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RESEARCH ARTICLE

Observations of Measurement of Thiopurine Metabolites to Monitor Response and Complications of Azathioprine Therapy in Neuromuscular Diseases

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Abstract

Azathioprine is an immunosuppressant, commonly used in autoimmune disorders. Due to its complex metabolism and genetic polymorphisms of metabolizing enzymes, there is wide variation in clinical response to standard dosage. We reviewed the records of 30 patients with neuromuscular disorders treated with Azathioprine. Thiopurine methyltransferase polymorphisms, thiopurine metabolites, CBC, and CMP were measured. The dosage of Azathioprine varied from 25 mg to 300 mg. The level of 6-thioguanine nucleotide ranged from 57 to 581 pmol/8 x 10^8 erythrocytes and 6-methylmercaptopurine from below 243 to 15,365 pmol/8 x 10^8 erythrocytes. In patients with normal or subtherapeutic metabolite levels, the dosage was increased when indicated, they showed improvement of symptoms, and when in toxic levels, the dosages were reduced. This study documents evidence of the usefulness of monitoring thiopurine metabolism particularly in patients at high risk, such as those taking drugs that decrease its metabolism, or increased dosages due to lack of response.

Key Words: Neuromuscular disorder, Azathioprine, Thiopurine metabolites, 6-thioguanine nucleotide (6-TGN), 6-methylmercaptopurine (6-MMP)

Introduction

Azathiopurine (AZA) is one of the most commonly used immunosuppressant drug as steroid sparing therapy or in combination in patients with neuromuscular diseases, who were poorly responsive or have significant side effects with therapy, because of the effectiveness and safety profile; [1, 2] however, due to its complex metabolism and genetic polymorphisms of the metabolizing enzymes there is wide variation in the clinical response to standard dosage.

Most absorbed as AZA is metabolized to mercaptopurine (MP) which can be further metabolized in two pathways, the oxidase hydrogenase pathway and the aldolase oxidase to form thiopuric acid which is secreted in the urine. MP can be methylated by thiopurine methyltransferase to form methyl mercapitopurine and by a group of enzymes, which are also called thioguanine nucleotides (TGN or 6-TGN), to produce TG monophosphate and triphosphate, which are the primary active metabolites to maintain therapeutic response. Elevated levels of these metabolites can increase toxicity.

Deficiency of thiopurine S-methyltransferase (TPMT) could produce elevated levels of metabolites and toxicity, and for this reason levels of this enzyme should be measured prior to initiation of therapy. Drugs such as salicylates may affect the effects of TPMT and allopurinol may lower levels of the enzyme and increase toxicity, on the other hand diuretics may increase the effect of TPMT. [3]

The American Gastroenterological Association recommends that thiopurine metabolites be monitored to guide treatment changes in active inflammatory bowel disease patients to optimize therapy and avoid thiopurine toxicity. Target levels of 6-TGN between 230 to 450 pmol/8 x 10^8 red cells were used in monotherapy to correlate with response to remission in inflammatory bowel disease, and higher levels predispose to bone marrow suppression. The levels of 6-methylmercaptopurine (6-MMP) above 5,700 pmol/8 x 10^8 have also been found to be useful to monitor for possible hepatotoxicity. [4] In a study of patients with myasthenia gravis the measurement of these metabolites was used in the evaluation of response to therapy. [5]

A standard protocol for monitoring thiopurine metabolism in neuromuscular disorders has not been established. And we reviewed the records of 30 patients with neuromuscular diseases seen in our clinic who received AZA and had the metabolites measured in addition to using standard monitoring methods and determine if those changes would be useful to manage therapy treatment.

Materials and Methods

We reviewed the records of patients with autoimmune neuromuscular disorders treated with AZA for over 3 months, in which the TPMT polymorphism was checked at the beginning of therapy. 6-TGN and 6-MMP levels were measured

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periodically every 3-4 months. Demographic information, AZA dosage, treatment response to monotherapy versus combination therapy and indication for dose adjustments were obtained.

