

Azathioprine in the Treatment of Autoimmune Blistering Diseases

Volker Meyer, MD, Stefan Beissert, MD*

KEYWORDS

- Azathioprine • Autoimmune blistering diseases • Pemphigus
- Bullous pemphigoid

HISTORY OF AZATHIOPRINE

The pharmaceutical precursor of azathioprine was originally 6-mercaptopurine. 6-Mercaptopurine was synthesized in 1951 by GB Elion and GH Hitchings using techniques reported by Fisher and Traube.¹ 6-Mercaptopurine was first used in 1953 in children with acute lymphatic leukemia.² Later, Calne,³ a young surgeon who later became professor of surgery at Cambridge, successfully treated dogs after renal transplantation with 6-mercaptopurine in 1960, but because of the high toxic potential of the drug it was not found suitable for long-term treatment of humans with solid-organ transplants. Subsequently, it was shown that one of the derivatives of 6-mercaptopurine found by Elion and Hitchings, BW 57-322, presented with less bone marrow toxicity⁴ but was as immunosuppressive as 6-mercaptopurine. To protect 6-mercaptopurine from being rapidly metabolized, an imidazole ring had been biochemically connected via the sulfur atom at position 6 of 6-mercaptopurine and thereby azathioprine was finally developed (Fig. 1). The first successful allogeneic renal transplant using a combination of azathioprine and corticosteroids was initiated in 1963.⁵ Azathioprine is now used in particular for the treatment of autoimmune diseases, such as autoimmune blistering diseases, inflammatory bowel disease, multiple sclerosis, and lupus, to name a few.

STRUCTURE AND METABOLISM

About 88% of azathioprine is ingested and 12% excreted via the gut.⁶ Nearly all the incorporated azathioprine is metabolized because only 2% is being excreted

A version of this article was previously published in *Dermatologic Clinics* 29:4.

The authors declare no conflict of interest.

Department of Dermatology, University of Muenster, Von-Esmarch-Str. 58, D-48149 Muenster, Germany

* Corresponding author.

E-mail address: beisser@uni-muenster.de

Immunol Allergy Clin N Am 32 (2012) 295–307

doi:[10.1016/j.iac.2012.04.009](https://doi.org/10.1016/j.iac.2012.04.009)

immunology.theclinics.com

0889-8561/12/\$ – see front matter © 2012 Elsevier Inc. All rights reserved.

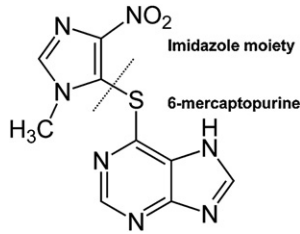


Fig. 1. Structure of azathioprine. The dashed line marks the cleavage site.

unchanged in the urine. The highest serum levels can be found approximately 2 hours after oral application and the half-life is approximately 5 hours. After uptake, azathioprine is nonenzymatically cleaved into its imidazole derivatives (methylnitroimidazole moiety) and 6-mercaptopurine (see **Fig. 1**). Three enzymes have been reported to compete for the cleavage of azathioprine (**Fig. 2**).⁷ Thiopurine S-methyltransferase (TPMT) is able to catabolize 6-mercaptopurine into the nontoxic 6-methyl mercaptopurine. Xanthine oxidase (XO) produces the nontoxic inactive metabolite 6-thiouric acid. A lack in one of these enzymes, XO and TPMT, leads to an increased production of toxic metabolites via the hypoxanthine phosphoribosyltransferase (HPRT) pathway. A lack of TPMT activity is normally caused by genetic mutations, whereas XO might be blocked by XO inhibitory drugs such as allopurinol, which is one of the most commonly prescribed drugs in Europe and North America. The enzyme HPRT metabolizes 6-mercaptopurine into 6-thiinosine 5-monophosphate (6-TIMP). This product is processed by TPMT to active methylated metabolites or is phosphorylated to 6-thiinosine triphosphate (6-TITP). 6-TITP is converted to 6-TIMP by inosine triphosphate pyrophosphohydrolase (ITPA). A lack of ITPA, often seen in Asian populations,⁸ leads to an increase in the level of toxic 6-TITP and induces corresponding side effects, such as leukopenia, gastrointestinal disturbances, or elevated liver function test results (for details concerning side effects see later discussion).

6-TIMP is converted by inosine monophosphate dehydrogenase and guanosine monophosphate synthetase into the principal active 6-thioguanine nucleotides. These nucleotides are converted by TPMT into inactive methylated metabolites.

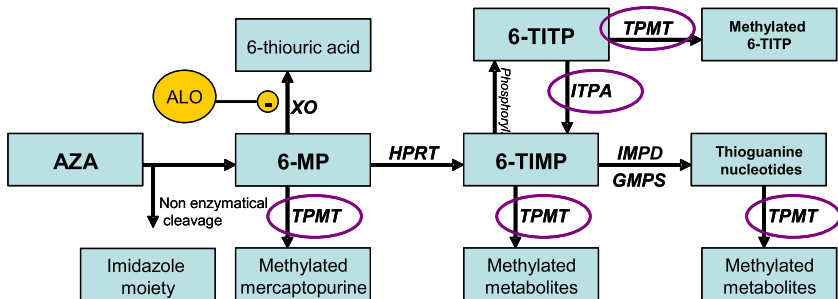


Fig. 2. Metabolism of azathioprine. Encircled enzymes indicate that tests are available for these enzymes for assessment of azathioprine's potential toxicity. ALO, allopurinol; AZA, azathioprine; GMPS, guanosine monophosphate synthetase; HPRT, hypoxanthine phosphoribosyltransferase; IMPD, inosine monophosphate dehydrogenase; ITPA, inosine triphosphate pyrophosphohydrolase; 6-MP, 6-mercaptopurine; 6-TIMP, 6-thiinosine monophosphatase; 6-TITP, 6-thiinosine triphosphatase; TPMT, thiopurine S-methyltransferase; XO, xanthine oxidase.

