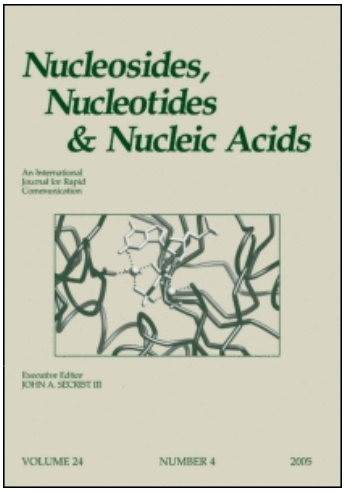

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ITPase Activity in Dry Blood Spots is Comparable with That in Fresh Erythrocytes

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ITPase ACTIVITY IN DRY BLOOD SPOTS IS COMPARABLE WITH THAT IN FRESH ERYTHROCYTES

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□ *Inosine triphosphate pyrophosphohydrolase (ITPase) catalyzing the pyrophosphohydrolysis of inosine triphosphate, deoxyinosine triphosphate and xanthosine triphosphate is involved in the metabolism and tolerance of thiopurine drugs. ITPase activity plays an important role in the prediction of toxicity to thiopurine therapy. Activities in dry blood spots were compared with fresh erythrocytes. Samples were incubated with inosine triphosphate, then inosine monophosphate was determined by a capillary electrophoresis method. Calculated enzyme activities obtained from dry blood spots were in good accordance with activity in fresh erythrocytes.*

Keywords Enzyme activity; ITPase; capillary electrophoresis; dry blood spots

INTRODUCTION

Inosine triphosphate pyrophosphohydrolase (ITPase) catalyzes the pyrophosphohydrolysis of inosine triphosphate (ITP) to inosine monophosphate (IMP). Deficiency of ITPase is a clinically benign condition characterized by abnormal accumulation of ITP in erythrocytes. ITPase deficiency has pharmacogenomic implications associated with metabolisms of 6-mercaptopurine (azathioprine) and may lead to toxicity in affected patients. Significant associations have been reported for flu-like symptoms, rash and pancreatitis.[1] The incidence of ITPase-deficient patients in population is over 10%.[2]

The aim of the study was to determine if ITPase activities in dry blood spots (DBS) are comparable with fresh erythrocytes and whether activities in DBS are stable enough to be transported by standard mailing.

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MATERIALS AND METHODS

All chemicals (analytical reagent grade) were obtained from Sigma (USA). Deionized water (18.3 MΩ.cm) was used for the preparation of all solutions.

Instrumentation and Separation Conditions

The experiments were performed on P/ACE 5510 with diode array detector (Beckman Instruments, USA) as described earlier.[3]

Enzyme Assay

Enzyme activities were measured in DBS and fresh erythrocytes. Blood samples in EDTA tubes were obtained from healthy volunteers (n = 40). Samples were immediately divided and one part was transferred to a paper card, the second was used for erythrocyte separation. DBS were kept for two (except of stability assay) days at laboratory temperature and then extracted/lysed with water (175 μL) while vortex mixing for 1 minute. Fresh erythrocyte lysates were prepared as described earlier.[3] The lysates were centrifuged (5000 × g, 10 minute) and 25 μL of supernatant was mixed with 100 mmol/L Tris buffer (150 μL, pH 9.0), 10 mmol/L dithiothreitol (10 μL) and 1 mol/L $MgCl_2$ (10 μL), and pre-incubated for 5 minutes at 37°C, 40 mmol/L ITP (10 μL) was added and incubated for 15 minutes at 37°C.[2] After incubation, the samples were deproteinated with 1 mol/L trichloracetic acid (20 μL), sonicated (30 seconds) and centrifuged (5000 × g, 1 minute). The supernatant was directly injected onto capillary or stored at −50°C. The measurement of haemoglobin in lysates (g/L) was performed on a Blood Analyzer Radiometer ABL 725 (Diamond Diagnostics, USA).

Stability Assay

Samples of DBS and fresh erythrocytes (n = 12) were kept for 1, 2, and 3 days at laboratory temperature and then processed as described above.

Statistical Analysis

Microsoft Excel spreadsheets (Microsoft Corporation) and QC Expert (TriloByte) were used for numerical data collection, graphs, and statistical analysis. Box plots, regression analysis, Bland-Altman plots and Student's t-test were applied for comparison of data. Results were considered statistically significant at $p < 0.05$.

