The Association Between Fasting Hypoglycemia and Methylated Mercaptopurine Metabolites in Children With Acute Lymphoblastic Leukemia

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Background. Symptomatic fasting hypoglycemia has been reported as an unusual side effect in patients with acute lymphoblastic leukemia (ALL) on maintenance therapy. We evaluated the relation of the red cell 6-mercaptopurine (6-MP) metabolite 6-methyl-mercaptopurine (6MMP) with hypoglycemia. Procedure. We retrospectively reviewed charts of three patients with ALL and symptomatic hypoglycemia while fasting who were noted to have high levels of 6MMP. All patients had an empiric trial of switching from evening to morning 6-MP administration, and two patients were subsequently switched to twice daily dosing. Results. Switching 6-MP from evening to morning administration reduced 6MMP levels yet preserved adequate levels of the active metabolite red cell 6-thioguanine nucleotide (6TGN). All patients had decreased hypoglycemic events when changed from evening to morning dosing. Two patients showed a rebound in 6MMP levels with return of hypoglycemic symptoms. Both were then switched to twice daily 6-MP dosing with one having a decrease in 6MMP and hypoglycemic symptoms. Conclusions. High levels of 6MMP are associated with symptomatic hypoglycemia which may be mitigated by switching to morning or twice daily 6-MP dose administration. Pediatric Blood Cancer 2014;61:1003–1006. © 2014 Wiley Periodicals, Inc.

Key words: 6-mercaptopurine; 6MMP; ALL; ALT; hypoglycemia; thiopurine metabolites

INTRODUCTION

The backbone of maintenance therapy (MT) for acute lymphoblastic leukemia (ALL) consists of oral 6-mercaptopurine (6-MP) and methotrexate (MTX). The target doses are 75 mg/m²/day of 6-MP and 20 mg/m²/week of MTX adjusted based on absolute neutrophil count. Other drugs administered during MT are monthly pulses of vincristine intravenously, 5 days of corticosteroid orally, either prednisone or dexamethasone, and every 3-month MTX administration in the spinal fluid.

6-MP is metabolized by one extracellular enzyme xanthine oxidase and two intracellular enzymes, thiopurine methyltransferase (TPMT) and hypoxanthine phosphoribosyltransferase [1]. It is metabolized in cells to the inactive nucleotide metabolite 6-methyl-mercaptopurine (6MMP), produced by the enzyme TPMT, and to the active, cytotoxic metabolite 6-thioguanine nucleotide (6TGN). High 6TGN levels correlate with therapeutic efficacy, whereas high 6MMP levels are associated with liver toxicity.

TPMT has genetic polymorphisms affecting its activity with approximately 89% of patients having full activity, 11% having intermediate activity, and less than 1% with absent activity. The higher the activity of TPMT the more 6-MP is converted to the 6MMP metabolite. TPMT activity is also induced by drugs including chemotherapy [2].

According Shih et al., patients with inflammatory bowel disease receiving 6-MP with preferential 6MMP metabolism were more likely to experience flu-like symptoms including headaches, fatigue, nausea, and myalgia. Although hypoglycemia was not documented in these patients we speculate that the symptoms might be due to symptomatic hypoglycemia suggesting a correlation between elevated 6MMP levels and hypoglycemia [3].

Fasting hypoglycemia has been found as a rare complication in about seven percent of children with ALL receiving 6-MP. It is more common in patients under the age of six where there may be reduced fat and glycogen storage, but the mechanisms remain unclear [4]. The symptoms for hypoglycemia, defined as a blood glucose level less than 50 mg/dl, include nausea, fatigue, headache, irritability, and dizziness. Bay et al. described a 3-year-old male who developed fasting hypoglycemia during MT in association with elevated alanine aminotransferase (ALT) [5]. They showed improvement with the addition of complex carbohydrates in the evening, a switch from evening to morning 6-MP dose administration, and a 6-MP dose reduction. Visavachaipan et al. [6] reported a patient with elevated ALT and fasting hypoglycemia who also improved with morning administration of 6-MP and use of cornstarch at night.

The liver is the primary site of glycogen storage and synthesis. When a patient fasts, glycogen in the liver is converted to glucose through glycogenolysis, the primary method for prevention of hypoglycemia. Once glycogen is depleted, glucose is then formed in the liver from certain amino acids, such as pyruvate, which are either oxidized or converted to glucose via gluconeogenesis. Alanine transaminase (ALT) is crucial for glucose production in the liver via the conversion of alanine to pyruvate. Pyruvate is subsequently converted into glucose with the energy supplied by ATP [7]. Increased TPMT levels and therefore elevated 6MMP in patients with ALL are known to be associated with elevated plasma ALT [8]. We speculate that 6MMP, through a yet unproven mechanism, impairs the conversion of alanine to pyruvate in the liver. We further speculate that the elevation of plasma ALT is a surrogate marker for the effect. In order to fully understand the mechanism, sophisticated biochemical analysis of the 6MMP effect on liver cell metabolism would be required, which it is beyond the scope of this article.

