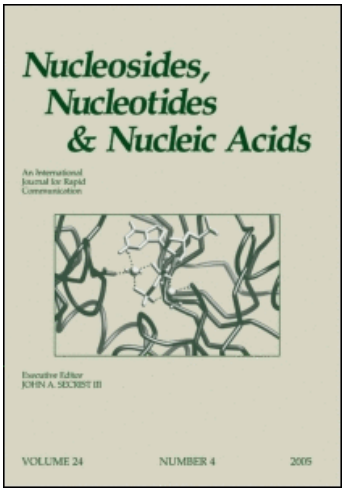

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S. Vikingsson[a]; B. Carlsson[a]; S. Almer[b]; C. Peterson[a]
[a] Division of Drug Research, Clinical Pharmacology, Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Linköping, Sweden [b] Division of Gastroenterology and Hepatology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden

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HOW SHOULD THIOPURINE TREATMENT BE MONITORED?—METHODOLOGICAL ASPECTS

S. Vikingsson,[1] B. Carlsson,[1] S. Almer,[2] and C. Peterson[1]
[1]Division of Drug Research, Clinical Pharmacology, Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Linköping, Sweden
[2]Division of Gastroenterology and Hepatology, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden

□ *Monitoring of thiopurine metabolites is important due to a complex metabolism with large interindividual variation, but the suitability of currently used methods has been questioned. The drawbacks include poor reproducibility, the inability to differentiate between the different analytes, as well as the use of a nontarget matrix. Further research should be directed toward measuring thiopurine metabolites in mononuclear cells, measuring the different nucleotides specifically, as well as measuring the incorporation of thioguanine into DNA. The studies should not be limited to thioguanosine nucleotides but include methylthioinosine nucleotides as well.*

Keywords TGN; methylthioinosine monophosphate; methyl mercaptopurine; inflammatory bowel disease; childhood leukemia

INTRODUCTION

The thiopurine drugs, azathioprine, mercaptopurine, and thioguanine, are widely used in the treatment of childhood acute lymphoblastic leukemia (ALL), and inflammatory bowel disease (IBD). They are prodrugs with a complex metabolism with large interindividual variation. This variation gives rise to substantial and unpredictable interindividual differences in the concentrations of the active metabolites during thiopurine therapy. In turn, this leads to variations in drug response resulting in lack of therapeutic effect as well as adverse events.

Low activity of the polymorphic enzyme thiopurine methyltransferase (TPMT) leads to abnormally high levels of thioguanosine nucleotides (TGN) in red blood cells (RBC), especially in homozygously deficient patients [1], with a frequency around 1 in 300 in Caucasians.[1,2] The high concentrations may cause adverse events that can be avoided by adjusting the dosage

Address correspondence to Svante Vikingsson, Clinical Pharmacology, University Hospital SE-58185, Linköping, Sweden. E-mail: svante.vikingsson@liu.se

based on TPMT activity determination prior to initiating treatment with thiopurines.

However, even when the TPMT activity is taken into account, a significant interindividual variation in the drug response remains. To help individualize the thiopurine treatment, monitoring of the active metabolites has been carried out in many centers for the last 20 years, but the clinical benefit of such methods has been questioned.[3] We believe that this lack of success mainly might be due to methodological aspects and the aim of this article is to discuss the methods used today, give possible explanations as to why they fail, and suggest how to improve them.

THE METHODS USED TODAY

The main strategy for the monitoring of thiopurine metabolites has been to measure TGN in RBC. A number of different methods have been described, the majority of which are based on hydrolysis of the TGN back to thioguanine using acid, possibly followed by some sort of sample clean-up.[4–6] Typically, the thioguanine is detected using UV-absorbance at either around 320 nm,[4] or 340 nm.[6] Most commonly used are the method developed by Lennard and Singleton,[4,7] the method developed by Derivieux and Boulieu,[6,8] or slightly modified versions thereof. Briefly the TGN are hydrolyzed using sulphuric acid, in the method by Lennard and Singleton, or perchloric acid, in the method by Dervieux and Boulieu, and analyzed by HPLC. In the method by Lennard and Singleton, the produced thioguanine is extracted by phenyl mercury adduct formation prior to injection onto the HPLC.