In patients with Lesch-Nyhan syndrome, reduced expression of HPRT can be detected, which makes these patients less suitable for azathioprine treatment. Although most of the therapeutic effects of azathioprine are dependent on the HPRT pathway, there is experimental evidence that the imidazole derivatives of azathioprine might also be effective.⁹

AZATHIOPRINE IN BULLOUS PEMPHIGOID

In 1971, Greaves and colleagues¹⁰ introduced azathioprine into the treatment of bullous pemphigoid. Before that, most patients had been treated with systemic corticosteroid monotherapy to prevent recurrent blister formation. It was reported by Greaves and colleagues¹⁰ that in 8 of 10 patients with bullous pemphigoid, no prednisone maintenance therapy was needed for preventing relapses, whereas azathioprine was given at a dose of 2.5 mg/kg body weight per day.

In a small prospective clinical trial in 1978, Burton and colleagues¹¹ examined azathioprine (2.5 mg/kg/d, $n = 12$) plus prednisone (30–80 mg/d) versus prednisone alone ($n = 13$). No significant difference between both groups was found concerning the overall disease control. The prednisone-sparing effect was statistically significant. In the azathioprine group, a cumulative average dose of 3688 mg of prednisone was used for more than 3 years, whereas 6732 mg was used in the prednisone monogroup for the same period. In 1993, Guillaume and colleagues¹² found no significant difference in disease control between prednisone monotherapy (1 mg/kg/d, $n = 31$) and azathioprine (100–150 mg/d, $n = 36$) plus prednisone therapy. However, the prednisone-sparing effect has not been investigated in this study.

In a national randomized trial in 2007, Beissert and colleagues¹³ compared treatment with methylprednisolone (0.5 mg/kg/d) with either azathioprine (2 mg/kg/d, $n = 38$) or mycophenolate mofetil (1 g twice a day, $n = 35$). The results showed no significant differences in the primary outcome (ie, complete healing of skin lesions) and a tendency to faster healing in the azathioprine group (azathioprine 23.8 ± 18.9 days vs mycophenolate mofetil 42.0 ± 55.9 days, P value is nonsignificant). Moreover, similar corticosteroid doses were used to control disease. Mycophenolate mofetil was significantly less liver toxic compared with azathioprine, which can be an advantage especially in elderly patients.

A Cochrane review concluded from the available study results that the addition of azathioprine in the treatment of bullous pemphigoid had not been established.¹⁴ The results of several studies concerning the treatment of bullous pemphigoid with azathioprine are summarized in **Table 1**.

AZATHIOPRINE IN PEMPHIGUS

The treatment of pemphigus vulgaris and pemphigus foliaceus usually does not differ significantly. Most investigators did not specify in their reports whether patients with pemphigus foliaceus or vulgaris had been treated.

The use of azathioprine for the treatment of pemphigus was introduced in 1969, when Krakowski and colleagues¹⁵ presented the first case report of a woman with pemphigus-vulgaris treated successfully with azathioprine. At the same time, Aberer and colleagues¹⁶ published a case series of 4 patients describing the use of azathioprine as “steroid saving and beneficial” in patients with pemphigus. In 1977, Lever and Schaumburg-Lever¹⁷ published a retrospective analysis of 63 patients with pemphigus. The investigators treated patients ($n = 12$) with prednisone monotherapy and the other patients in this cohort ($n = 51$) with a combination of azathioprine, cyclophosphamide, or methotrexate and prednisone. In this report, azathioprine was described as “steroid saving and effective.”

Table 1 Use of azathioprine in bullous pemphigoid (case reports are not included)				
Investigators	Year	N	AZA Dose	Main Outcome
Greaves et al ¹⁰	1971	10	2.5 mg/kg/d	8/10 symptom free, no prednisone needed
van Dijk & van Velde ⁴⁴	1973	5	75–250 mg/d	4/5 excellent response
Burton & Greaves ⁴⁵	1974	12	2.5 mg/kg/d	12/12 excellent response
Ahmed et al ⁴⁶	1977	15	1.5 mg/kg/d	Reduces duration of maintenance, steroid sparing
Burton et al ¹¹	1978	12	2.5 mg/kg/d	Steroid sparing, but control of disease not significantly different from steroid alone
Guillaume et al ¹²	1993	36	100–150 mg/d	No significant difference to steroid monotherapy concerning disease control
Beissert et al ¹³	2007	73	2 mg/kg/d	No significant differences in primary outcome between AZA + MP and MMF + MP, tendency of faster healing in AZA-treated patients

Abbreviations: AZA, azathioprine; MMF, mycophenolate mofetil; MP, methylprednisolone; N, number of patients treated with azathioprine.

The investigators’ therapeutic approach is still known as Lever’s regime. Initially, patients receive a high dose of prednisone (up to 2 mg/kg body weight) in combination with azathioprine 2.0 to 2.5 mg/kg. After cessation of new blister formation and reepithelization of erosions, the prednisone dose is reduced to 40 mg/d, while the azathioprine dose remains unchanged. Further proceedings depend on the individual’s clinical development. Normally, the prednisone dose is gradually reduced over a period of several months.

