Biochemical and Biophysical Research Communications 441 (2013) 114–119

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

HIV-1 TAT-mediated protein transduction of human HPRT into deficient cells

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ARTICLE INFO

Article history:
Received 3 October 2013
Available online 12 October 2013

Keywords:
Lesch–Nyhan disease
HPRT
Recombinant proteins
Protein purification
TAT transduction domain
Enzyme replacement therapy

ABSTRACT

Lesch–Nyhan disease (LND) is a severe and incurable X-linked genetic syndrome caused by the deficiency of hypoxanthine–guanine phosphoribosyltransferase (HPRT), resulting in severe alterations of central nervous system, hyperuricemia and subsequent impaired renal functions. Therapeutic options consist in supportive care and treatments of complications, but the disease remains largely untreatable. Enzyme replacement of the malfunctioning cytosolic protein might represent a possible therapeutic approach for the LND treatment. Protein transduction domains, such as the TAT peptide derived from HIV TAT protein, have been used to transduce macromolecules into cells *in vitro* and *in vivo*. The present study was aimed to the generation of TAT peptide fused to human HPRT for cell transduction in enzyme deficient cells. Here we document the construction, expression and delivery of a functional HPRT enzyme into deficient cells by TAT transduction domain and by liposome mediated protein transfer. With this approach we demonstrate the correction of the enzymatic defect in HPRT deficient cells.

Our data show for the first time the feasibility of the enzyme replacement therapy for the treatment of LND.

1. Introduction

Human hypoxanthine–guanine phosphoribosyltransferase (HPRT or HGPRT) is a key enzyme in purine metabolism. Mutations in its gene, HPRT1, cause the Lesch–Nyhan disease (LND). Affected patients suffer from hypereuricaemia, neurological manifestations including dystonia, chorea, pyramidal signs and a distinctive self-injurious behaviour [1]. An intriguing aspect of the disease is how the housekeeping gene HPRT1 can cause neurobehavioral and renal problems. Treatment of renal problems in the early childhood does not prevent the development of dysfunctions in the brain, suggesting that the HPRT1 gene might have a role during the neuronal development. This is in line with recent findings showing that HPRT1 deficient neuronal cells manifest impairment in developing dopaminergic neurons [2]. Furthermore, HPRT1 gene expression seems to be crucial for the establishment of the purinergic signalling during development of neuronal pathways [3,4]. These discoveries define new potential molecular targets for modulation of this intractable neurological phenotype, thus opening to a new prospective of therapeutic intervention.

To date, patient treatment is limited to the control of uric acid, and the reduction of alterations in mood and behaviour. The latter aspect of the disease has been recently investigated by our research group that evaluated the effects of S-Adenosylmethionine in a group of LND patients. Our results show an improvement of the self-injurious behaviour in a sub-group of patients, indicating the potential benefit of this compound for the abnormal behaviour of the patients [5]. Nevertheless the neurological abnormalities remain incurable.

Therapeutic interventions for the re-establishment of a functional HPRT enzyme in the central nervous system are still challenging. Problems concerning the enzyme delivery to the affected cells, the blood–brain barrier penetration, safety issues, uncertainty regarding the ideal timing for intervention, and the lack of knowledge in the molecular mechanisms of neurological phenotype, remain pivotal in designing effective therapeutic approaches. Regarding the delivery of the therapeutic enzymes much has been learned by the enzyme replacement therapy, a well-established approach for treating lysosomal storage diseases. At the moment there are a group of nine commercially available glycoprotein products, for the treatment of Fabry disease, Hurler–Scheie (Mucopolysaccharidosis I H-S, MPS IH-S) and Scheie (MPS IS) syndromes, Hunter syndrome (MPS II), Maroteaux–Lamy (MPS VI) syndrome, and Pompe disease. Treatments for Niemann–Pick B disease, Metachromatic leukodystrophy and α-mannosidosis are at the preclinical stage [6,7]. Each compound is intended to augment or replace the activity of a specific endogenous catabolic enzyme within cellular lysosomes. Essential for this therapeutic approach is the entry of the enzyme into diseased cells by receptor-mediated mechanisms. Over time, chimeric enzymes have been built to improve targeting to specific cell

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0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.029

receptors [8], or by passing the need of a specific receptor using the 11-amino-acid human immunodeficiency virus (HIV) TAT protein transduction domain. This modification was proven to extend the range of tissues corrected by infused enzymes [9].

TAT peptide is part of the Protein Transduction Domains (PTDs) group of peptides that can cross biological membranes in a receptor-independent manner [10]. PTDs can deliver various cargo molecules into practically all cells both *in vitro* and *in vivo* [11]. Furthermore it has been shown for TAT chimeric proteins the ability to cross the blood–brain barrier in mice [12].

Taken together, these findings raise the possibility to treat LND by an enzyme replacement approach.

