Biometals
DOI 10.1007/s10534-015-9873-5

Deferasirox-TAT(47–57) peptide conjugate as a water soluble, bifunctional iron chelator with potential use in neuromedicine

Dibakar Goswami · Hector A. Vitorino · Roxana Y. P. Alta · Daniel M. Silvestre · Cassiana S. Nomura · M. Teresa Machini · Breno P. Espósito

Received: 15 June 2015 / Accepted: 28 June 2015

Abstract Deferasirox (DFX), an orally active and clinically approved iron chelator, is being used extensively for the treatment of iron overload. However, its water insolubility makes it cumbersome for practical use. In addition to this, the low efficacy of DFX to remove brain iron prompted us to synthesize and evaluate a DFX-TAT(47–57) peptide conjugate for its iron chelation properties and permeability across RBE4 cell line, an in vitro model of the blood–brain barrier. The water-soluble conjugate was able to remove labile iron from buffered solution as well as from iron overloaded sera, and the permeability of DFX-TAT(47–57) conjugate into RBE4 cells was not affected compared to parent deferasirox. The iron bound conjugate was also able to translocate through the cell membrane.

Keywords Deferasirox · Iron · Tat · Peptide · Blood–brain barrier · Overload · Chelation

D. Goswami · H. A. Vitorino · R. Y. P. Alta · D. M. Silvestre · C. S. Nomura · B. P. Espósito (✉)
Departamento de Química Fundamental, Universidade de São Paulo, Av. Lineu Prestes 748, São Paulo 05508-000, Brazil
e-mail: breno@iq.usp.br

D. Goswami
Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

M. T. Machini
Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Av. Lineu Prestes 748, São Paulo 05508-000, Brazil

Introduction

Over the last few years, the onset and complications of neurodegenerative diseases e.g. Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis have been strongly associated with oxidative stress-induced cell death (Andersen 2004). The brain is particularly vulnerable to such ROS-dependent damages because of its high metabolic rate and low capacity of cellular regeneration (Lee et al. 2008).This has been accepted as the link between neurodegeneration and brain iron overload. Iron is essential for several biochemical processes including synthesis of neurotransmitters, and so is the most abundant and widely distributed transition metal ion in a normal brain (Batista-Nascimento et al. 2012). However, disturbance of iron homeostasis and/or aging of brain can cause brain iron overload. Since "free" iron participates in Fenton reactions to produce ROS in presence of oxygen, it poses a threat to the normal functions of the brain.

Treatment of brain iron overload using metal chelators has widely been recognised as a potent

Published online: 12 July 2015

therapy to restore normal brain functions. Details of the current chelators undergoing trial/research have been reviewed recently (Mounsey and Teismann 2012). A combination of BBB-permeable chelator (deferiprone) and antioxidant (*N*-acetyl cysteine) has also been successfully employed (Sripetchwandee et al. 2014). Apart from these, a well-known strategy for brain delivery of non-BBB permeable chelators is to conjugate the chelator to a BBB-permeable moiety acting as a carrier (Gabathuler 2010). For this purpose, glycoproteins and peptides with BBB-permeability have drawn considerable attention as delivery vectors for neurotherapeutics into the brain (Schwarze et al. 1999; Mazel et al. 2001; Rousselle et al. 2000). The advantages of the cell penetrating peptides (CPPs) to overcome multi-drug resistance present in BBB have also been well explored (Kurrikoff et al. 2011). Our group has recently introduced conjugates between CPPs and the iron chelator desferrioxamine (DFO), e.g. DFO-TAT(47–57) and DFO-penetratin, as a means to load a BBB model with the otherwise cell-impermeant DFO (Goswami et al. 2014).

Deferasirox (DFX), an orally active tridentate iron chelator, has been clinically studied (Bruin et al. 2008) and recently approved for therapy. It has satisfactory performance when applied to iron-overloaded patients undergoing long term blood transfusions (Choudhury and Naithani 2007; Yang et al. 2007). However, despite its good cell permeability, it is practically insoluble in water, being classified as a Class II drug (poorly soluble, highly permeable) according to the Biopharmaceutics Classification System (BCS) (Food and Drug Administration 2015). Also, due to its insolubility in water, the method advised to patients for taking the medicine orally is quite cumbersome (Novartis 2015). In addition, reports suggest that although DFX is able to reduce both hepatic iron storage and serum ferritin concentration, it is not so efficient to treat brain iron overload (Finkenstedt et al. 2010; Tai et al. 2014). A recent study (Kamalinia et al. 2013) has demonstrated the use of lactoferrin-DFX conjugate for efficient BBB permeation. In view of this, we envisaged that conjugation of DFX with TAT(47–57), a BBB-permeable CPP (Langel 2002), would increase the water solubility of the conjugates as well as its efficiency in case of brain iron overload. Herein, we report the synthesis of DFX conjugate with TAT(47–57) peptide, its iron binding properties, and ability to reduce iron overload from a BBB model in vitro.