There are only a few prospective randomized trials reported on pemphigus. Beissert and colleagues¹⁸ examined 38 patients with pemphigus vulgaris or pemphigus foliaceus and found no significant differences between treatment with azathioprine and mycophenolate mofetil, both in combination with methylprednisolone, concerning remission of disease and corticosteroid-sparing effects. The patients treated with azathioprine received a median methylprednisolone dose of 8.916 ± 29.844 mg. In the mycophenolate mofetil-treated group, patients received a median of 9.334 ± 13.280 mg methylprednisolone ($n =$ nonsignificant). The mean duration of follow-up was 438 days in both groups. However, the time needed to achieve disease control in 50% of the patients was about 30 days in the azathioprine group compared with 75 days in the mycophenolate mofetil group. Perhaps the inhibition of at least 3 enzymatic pathways by azathioprine (see Fig. 2) is able to induce a faster response in comparison with mycophenolate mofetil, which inhibits primarily 1 signaling pathway. Nevertheless, after 200 days of treatment, the patients in the mycophenolate mofetil group had a remission rate of 90%, whereas those who were treated with azathioprine had a remission rate of 75%. After 600 days, this trend persisted because 20% of patients with pemphigus were still not achieving effective control with azathioprine compared with 10% of patients using mycophenolate. The recurrence rate was similar in both groups.

In another study with 120 patients analyzed, Chams-Davatchi and colleagues¹⁹ found no significant difference concerning disease remission between azathioprine and mycophenolate mofetil, but the patients treated with azathioprine showed significantly less steroid consumption. These findings indicate that azathioprine is well suited for first-

line treatment of pemphigus. Rose and colleagues²⁰ compared dexamethasone-cyclophosphamide pulse therapy with oral methylprednisolone/azathioprine therapy in pemphigus. A tendency was found in favor of methylprednisolone/azathioprine concerning complete remissions.

In IgA pemphigus, azathioprine showed no considerable effect. Dapsone is usually the first-line treatment.²¹ For pemphigus vegetans, azathioprine seems to work in individual cases.²² For treatment of refractory patients, disease control has been reported under the corticosteroid/azathioprine regimen using additionally photopheresis or retinoids.^{23,24}

In paraneoplastic pemphigus, therapy for the underlying malignancy is essential. Concomitant treatment with azathioprine and other immunomodulatory drugs has been reported, but it is difficult to conclude whether any of them will reliably affect the prognosis of the disease because its mortality approaches 90%.²⁵ Lam and colleagues²⁶ presented a case of a 77-year-old man with chronic conjunctivitis, acanthosis nigricans, and pemphigus-like mucocutaneous lesions. Further examinations revealed an underlying bronchogenic squamous cell carcinoma (SCC). Although skin lesions resolved with oral prednisone and azathioprine (100 mg/d) therapy, the conjunctivitis and mucous membrane erosions persisted. Verrini and colleagues²⁷ described another patient with paraneoplastic pemphigus who showed good response to azathioprine (100 mg/d) but died after a short time after initiation of treatment.

The importance of systemic corticosteroids in the treatment of pemphigus is clearly documented. Because the reports show a tendency in favor of azathioprine concerning corticosteroid-sparing effects and no significant differences regarding disease control, the authors suggest using azathioprine as first-line therapy in mild to moderate cases of pemphigus. Mycophenolate mofetil seems to be a valid second-line choice. In severe and rapidly progressing cases, dexamethasone/cyclophosphamide pulse therapy should be considered.

Because pemphigus is a chronic disease, long-term follow-up studies (>3 years) are clearly needed. A Cochrane review concluded from the available study results that the optimal immunomodulatory agent in the treatment of pemphigus has not yet been found. Although azathioprine and cyclophosphamide did show advantages concerning the steroid-sparing effect, mycophenolate showed superior disease control.²⁸

The results of several studies concerning the treatment of pemphigus with azathioprine are shown in **Table 2**.

AZATHIOPRINE IN OTHER AUTOIMMUNE BLISTERING DISEASES

Several case reports show that azathioprine can be effective in cicatricial pemphigoid. These reports demonstrate that azathioprine is able to control disease and prevent progression. However, azathioprine showed no beneficial effect on already existing scars and cicatrizing vegetation. Unfortunately, there are no randomized or blinded trials available. The results of relevant case reports concerning the treatment of cicatricial pemphigoid are shown in **Table 3**.

Pemphigus can also develop in children, which is a rare event in Europe and North America. Some reports center around therapy for juvenile pemphigus with azathioprine and demonstrate the efficiency of azathioprine in children with pemphigus.²⁹ The results of important case reports concerning the use of azathioprine in juvenile pemphigus are shown in **Table 4**.