In the present study we show the construction, expression and purification of a recombinant enzyme containing the HPRT domain fused to the TAT peptide. Moreover, we describe *in vitro* assays for the biochemical characterisation, delivery and evaluation of the functional complementation in HPRT deficient cells. These findings have a direct implication for the enzyme replacement therapy approach in LND.

2. Material and methods

2.1. Bacterial expression constructs

The coding sequence of HPRT1 gene (NM_0001942) was amplified from cDNA derived from healthy donors by RT-PCR using specific primers (5′-ACGCGTCGACATGGCGACCCGCAGCCCT-3′, 5′-ATAGTTTAGCGGCCGCTTAGGCTTTGTATTTTGC-3′) and cloned into the Sal I, Not I sites of the pET 28 b TAT-2.1 bacterial vector. In the generated pTAT-HPRT construct, HPRT1 gene is cloned downstream the TAT domain. A 6× histidine sequence, located upstream of the TAT-HPRT sequence, was used for protein purification.

The second bacterial construct, pHPRT, was obtained by cloning the amplified HPRT1 gene using the primers 5′-GGAATTCCATA TGGCGACCCGCAGCCCTGGC-3′ and 5′-CCGTCGAATTCCAGGCTTTG-TATTTTGCTTTTCC-3′, into the NdeI, EcoRI restriction sites of the pET 28b TAT-2.1 where the TAT transduction domain was previously removed by enzymatic digestion. The resulting plasmid contains double 6× histidine tags placed at 5′ and 3′ end of the HPRT gene sequence. All the vectors were checked by sequencing.

2.2. Protein expression and purification

The bacterial expression constructs were introduced into the BL21 (DE) strain of *Escherichia coli* by heat shock and plated on agar plates containing kanamycin (25 μg/ml) and chloramphenicol (34 μg/ml). Protein expression was induced using 0.5 mM of Isopropyl β-D-1-thiogalactopyranoside (IPTG) for 3 h at 37 °C. Bacteria produced the recombinant HPRT protein (rHPRT) in soluble form, while rTAT-HPRT has been found insoluble associated with cell pellets. Therefore, two different strategies were designed for purification of the recombinant proteins. Overall, the purification procedures consist of a lysis step where bacteria disruption was obtained by sonication and enzymatic digestion for 1 h at RT. Afterward, bacterial lysates were centrifuged at 6000*g* and the clarified suspension was loaded on Nickel based affinity column. After several washes the purified proteins were eluted from the column by the addition of increasing concentration of Imidazole.

In the case of rTAT-HPRT, the bacterial lysis was performed in a buffer A (6 M Guanidinium, 20 mM phosphate buffer pH 7.8 and 0.5 M NaCl), while buffer B (8 M Urea, 20 mM phosphate buffer pH 7.8, 0.5 M NaCl) was used for column equilibration and protein binding. The column was washed with buffer B at pH 6, then slowly with Buffer C (50 mM NaH_2PO_4 pH 8.0, 0.5 M NaCl, 20 mM Imidazole) for protein renaturation. The purified refolded TAT protein was then eluted with 50 mM NaH_2PO_4 pH 8.0, 0.5 M NaCl, 1 M Imidazole.

The lysis buffer for rHPRT protein purification contained 50 mM Na_2HPO_4 and 500 mM NaCl, supplemented with Lysozyme (10 μg/ml), DNAsi I (5 μg/ml) and protease inhibitors (Complete Mini EDTA-Free, Roche Diagnostic, Switzerland). Column binding and washes were performed with 25 mM NaH_2PO_4 pH 8.0, 0.5 M NaCl, 80 mM Imidazole pH 6, and elution of the purified rHPRT was obtained with 25 mM NaH_2PO_4 pH 8.0, 0.5 M NaCl, 500 mM Imidazole pH 6.

Protein concentration and desalting was performed using the Centricon devices (Millipore, Billerica, MA, USA). Purity of the proteins was checked by Coomassie Blue staining of 15% SDS–PAGE gels. Protein identification was performed by immuno-staining of PDVF membranes using a polyclonal anti-HPRT (sc-20975, Santa Cruz, California, USA) antibody and a monoclonal anti-His-probe (sc-8036 Santa Cruz, California, USA) antibody.

2.3. Enzyme kinetics

The activity of the recombinant proteins was recorded using the method described in [13] based on spectrophotometric assay. Briefly, 300 ng of recombinant proteins were added to a reaction mixture containing 100 mM Tris HCl at pH 7.5, 12 mM $MgCl_2$, 1 mM 5′-Phosphorylribose 1-Pyrophosphate and increasing concentrations of guanine or hypoxanthine (0–100 μM). The formation of GMP or IMP was recorded at 37 °C for 2 min in a spectrophotometer at 254 nm and 245 nm respectively. The change in extinction coefficient for hypoxanthine to IMP is 1770 M^{-1} cm^{-1} and for guanine to GMP is 5146 M^{-1} cm^{-1}. The Michaelis–Menton K_m and V_{max} were calculated by non-linear regression using a website for statistical computation (http://vassarstats.net/).

