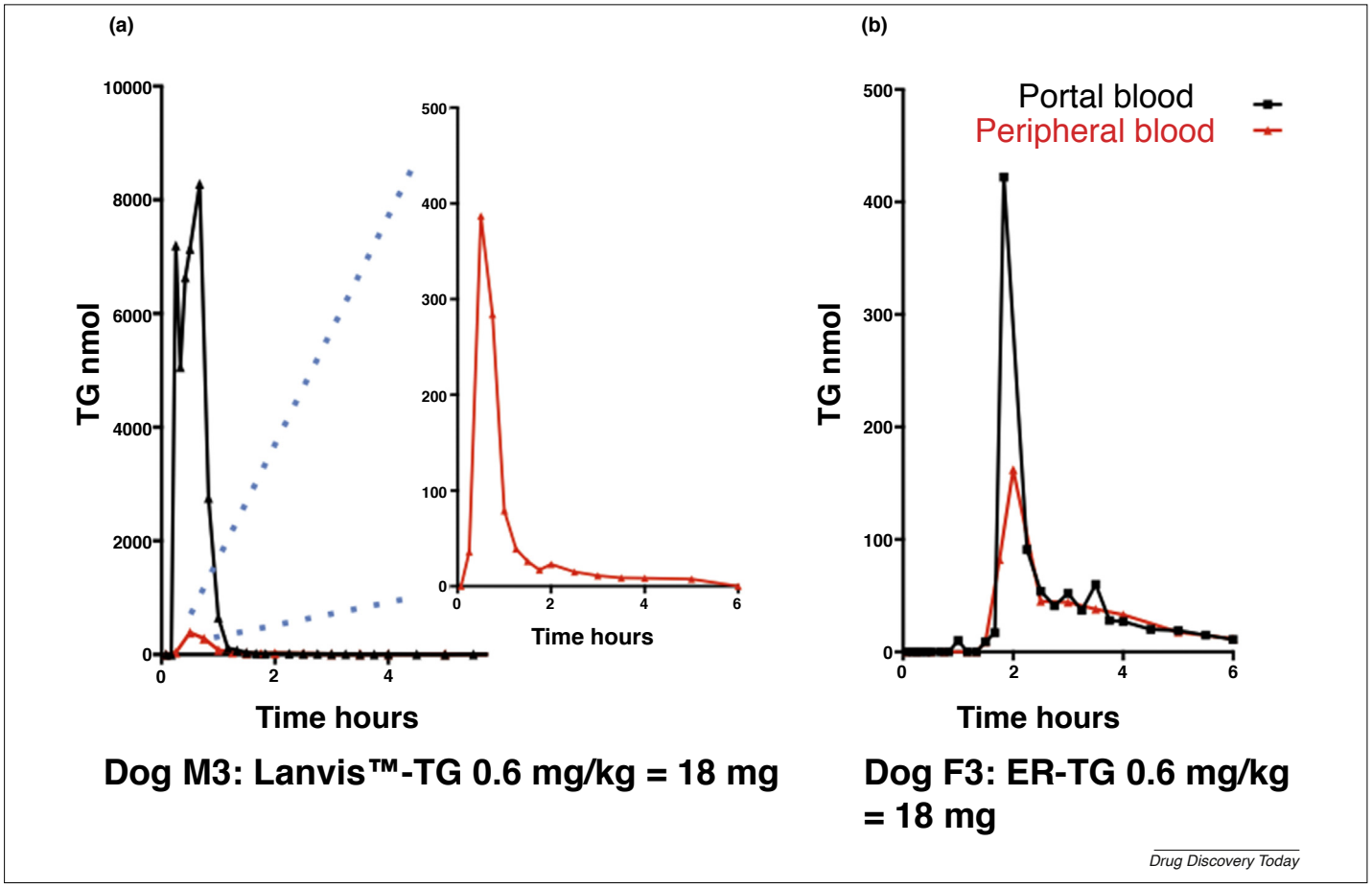

FIGURE 1

Portal blood and peripheral blood pharmacokinetics over 6 h. **(a)** Data from a single experiment with a 30 kg male dog fed 18 mg Lanvis™-thioguanine (TG) with 300 g fresh minced beef. **(b)** A 30 kg female dog fed 18 mg extended-release (ER)-TG with 300 g fresh minced beef.

each dose) [18,19]. This is because the NRH that occurs infrequently with low-dose TG is not associated with the clinically significant complication of portal hypertension [19]. Furthermore, it is becoming increasingly well documented that NRH is not irreversible. It regresses over time with cessation of the causative agent [20].