AZATHIOPRINE DURING PREGNANCY

The use of immunomodulatory agents during pregnancy should always be extremely well considered. Azathioprine is able to cross the human placenta.³⁰ The fetal liver

Table 2**Use of azathioprine in pemphigus (most case reports are not included)**

Investigators	Year	N	AZA Dose	Main Outcome
Krakowski et al ¹⁵	1969	1	75–150 mg	Control of disease after 4 mo
Aberer et al ¹⁶	1969	4	1–3 mg/kg/d	Steroid saving and beneficial
Burton et al ⁴⁷	1970	4	2.5 mg/kg/d	3 of 4 with excellent response, but relapse after discontinuation of AZA; 1 patient dropped out because of severe side effects
Roenigk & Deodhar ⁴⁸	1973	10	50–250 mg/d	7, excellent; 2, good; 1, fair
van Dijk & van Velde ⁴⁴	1973	5	50–200 mg/d	5/5 excellent response
Lever & Schaumburg-Lever ¹⁷	1977	6	50–150 mg/d	Steroid saving and effective
Lever & Schaumburg-Lever ⁴⁹	1984	21	100 mg/d	Safe and effective, disease control in 21
Aberer et al ¹⁶	1987	27	1–3 mg/kg/d	45% free of disease; 38% clinically free of disease, but with raised antibody titers; 17% with controlled disease
Tan-Lim & Bystryl ⁵⁰	1990	12	50–150 mg/d	AZA in combination with plasmapheresis decreased antibodies faster than without plasmapheresis
Mourellou et al ⁵¹	1995	15	100 mg/d	Remission in 14 patients, less mortality compared with steroid monotherapy
Carson et al ⁵²	1996	72	50–250 mg/d	AZA significantly reduced mortality, steroid sparing, 37% remission, 33% stable disease
Scully et al ⁵³	1999	17	1–3 mg/kg/d	Not pointed out clearly. 2 deaths reported under treatment with AZA
Ljubojevic et al ⁵⁴	2002	129	100–150 mg/d	Reduction of mortality compared with patients with steroid monotherapy
Beissert et al ¹⁸	2006	18	2 mg/kg/d	Steroid-sparing effect of AZA similar to that of MMF. Quicker response to therapy with AZA than with MMF. No significant differences in overall outcome
Rose et al ²⁰	2005	11	2–2.5 mg/kg/d	Tendency in favor of AZA concerning complete remissions compared with cyclophosphamide pulse therapy
Chams-Davatchi et al ¹⁹	2007	30	2.5 mg/kg/d	AZA most effective compared with CYP and MMF or cortisone monotherapy, but no significant differences in complete remission
Chaidemenos et al ⁵⁵	2011	19	100 mg/d	Notable steroid-sparing effect of AZA; high dose of prednisone leads significantly faster to remission than low dose of prednisone combined with AZA

Abbreviations: AZA, azathioprine; CYP, cyclophosphamide; MMF, mycophenolate mofetil; N, number of patients treated with azathioprine.

Table 3
Use of azathioprine in cicatricial pemphigoid

Investigators	Year	N	AZA Dose	Main Outcome
Dantzig ⁵⁶	1973	1	150 mg/d	Remission of disease
Braun-Falco et al ⁵⁷	1981	1	100 mg/d	Among several trials of treatment, only AZA effective
Mondino & Brown ⁵⁸	1983	9	1.5 mg/kg/d	10 of 18 eyes showed no progress
Pawlofsky et al ⁵⁹	1985	1	150 mg/d	Successful treatment
Tauber et al ⁶⁰	1991	11	2 mg/kg/d	AZA failed to control disease in 9% of cases, diaminodiphenylsulfone recommended for treatment
Lugovic et al ⁶¹	2007	1	2 mg/kg/d	AZA effective for treatment of inflammation and blistering, but no effect on scarring

Abbreviations: AZA, azathioprine; N, number of patients treated with azathioprine.

does not possess the enzymes to convert azathioprine into its active metabolites, suggesting that the fetus might have a certain protection against azathioprine-induced cellular toxicity.³¹ Most large studies have shown that azathioprine during pregnancy is tolerated, although the rate of congenital malformations seems to be 3% to 9% in infants exposed antenatally.³² Reported malformations include myelomeningocele, preaxial polydactyly, microcephaly, thymic atrophy, hypospadias, and adrenal hypoplasia.^{31,32}

SIDE EFFECTS OF AZATHIOPRINE

Azathioprine is generally well tolerated. In a study of Japanese patients (n = 114) treated with azathioprine 50 mg/d for inflammatory bowel disease, 33% developed side effects over a period of 48 months of investigation.³³ The most common side effect of azathioprine is leukopenia (white blood cells $\leq 3000/\mu\text{L}$), with 18 (15.8%) affected patients. Of these patients, 15 experienced mild leukopenia with no necessary change of dosage. Two patients (1.8%) had severe leukopenia ($<1000/\mu\text{L}$), followed by a decrease in the dose of azathioprine. Although the mild forms of

Table 4
Use of azathioprine in juvenile pemphigus

Investigators	Year	Patients' Age (y)	N	AZA Dose	Main Outcome
Harrington et al ⁶²	1978	15	1	50–100 mg/d	Good response
Lynde et al ⁶³	1984	15	1	50–125 mg/d	Complete remission after 2.5 y
Fine et al ⁶⁴	1988	13	1	50–125 mg/d	Complete remission in combination with plasmapheresis after 12 mo
Wananukul & Pongprasit ⁶⁵	1999	11–14	3	2 mg/kg/d	2 patients, controlled disease; 1 patient, complete remission
Harangi et al ⁶⁶	2001	10	1	2 mg/kg/d	After 4.5 y of treatment, complete remission

Abbreviations: AZA, azathioprine; N, number of patients treated with azathioprine.

leukopenia occurred after an average treatment period of 13.7 months (mean; range 1–47 months), the severe forms of leukopenia occurred in less than 1 month.