We consider patients who are refractory or not responding to treatment if symptoms persisted or worsened. Steroid dependence refers to patients who required systemic steroids to maintain disease control.

Patients underwent standard laboratory follow-up, including complete blood count (CBC), total lymphocyte count, liver function tests and erythrocyte mean corpuscular volume (MCV) levels to monitor for side effects. Leukopenia was defined as a total white blood count of below 3.5 x 10^3 /µL.

Measurement of 6-MP Metabolites

All patients underwent thiopurine methyltransferase, 6-TGN and 6-MMP measurements using PRO-Predict (Prometheus Inc., San Diego, CA). Heparinized blood samples were collected and sent to Prometheus Inc. commercial laboratory, and the test provided RBC levels of 6-TGN and 6-MMP in picomoles per 8 x 10^8 erythrocytes using reversed-phase high performance liquid chromatography as reported by the analyzing laboratory. A 6-TGN level of over 230 was considered therapeutic in a level of over 400 was considered potentially myelotoxic, and a 6-MMP level of over 5,700 was considered potentially hepatotoxic.

Data Analysis

Descriptive statistics, frequencies, and group statistics were determined using SPSS program version (SPSS, Chicago, IL) and analysis included independent t-tests to compare mean 6-TGN and 6-MMP levels between patients who were receiving different dosages of AZA. Difference in means was considered statistically significant when the two-tailed P value was below 0.05. All measurements, where applicable, were reported in range and means.

Results

The records of 30 patients were reviewed for this study. These included 12 males and 18 females, none had TPMT deficiency. The age ranges from 36 to 84 (70.0 + 13.7) and the dosage was standard 2 mg/kg and this was adjusted according to the patients' response. The patient's characteristics were summarized in table 1.

Table 1. Patient's characteristics.

Age		70.0 ± 13.7 (36-84)
Female: Male		18:12
AZA dosage		25-300 mg
Mono: Combination		9:21
Diagnosis	Myasthenia Gravis	18
	CIDP	4
	Polymyositis	3
	Dermatomyositis	2
	Miscellaneous	3

There was no linear correlation between AZA dosage and 6-TGN levels in this study. Within the 30 patients, 18 (60%) were diagnosed with myasthenia gravis and 4 (13%) chronic inflammatory demyelinating polyneuropathy and 3 (10%) polymyositis, 2 (7%) dermatomyositis and 3 (10%) with miscellaneous diagnoses including fasciitis with polymyositis and peripheral neuropathy with monoclonal gammopathy of undermined significance and one patient with a ganglioneuropathy.

Nine (30%) patients used AZA as monotherapy and the others (70%) combination with prednisone, or other therapies including IVIG and plasma exchange. Of the 9 patients with AZA monotherapy, 6 have 6-TGN levels of below 200 pmol/8 x 10^8, and 2 had 6-TGN levels between 200 pmol/8 x 10^8 and 400 pmol/8 x 10^8, and one had 6-TGN level of over 400 pmol/8 x 10^8. Of the 21 patients who received combination therapy with prednisone or other methods, the levels were below 200 pmol/8 x 10^8 in 10 patients, between 200-400 pmol/8 x 10^8 in 10 patients and 1 were above 400 pmol/8 x 10^8.

The dosage varied from 25 mg to 300 mg a day. The level of 6-TGN ranged from 57 to 581 and the 6-MMP from 243 to 15365. There was no linear distribution of AZA dosage or values. Four patients had 6-MMP levels of over 5700 and none of them had an abnormal liver function test. One patient had transient elevated liver function taking 150 mg a day which resolved without changing therapy.

Some symptomatic patients in which the 6-TGN levels were normal or below therapeutic received increased dosages of AZA resulting in improvement of symptoms.

As can be seen in table 2, the following are the observations of the various patients, in which doses were adjusted

According to the Metabolites:

Case # 1 is a 69-year-old patient with generalized acetylcholine receptor positive myasthenia gravis who developed increased symptoms of weakness and dysphagia on azathioprine alone in the dosage of 150 mg daily, and the dose was increased to 175 mg daily. The metabolites remained stable and nontoxic, and the hematology profile was normal. The patient responded well.