The slow pharmacodynamic action of the thiopurine pro-drugs could be explained by the known mechanism of action on circulating activated T-lymphocytes. TGTP competes with the GTP second messenger in binding of intracellular RAC1, inducing apoptosis and inhibiting proliferation of the circulating systemic activated T lymphocytes that otherwise home to the gut [21,22]. Thus, as with the newer drugs that antagonise gut homing of activated lymphocytes such as the anti-α4β7 monoclonal vedolizumab, systemic TGTP is not rapidly effective in dampening inflammation that is already present in the gut. Here, evidence is presented to underpin the development of formulations of TG that act rapidly and without the risk of excessive drug in the portal circulation. These novel controlled-release formulations will (i) avoid NRH and (ii) have a fast onset of action because their main site of action is local at the site of IBD.

Liver vascular toxicity and TG portal vein C_{max}

We have previously published that liver vascular toxicity of TG is dose-dependent. This was determined in an animal model of sinusoidal obstructive syndrome, which is the animal model counterpart of NRH. Importantly, it was demonstrated that the dose-dependent liver vascular toxicity was not related to a cumulative dose of TG, and that the liver toxicity was associated with the concentration of thioguanine nucleotides in the portal circulation [23]. Furthermore, in the *Winnie* mouse model of spontaneous colitis, splitting a daily dose to minimise high portal thionucleotide concentrations versus gavaging an equivalent once-daily dose of oral TG was as effective in treating the colitis [23] (*Winnie* mice have a single nucleotide polymorphism to cause a defective barrier function, which underlies UC). The model recapitulates responses to standard therapies used in UC [24–26]. In patients with IBD, the risk of clinically significant liver vascular toxicity – NRH – is also avoided with low-dose or split-dose prescribing of TG [18,19] and does not increase over time with low-dose TG therapy.

Similar to splitting an oral dose, maximum portal concentrations (C_{max}) of TG or thioguanine nucleotides are reduced when using oral extended-release formulations (data presented at the Controlled Release Society ASM 2013 in Hawaii). Specifically, we manufactured an extended-release tablet of TG (ER-TG) that was predicted *in vitro* to release over 3 h (this release pattern over 3 h compares with Lanvis™-TG, a licensed tablet of TG available in Australia, which disintegrates within 1 h of ingestion). In these experiments (UQ IAEC MED/MMRI/167/12), portal TG concentrations were measured in greyhound dogs fed ER-TG. Briefly, the portal vein and a cutaneous brachial vein were cannulated in three pairs of adult greyhounds. Each