WHY WE NEED NEW METHODS

RBC are not the target of thiopurines and it is questionable if the metabolite concentration in RBC is a good surrogate marker for drug effects. Even though it has been shown that extremely high TGN concentrations in RBC correlate with adverse events, and that the methods are effective for checking compliance, a number of studies have failed to establish a correlation to clinical effects.[3] In a recent meta-analysis by Osterman et al.,[9] a correlation between remission and TGN concentrations was found, but TGN concentration only partially explained remission.

WHERE DO THE PROBLEMS ARISE?

There are a number of methodological disadvantages with the methodology used so far that might contribute to the lack of observed correlation. First, the acid hydrolysis methods have shown poor reproducibility. In the

modified Lennard and Singleton method [4] used in our laboratory, the observed inter-day CV was more than 20% at concentrations between 58 and 660 pmol/8 × 10^8 RBC.[10]

The hydrolysis step is another possible source of error. Differences in hydrolysis efficiency make comparisons between results obtained by different methods difficult. This was nicely shown by Shipkova et al.[11] in a comparison between the methods developed by Lennard and Singleton,[4,7] and Dervieux and Boulieu.[6,8] Especially the method developed by Lennard and Singleton seemed to suffer from incomplete hydrolysis.

Second, at least 9 different substances could possibly contribute to the thioguanine reported as TGN, including the free base, the riboside, the three nucleotides (mono-, di-, and triphosphate), as well as the deoxy analogs. However, earlier studies showed that the free base[4,10] and the riboside[10] are not present in RBCs, and therefore do not contribute to TGN. Deoxyribonucleotides are present at such low concentrations that their contribution toward the total TGN probably is negligible.[12] Instead TGN is mainly made up by thioguanosine mono- (TGMP), di- (TGDP), and triphosphates (TGTP).[10,13–15]

It is likely that these substances differ in their clinical effects, and therefore it is possible that individual patients might be more or less skewed toward the more potent substances, affecting the clinical outcome. Some evidence from studies comparing TGMP, TGDP, and TGTP concentrations suggest that this is indeed the case.[10,13,15] More specifically, this indicates that the concentration of TGN might not accurately reflect the concentrations of TGTP and deoxyTGTP, which are the substances believed to be incorporated into RNA and DNA, respectively.

The third major drawback is that TGN is measured in RBC. The RBC lack inosine monophosphate dehydrogenase[16] and cannot form TGN from azathioprine or mercaptopurine. Instead, the TGN present in the RBC are hypothesized to be produced by phosphorylation of thioguanine absorbed from tissues capable of converting 6-mercaptopurine into thioguanine.[17] Individual differences between patients in the transport of these substances might therefore give rise to differences in the ratio between the TGN concentration in the targeted cells and the TGN concentration measured in RBC, and thus explain the weak or absent correlation between TGN concentration and clinical response.

Taken together, it seems likely that even though the concentration of one or more of these substances in the target cells correlate well with the clinical outcome it would be very difficult to observe this when measuring TGN in RBC.

THE IDENTITY OF meTIMP AND ITS IMPORTANCE

Because the thiopurines are prodrugs, a number of other metabolites are produced. Of these, especially methylthioinosine monophosphate

(meTIMP) and methylmercaptopurine (meMP) as measured by various acid hydrolysis methods, have attracted a research interest, especially regarding adverse events.

When acid hydrolysis methods are used, the analytes reported as meTIMP are the same as those reported as meMP. Mercaptopurine is the base in thioinosine nucleotides making meTIMP and meMP, indistinguishable. The substance is hydrolyzed back to 4-amino-5-(methylthio)carbonyl imidazole (AMTCI) during the measurement.[8] A name analogous with TGN is methylthioinosine nucleotides (meTIN), which will be used in the reminder of this article.

In patients treated orally, neither meMP nor meMP riboside was found in RBC.[18] It has also been shown that meTIMP does contribute to the total meTIN, but other, not yet identified substances also contributes.[10] Earlier research showed that very high meTIN values did predict adverse events,[19] as well as that meTIN was increased instead of TGN in patients not responding to treatment.[20] Both these studies indicate the importance of further studies of meTIN.

WHAT ARE THE ALTERNATIVES?

