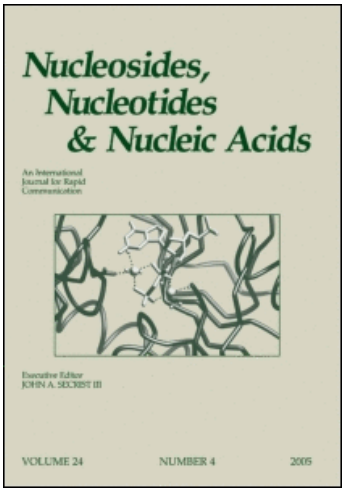

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Determination of ITPase Activity in Erythrocyte Lysates Obtained for Determination of Tpmt Activity

J. Bierau[a]; J. A. Bakker[ab]; M. Lindhout[a]; A. H. van Gennip[a]
[a] Laboratory of Biochemical Genetics, Department of Clinical Genetics, Maastricht University Hospital, Maastricht, the Netherlands [b] Department of Clinical Chemistry, Maastricht University Hospital, Maastricht, the Netherlands

To cite this Article Bierau, J. , Bakker, J. A. , Lindhout, M. and van Gennip, A. H.(2006) 'Determination of ITPase Activity in Erythrocyte Lysates Obtained for Determination of Tpmt Activity', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 1129 — 1132
To link to this Article: DOI: 10.1080/15257770600894253
URL: http://dx.doi.org/10.1080/15257770600894253

Nucleosides, Nucleotides, and Nucleic Acids, 25:1129–1132, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600894253

Taylor & Francis
Taylor & Francis Group

DETERMINATION OF ITPase ACTIVITY IN ERYTHROCYTE LYSATES OBTAINED FOR DETERMINATION OF TPMT ACTIVITY

J. Bierau □ *Laboratory of Biochemical Genetics, Department of Clinical Genetics, Maastricht University Hospital, Maastricht, the Netherlands*

J. A. Bakker □ *Laboratory of Biochemical Genetics, Department of Clinical Genetics, and Department of Clinical Chemistry, Maastricht University Hospital, Maastricht, the Netherlands*

M. Lindhout and A. H. van Gennip □ *Laboratory of Biochemical Genetics, Department of Clinical Genetics, Maastricht University Hospital, Maastricht, the Netherlands*

□ *The indication for the determination of both thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphohydrolase is identical (i.e., adverse drug reactions toward mercaptopurines). Therefore, we tested whether or not our standard procedure to prepare erythrocyte lysates for measurement of TPMT activity, which includes treatment with Chelex 100 (a chelating resin), was suitable for the measurement of ITPase activity. It also was tested to see if ITPase activity differs in EDTA and Heparin anti-coagulated blood samples. We found that there was no difference between the ITPase activity in erythrocyte lysates prepared from EDTA or Heparin anti-coagulated blood. Treatment with a chelating resin or omission of magnesium from the assay procedure resulted in decreased and nearly absent ITPase activity, respectively. We conclude that untreated erythrocyte lysates obtained for determination of TPMT activity are suitable for determination of ITPase activity. However, after treatment with Chelex 100 the erythrocyte lysates become unsuitable for determination of ITPase activity.*

Keywords Inosine triphosphate pyrophosphohydrolase; Thiopurine methyl transferase; Mercaptopurine therapy; Pharmacogenetics

INTRODUCTION

Thiopurine methyltransferase (TPMT) deficiency is a well-established risk factor in mercaptopurine therapy. Diminished or absent TPMT

Address correspondence to J. Bierau, Laboratory of Biochemical Genetics, Department of Clinical Genetics, Maastricht University Hospital, P.O. Box 5800, 6202 AZ, Maastricht, the Netherlands. E-mail: jorgen.bierau@gen.unimaas.nl

activity leads to an accumulation of cytotoxic mercaptopurine metabolites and causes damage to normal dividing cells as well as malignant cells. Recently, it became apparent that diminished or absent inosinetriphosphate pyrophosphohydrolase (ITPase) activity is a risk factor as well.[1] Normally, ITPase catalyzes the pyrophosphohydrolysis of ITP to IMP. Deficiency of ITPase leads to an intracellular accumulation of ITP. In case of treatment with mercaptopurines, ITPase deficiency most likely leads to an accumulation of thio-inosine-5′-triphosphate, which may compete with ATP and GTP in normal cellular processes, thus, causing toxicity. For patients with unexplained adverse reactions towards mercaptopurines but having normal TPMT activity, the measurement of ITPase activity clearly is indicated. Ideally, both TPMT and ITPase activities should be determined prior to the start of mercaptopurine therapy. We investigated in both EDTA and Heparin anti-coagulated blood samples whether or not ITPase activity can be determined in erythrocyte lysates that have been obtained for the determination of TPMT activity, and to what extend the preparation procedures can be combined. The normal preparation for routine TPMT determinations involves treatment with a chelating agent. Therefore, the effects of Chelex 100 (a chelating resin) and the omission of magnesium from the reaction mixture on the ITPase activity were investigated.

MATERIALS AND METHODS

Chemicals: ITP, IMP, and Chelex 100 sodium were purchased from Sigma (Zwijndrecht, the Netherlands). $MgCl_2$ was purchased from Merck (Amsterdam, the Netherlands). All chemicals were of analytical grade.

Patient material: The effect of the subsequent steps in the work-up procedure for TPMT analysis on the ITPase activity in erythrocyte lysates was studied in EDTA- and Heparin anti-coagulated blood samples from the same patient obtained at the same time. Linearity of the assay was tested using a lysate of pooled erythrocytes from multiple donors.