Although limited by the number of patients, we have only observed symptomatic hypoglycemia in patients with very high levels of 6MMP. Studies have shown that dividing the 6-MP dose to morning and evening rather than the standard of giving 6-MP in the...
evening will lower the levels of 6MMP without changing levels of the active metabolite 6TGN [9]. This implies that giving the whole dose of 6-MP in the morning may further reduce the 6MMP levels. We hypothesized that changing the time of 6-MP dose from evening to morning would give the lowest level of 6MMP yet still preserve adequate levels of 6TGN.

PATIENTS AND METHODS

The patients we studied included two males and one female with ALL on MT treated at the Children’s Hospitals and Clinics of Minnesota from June 2006 to date. All were treated in accordance with Children’s Oncology Group (COG) AALL0331 protocol for non-high-risk ALL in first remission. Each patient exhibited symptomatic hypoglycemia while on 6-MP MT. We retrospectively reviewed the patients’ charts by obtaining levels of glucose, 6-MP dosage, ALT, and ANC levels from their electronic medical records. TPMT activity and erythrocyte metabolites of 6MMP and 6TGN were also obtained (Prometheus Labs, San Diego, CA). In addition, glucose strips and continuous glucose monitoring were used to measure and record home glucose levels. Patients had not received red blood cell transfusions a month prior to determinations that might affect their TPMT, 6MMP, and 6TGN results. These data were entered into excel spreadsheets and graphed for analysis. Symptomatic hypoglycemia was defined as a blood glucose level of below 50 mg/dl with symptoms such as nausea, irritability, pallor, and lightheadedness. Additional interventions included intake of complex carbohydrates such as cornstarch during the day and before bedtime.

The patients exhibited symptomatic hypoglycemia while on MT and had an empiric trial of switching from evening to morning 6-MP administration in order to reduce their hypoglycemic episodes. In patients number two and three 6MMP levels rebounded and hypoglycemic symptoms returned. We then switched their 6-MP administration from morning to twice daily, morning and evening, at the same total average daily dose. We initially chose to change to a single morning dose rather then split dosing to maximize adherence. Adherence to therapy may be worse with twice as many 6-MP doses and non-adherence to 6-MP is one of the major reasons for ALL relapse.

Statistical Analysis

As there are only three eligible patients, the analysis is descriptive only comparing symptoms and laboratory values before and after changing from evening to morning 6-MP dosing. We plotted the variation of 6MMP, 6TGN, and ALT levels before and after 6-MP dose schedule changes (i.e., evening to morning and morning to twice daily dosing).

Patient Histories

Patient #1 was diagnosed at age one in with standard risk B-cell ALL. During interim maintenance including oral 6-MP, he developed symptoms suggestive of morning hypoglycemia: shakiness, pallor, and fatigue. The symptoms worsened following completion of steroid pulses but resolved with feeding. He was seen by Endocrinology and was noted to be hypoglycemic with blood glucose of 20 mg/dl. They instituted home finger stick glucose monitoring and nighttime nasogastric feedings, and he had an improvement in symptoms. He was noted to have transient hair loss associated with the onset of hypoglycemia. At month 3.1 of MT he was switched from evening to morning 6-MP administration with further symptom improvement. He continued on morning 6-MP dosing until the completion of therapy.

Patient #2 was diagnosed at 9 years of age with standard risk B-cell ALL. A few weeks after starting MT, she started having spells of light-headedness, tachycardia, and headache but the complications improved after eating. She had substantial hair loss during month 5 of MT. A glucose level of 41 mg/dl was noted at month 5.6 of MT when fasting for a sedated lumbar puncture. She was then switched to morning 6-MP administration, which resulted in improvement of symptoms and a sharp reduction in 6MMP (Fig. 1). At month 8.1 of MT hypoglycemia returned with a concomitant rise in 6MMP levels. However, morning blood glucose levels transiently improved with introducing simple carbohydrate intake at night. At month 9.3 of MT, during a 5-day dexamethasone pulse, her home continuous glucose monitoring system (CGMS) recorded elevated glucose levels in the 200–300 mg/dl range. She was given a few doses of insulin and instructed to start metformin with the next pulse. She was also switched from morning 6-MP to twice daily split dosing of 6-MP at the same weekly dose, continuing until the end of therapy. This change resulted in a decline of 6MMP and improvement of symptoms (Fig. 1).

