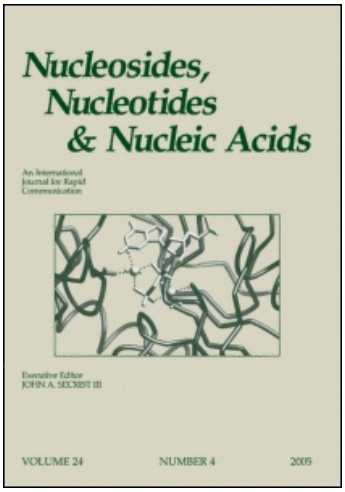

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FATAL MYELOTOXICITY AFTER AZATHIOPRINE TREATMENT

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□ *Azathioprine and 6-mercaptopurine have been used for many years in the treatment of inflammatory bowel disease. Approximately 0.3% of the population are homozygous for variant alleles associated with extremely low thiopurine S-methyltransferase enzyme activity. We describe the case of a young patient with ulcerative colitis, homozygous for TPMT*3A alleles, who suffered fatal azathioprine-induced myelotoxicity after standard dosing with azathioprine. Screening for decreased activity of TPMT in patients prior to azathioprine treatment is advised to minimize the risk of drug-induced toxicity.*

Keywords Ulcerative colitis; azathioprine; myelotoxicity; poor metabolizer; pharmacogenetics; thiopurine S-methyltransferase

INTRODUCTION

The thiopurine drugs, azathioprine and 6-mercaptopurine, have been used for many years in the treatment of inflammatory bowel disease (IBD). Both prodrugs undergo metabolic activation via hypoxantine-guanine phosphoribosyltransferase into 6-thioguanine nucleotides which are incorporated into the DNA as false bases and induce the therapeutic and cytotoxic activity. Two competitive pathways, catalysed by thiopurine S-methyltransferase (TPMT) and xanthine oxidase, mediate the degradation to inactive metabolites 6-methylmercaptopurine and 6-thiouric acid, respectively. Although the drugs are usually well tolerated, typical adverse drug reactions as pancreatitis, fever, nausea, hepatitis can occur. Bone

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marrow suppression is dose-dependent and is often a delayed side effect requiring regular complete blood count monitoring. We describe a patient with ulcerative colitis who developed azathioprine-induced pancytopenia with subsequent gas gangrene as a result of TPMT deficiency.

CASE REPORT

A male, born 1984, was generally healthy until September 2003, when he started to complain of tenesmus, bloody diarrhea, and weight loss of 3 kg. He underwent colonoscopy with finding of mild to moderate left-sided ulcerative colitis. Signs of inflammation were also revealed in the laboratory screen. The patient began to take mesalazine 4 g per day. The dose was decreased to 2 g per day after inducing remission in November 2003.

One year later in September 2004, a relapse with endoscopic finding of extensive, active colitis occurred. The patient was treated with prednisolone 60 mg a day and after a substantial improved of the clinical conditions the dose was gradually decreased by 10 mg every 2 weeks.

In January 2005, ulcerative colitis reactivated on daily doses of prednisolone 10 mg and mesalazine 4 g per day. Body weight loss of 10 kg within a few weeks occurred and laboratory signs of inflammation (C-reactive protein, 125mg/L; platelet count, 640 $\times$ 10^9/L) supported the clinical impression. Further, the patient showed symptoms of anemia (hemoglobin 9.3g/dL) and protein-caloric malnutrition (total protein, 53 g/L; albumin, 19 g/L). Endoscopic examination revealed serious inflammatory and postinflammatory changes on the mucosa of large intestine. The patient was admitted to hospital for intensive treatment. Prescribed medication: methylprednisolone 60 mg intravenously, ciprofloxacin, metronidazol, parenteral nutrition, and enteral support. Several days later the patient's state substantially improved. Due to the corticosteroid-dependent course of the disease, a decision to commence treatment with azathioprine was made with plans to discontinue corticosteroids and/or mesalazine after the clinical effects of azathioprine treatment appeared. Methylprednisolone at a dose of 48 mg daily was maintained, and azathioprine 100 mg a day was added (body weight 65 kg). The patient was discharged from hospital for home care in a very good condition. Upon discharge the patient's laboratory values were as follows: C-reactive protein, 3mg/L; leucocyte count, 12.6 $\times 10^9$/L; neutrophil count, 12.0 $\times 10^9$/L; hemoglobin, 11.5 g/dL; platelet count, 332 $\times 10^9$/L.

In the following weeks the patient was clinically well and kept taking the recommended medication. Liver function tests were within normal limits, but leucocyte counts slowly decreased over time; these were however within the normal range up to the week 5 after the start of azathioprine treatment. At week 5, the level of leucocytes dropped to 2.3x109/L; therefore, the

patient was contacted to discontinue azathioprine, and subsequent blood count monitoring was advised. Five days later he felt well. He only had a small erosion on his right thigh, which he had suffered several days before from climbing over a fence. During the following hours, agonizing pain and oedema of the right thigh suddenly developed.