2.4. Transfection and transduction of the recombinant proteins in human cells

HPRT deficient leukemia T-cells (CEM/HPRT-) and HeLa cells were grown in RPMI medium containing 10% Foetal Bovine Serum (FBS, Life Technologies, Ltd, Carlsbad, California, USA) and antibiotics (100 μg/ml streptomycin, 100 U/ml penicillin). HPRT recombinant protein was introduced by lipofection into CEM-HPRT-cells seeded in 12-well plates, at concentration of 15×10^3 cells/well in complete medium. Transfection complexes were prepared using Lipofectamine 2000 (Life Technologies, Ltd, Carlsbad, California, USA) according to the manufacture instructions. Twenty-four hours after cell seeding, the transfection mixture was added to the medium deprived of serum for an overnight.

For the transduction of rTAT-HPRT1, HeLa and CEM/HPRT-cells were incubated with the recombinant protein in the medium without serum at the time of seeding. Cells were cultured for further 48 h, after serum addition to the culture medium. The presence of recombinant proteins in the treated cells was evaluated by Western blot. The activity of the rHPRT enzyme in the cells was assessed by the 6-thioguanine (6TG) assay.

2.5. Effects of HPRT recombinant proteins on cell proliferation: 6TG assay

The assay is based on the ability of HPRT deficient cells to grow in the presence of the toxic purine analogue 6TG. This phenotype can be reverted by the introduction of a functional HPRT enzyme. CEM/HPRT-cells were exposed to 1 μg/ml of 6TG (Sigma–Aldrich, St. Louis, MO, USA) after treatment with the recombinant proteins. The effects on cell proliferation were evaluated by counting cells with a hemocytometer just before and 48 h after treatment with the drug.

2.6. Western blot analysis

The ability of the TAT basic domain to mediate the introduction into cells of heterologous HPRT, or the liposome to transfect the recombinant protein, was evaluated by Western blot. Cells were washed 2 times in a Dulbecco's Phosphate buffered saline (PBS, Sigma–Aldrich) and lysed in 2× Laemmli sample buffer. Total proteins were separated by 15% SDS–PAGE and transferred to PVDF membrane by electroblotting. The membranes were incubated for 1 h in block solution containing 5% Bovine serum albumin (Sigma–Aldrich) dissolved in Tris-buffered saline (TBS), and washed three times with TBS containing Tween-20 (Sigma–Aldrich). After washing, the membranes were incubated with mouse anti-histidine antibody or rabbit anti-HPRT for an overnight at 4 °C. Afterwards membranes were washed three times with TBS containing Tween 20. The membranes were then incubated with goat anti-rabbit immunoglobulins (Sigma, dilution 1:10,000) for 1 h. Immunoreactive bands were visualized using a SuperSignal West Pico Chemiluminescent Substrate (ThermoScientific, Rockford, IL, USA).

3. Results

3.1. Construction and purification of rHPRT and rTAT-HPRT fusion proteins

In order to verify the feasibility of the enzyme replacement therapy for LND, we constructed two expression plasmids containing the HPRT domain and the HPRT linked to the TAT transduction domain. The transgenes were cloned in the pET28b derived-vector to produce recombinant rTAT-HPRT and rHPRT proteins in bacteria (Fig. 1A and B).

E. coli BL21 (DH3) strain was used for the expression of recombinant proteins. Many proteins containing the TAT domain result insoluble and are purified from the bacterial inclusion bodies to increase protein yields and transduction potential [14]. Also rTAT-HPRT protein formed insoluble aggregates that were extracted from bacteria using a denaturing buffer. After centrifugation, the clarified extracts were loaded on nickel affinity column for protein binding. In our experimental conditions, rTAT-HPRT protein solubilisation in aqueous buffers was achieved by removal of the denaturant when the protein was still bound to the column using a buffer exchange. Afterwards, the recombinant protein was eluted from the column by increasing concentration of nickel competing agent. Fractions containing the fusion protein were pooled and salts removed. We produced a second protein the rHPRT devoid of the TAT transduction domain. This protein on the contrary of rTAT-HPRT was successfully produced and purified under standard native conditions. As shown in Fig. 1C the rTAT-HPRT and rHPRT were purified nearly to the homogeneity, yielding to 95% pure protein bands as determined by SDS–PAGE.