The next common side effects were gastrointestinal disturbances, including vomiting, nausea, and diarrhea. Eleven patients (9.6%) experienced such symptoms, which occurred shortly (<1 month) after initiation of therapy and were all self-limited after a few days. Further side effects were elevated liver function test results. One patient (0.9%) had a severe increase in liver enzyme levels with more than 20-fold increase of transaminases levels compared to normal levels after 2 weeks of treatment. After azathioprine had been discontinued, liver enzymes returned to normal levels. Three patients (2.6%) had flulike symptoms (ie, fever, headache, rash, arthralgia, myalgia, and malaise) that were self-limited after discontinuation of azathioprine. Two more patients showed mild loss of hair that did not lead to discontinuation of therapy.

The side effects observed in the Japanese patients were identical to those observed in white patients but developed after half the azathioprine dose that leads to similar side effects in white patients. Japanese patients showed TPMT gene polymorphisms less frequently compared with white patients or Africans. Although, for instance, TPMT activity-reducing *TPMT*3A* alleles were detected in 4.5% of British white patients, Kumagai and colleagues³⁴ could not find any individual in a cohort of 522 Japanese patients who carried that allele. **Table 5** shows the frequency of variant TPMT alleles in different ethnic populations. The most frequent TPMT gene polymorphisms can be found in Africans and white patients, whereas Asians have a low prevalence of TPMT gene polymorphisms. In white patients, approximately 1 in 300 patients has a distinctly reduced TPMT activity, and about 11% of white patients show a moderately reduced activity, resulting in increased production of toxic metabolites (eg, thioinosine monophosphates) when taking azathioprine.³⁵ Myelosuppression in patients with reduced TPMT activity develops more rapidly.³⁶ Uchiyama and colleagues⁸ examined the relationship between the incidence of azathioprine-associated adverse effects and either the incidence of TPMT or ITPA gene polymorphisms (for the metabolic mechanism of ITPA see **Fig. 2**). In 5 of 6 patients who had developed an acute bone marrow toxicity, a mutation (94C>A) of the ITPA gene was detectable. About 75% of patients with agranulocytosis had the 94C>A allele. In red blood cells of patients with the homozygous 94C>A missense mutation, ITPA activity was below detectable levels. The frequency of 94C>A alleles was 31 of 200 in Japanese individuals (15.5%), which is

Table 5 Frequency of variant TPMT alleles in different ethnic populations				
Population	n	TPMT*2 (%)	TPMT*3A (%)	TPMT*3C (%)
British	398	0.5	4.5	0.3
French	382	0.5	5.7	0.8
Americans	282	0.2	3.2	0.2
South-west Asians	198	0	1	0
African Americans	248	0.4	0.8	2.4
Kenyans	202	0	0	5.4
Ghanaians	434	0	0	7.6
Chinese	384	0	0	2.3
Japanese	1044	0	0	1.6

Abbreviation: n, number of alleles.
Adapted from Kumagai K, Hiyama K, Ishioka S, et al. Allelotype frequency of the thiopurine methyltransferase (TPMT) gene in Japanese. *Pharmacogenetics* 2001;11(3):277.

2.6-fold higher than in white patients.^{8,37} Because of the high correlation between the side effects of azathioprine therapy and the prevalence of ITPA polymorphism genes in Japanese, it is suggested that in Japanese patients, instead of determination of TPMT gene polymorphisms or activity, a screen for ITPA gene mutations or activity should be introduced.⁸ In white patients and Africans, monitoring for deficiency in TPMT activity is recommended before azathioprine is introduced.

With regard to the observed side effects of azathioprine, laboratory monitoring, including blood cell counts and serum analysis (especially liver enzymes), is recommended weekly for the first 8 weeks of treatment. After 8 weeks of treatment, the interval of laboratory monitoring can be extended to once per month.

The acute toxicity of azathioprine is low. A renal transplant patient who received a massive single overdose of azathioprine of 7500 mg experienced nausea, vomiting, and diarrhea followed by mild leukopenia, mild abnormalities in liver functions, as well as improved renal function.³⁸

Like all immunosuppressive drugs, long-term intake of azathioprine raises the risk of immunosuppression-induced malignancies. In a large 20-year follow-up study, patients with rheumatoid arthritis receiving azathioprine had a 10-fold increased risk for developing immunosuppression-associated myeloproliferative disorders compared with the general population. Patients with rheumatoid arthritis without a history of azathioprine treatment had only a 5-fold increased risk of developing myeloproliferative disorders.³⁹ The risk of developing nonmelanoma skin cancers (NMSCs; SCCs and basal cell carcinomas [BCCs]) was 2-fold increased in the azathioprine-treated group with rheumatoid arthritis compared with the general population. There was a significant increase in cancer development in relation to the cumulative lifetime intake of azathioprine. In 8.6% of the patients with a lifetime intake of up to 50 g azathioprine, different kinds of cancer developed within 20 years. In patients with a cumulative dose of 200 g azathioprine, different kinds of cancer developed in more than 20% of cases within 20 years.³⁹

There is strong evidence for an additive carcinogenic effect of UV radiation, especially UV-A (320–400 nm), in combination with azathioprine treatment concerning the development of NMSCs. Ramsay and colleagues⁴⁰ examined transplant recipients of Fitzpatrick skin types I to IV. From 361 patients, 187 (51.8%) developed histologically diagnosed NMSCs. The ratio of SCC to BCC was reversed in these patients from 1:3.7 before transplantation to 2:1 after transplantation.⁴⁰ NMSC increased with the duration of medical immunosuppression. Among the transplant recipients, 29.1% developed 1 or more NMSCs when immunosuppression did not exceed 5 years, but immunosuppression for 10 to 20 years led to an incidence of 72.4% of NMSCs in the treated patients. However, the patients received not only azathioprine (on average 100 mg daily) but also 225 mg of cyclosporine and 7 mg of prednisone daily (follow-up 7.1 years).