On admission to our hospital his blood count was as follows: leucocyte count, 0.4×10^9/L; neutrophil count, 0.1×10^9/L; haemoglobin, 12.3 g/dL; platelet count, 147×10^9/L. Further, symptoms of rhabdomyolysis were discovered (creatine kinase,141 μkat/L; myoglobin, 1,784 mg/L), as well as hypoproteinemia (total protein, 27 g/L; albumin, 14 g/L) and severe inflammatory parameters (C-reactive protein, 118 mg/L).

The reason for the edema and pain of the right thigh was gas gangrene. On the day of admission to our hospital, incision and necrosectomy were performed and intensive supportive and ATB therapy was applied (combination of clindamycin 600 mg/6 hours, vancomycin 500 mg/6 hours, meropenem 500 mg/6 hours, fluconazol 200mg/12 hours). However, despite intensive treatment with growth factors, worsening of the pancytopenia developed progressively reaching following levels: leucocyte count, 0.2×10^9/L; neutrophil count, 0.1×10^9/L; haemoglobin, 6.5 g/dL; platelet count, 7×10^9/L. Despite treatment his health deteriorated and multi-organ failure appeared. He died 28 days following his hospitalization.

RESULTS

A blood sample was taken for genetic screening of TPMT. Genomic DNA was extracted using a QIAmp DNA Blood Mini Kit (QIAGEN GmbH, Germany). PCR amplification was run in a MyCycler (Bio-Rad, USA) with primers and protocol as described previously.[1] Subsequent RFLP analysis was aplied for TPMT*3B, and TPMT*3C, while allele specific PCR was run for TPMT*2. The fragments were separated in 3% agarose gel and visualized by staining with ethidium bromide. The primers were ordered at Sigma-Aldrich (USA), all other components of PCR reaction mix and Top Vision agarose were purchased from Fermentas (Lithuania). Results of the examination explained why severe bone marrow suppression occurred in the patient—he was a homozygous carrier of *TPMT*3A* allele that is associated with complete enyzme deficiency and phenotype of "poor metabolizer."

DISCUSSION

Gas gangrene occurs most frequently in contaminated wounds following trauma or surgery. It is caused by a wide variety of *Clostridium* species, the most common being *Clostridium perfringens* and *Clostridium septicum.*

Causative agents are anaerobic, spore-forming, Gram-positive bacilli and they produce local and systemic toxins. Spontaneous, nontraumatic clostridial myonecrosis is uncommon and is usually associated with gastrointestinal and haematological malignancy, diabetes mellitus and peripheral vascular disease.[2,3] Clostridial myonecrosis is rapidly progressive consisting of muscle necrosis and systemic toxicity. It is usually seen in elderly and immunocompromised patients. Clostridial myonecrosis is a rare disease, the estimated incidence is 900–1,000 cases per year in the United States.[4]

The sudden onset of severe pain is often the first symptom experienced by the patient. Typically, the pain is out of proportion to the physical examination findings and is minimally relieved by medications. Initial physical examination of the site may be normal, within minutes to hours; localized tense edema, pallor, and tenderness are seen.[3]

Treatment includes early operative debridement and antibiotic therapy. Because of polymicrobial nature of some necrotizing soft tissue infections, empiric antibiotic coverage is implemented. Antibiotic combinations used to treat gas gangrene usually include penicillin and clindamycin. Clindamycin has the ability to reduce the production of exotoxins produced by *Clostridium* species.[4,5] Despite optimal management, the mortality rate is high, 67–100% with most subjects dying within 24 hours after the clinical onset.[3]

The case report, which we describe here, is to our knowledge the first case of clostridial myonecrosis in a subject with ulcerative colitis. It illustrates the potentially serious consequences of unrecognized deficiency of TPMT activity in patients receiving azathioprine treatment. In the general Caucasian population, nearly 90% of the people have high TPMT activity; 10% are heterozygous carriers of one variant allele resulting in a decreased TPMT activity, and 0.3% of the population are homozygotes for variant alleles leading to complete TPMT deficiency or extremely low enzyme activity.[6] Administration of usual doses of azathioprine to TPMT deficient patients leads to massive intracellular overload with 6-thioguanine nucleotides as a consequence of impaired drug inactivation to 6-methylmercaptopurine.

Several studies reported that screening of TPMT genotype/phenotype does not predict the development of azathioprine-induced myelotoxicity,[7–9] while other authors found a substantial correlation between low enzyme activity and the development of myelotoxicity.[10,11] Colombel et al. reported that assessment of TPMP genotype or activity can reduce the risk of myelotoxicity in approximately one-third of patients.[12] These results indicate that screening for TPMT deficiency is not a sufficient approach to establish whether the treatment will be well tolerated or not, but screening is a reasonable way to identify patients with a high risk of severe myelotoxicity due to inherited deficiency of TPMT activity. Therefore, the use of TPMT genotyping or phenotyping prior to azathioprine therapy is recommended as a supplement to other routine and still essential

measures for ensuring safe azathioprine use, including monitoring of blood cell counts.

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