3.2. Determination of the kinetic parameters for HPRT domain

The enzymatic activity of the HPRT domain in the recombinant proteins was evaluated *in vitro* by measuring the kinetic parameters of the enzyme. This was determined using the ability of the

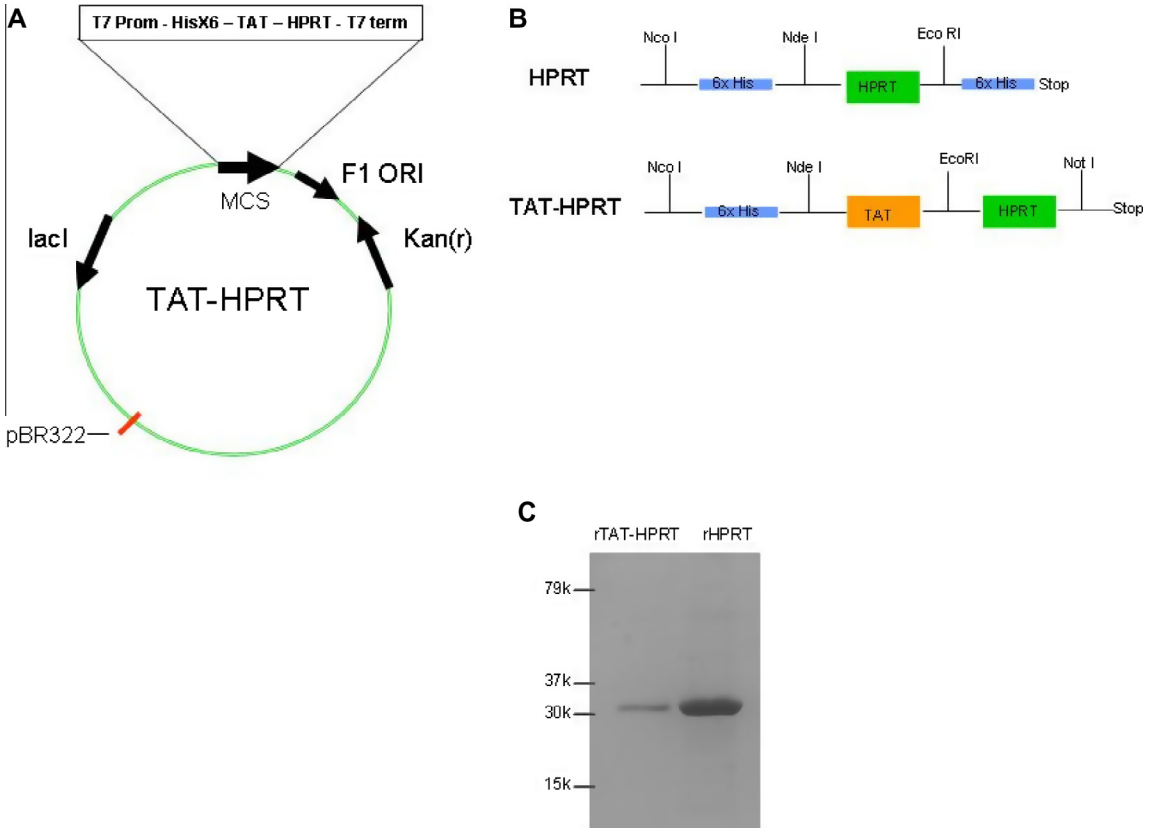

Fig. 1. Construction, production and purification of rTAT-HPRT and rHPRT. The expression vector pET-TAT HPRT is based on pET-28b. The coding frame is represented by an open box along with the 6His tag and the HIV-1 Tat peptide (Panel A). rHPRT was obtained by TAT removal and insertion of the in-frame HPRT sequence. Restriction enzymes used for cloning are shown in Panel B. Expression of the recombinant proteins was obtained after 3 h IPTG-induction at 37 °C. rTAT-HPRT localised in the bacterial pellet while rHPRT is present in the cytoplasm. Proteins were purified by affinity chromatography, and concentrated by desalting. Panel C shows 1 and 3 μg of rTAT-HPRT and rHPRT, respectively loaded in a standard SDS–PAGE that was stained with Coomassie blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