A number of other approaches for measuring thiopurine metabolites have been published. A number of attempts have been made to specifically measure TGMP, TGDP, and TGTP,[10,15,21] Most of these have been modifications of an extraction protocol developed by Rabel et al.[22]

Another strategy is to enzymatically remove the phosphate groups from the nucleotides and measure them as nucleosides. Such a method was developed by Giverhaug et al.[18] for the measurement of meTIN, but essentially this would be the same type of measurement as those using acid hydrolysis, because the free base was not present.[18] However, in 2002, Dervieux et al. published a method using this technique to measure meTIN in lymphocytes from patients receiving infusions of mercaptopurine as treatment for acute lymphoblastic leukemia (ALL).[23] This is a very interesting approach but we have not found any clinical studies using this type of method for measurements of TGN in lymphocytes.

A third approach is to measure the amount of thioguanine bases that are incorporated into the DNA. Since incorporation of thioguanine into DNA is believed to be one of the main mechanisms for thiopurine effects, this might be a way to clinically measure treatment effect. Three methods for quantifying thioguanine incorporation into DNA have been published,[24–26] but to our knowledge, studies on the correlation to patient outcomes are lacking.

CONCLUSIONS

The methods used in clinical routine today are not good enough, probably due to poor reproducibility, the inability to differentiate between different analytes, as well as the use of an unsuitable matrix. However, we believe that there is a possibility to develop a useful method, and believe that research should be directed toward measuring the nucleotides in mononuclear cells, measuring the different nucleotides specifically, as well as measuring the incorporation of thioguanine into DNA. The studies should not be limited to TGN, but include meTIN and other metabolites as well.

REFERENCES

1. Lennard, L.; Van Loon, J.A.; Weinshilboum, R.M. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. *Clin. Pharmacol. Ther.* **1989**, 46, 149–154.
2. Pettersson, B.; Almer, S.; Albertioni, F.; Soderhall, S.; Peterson, C. Differences between children and adults in thiopurine methyltransferase activity and metabolite formation during thiopurine therapy: possible role of concomitant methotrexate. *Ther. Drug. Monit.* **2002**, 24, 351–358.
3. Herrlinger, K.R.; Fellermann, K.; Fischer, C.; Kreisel, W.; Deibert, P.; Schoelmerich, J.; Fleig, W.E.; Ruhl, A.; Reinshagen, M.; Greinwald, R.; Stange, E.F.; Schwab, M. Thioguanine-nucleotides do not predict efficacy of tioguanine in Crohn's disease. *Aliment Pharmacol. Ther.* **2004**, 19, 1269–1276.
4. Lennard, L.; Singleton, H.J. High-performance liquid chromatographic assay of the methyl and nucleotide metabolites of 6-mercaptopurine: quantitation of red blood cell 6-thioguanine nucleotide, 6-thioinosinic acid and 6-methylmercaptopurine metabolites in a single sample. *J. Chromatogr.* **1992**, 583, 83–90.
5. Erdmann, G.R.; France, L.A.; Bostrom, B.C.; Canafax, D.M. A reversed phase high performance liquid chromatography approach in determining total red blood cell concentrations of 6-thioguanine, 6-mercaptopurine, methylthioguanine, and methylmercaptopurine in a patient receiving thiopurine therapy. *Biomed. Chromatogr.* **1990**, 4, 47–51.
6. Dervieux, T.; Boulieu, R. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. *Clin. Chem.* **1998**, 44, 551–555.
7. Lennard, L. Assay of 6-thioinosinic acid and 6-thioguanine nucleotides, active metabolites of 6-mercaptopurine, in human red blood cells. *J. Chromatogr.* **1987**, 423, 169–178.
8. Dervieux, T.; Boulieu, R. Identification of 6-methylmercaptopurine derivative formed during acid hydrolysis of thiopurine nucleotides in erythrocytes, using liquid chromatography-mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance assay. *Clin. Chem.* **1998**, 44, 2511–2515.
9. Osterman, M.T.; Kundu, R.; Lichtenstein, G.R.; Lewis, J.D. Association of 6-thioguanine nucleotide levels and inflammatory bowel disease activity: a meta-analysis. *Gastroenterology* **2006**, 130, 1047–1053.
10. Vikingsson, S.; Carlsson, B.; Almer, S.H.; Peterson, C. Monitoring of thiopurine metabolites in patients with inflammatory bowel disease-what is actually measured? *Ther. Drug Monit.* **2009**, 31, 345–350.
11. Shipkova, M.; Armstrong, V.W.; Wieland, E.; Oellerich, M. Differences in nucleotide hydrolysis contribute to the differences between erythrocyte 6-thioguanine nucleotide concentrations determined by two widely used methods. *Clin. Chem.* **2003**, 49, 260–268.
12. Boulieu, R.; Bory, C.; Gonnet, C. Liquid-chromatographic measurement of purine nucleotides in blood cells. *Clin. Chem.* **1985**, 31, 727–731.
13. de Boer, N.K.; Derijks, L.J.; Keizer-Garritsen, J.J.; Lambooy, L.H.; Ruitenbeek, W.; Hooymans, P.M.; van Bodegraven, A.A.; de Jong, D.J. Extended thiopurine metabolite assessment during 6-thioguanine therapy for immunomodulation in Crohn's disease. *J. Clin. Pharmacol.* **2007**, 47, 187–191.