Most of the observed NMSCs in immunosuppressed transplant recipients developed on sun-exposed areas (80% of SCCs, 71% of BCCs, and 64% of keratoacanthomata). One of the active metabolites of azathioprine is thioguanine nucleotides. These nucleotides are precursors for 6-thioguanine, which can be incorporated into DNA on replication. Normally, DNA does not significantly absorb UV-A wavelengths (320–400 nm), whereas thiopurines do. 6-Thioguanine nucleotides have an absorbance maximum at 342 nm.⁴¹ Moreover, 6-mercaptopurine as a metabolite of azathioprine generates reactive oxygen species (ROS) when exposed to UV-A.⁴² O'Donovan and colleagues⁴¹ demonstrated that biologically relevant doses of UV-A generate ROS in cultured cells with 6-thioguanine-substituted DNA. In experimental cell lines (HCT116, human colorectal carcinoma cells) containing about 0.01% 6-thioguanine-substituted DNA,

irradiation with a nontoxic dose of 1 kJ/m² UV-A led to a 3-fold increase in adenine phosphoribosyltransferase (*aprt*) mutation frequencies compared with nonsubstituted DNA. Neither 6-thioguanine nor UV-A alone was detectably mutagenic. In normal DNA without substitutes, 500 kJ/m² of UV-A irradiation was required to induce a similar increase in *aprt* mutation frequency. O'Donovan and colleagues⁴¹ measured the amount of 6-thioguanine in DNA extracted from clinically normal skin of 3 azathioprine-treated patients. Around 0.02% of 6-thioguanine-substituted DNA was found. In a control group of patients who did not receive azathioprine, no substituted DNA was detectable. Furthermore, azathioprine-treated patients showed a reduction of the minimal erythema dose for UV-A but not for UV-B. These findings indicate that the mutagenic effect of UV radiation is increased under azathioprine treatment. UV-A seems to play a more important role for photocarcinogenesis in these patients than UV-B.

In a mouse model, Reeve and colleagues⁴³ showed that azathioprine but not cyclophosphamide increased the incidence of skin tumors significantly after UV irradiation. Based on these results, it is recommended that patients undergoing therapy with azathioprine should use effective sunscreens and avoid unnecessary long-term exposure to the sun. Patients who require long-term treatment with azathioprine should undergo a clinical examination of the skin twice a year.

SUMMARY

Although there are still no standard guidelines for the treatment of autoimmune blistering diseases, azathioprine has shown good efficacy in acquired autoimmune blistering diseases. Azathioprine is normally well tolerated. Side effects of azathioprine normally occur in mild variants. Severe reactions are often caused by reduced TPMT or ITPA activity. Therefore, screening for the activity of TPMT should be conducted in white patients and Africans, whereas the Japanese should be screened for the activity of ITPA before therapy with azathioprine is started. Due to its potential steroid-sparing effect and the relatively rare side effects, azathioprine is clinically meaningful for the treatment of pemphigus, and recent developments in targeting B cells in autoimmune blistering disorders must prove their superiority against azathioprine.

REFERENCES

1. Elion GB. The purine path to chemotherapy. *Science* 1989;244(4900):41–7.
2. Burchenal JH, Murphy ML, Ellison RR, et al. Clinical evaluation of a new antimetabolite, 6-mercaptopurine, in the treatment of leukemia and allied diseases. *Blood* 1953;8(11):965–99.
3. Calne RY. The rejection of renal homografts. Inhibition in dogs by 6-mercaptopurine. *Lancet* 1960;1(7121):417–8.
4. Calne RY, Murray JE. Inhibition of the rejection of renal homografts in dogs by Burroughs Wellcome 57–322. *Surg Forum* 1961;12:118–20.
5. Calne RY, Loughridge LW, Pryse-Davies J, et al. Renal transplantation in man: a report of five cases, using cadaveric donors. *BMJ* 1963;2(5358):645–51.
6. Berlit P. Azathioprin, internist. *Praxis* 1995;35:661–5.
7. Karran P, Attard N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. *Nat Rev Cancer* 2008;8(1):24–36.
8. Uchiyama K, Nakamura M, Kubota T, et al. Thiopurine S-methyltransferase and inosine triphosphate pyrophosphohydrolase genes in Japanese patients with