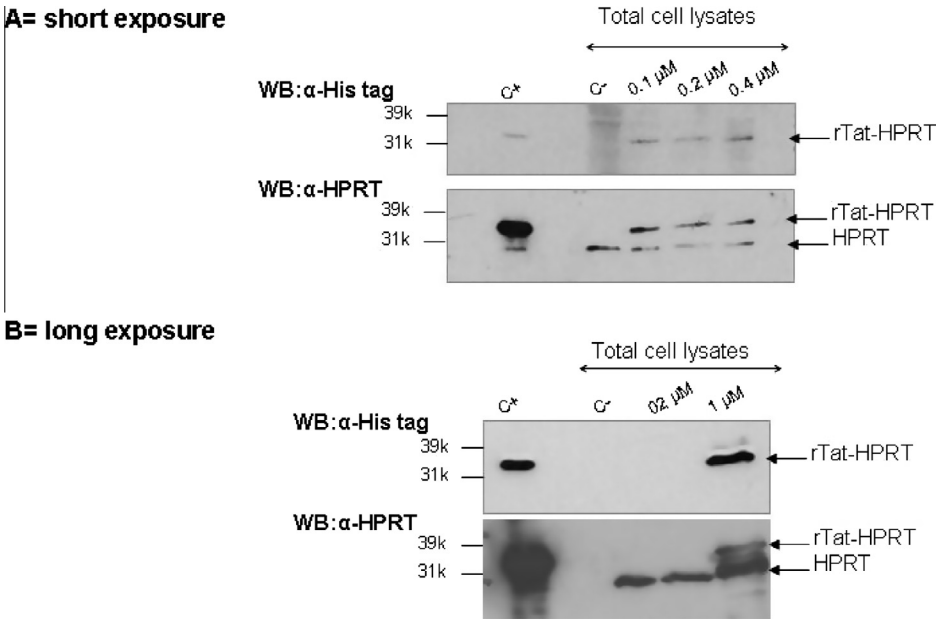

Fig. 2. TAT mediated-HPRT delivery into cells. Purified rTAT-HPRT was added to the HeLa cells at the indicated concentrations in PBS (1 h, Panel A = short exposure) or in medium without serum (overnight, Panel B = long exposure). After treatment, cells were incubated with Trypsin–EDTA, washed with PBS and lysed with 2× Laemmli loading buffer. Intracellular delivery of the recombinant HPRT enzyme was determined by Western blot using α-His tag or α-HPRT antibodies. Data show that the delivery of recombinant HPRT is rapid (within 1 h at 0.1 μM, Panel A) and sustained at high concentration of recombinant protein (1 μM, Panel B). C+: positive control (rTAT-HPRT); C−: Untreated cells; HPRT: endogenous enzyme.

HPRT domain to form nucleoside mono-phosphate and pyrophosphates, when hypoxanthine or guanine reacts with phosphoribosyl pyrophosphate. The Lineweaver–Burk plot was used to determine the K_m and V_{max}, for both hypoxantine and guanine (Supplementary Fig. 1). The K_m and V_{max} for hypoxanthine were 0.02 mM and 2.5 μmol/min/mg, respectively. Similar values were measured for guanine where the calculated K_m was equal to 0.095 mM and V_{max} equal to 40 μmol/min/mg. The measured kinetic parameters of the HPRT domain were similar to published values [13,15].

The activity of the rTAT-HPRT fusion protein was also determined to exclude any functional interference of the peptide on the enzyme kinetic. We found no significant differences between the two produced proteins in the K_m and V_{max} for either hypoxanthine or guanine (data not shown) indicating that in both proteins the HPRT enzyme is active.

3.3. Transduction of TAT-HPRT and functional complementation of HPRT deficiency in vitro

The ability of TAT peptide to deliver HPRT enzyme into cells was assessed by determining the presence of the protein in the total cell lysates. HeLa cells were treated with increasing protein concentrations for short (1 h in PBS) or long incubation time (overnight, in medium without serum). After treatment, cells were detached by a prolonged trypsin treatment to remove the eventual extracellular rTAT-HPRT bound to cell membrane. In both experimental conditions, the presence of HPRT protein into cell lysates has been demonstrated (Fig. 2A and B). These data suggest that TAT-mediated intracellular delivery of the enzyme is rapid (within an hour) and efficient since it occurs at low concentrations (0.1 μM). Still, prolonged presence of the enzyme was limited to high dose treatment (1 μM).

We next tested the activity of the transduced HPRT domain by means of the 6TG resistance assay. This test is based on the ability of the HPRT enzyme to transform 6TG in the toxic nucleoside analogue 6-thioguanine monophosphate that is included in DNA during cell replication. Proliferating cells with functional HPRT are poisoned and will die after 6TG treatment, while cells HPRT deficient will survive. The Fig. 3 illustrates the survival curves to 6TG treatment of two human cell lines with functional (HeLa) or defective endogenous HPRT protein (CEM/HPRT-). Massive cell death was recorded after 48 h exposure of HeLa cells to the drug, while CEM/HPRT-showed no sign of toxicity.

In these experimental conditions, transduction of CEM/HPRT-cells with an active HPRT enzyme should be able to reverse the response to 6TG treatment. To determine the activity of the chimeric enzyme, CEM/HPRT-cells were treated overnight with rTAT-HPRT (1 μM) in medium without serum. The next day (Day 1), in order to assess possible cytotoxic effect of the recombinant proteins,