Case # 2, is a 36-year-old female with fasciitis and perimyositis with marked swelling in the legs and a high CK. She was on tapering dose of prednisone because of severe cushingoid features. The patient was then placed on azathioprine, and at 100 mg daily she got worse, and the dosage was increased to 200 mg daily. The metabolites remained within the therapeutic range, and the patient had marked improvement. Hematology profile remained normal, and the metabolites were stable.

Case # 3, is a 60-year-old female with anti-MuSK positive myasthenia gravis, doing well on 200 mg of azathioprine daily alone, with normal hemogram, but metabolites were toxic, and for this reason the dosage was decreased to 150 mg daily, and the patient did well, and remained stable. There was a mild increase at 6-MMP, but the dosage was not changed, and the hemogram remained normal, and the patient did very well.

Table 2. Selected Patient's information.

	Clinical characteristics	AZA dose	6-TGN level (230-400 pmol/8 x 10^8)	6-MMP level (< 5200 pmol/ 8 x 10^8)	Decision/response to change of drug dosage
Case 1	69 yo F, MG with ptosis & generalized fatigue	150 mg daily	360 pmol/8 x 10^8	15365 pmol / 8 x 10^8	Pt with ptosis and generalized weakness had excellent response to increased AZA dose from 150 mg to 175 mg q d and her 6-TGN was stable, and 6-MMP levels decreased.
		175 mg daily	327 pmol/8 x 10^8	7325 pmol / 8 x 10^8	
Case 2	36 yo F, Fasciitis and perimyositis proven by bx with severe limb swelling and pain	100 mg daily	265 pmol/8 x 10^8	776 pmol / 8 x 10^8	Pt with pain and swelling in the legs which improved with increased AZA dosage from 100 mg to 200 mg, and the metabolites remained within therapeutic levels.
		200 mg daily	273 pmol/ 8 x 10^8	1398 pmol / 8 x 10^8	
Case 3	61 yo F, MuSK Ab+ MG with generalized weakness & mild respiratory failure	200 mg daily	279 pmol/8 x 10^8	14984 pmol / 8 x 10^8	Due to toxic levels, the dosage was reduced from 200 to 150 mg q d and pt felt better although there were no obvious clinical changes. Surprisingly 6-MMP levels increased, but 6-TGN levels remained stable.
		150 mg daily	288 pmol/8 x 10^8	16776 pmol / 8 x 10^8	
Case 4	80 yo M, MG with ptosis and weakness on allopurinol	25 mg daily	186 pmol /8 x 10^8	281 pmol / 8 x 10^8	Due to increased ptosis and weakness, the AZA dosage was increased to 50 mg with excellent response, yet the metabolites did not raise.
		50 mg daily	290 pmol / 8 x 10^8	290 pmol / 8 x 10^8	
Case 5	60 yo F, MG s/p thymectomy for thymoma, with generalized weakness, ptosis and mild respiratory failure	250 mg daily	267 pmol / 8 x 10^8	794 pmol / 8 x 10^8	Pt had generalized weakness, ptosis with respiratory failure requiring plasma exchange. She had good response to increased dosage. The 6-TGN levels increased, but the WBC remained normal.
		275 mg daily	552 pmol / 8 x 10^8	2011 pmol / 8 x 10^8	
Case 6	56 yo F, polymyositis with HMGR Ab +	150 mg daily	118 pmol / 8 x 10^8	4387 pmol / 8 x 10 ^{8}	Increased AZA from 150 to 175 mg because the patient was symptomatic, and she showed some improvement, but remained weak and developed severe nausea at higher doses, but metabolites remained therapeutic. Eventually it was decided to change to mycophenolate.
		175 mg daily	129 pmol / 8 x 10^8	2151 pmol / 8 x 10^8	
Case 7	70 yo F, MG with double vision, weakness and dysphagia	150 mg daily	546 pmol / 8 x 10^8	4590 pmol / 8 x 10^8	Pt was on AZA and IVIG but remained weak, so the dosage was increased, and the metabolites raised. Then pt developed leukopenia with WBC of 2.5 × 109/L, then the dose was reduced to 175 mg q d. Pt remained strong and WBC raised to normal.
		200 mg daily	658 pmol / 8 x 10^8	18530 pmol / 8 x 10^8	

