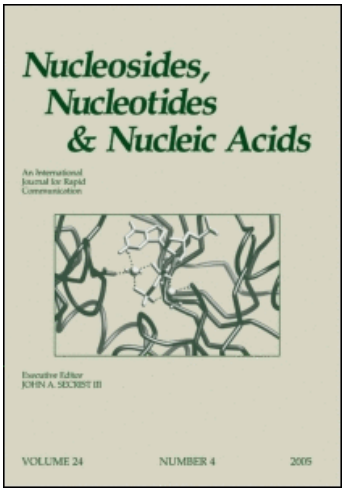

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 27 October 2004

To cite this Article Carlucci, F. , Tabucchi, A. , Aiuti, A. , Rosi, F. , Floccari, F. , Pagani, R. and Marinello, E.(2004) 'Evaluation of ADA Gene Expression and Transduction Efficiency in ADA/SCID Patients Undergoing Gene Therapy', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1245 — 1248
To link to this Article: DOI: 10.1081/NCN-200027508
URL: http://dx.doi.org/10.1081/NCN-200027508

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1245–1248, 2004

Evaluation of ADA Gene Expression and Transduction Efficiency in ADA/SCID Patients Undergoing Gene Therapy

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ABSTRACT

A capillary electrophoresis (CE) method was developed for ADA/SCID diagnosis and monitoring of enzyme replacement therapy, as well as for exploring the transfection efficiency for different retroviral vectors in gene therapy.

Key Words: Gene therapy; ADA/SCID; Capillary electrophoresis.

INTRODUCTION

SCID is a group of genetic disorders characterized by a block in T lymphocyte differentiation, variably associated with abnormal development of other lymphocyte lineages, B or NK lymphocytes or more rarely of the myeloid lineage. About 20% of SCIDs are caused by adenosine deaminase (ADA)-deficiency which leads to accumulation of deoxyadenosine nucleotides (dAXP) known to be toxic for lymphoid

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DOI: 10.1081/NCN-200027508
1525-7770 (Print); 1532-2335 (Online)

tissue. ADA/SCID has long been considered a model disease for gene therapy trials since it's caused by a defect in a single gene. An improved protocol for gene transfer into autologous bone marrow (BM) $CD34^+$ cells, combined with non-myeloablative conditioning was designed.[1] To explore the transfection efficiency for different retroviral vectors and fully evaluate the efficacy of gene therapy (GT), the measurement of ADA activity in transduced cells and in red blood cell (RBC) is essential. Moreover the evaluation of ADA activity in plasma is necessary in enzyme replacement therapy with PEG-ADA. A suitable capillary electrophoresis (CE) method was developed.

MATERIALS AND METHODS

ADA was evaluated by the quantitation of substrate disappearance and reaction product formation separated by CE. Separations were performed in 42 cm × 50 μm i.d.; uncoated capillary the conditions were electrolyte 20 mmol/L Na-borate (pH 10.00), voltage 10 kV, UV analysis at 254 nm. The assay mixture contained 50 mmol/L Tris (pH 7.2), 0.4 mmol/L adenosine and suitable sample amount (10 μg of protein for lymphocytes, 20 μL of packed RBC for erythrocytes and 20 μL of plasma in the case of plasma activity). The activity was expressed as nmol/h/mg protein for lymphocytes and as μmol/h/mL packed RBC or plasma for erythrocytes and plasma respectively.