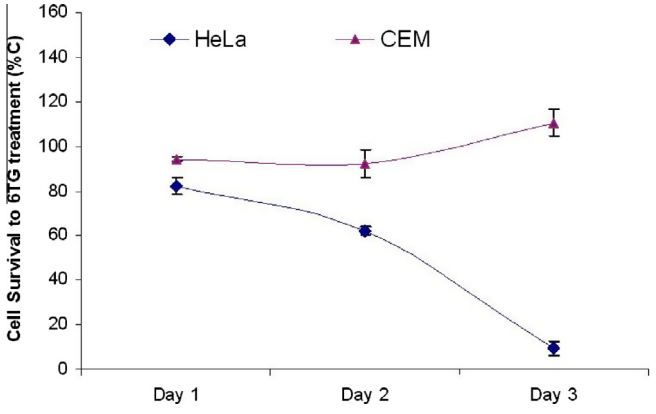

Fig. 3. 6TG resistance assay. HPRT positive (HeLa) and HPRT deficient cells (CEM cells) were seeded at 50% confluence and after 24 h were exposed to 6TG at a final concentration of 1 μg/ml. Cells were counted by haemocytometer just before treatment (Day 1) and in the subsequent 48 h (Days 2–3). Cell viability was assessed by Trypan blue exclusion assay. The panel shows that CEM/HPRT deficient cells "resist" to the toxicity of 6TG and survive. The results are means ± S.E.M. of three separate experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cells were counted and lysed for the detection of the recombinant enzyme by Western blot.

The rest of the cultures were treated with 6TG and incubated for a further 48 h. The same procedure was repeated at Day 3. We recorded a significant reduction in the number of surviving cells during the 2 days of treatment with 6TG in CEM/HPRT-cell cultures exposed to rTAT-HPRT compared to untreated cells (Fig. 4A). Incubation of cells with rHPRT alone on the other hand did not affect cell survival to the 6TG treatment (data not shown) indicating that the effect is rTAT-HPRT mediated. In addition to cell survival decrease, we observed a prolonged presence of rTAT-HPRT throughout the time interval of the experiment as detected by Western blot. The presence and the measured activity of HPRT in these cells, strongly suggested that TAT peptide mediates the transduction of a functional protein into cells. To prove that the recorded effect is mediated by rTAT-HPRT protein, liposomes/rHPRT complexes were used.

It is widely recognised the ability of liposomes to deliver DNA/proteins into cells. Similarly to TAT peptide, liposomes/rHPRT complexes induce significant decrements in cell survival in CEM/HPRT-cells treated with 6TG (Fig 4B).

Altogether these results demonstrate that rTAT-HPRT is efficiently transduced into cells and that it is able to mediate the correction of the CEM/HPRT-response to 6TG treatment.

4. Discussion

Here we show the construction, expression and purification of a functional human HPRT enzyme delivered by TAT peptide or by liposomes into deficient cells. We provide evidences of the enzymatic activity *in vitro* of the purified transferase and of its ability to induce the 6TG-mediated cytotoxicity in HPRT deficient cells. Our data suggest that enzyme replacement for HPRT deficiency might be possible.

The membrane-translocating property of the HIV-1 TAT peptide has been used for more than two decades for the delivery of different cargos into cells, in a non-invasive way [11]. Different types of enzymes have been transduced *in vitro* and *in vivo*: kinases [16], catalases, phosphatases, oxidases and dismutases [8]. Although experimental conditions for successful cell transduction varied with the type of protein/peptide, delivery of functional enzymes occurs in an interval of concentrations between 0.5 μM and 4 μM. Our results are in line with this interval, suggesting a similar mechanism of delivery for these proteins. We show significant levels of recombinant HPRT enzyme in HeLa and CEM/HPRT-cells after short or long treatment with recombinant TAT protein. However, only high dose (1 μM) of rTAT-HPRT resulted in persistent levels of the enzyme in cell lysates. A possible explanation for this finding can be provided by the TAT-mediated mechanism of transduction across the plasma membrane. It is widely accepted that TAT binds first to various extracellular cell surface receptors (integrins, heparan sulfate proteoglycans, etc.) [17] and enters the cells via endocytosis [18]. Therefore, cells must be treated long enough with sufficient TAT protein for cell transduction to occur. Detection at low doses of the rTAT-HPRT in cell lysates after short exposure might be explained as proteins that are still extracellularly bound or stuck to the surface of cells. Aware of these possibilities, we used a prolonged trypsin treatment and PBS washes before cell lysis to remove any TAT protein bound extracellularly. We are thus confident that the enzyme we detected in the total cell lysates is the transduced intracellular rTAT-HPRT protein.

In addition we showed that the delivered rTAT-HPRT enzyme induces cell death after 6TG treatment in cells that are incapable to metabolize the drug. The same occurred in cells treated with cationic liposome/rHPRT protein complexes. These data strongly suggest that the effect on cell viability is rHPRT-mediated. Although treatment with rHPRT alone does not induce toxic effects, we can not exclude that what we have recorded can be ascribed to non-specific toxic effect of TAT. It has been suggested that protein transduction domains induce toxicity at concentrations above 100 μM [19]. In our experimental conditions, treatment with 1 μM TAT-protein should be therefore considered safe.