Standard sample preparation for TPMT analysis: EDTA or Heparin anti-coagulated blood was centrifuged at 10 minutes at 800 g at ambient temperature. The plasma and the buffycoat were removed and the erythrocytes were resuspended to the original volume in 0.9% saline. Subsequently, the erythrocytes were washed twice by centrifugation under the same conditions. The resulting pellet containing the erythrocytes was resuspended in an approximately equal volume of 0.9% saline. Subsequently, an aliquot was taken for the determination of the number of erythrocytes. An erythrocyte lysate was obtained by diluting the erythrocyte sample with 4 volumes of ice-cold de-ionized water. After 10 minutes of incubation on ice cell debris was removed via centrifugation. ITPase activity was determined in the thus obtained lysate. To follow the standard preparation procedure for

a TPMT activity determination, a 1 ml aliquot of the erythrocyte lysate was treated with approximately 70 mg Chelex 100, a chelating resin, at 4°C for 1 hour under constant gentle stirring. By stopping the stirring, the resin precipitated and the supernatant was now used for the determination of ITPase activity.

ITPase activity assay: The ITPase activity assay was adapted from the assay described by Sumi and colleagues.[2] To 185 μl of a solution containing 100 mM Tris-HCl pH 8.5, 540 μM dithiothreitol, 54 mM $MgCl_2$, 2.16 mM ITP in a reaction vial placed on ice 25 μl of erythrocyte lysate was added. The reaction mixture was than incubated in a shaking water bath at 37°C for 30 minutes. The reaction was stopped by perchloric acid precipitation with subsequent neutralization with K_2CO_3. The sample was cleared by centrifugation and the supernatant was used for HPLC analysis. ITP and IMP were quantified by using ion-pair based reversed-phase HPLC method with UV detection. ITPase activity was expressed as nmoles of IMP formed from ITP in one hour per 10^6 erythrocytes. Reactions were performed in duplicate.

RESULTS

Under standard reaction conditions, a good linearity was observed to at least the equivalent of 19.6×10^6 erythrocytes added to the assay mixture ($y = 26.65x$, $R^2 = 0.9983$, 4 concentrations, triplicate measurements). Table 1 shows that under standard reaction conditions, the ITPase activity was identical in erythrocyte lysates prepared from EDTA and Heparin anti-coagulated blood obtained from the same patient. Omission of magnesium from the reaction mixture had a detrimental effect on the ITPase activity measured. Treatment with Chelex 100 prior to the ITPase assay resulted in

TABLE 1 The ITPase Activity in Erythrocyte Lysates Prepared from EDTA- and Heparin Anti-Coagulated Blood Samples from the Same Patient, with and without Pretreatment with Chelex 100 and in the Presence and Absence of Mg^{2+}

	EDTA-blood		Heparin-blood		Pool sample	
Reaction conditions	Specific activity*	% Activity	Specific activity*	% Activity	Specific activity*	% Activity
Standard assay	11.50	100	11.21	100	5.83	100
No Mg^{2+} added	0.06	0.5	0.07	0.7	0.01	0.2
Standard assay after Chelex treatment	7.34	64	8.47	76	5.06	88
No Mg^{2+} added after Chelex treatment	0.07	0.6	0.02	0.2	not done	—

*Specific ITPase activity is expressed as nmol IMP/10^6 RBC/hr.

a lower ITPase activity in both EDTA and Heparin anti-coagulated blood. Repetition of the experiment showed that the loss of activity was 12–36%. Treatment with Chelex 100 in combination with omission of magnesium from the reaction mixture resulted in very low ITPase activity and was indistinguishable from the samples in which only magnesium was omitted. As shown in Table 1, the same trend was observed when these experiments were repeated using the erythrocyte pool. Linearity with respect to the amount of erythrocyte lysate added remained (data not shown).

DISCUSSION

The present results indicate that untreated erythrocyte lysates prepared from either EDTA or Heparin anti-coagulated blood samples are suitable for the measurement of ITPase activity. However, both treatment with Chelex 100 and omission of magnesium from the reaction mixture resulted in a strongly decreased ITPase activity. The fact that ITPase is highly dependant on the concentration of Mg^{2+} is known,[3] however, the fact that pretreatment of erythrocyte lysates with Chelex 100 caused diminished ITPase activity at a saturating concentration of Mg^{2+}[3] is a new finding. We conclude that samples treated with Chelex 100 are not suitable for measurement of ITPase activity. Apparently, the presence of EDTA in whole blood does not affect ITPase activity.

REFERENCES

1. Marinaki, A.M.; Ansari, A.; Duley, J.A.; Arenas, M.; Sumi, S.; Lewis, C.M.; El-Monsor, Shobowale-Bakre, el-M.; Escudero, E.; Fairbanks, L.D.; Sanderson, J.D. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). *Pharmacogenetics* **2004**, 14, 181–187.
2. Sumi, S.; Marinaki, A.M.; Arenas, M.; Fairbanks, L.; Monsor, Shobowale-Bakre, el-M.; Rees, D.C.; Swee Lay, Thein, S.L.; Ansari, A.; Sanderson, J.; De Abreu, R.; Simmonds, H.A.; Duley, J.A. Genetic basis of inosine triphosphate pyrophosphatase deficiency. *Hum. Genet.* **2002**, 111, 360–367.
3. Holmes, S.L.; Turner, B.M.; Hirschhorn, K. Human inosine triphosphatase: Catalytic properties and population studies. *Clin. Chim. Acta* **1979**, 97, 143–153.