14. Lavi, L.E.; Holcenberg, J.S. A rapid and sensitive high-performance liquid chromatographic assay for 6-mercaptopurine metabolites in red blood cells. *Anal. Biochem.* **1985**, 144, 514–521.
15. Neurath, M.F.; Kiesslich, R.; Teichgraber, U.; Fischer, C.; Hofmann, U.; Eichelbaum, M.; Galle, P.R.; Schwab, M. 6-thioguanosine diphosphate and triphosphate levels in red blood cells and response to azathioprine therapy in Crohn's disease. *Clin. Gastroenterol. Hepatol.* **2005**, 3, 1007–1014.
16. Montero, C.; Duley, J.A.; Fairbanks, L.D.; McBride, M.B.; Micheli, V.; Cant, A.J.; Morgan, G. Demonstration of induction of erythrocyte inosine monophosphate dehydrogenase activity in Ribavirin-treated patients using a high performance liquid chromatography linked method. *Clin. Chim. Acta* **1995**, 238, 169–178.
17. Duley, J.A.; Florin, T.H. Thiopurine therapies: problems, complexities, and progress with monitoring thioguanine nucleotides. *Ther. Drug. Monit.* **2005**, 27, 647–654.
18. Giverhaug, T.; Bergan, S.; Loennechen, T.; Rugstad, H.E.; Aarbakke, J. Analysis of methylated 6-mercaptopurine metabolites in human red blood cells: comparison of two methods. *Ther. Drug Monit.* **1997**, 19, 663–668.
19. Hindorf, U.; Lindqvist, M.; Hildebrand, H.; Fagerberg, U.; Almer, S. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. *Aliment Pharmacol. Ther.* **2006**, 24, 331–342.
20. Dubinsky, M.C.; Yang, H.; Hassard, P.V.; Seidman, E.G.; Kam, L.Y.; Abreu, M.T.; Targan, S.R.; Vasiliauskas, E.A. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. *Gastroenterology* **2002**, 122, 904–915.
21. Keuzenkamp-Jansen, C.W.; De Abreu, R.A.; Bokkerink, J.P.; Trijbels, J.M. Determination of extracellular and intracellular thiopurines and methylthiopurines by high-performance liquid chromatography. *J. Chromatogr. B* **1995**, 672, 53–61.
22. Rabel, S.R.; Stobaugh, J.F.; Trueworthy, R. Determination of intracellular levels of 6-mercaptopurine metabolites in erythrocytes utilizing capillary electrophoresis with laser-induced fluorescence detection. *Anal Biochem* **1995**, 224, 315–322.
23. Dervieux, T.; Chu, Y.; Su, Y.; Pui, C.H.; Evans, W.E.; Relling, M.V. HPLC determination of thiopurine nucleosides and nucleotides in vivo in lymphoblasts following mercaptopurine therapy. *Clin. Chem.* **2002**, 48, 61–68.
24. Tidd, D.M.; Dedhar, S. Specific and sensitive combined high-performance liquid chromatographic-flow fluorometric assay for intracellular 6-thioguanine nucleotides metabolites of 6-mercaptopurine and 6-thioguanine. *J. Chromatogr.* **1978**, 145, 237–246.
25. Warren, D.J.; Andersen, A.; Slordal, L. Quantitation of 6-thioguanine residues in peripheral blood leukocyte DNA obtained from patients receiving 6-mercaptopurine-based maintenance therapy. *Cancer Res.* **1995**, 55, 1670–1674.
26. Olesen, K.M.; Hansen, S.H.; Sidenius, U.; Schmiegelow, K. Determination of leukocyte DNA 6-thioguanine nucleotide levels by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B* **2008**, 864, 149–155.