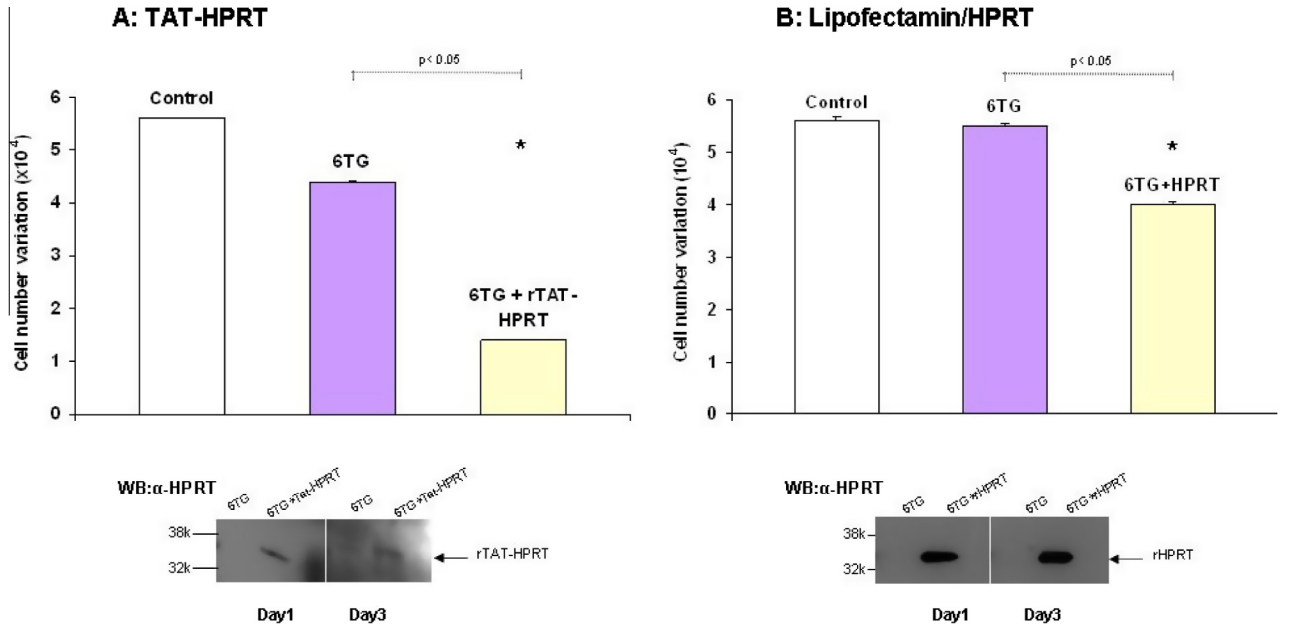

Fig. 4. Recombinant proteins mediate the correction of the CEM/HPRT-response to 6TG. Recombinant proteins were added to the culture medium without serum, alone (rTAT-HPRT) or mixed with Lipofectamin2000 (rHPRT) at a final protein concentration of 1 μM. After an overnight exposure, cells were counted to determine the cytoxicity of the treatment (Day 1) and incubated with 1 μg/ml 6TG for a further 48 h (Day 3). Cell viability was determined by Trypan blue exclusion assay and intracellular delivery of the enzymes by Western blot. Panels A and B show the results for rTAT-HPRT and Lipofectamin/rHPRT respectively. In both cases a significant reduction in cell survival was measured in cells treated with the recombinant proteins plus 6TG (yellow bars), compared to 6TG-treated (violet, bar) or untreated cells (white bars). Furthermore, HPRT signal was detected in lysates of cells exposed to TAT or liposome complexes during the 48 h of 6TG treatment. The results are means ± S.E.M. of three separate experiments. Data significance was tested by student's *t* test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Altogether our data strongly suggest the possibility to use a protein transduction domain for the phenotype correction of HPRT defective cells. To our knowledge, this is the first report on an enzyme replacement approach applied to the treatment of HPRT deficiency. It is a step forward in the development of effective treatments for this disease.

In summary the present study demonstrated for the first time that exogenous human HPRT fused with HIV-1 TAT peptide, can be directly transduced into HPRT deficient CEM cells, and that the resulting intracellular HPRT activity levels are significantly increased. Thus, we conclude that rTAT-HPRT fusion protein may offer a new therapeutic tool for Lesch Nyhan disease.

Acknowledgments

The authors thank Prof. Dowdy, University of California, La Jolla, San Diego, USA for providing us with pET 28 b TAT-2.1 bacterial vector and Dr. Paola Ferraro (Department of Biology, University of Padua, I-35131 Padua, Italy) for providing the CEM/HPRT-cell line.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.029.