- inflammatory bowel disease in whom adverse drug reactions were induced by azathioprine/6-mercaptopurine treatment. *J Gastroenterol* 2009;44(3):197–203.
9. Szawlowski PW, Al-Safi SA, Dooley T, et al. Azathioprine suppresses the mixed lymphocyte reaction of patients with Lesch-Nyhan syndrome. *Br J Clin Pharmacol* 1985;20(5):489–91.
 10. Greaves MW, Burton JL, Marks J, et al. Azathioprine in treatment of bullous pemphigoid. *BMJ* 1971;1(5741):144–5.
 11. Burton JL, Harman RR, Peachey RD, et al. Azathioprine plus prednisone in treatment of pemphigoid. *BMJ* 1978;2(6146):1190–1.
 12. Guillaume JC, Vaillant L, Bernard P, et al. Controlled trial of azathioprine and plasma exchange in addition to prednisolone in the treatment of bullous pemphigoid. *Arch Dermatol* 1993;129(1):49–53.
 13. Beissert S, Werfel T, Frieling U, et al. A comparison of oral methylprednisolone plus azathioprine or mycophenolate mofetil for the treatment of bullous pemphigoid. *Arch Dermatol* 2007;143(12):1536–42.
 14. Kirtschig G, Middleton P, Bennett C, et al. Interventions for bullous pemphigoid [review]. *Cochrane Database Syst Rev* 2010;10:CD002292.
 15. Krakowski A, Covo J, Rozanski Z. Pemphigus vulgaris. *Arch Dermatol* 1969;100(1):117.
 16. Aberer W, Wolff-Schreiner EC, Stingl G, et al. Azathioprine in the treatment of pemphigus vulgaris. A long-term follow-up. *J Am Acad Dermatol* 1987;16(3 Pt 1):527–33.
 17. Lever WF, Schaumburg-Lever G. Immunosuppressants and prednisone in pemphigus vulgaris: therapeutic results obtained in 63 patients between 1961 and 1975. *Arch Dermatol* 1977;113(9):1236–41.
 18. Beissert S, Werfel T, Frieling U, et al. A comparison of oral methylprednisolone plus azathioprine or mycophenolate mofetil for the treatment of pemphigus. *Arch Dermatol* 2006;142(11):1447–54.
 19. Chams-Davatchi C, Esmaili N, Daneshpazhooh M, et al. Randomized controlled open-label trial of four treatment regimens for pemphigus vulgaris. *J Am Acad Dermatol* 2007;57(4):622–8.
 20. Rose E, Wever S, Zilliken D, et al. Intravenous dexamethasone-cyclophosphamide pulse therapy in comparison with oral methylprednisolone-azathioprine therapy in patients with pemphigus: results of a multicenter prospectively randomized study. *J Dtsch Dermatol Ges* 2005;3(3):200–6.
 21. Zillikens D, Miller K, Hartmann AA, et al. IgA pemphigus foliaceus: a case report. *Dermatologica* 1990;181(4):304–7.
 22. Monshi B, Marker M, Feichtinger H, et al. Pemphigus vegetans—immunopathological findings in a rare variant of pemphigus v vulgaris. *J Dtsch Dermatol Ges* 2010;8(3):179–83.
 23. Faulde MK, Sörhage B, Ksoll A, et al. Complete remission of drug resistant Pemphigus vegetans treated by extracorporeal photopheresis. *J Eur Acad Dermatol Venereol* 2007;21:822–49.
 24. Ichimiya M, Yamamoto K, Muto M. Successful treatment of pemphigus vegetans by addition of etretinate to systemic steroids. *Clin Exp Dermatol* 1998;23(4):178–80.
 25. Kimyai-Asadi A, Jih MH. Paraneoplastic pemphigus. *Int J Dermatol* 2001;40(6):367–72.
 26. Lam S, Stone MS, Goeken JA, et al. Paraneoplastic pemphigus, cicatricial conjunctivitis, and acanthosis nigricans with pachydermatoglyphy in a patient with bronchogenic squamous cell carcinoma. *Ophthalmology* 1992;99(1):108–13.

27. Verrini A, Cannata G, Cozzani E, et al. A patient with immunological features of paraneoplastic pemphigus in the absence of a detectable malignancy. *Acta Derm Venereol* 2002;82(5):382–4.
28. Martin LK, Agero AL, Werth V, et al. Interventions for pemphigus vulgaris and pemphigus foliaceus. *Cochrane Database Syst Rev* 2009;1:CD006263. DOI: 10.1002/14651858.CD006263.pub2.
29. Mensing H, Brehm H, Nasemann T. Bullöses Pemphigoid im Kindesalter. *Hautarzt* 1984;35:254–8 [in German].
30. Ostensen M. Treatment with immunosuppressive and disease modifying drugs during pregnancy and lactation [review]. *Am J Reprod Immunol* 1992;28(3–4): 148–52.
31. Janssen NM, Genta MS. The effects of immunosuppressive and anti-inflammatory medications on fertility, pregnancy, and lactation. *Arch Intern Med* 2000;160(5): 610–9.
32. Tendron A, Gouyon JB, Decramer S. In utero exposure to immunosuppressive drugs: experimental and clinical studies. *Pediatr Nephrol* 2002;17(2):121–30.
33. Takatsu N, Matsui T, Murakami Y, et al. Adverse reactions to azathioprine cannot be predicted by thiopurine S-methyltransferase genotype in Japanese patients with inflammatory bowel disease. *J Gastroenterol Hepatol* 2009;24(7):1258–64.
34. Kumagai K, Hiyama K, Ishioka S, et al. Allelotype frequency of the thiopurine methyltransferase (TPMT) gene in Japanese. *Pharmacogenetics* 2001;11(3):275–8.
35. Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. *Clin Pharmacol Ther* 1989;46(2):149–54.
36. Anstey A, Lennard L, Mayou SC, et al. Pancytopenia related to azathioprine—an enzyme deficiency caused by a common genetic polymorphism: a review. *J R Soc Med* 1992;85(12):752–6.
37. Sumi S, Marinaki AM, Arenas M, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. *Hum Genet* 2002;111(4–5):360–7.
38. Carney DM, Zukoski CF, Ogden DA. Massive azathioprine overdose. Case report and review of the literature. *Am J Med* 1974;56(1):133–6.
39. Silman AJ, Petrie J, Hazleman B, et al. Lymphoproliferative cancer and other malignancy in patients with rheumatoid arthritis treated with azathioprine: a 20 year follow up study. *Ann Rheum Dis* 1988;47(12):988–92.
40. Ramsay HM, Fryer AA, Hawley CM, et al. Non-melanoma skin cancer risk in the Queensland renal transplant population. *Br J Dermatol* 2002;147(5):950–6.
41. O'Donovan P, Perrett CM, Zhang X, et al. Azathioprine and UVA light generate mutagenic oxidative DNA damage. *Science* 2005;309:1871–4.
42. Hemmens VJ, Moore DE. Photochemical sensitization by azathioprine and its metabolites—I. 6-Mercaptopurine. *Photochem Photobiol* 1986;43(3):247–55.
43. Reeve VE, Greenoak GE, Gallagher CH, et al. Effect of immunosuppressive agents and sunscreens on UV carcinogenesis in the hairless mouse. *Aust J Exp Biol Med Sci* 1985;63(Pt 6):655–65.
44. van Dijk TJ, van Velde JL. Treatment of pemphigus and pemphigoid with azathioprine. *Dermatologica* 1973;147(3):179–85.
45. Burton JL, Greaves MW. Azathioprine for pemphigus and pemphigoid—a 4 year follow-up. *Br J Dermatol* 1974;91(1):103–9.
46. Ahmed AR, Maize JC, Provost TT. Bullous pemphigoid. Clinical and immunologic follow-up after successful therapy. *Arch Dermatol* 1977;113(8):1043–6.
47. Burton JL, Greaves MW, Marks J, et al. Azathioprine in pemphigus vulgaris. *BMJ* 1970;3(5714):84–6.