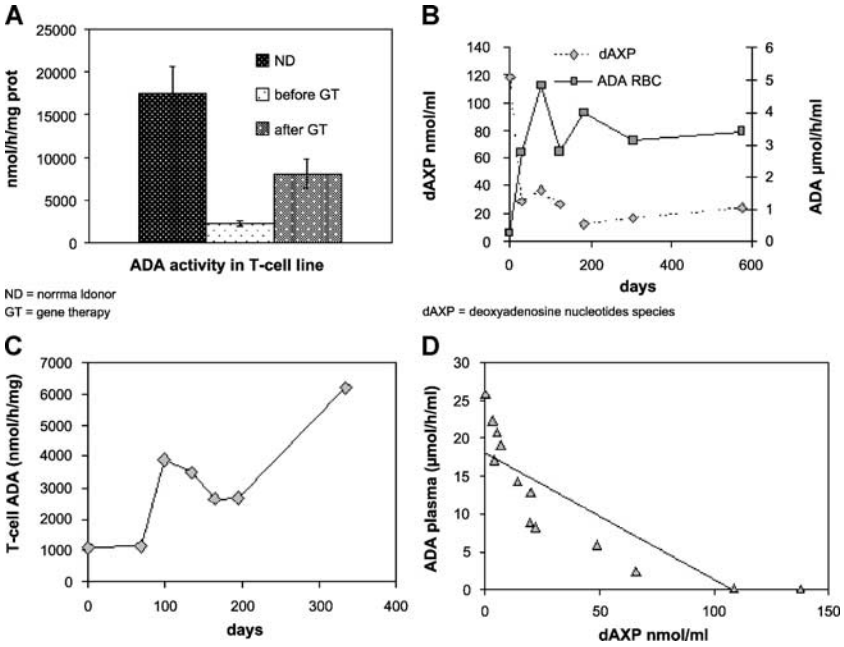

Figure 1. A: ADA activity in T-cell line of normal donors (ND) and before and after gene therapy. B: behaviour of toxic metabolite (dAXP) and ADA activity in RBC long time after gene therapy. C: ADA activity in T-Cell long time after gene therapy. D: correlation between ADA activity in plasma and toxic metabolite in RBC.

RESULTS

The correlation coefficient of peak area versus concentration exceeded 0.998 for all compounds (substrates and products) over the range 2–500 μmol/L. The linearity of reaction product formation with respect to incubation time was checked in the range 0–60 min. for a incubation mixture containing 25 μg of sample proteins; the linearity of total activity with respect to sample amounts (range 0–50 μg of protein) in the incubation mixture was also analysed (both $r > 0.99$). The intra- and inter-assay variability showed a relative standard deviation (RSD) < 3%.

The method permitted a complete screening of gene therapy protocols, evidencing the restoration of intracellular ADA activity in T-cell line from almost undetectable levels to close 50% of controls. After stem-cell gene therapy ADA activity in T-cell line rose from 992 ± 340 to 8010 ± 2362 nmol/h/mg prot (Fig. 1A) and restoration of ADA activity was evident also in RBC (Fig. 1B). The levels and stability of the vector ADA gene expression in T-cell long time after stem-cell gene therapy is evident from the Fig. 1C. ADA activity in RBC increased from undetectable levels to 20–30% of healthy controls, this behaviour was coupled with the reduction of toxic deoxyadenosine nucleotides evaluated according to Carlucci et al.[2] (Fig. 1B). In RBC of healthy subjects, ADA showed a value of 18.23 ± 10.19 nmol/h/mL packed RBC (n=10); in patients at diagnosis it was almost undetectable. Since improvement in immune function follows correction of metabolic abnormalities,[1] the goal of enzyme replacement therapy with PEG-ADA is to reduce toxic deoxyadenosine nucleotides, maintaining plasma ADA activity in the range of 15–35 μmol/h/mL. We demonstrated that plasma ADA activity negatively correlated with dAXP level in RBC (Fig. 1D).

DISCUSSION

A variety of HPLC methods has been developed for research and diagnostic purposes, but two-dimensional thin-layer chromatography is also widely used.[3] Capillary electrophoretic separation techniques, with their high separation efficiency, flexibility, and high sample throughput, can be useful for the diagnosis of inborn errors of purine and pyrimidine metabolism[4] We demonstrated that the technique is a valuable, fast and inexpensive screening tool in the evaluation of ADA activity. CE is also particularly versatile with respect to other separative techniques, allowing shifts from one separation method to another in a few minutes with limited reagent consumption (3 mL every 20–30 runs) and theoretically indefinite column life. After 550 runs with the same capillary for enzyme determinations we failed to find variation in separation efficiency (CV < 5%). The method evidenced to be useful to quickly assess the expression of ADA in cells from SCID patients and represents an important tool for the follow-up of patients treated with enzyme replacement therapy or in clinical gene transfer protocols.

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