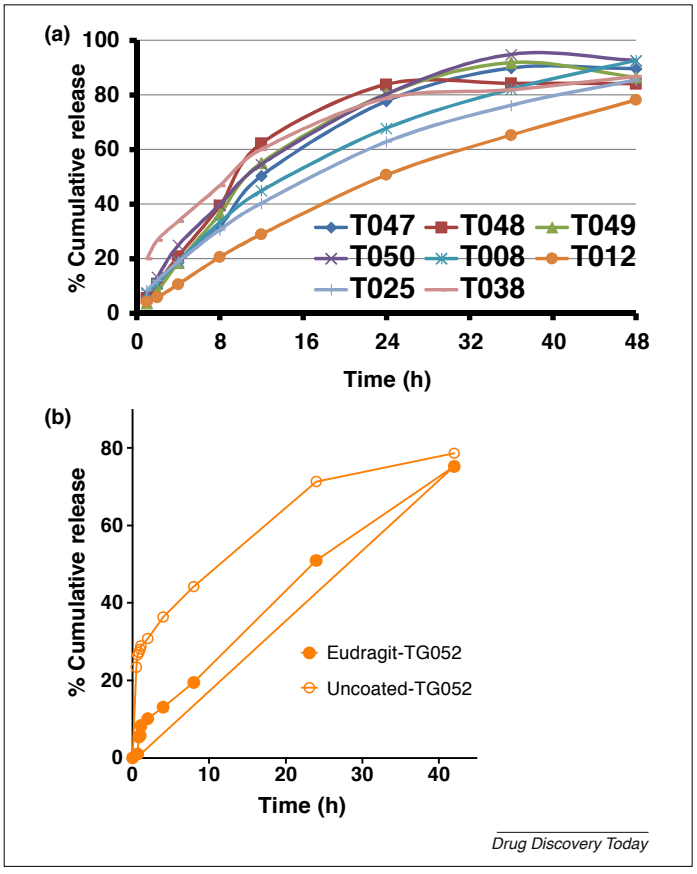

FIGURE 2

In vitro cumulative release of thioguanine (TG) from **(a)** eight distinctive 200 mg tablet formulations containing 20 mg TG; **(b)** 'T052' uncoated and hand-coated Eudragit® S100 100 mg tablets containing 20 mg TG.

pair of dogs was fed, after an overnight fast, 0.6 mg/kg TG (together with a meal of lean minced beef) either in the ER-TG or Lanvis™-TG formulations. Venous and portal blood were drawn at regular intervals for 24 h. Following protein precipitation with an acetonitrile/acetone solution containing an isotope-labelled internal standard, quantitation of plasma TG was achieved using liquid chromatography tandem mass spectrometry on a Sciex 4000 QTRAP®.

Peak portal vein TG C_{max} concentrations ranged from 224 nM to 430 nM for ER-TG compared with 2870 nM to 8100 nM for Lanvis™-TG. Portal vein TG area under the curve (AUC) with ER-TG ranged from 36 to 80% of AUC Lanvis™-TG (Mann–Whitney $P < 0.05$). Mean AUC in peripheral blood with ER-6TG was 76% of AUC with Lanvis™-TG (*P* not significant). Figure 1 illustrates one of the experiments. The data show reduced AUC with ER-TG, suggesting that the extended release could increase first-pass small intestinal metabolism of TG with ER-TG.

Rectal TG has a rapid action

We published that daily rectal TG but not rectal MP enemas improve distal colitis in the *Winnie* mouse model of UC [27]. The improvement was rapid. Diarrhoea improved by 10 days. At sacrifice on day 14 there was a significant improvement in colon weight and length (a reliable indicator of ameliorated colitis). Strikingly, blinded histological scoring confirmed less inflammation in the distal colonic segment but not more-proximal colonic segments with TG after only 14 days. There was no immunosuppression consistent with the colon having a low capacity to absorb TG or that metabolism was local [27,28]. It was inferred that the local metabolism involved bacterial conversion as well as mucosal conversion of pro-drug, because the rapid beneficial effect of TG also occurred in *Winnie* crossed with hypoxanthine-guanine phosphoribosyltransferase-deficient mice that lack the host enzyme required for the conversion of TG to active drug (unpublished data).

In contrast to TG, it was interesting that an equivalent daily dose of rectal MP over 14 days or larger oral daily gavaged doses of MP over 1 month did not improve colitis. This is probably because the conversion of MP to active drug, whether by bacteria, mucosa, liver or circulating lymphocytes, is rate-limited by inosine monophosphate dehydrogenase [17].