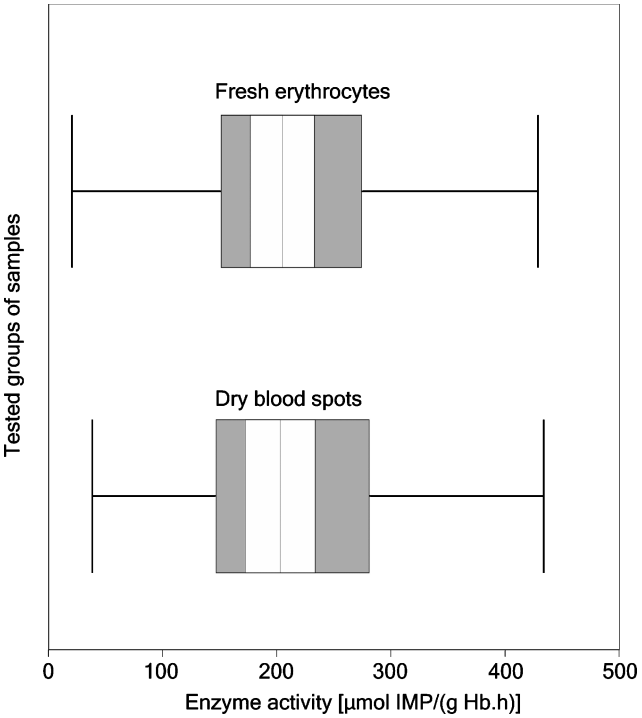

FIGURE 1 Box plots of ITPase activities in fresh erythrocytes and DBS.

RESULTS AND DISCUSSION

ITPase enzyme activities in DBS and fresh erythrocytes were compared. Basic statistics was applied and both data groups showed normal distributions with similar medians, 25th and 75th percentiles (Figure 1). t-tests showed good agreement of means for unpaired data. Regression analysis of the data fitted the equation $y = 0.9758x + 3.4598$ with a strong correlation ($r = 0.966$; Figure 2). Mean difference and standard deviation of the Bland-Altman plot were 1.81 and 17.16 μmol IMP/(g Hb.h), respectively (Figure 3). Obtained difference and standard deviation are considered acceptable for diagnostics purposes.

The stability of ITPase in DBS and fresh erythrocytes was tested at laboratory temperature within 3 days of blood collection. The calculated activities in DBS decreased by 3.1, 4.9 and 17.7% at 1, 2, and 3 days, respectively.

In conclusion, DBS samples can be used for measuring ITPase activity in order to predict adverse thiopurine drug reactions due to ITPase deficiency. The DBS can be transported by standard mailing within two days of collection.

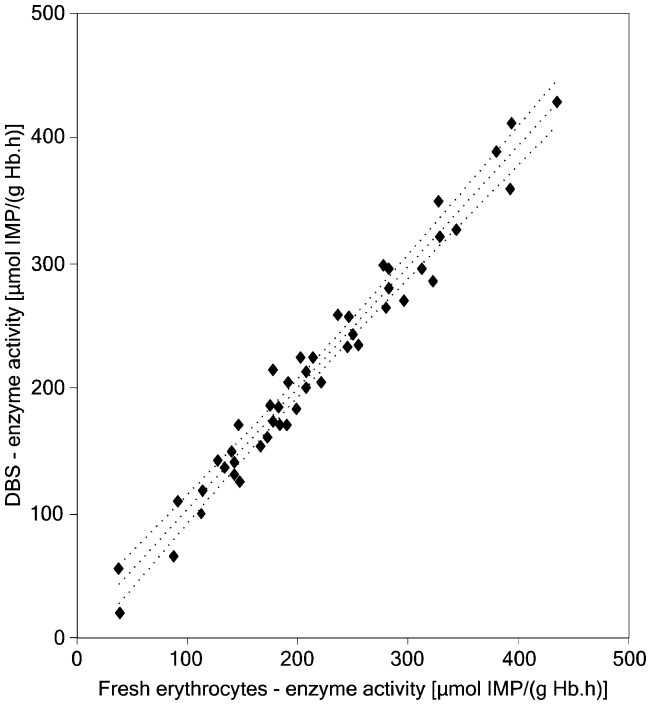

FIGURE 2 Regression plot of ITPase activities in fresh erythrocytes and DBS.

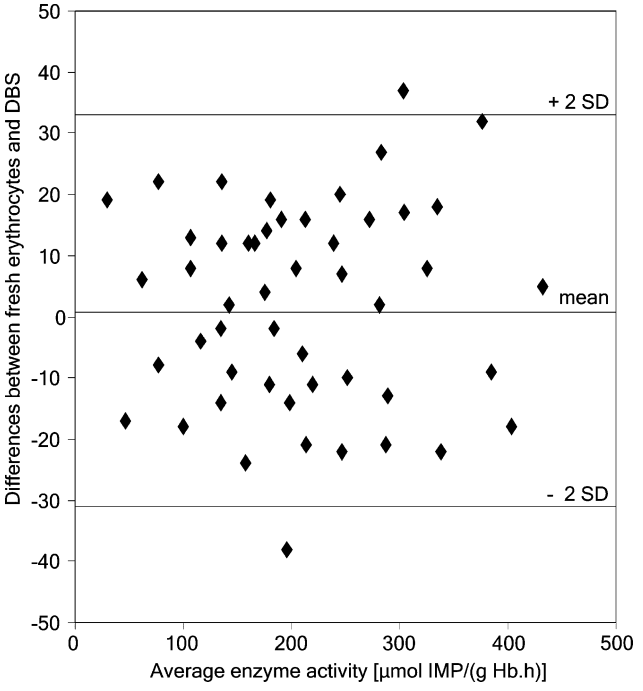

FIGURE 3 Bland-Altman plot-comparison of ITPase activities in fresh erythrocytes and DBS.

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