Patient #3 was diagnosed at age two and a half with standard risk B-cell ALL. At month 1.3 of MT he developed an acute onset of lethargy and altered level of consciousness. The MRI of his brain and EEG were normal. He was subsequently noted to have severe hypoglycemia with glucose levels ranging from 30 to 40 mg/dl. After discussion, 6-MP administration was switched to the morning, significantly reducing 6MMP levels (Fig. 1). He had no further episodes of hypoglycemia until fasting for a lumbar puncture at month 6.3 of MT. Endocrinology recommended use of cornstarch in the evening. With these changes, his home glucose monitoring system reported a maximum glucose level of 200 mg/dl during a dexamethasone pulse with no rebound of hypoglycemia afterwards. At month 25 of MT, he developed hypoglycemic symptoms with glucose levels near 50 mg/dl following dexamethasone pulses. Parents had difficulty in giving cornstarch to the

![Figure 1.](image)

**Fig. 1.** 6MMP levels before and after 6-MP schedule changes. Arrows with solid heads indicate where patients were switched from evening to morning dosing. The arrow with thin line head indicates where Patient #2 and #3 were switched from morning to twice daily dosing.
patient, so he was switched from cornstarch to whey protein shakes without improvement in symptoms. 6MMP levels were noted to rise at the same time (Fig. 1). He was switched to BID 6-MP dosing during month 27 of MT. There was only a minimal and transient decrease in 6MMP without resolution of hypoglycemia symptoms. He remained on BID dosing until completing therapy.

RESULTS

All three patients showed reduction in 6MMP levels following the switch from evening to morning 6-MP dose administration (Fig. 1). Patients number two and three showed a rebound in 6MMP. Only patient two responded with decreased 6MMP and hypoglycemia symptoms after switched from morning to twice a day split dosing (Fig. 1). 6TGN levels temporarily fell shortly after the switch from evening to morning in patient number one and two but returned to previous levels (Fig. 2). These are in the range of unpublished data from the CCG1922 study of 200 patients following 3 months of MT which showed a median 6TGN level of 258 pmol/8 x 10^8 RBC and a 25th percentile of 168 pmol/8 x 10^8 RBC (Bostrom B, unpublished work, 2013). ALT levels also improved with the change in timing of 6-MP dose administration from evening to morning (Fig. 3). TPMT enzyme activity fluctuated but did not appear to change in concert when 6-MP was switched from evening to morning dosing.

CONCLUSIONS

Many publications identified hypoglycemia as an adverse but reversible side effect of MT for ALL that generally improves 3–4 months after completion of therapy but could not identify why it occurred [4–6]. Our patients had symptomatic hypoglycemia associated with 6-MP therapy similar to that found by other investigators. Our investigation suggested that hypoglycemia in children is associated with elevated levels of 6MMP, and we demonstrated that 6MMP can be reduced by changing the timing of 6-MP dose administration to the morning. This fall in 6MMP is associated with reduced symptomatic hypoglycemia from fasting. In patient two, following 6MMP rebound with return of hypoglycemia there again was a reduction in 6MMP following switch to BID dosing which did not occur in patient three. Traditionally 6-MP is given in the evening because plasma pharmacokinetic studies showed better absorption on an empty stomach, which is easily done at night [10]. Also, a non-randomized single institution study showed improved survival when 6-MP was given in the evening [11]. We showed that switching from evening to morning administration did not negatively impact the 6TGN level, which is felt to be the most important anti-leukemic metabolite. A large randomized study comparing evening versus split dose morning and evening 6-MP therapy showed that the split dose did not affect the outcome. This suggests that the time of day 6-MP is given is not important for an anti-leukemic effect as long as the dose is adjusted based on the ANC [9].

The levels of the active metabolite 6TGN are inversely related to TPMT activity as TPMT catabolizes 6-MP to 6MMP reducing 6-MP available for production of 6TGN [1]. The reason for the decrease in 6MMP levels with change in 6-MP dosing schedule is not known. Presumably, TPMT activity would be affected by the change in 6-MP dose schedule to explain the decrease in 6MMP. However, we did not see a concomitant increase in 6TGN that would be expected if TPMT activity were reduced by the change in schedule. In fact, there was an initial decrease of 6TGN in patient number one and three shortly after the switch, but their levels rapidly returned to baseline. There was no noticeable change in TPMT activity in our patients after changing the 6-MP dosing schedule. An extensive review of the literature on TPMT revealed no studies that showed a diurnal variation of TPMT activity in the blood or liver.

Due to hypoglycemia’s transient nature and symptoms similar to those of acute viral illness, patients with subclinical fasting hypoglycemia may be difficult to diagnose. The standard of care for patients in MT is to measure ALT every 3 months. Our approach in patients with persistently elevated ALT is to measure 6MMP and 6TGN. If the 6MMP level is elevated, we screen for morning hypoglycemia with a fasting blood glucose. This can be done at the time of a sedated procedure, or by the use of home blood glucose monitoring with strips. In patients with morning hypoglycemia, the
CGMS could be considered to document glucose levels before, during, and after a corticosteroid pulse as patients may develop rebound hypoglycemia. We suggest that patients with a glucose level of less than 50 mg/dl or between 50 and 70 mg/dl with symptoms that do not improve with complex carbohydrates in the evening should consider a trial of switching 6-MP from evening to morning or to twice daily administration.

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REFERENCES