Controlled release oral TG tablets could deliver TG locally to the distal intestine

We made novel controlled-release oral formulations of TG that provide near-zero-order *in vitro* kinetic release of TG over 24–48 h in controlled conditions at pH 7.5. The formulation examples were made using direct compression and wet granulation tableting techniques [29]. The physical properties of the tablets and their dissolution characteristics were stable over at least 2 years [29]. *In vitro* drug release studies were conducted using US Pharmacopeia (USP)-accepted methods in gastric and intestinal environments to mimic conditions in the gastrointestinal tract. The release rate was conducted using USP-II paddle apparatus at 50 rpm. In more detail, tablets were placed in individual vessels that were immersed in 750 ml of 0.1 N HCl at 37 °C. After 1 h of operation, 200 ml of tribasic sodium phosphate buffer was added to the vessels. The pH was adjusted to 7.5. The operation of adding the buffer and adjusting the pH was completed within 5 min. The dissolution apparatus was continued for another 48 h. Figure 2a illustrates examples of cumulative release of TG at pH 7.5 with eight different 200 mg tablet formulations that contained 20 mg TG. Gastric pH was associated with significant release of TG over 1 h (data not shown), but its dissolution in the gastric condition could be prevented by coating tablets with Eudragit® S100. Figure 2b shows that coating 'T052' 100 mg tablets with Eudragit® S100 inhibited release of TG at gastric pH but enabled controlled release over the next 36–48 h.

In Australia, physicians are permitted to prescribe off-label formulations of approved pharmaceutical drugs if they utilise a registered compounding chemist to make the formulations. We have prescribing experience with daily TG suppositories in patients with proctitis and with daily TG enemas in patients with pouchitis or distal UC. In contrast to oral thiopurines currently in the market place, the treatment response in >20 patients with suppository or enema TG was rapid within 7–14 days. Thus, the experience supports the contention that controlled-release drug formulations that would deliver TG to the colon should have a rapid onset of action. Furthermore, systemic levels of TGN in blood measured routinely as part of therapeutic drug monitoring were ~20% of those expected with equivalent doses of oral Lanvis™-TG. This is consistent with less TG entering the portal circulation when delivered via the colon.

Rational prescribing in health budgets

There is clearly a clinical need for fast-acting, safe, effective, well-tolerated and economical once-daily oral immunomodulating therapies for IBD. This is true for first- and second-world countries and for those countries with third-world health economies. Currently, most key opinion leader perspectives, and most treatment trials in IBD, are industry-driven. It is hoped that the evidence reviewed in this article will provide the rationale and motivation for properly resourced clinical trials, which are hypothesis-driven, so that a controlled-release formulation of TG might be quickly brought to the marketplace for treatment of IBD. This is because:

- the currently available oral immunomodulating therapies – AZA, MP, methotrexate – have a slow onset of action and are often not well-tolerated;
- although anti-TNFα drugs are generally very effective and fast in their action they require parenteral administration; there are also substantive safety concerns with them, including lymphoma [30] and metastatic melanoma; moreover, there is a clinically significant risk of infection, for example tuberculosis [31,32] and histoplasmosis [33]; this risk is amplified in the setting of third-world hygiene; the parenteral monoclonal therapies that target gut-homing of lymphocytes, while appearing safer, are also slow in their onset of action, as well as expensive;
- health expenditure runs from 10–20% of gross national expenditure in many countries; prescribing the latest drugs places a considerable burden on the health budget; this is amply exemplified by one of the anti-TNFα therapies, adalimumab, which is the world's current highest-earning drug; after thiopurine failure, the more costly monoclonal antibody therapies are initiated in most first-world countries, and also in many medical centres in poorer countries; the associated pharmaceutical expenditure can only be rationally sustained by negative offsets in other areas of health or the global budgets of these countries.

Concluding remarks

All of the licenced thiopurine compounds were developed and clinically tested in the 1950s and are out of patent. To date, the pharmaceutical companies with registered thiopurines have not been prepared to fund clinical trials in IBD. Consequently, there is little financial appetite to fund new clinical trials with a new generation of thiopurines. However, it is hoped that this situation will change with increased awareness of the requirement for rational health budgeting [34], and the prospect of intellectual property protection for controlled- and/or colonic-release TG, which has a rapid action and is well tolerated.

Conflicts of interest

The corresponding author is a director of an Australian startup company ProdrugXtend Pty, which owns a PCT relating to thiopurines: Novel formulation and treatment methods.

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