48. Roenigk HH Jr, Deodhar S. Pemphigus treatment with azathioprine. Clinical and immunologic correlation. *Arch Dermatol* 1973;107(3):353–7.
49. Lever WF, Schaumburg-Lever G. Treatment of pemphigus vulgaris. Results obtained in 84 patients between 1961 and 1982. *Arch Dermatol* 1984;120(1):44–7.
50. Tan-Lim R, Bystryń JC. Effect of plasmapheresis therapy on circulating levels of pemphigus antibodies. *J Am Acad Dermatol* 1990;22(1):35–40.
51. Mourellou O, Chaidemenos GC, Koussidou T, et al. The treatment of pemphigus vulgaris. Experience with 48 patients seen over an 11-year period. *Br J Dermatol* 1995;133(1):83–7.
52. Carson PJ, Hameed A, Ahmed AR. Influence of treatment on the clinical course of pemphigus vulgaris. *J Am Acad Dermatol* 1996;34(4):645–52.
53. Scully C, Paes De Almeida O, Porter SR, et al. Pemphigus vulgaris: the manifestations and long-term management of 55 patients with oral lesions. *Br J Dermatol* 1999;140(1):84–9.
54. Ljubojević S, Lipozencić J, Brenner S, et al. Pemphigus vulgaris: a review of treatment over a 19-year period. *J Eur Acad Dermatol Venereol* 2002;16(6):599–603.
55. Chaidemenos G, Apalla Z, Koussidou T, et al. High dose oral prednisone vs. prednisone plus azathioprine for the treatment of oral pemphigus: a retrospective, bi-centre, comparative study. *J Eur Acad Dermatol Venereol* 2011;25(2):206–10.
56. Dantzig P. Circulating antibodies in cicatricial pemphigoid. *Arch Dermatol* 1973;108(2):264–6.
57. Braun-Falco O, Wolff HH, Ponce E. Disseminated cicatricial pemphigoid. *Hautarzt* 1981;32(5):233–9.
58. Mondino BJ, Brown SI. Immunosuppressive therapy in ocular cicatricial pemphigoid. *Am J Ophthalmol* 1983;96(4):453–9.
59. Pawlowsky C, Simon M Jr, Fartasch M, et al. Disseminated cicatricial pemphigoid. *Dermatologica* 1985;171(4):259–63.
60. Tauber J, Sainz de la Maza M, Foster CS. Systemic chemotherapy for ocular cicatricial pemphigoid. *Cornea* 1991;10(3):185–95.
61. Lugović L, Buljan M, Situm M, et al. Unrecognized cicatricial pemphigoid with oral manifestations and ocular complications. A case report. *Acta Dermatovenereol Croat* 2007;15(4):236–42.
62. Harrington I, Sneddon IB, Walker AE. Pemphigus vulgaris in a 15-year-old girl. *Acta Derm Venereol* 1978;58(3):277–9.
63. Lynde CW, Ongley RC, Rigg JM. Juvenile pemphigus vulgaris. *Arch Dermatol* 1984;120(8):1098–9.
64. Fine JD, Appell ML, Green LK, et al. Pemphigus vulgaris. Combined treatment with intravenous corticosteroid pulse therapy, plasmapheresis, and azathioprine. *Arch Dermatol* 1988;124(2):236–9.
65. Wananukul S, Pongprasit P. Childhood pemphigus. *Int J Dermatol* 1999;38(1):29–35.
66. Harangi F, Várszegi D, Schneider I, et al. Complete recovery from juvenile pemphigus vulgaris. *Pediatr Dermatol* 2001;18(1):51–3.