Case # 4 is an 80-year-old male with acetylcholine receptor positive generalized myasthenia gravis who was on allopurinol. He was on tapering doses of azathioprine down to 25 mg daily for 3 months, and he had increased ptosis and weakness, and the metabolite levels were nontoxic, and the hemogram was normal. The dosage was increased to 50 mg daily, and he improved significantly. The hemogram remained normal, and the metabolites remained between therapeutic range.

Case # 5 is a 60-year-old female with acetylcholine receptor positive myasthenia gravis, post thymectomy with generalized weakness and ptosis. Because of worsening cushingoid features on prednisone, she underwent rescue plasma exchange therapy. At that time, she was on 250 mg daily of azathioprine. The metabolites were normal before pheresis, and for this reason the azathioprine was increased to 275 mg daily. She had a mild increase in the 6-TGN levels but remained stable and white count was normal up to 4 months after pheresis, and it was decided to leave the patient on the same dosage, and she remained stable. The metabolites normalized, and the hemogram remained normal.

Case 6 is a patient with necrotizing myositis who was not doing well with increased weakness while on azathioprine 150 daily. The metabolites were within therapeutic range, and the dosage was increased to 175 mg daily, and the symptoms improved. The metabolites remained stable, and she did very well, but developed nausea, and could not tolerate azathioprine, and this medication was discontinued, and replaced by Cellcept, and she did well on this medication.

Case 7 is a 70-year-old female with acetylcholine receptor positive generalized myasthenia gravis. She was getting weaker on azathioprine and required rescue therapy with IVIG. The

metabolites were normal, and the dosage of azathioprine was increased from 150 mg to 200 mg daily. After 3 months, the metabolites increased, and the white count became lower, and for that reason the azathioprine was reduced to 175 mg daily, and she did very well on this dosage. The metabolites stabilized below toxic levels, and her white count returned to normal.

Discussion

AZA is one of the most commonly used immunosuppressant drugs as steroid sparing therapy or in addition to steroids in patients who are poorly responsive. The response to AZA treatment varies and some patients require higher dosages or in those with drugs that affect TPMT activity, and the dosage was adjusted.

As observed in the table 2, we found that the addition of clinical assessment and measurement of 6-TGN and 6-MMP helped in the dosage adjustment in these patients. It seemed that the levels correlated with hemogram, and only one patient developed leukopenia, and high levels of metabolites, in retrospect, the dosage should have been decreased. Otherwise, the measurement helped. Other studies have investigated thiopurine metabolites in relation to efficacy and side effects in inflammatory bowel disease, and it was found that 6-TGN levels of over 230 pmol were associated with disease remission and a level of over 400 was associated with bone marrow suppression and similar levels of 6-MMP over 5700 pmol correlation with liver toxicity. [1, 2, 4, 6, 7] In one study of myasthenia gravis adjustment of AZA according to the metabolites level was found to be useful. [5]

Thiopurine metabolites are not frequently analyzed during AZA therapy; however, from our cases, the measurements could help as a guidance to determine if dosages could be changed in those not responding to therapy, monitor the risks of hepatotoxicity or bone marrow suppression and adjust the dosage in order to optimize therapy.

We recommend that in addition to the standard measurement of TPMT before therapy, monitoring of thiopurine metabolites is very useful in cases that are either not responding or requiring higher than standard dosage. We also recommend to monitor carefully, particularly in patients who receive the drugs that may affect thiopurine metabolism such as allopurinol.

Abbreviations

AZA - azathioprine

MP - mercaptopurine

TPMT - thiopurine S-methyltransferase

6-TGN - 6-thioguanine nucleotide

6-MMP - 6-methylmercaptopurine

CBC - complete blood count

MCV - mean corpuscular volume

CIDP - chronic inflammatory demyelinating polyneuropathy

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