References

[1] G.H. Guibinga, F. Murray, N. Barron, HPRT-deficiency dysregulates cAMP-PKA signaling and phosphodiesterase 10A expression: mechanistic insight and potential target for Lesch–Nyhan disease?, PLoS One 8 (2013) e63333
[2] G.H. Guibinga, S. Hsu, T. Friedmann, Deficiency of the housekeeping gene hypoxanthine–guanine phosphoribosyltransferase (HPRT) dysregulates neurogenesis, Mol. Ther. 18 (2010) 54–62.
[3] L. Mastrangelo, J.E. Kim, A. Miyanohara, T.H. Kang, T. Friedmann, Purinergic signaling in human pluripotent stem cells is regulated by the housekeeping gene encoding hypoxanthine guanine phosphoribosyltransferase, Proc. Natl. Acad. Sci. USA 109 (2012) 3377–3382.
[4] M. Göttle, H. Burhenne, D. Sutcliffe, H.A. Jinnah, Purine metabolism during neuronal differentiation: the relevance of purine synthesis and recycling, J. Neurochem. (2013), http://dx.doi.org/10.1111/jnc.12366.
[5] D. Dolcetta, P. Parmigiani, L. Salmaso, R. Bernardelle, U. Cesari, G. Andrighetto, G. Baschirotto, W.L. Nyhan, U. Hladnik, Quantitative evaluation of the clinical effects of s-adenosylmethionine on mood and behavior in lesch–nyhan patients, Nucleosides Nucleotides Nucleic Acids 32 (2013) 174–188.
[6] T.A. Ratko, A. Marbella, S. Godfrey, N. Aronson, Enzyme-Replacement Therapies for Lysosomal Storage Diseases [Internet], Rockville (MD): Agency for Healthcare Research and Quality (US), Technical Briefs, No. 12, 2013, http://www.ncbi.nlm.nih.gov/books/NBK117219.
[7] S. Bruni, L. Loschi, C. Incerti, O. Gabrielli, G.V. Coppa, Update on treatment of lysosomal storage diseases, Acta Myol. 26 (2007) 87–92.
[8] J.H. Grubb, C. Vogler, W.S. Sly, New strategies for enzyme replacement therapy for lysosomal storage diseases, Rejuvenation Res. 13 (2010) 229–236.
[9] K.O. Orii, J.H. Grubb, C. Vogler, B. Levy, Y. Tan, K. Markova, B.L. Davidson, Q. Mao, T. Orii, N. Kondo, W.S. Sly, Defining the pathway for TAT-mediated delivery of beta-glucuronidase in cultured cells and MPS VII mice, Mol. Ther. 12 (2005) 345–352.
[10] J.S. Wadia, S.F. Dowdy, Modulation of cellular function by TAT mediated transduction of full length proteins, Curr. Protein Pept. Sci. 4 (2003) 97–104.
[11] X. Zhang, X. Zhang, F. Wang, Intracellular transduction and potential of Tat PTD and its analogs: from basic drug delivery mechanism to application, Expert Opin. Drug Deliv. 9 (2012) 457–472.
[12] E. Kilic, U. Kilic, D.M. Hermann, TAT fusion proteins against ischemic stroke: current status and future perspectives, Front. Biosci. 11 (2006) 1716–1721.
[13] R. Sampat, R. Fu, L.E. Larovere, R.J. Torres, I. Ceballos-Picot, M. Fischbach, R. de Kremer, D.J. Schretlen, J.G. Puig, H.A. Jinnah, Mechanisms for phenotypic variation in Lesch–Nyhan disease and its variants, Hum. Genet. 129 (2011) 71–78.
[14] H. Nagahara, A.M. Vocero-Akbani, E.L. Snyder, A. Ho, D.G. Latham, N.A. Lissy, M. Becker-Hapak, S.A. Ezhevsky, S.F. Dowdy, Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27Kip1 induces cell migration, Nat. Med. 4 (1998) 1449–1452.
[15] Y. Xu, J. Eads, J.C. Sacchettini, C. Grubmeyer, Kinetic mechanism of human hypoxanthine–guanine phosphoribosyltransferase: rapid phosphoribosyl transfer chemistry, Biochemistry 36 (1997) 3700–3712.
[16] M.S. Jeong, D.W. Kim, M.J. Lee, Y.P. Lee, S.Y. Kim, S.H. Lee, S.H. Jang, K.S. Lee, J. Park, T.C. Kang, S.W. Cho, O.S. Kwon, W.S. Eum, S.Y. Choi, HIV-1 TAT-mediated protein transduction of human brain creatine kinase into PC12 cells, BMB Rep. 41 (2008) 537–541.
[17] A. Chauhan, A. Tikoo, A.K. Kapur, M. Singh, The taming of the cell penetrating domain of the HIV TAT: myths and realities, J. Controlled Release 117 (2007) 148–162.
[18] J.P. Rayapureddi, W.J. Tomamichel, S.T. Walton, R.M. Payne, TAT fusion protein transduction into isolated mitochondria is accelerated by sodium channel inhibitors, Biochemistry 49 (2010) 9470–9479.
[19] S.W. Jones, R. Christison, K. Bundell, C.J. Voyce, S.M. Brockbank, P. Newham, M.A. Lindsay, Characterisation of cell-penetrating peptide-mediated peptide delivery, Br. J. Pharmacol. 145 (2005) 1093–1102.