Materials and methods

Materials

All fluorenylmethyloxycarbony (Fmoc)-amino acids and *N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl) uroniumhexafluorophosphate, *O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluroniumhexafluorophosphate (HBTU) were purchased from ChemPep Inc. (USA). Fmoc-Arg(Pmc) was purchased from Novabiochem (USA). DFX (Exjade®) was donated by Novartis, and was further purified (see below). *N,N′*-Diisopropylcarbodiimide (DIC) was purchased from Advanced ChemTech (USA). 1-Hydroxybenzotriazole (HOBt) was purchased from Bachem (USA). *N,N′*-Diisopropylethylamine (DIPEA) and ninhydrin were obtained from Applied Biosystems (USA). Piperidine, trifluoroacetic acid (TFA), thioanisole, ethanedithiol (EDT), HEPES, nitrilotriacetic acid (NTA), ferrous ammonium sulfate (FAS), ascorbic acid, calcein, calcein-AM (the cell-permeant form of calcein) and G418 were obtained from Sigma-Aldrich. Phenol was purchased from LabSynth (Brazil). Dihydrorhodamine hydrochloride (DHR) was obtained from Biotium. All the reagents were of analytical grade and used as received without further purification. The solvents dichloromethane (DCM) and methanol (MeOH) (analytical grade) were purchased from Merck (Germany), whereas DMSO (analytical grade) was obtained from Sigma-Aldrich. DMF (analytical grade) and acetonitrile (ACN) (chromatographic grade) were purchased from Vetec Fine Chemicals Ltd. (Brazil). Alpha Minimum Eagle Medium 1:1 Ham's F10 Medium (αMEM-F10) was purchased from Vitrocell (Brazil).HBS (HEPES Buffered Saline; NaCl 150 mM, HEPES 20 mM; pH 7.4) treated with Chelex-100® (Sigma, 1 g/100 mL) was used throughout the experiments.

Purification of DFX from Exjade®

A suspension of finely powdered Exjade® (500 mg) was suspended in 200 mL of aqueous MeOH (50 %) and stirred for 30 min. After that, 50 mL of 5 M aqueous NaOH was added and further stirred for 3 h. The suspension was then filtered and the filtrate was acidified (pH $\sim$ 2) with dropwise addition of conc. HCl at 0 °C. A microcrystalline white ppt of pure DFX appeared, which was filtered and dried under vacuum

(380 mg). ^{1}H NMR (Bruin et al. 2008) (500 MHz, DMSO-d_6) δ 6.86–6.88 (m, 1H), 6.99–7.04 (m, 3H), 7.36–7.40 (m, 2H), 7.54–7.57 (m, 3H), 7.97–8.07 (m, 3H), 10.04 (s, 1H), 10.79 (s, 1H), 13.19 (broad s, 1H).

Synthesis, purification and characterization of DFX-TAT(47–57) conjugate (conjugate **1**)

TAT(47–57) (YGRKKRRQRRR) peptide was synthesized manually on Fmoc-Arg(Pmc)-Wang resin (0.1 g, 0.61 mmol/g) using the Fmoc/^{t}Bu strategy described previously (Goswami et al. 2014). After assembly, the terminal Fmoc group was deprotected with 20 % piperidine in DMF. Conjugation of the –COOH group of DFX with the terminal –NH_2 of the resin-bound peptide was achieved using 2.5 M excess (relative to the resin capacity) of a solution of DFX, DIC and HOBt dissolved in a minimal amount of DCM-DMF 1:1. A few drops of DIPEA were also added. The reaction was complete at RT after 60 min (as evident from ninhydrin test). Side-chain protected conjugate was cleaved from the resin and fully deprotected to get DFX-TAT(45-57) conjugate (conjugate **1**) using 0.84 mL TFA with 0.06 g phenol, 0.04 mL distilled water, 0.04 mL thioanisole, and 0.02 mL ethanedithiol as scavengers at 37 °C for 3.5 h. The crude conjugate was precipitated by dry diethyl ether, separated from the reaction medium by centrifugation and removal of the supernatant, washed with ether two twice, dissolved in 0.1 % aq. TFA, and freeze-dried (yield 0.042 g). The major product was characterized (by LC–MS) as conjugate **1**.

Purification of the crude conjugate was accomplished using Waters Model 600E preparative RP-HPLC consisting of a Waters Delta 600 quaternary pump, Waters 2487 dual absorbance UV detector, Rheodyne 3725i-119 manual sample injector, Waters 600 gradient controller, Kipp&Zonen 124 SE recorder and a Vydac C18 preparative column. The eluate profile was monitored at a wavelength of 210 nm. The flow rate was maintained at 9.0 mL/min using 0.1 % TFA/water as solvent A and 60 % CH_3CN/0.09 % TFA as solvent B. For conjugate **1**, the following linear gradient was applied: 0 min 25 % B, 15 min 40 % B, 55 min 60 % B.

Conjugate **1** was characterized by LC/ESI–MS on a Shimadzu liquid chromatographer (Kyoto, Japan) composed of two LC-20AD pumps, a DGU-20A_3 degasser, a CTO-20A column oven, a C18 Shim-pack GVP-ODS pre-column, a C18 Shim-pack VP-ODS column and SDP-20AV detector coupled to an AmaZon X electrospray mass spectrometer (Bruker Daltonics, Fahrenheitstrasse, Germany). The software HyStar 3.2 was used in the analysis of mass spectra obtained. The solvents used for chromatography were: solvent A 0.1 % TFA/H_2O and B 60 % ACN/0.09 % TFA/H_2O, with a linear gradient from 5 to 95 % B in 30 min. Amino acid analysis of the purified and hydrolysed (6 M HCl, 110 °C, 24 h) conjugate was conducted in a Dionex®BioLC Chromatography system (Dionex, USA) employing a ion exchange method on a AminoPac PA10 (2.0 × 25 cm) column and an electrochemical detector ED50. The logP_{OW} (octanol/water) of both DFX and DFX-TAT(47–57) conjugate (**1**) was calculated using ACD Chem Sketch (freeware).

Conjugate **1** was found to be highly water soluble (>10 mg/mL) under normal conditions without using any external agitation. This was highly encouraging since it fulfilled one of our primary goals to prepare a water-soluble conjugate. Also, all the iron chelation experiments could be done exclusively in aqueous and/or buffer media without contaminations from organic solvents required to dissolve a lipid-soluble drug like DFX. For all the experiments, a stock solution (1 mM) of conjugate **1** in HBS was always used. For DFX, a stock solution (10 mM) of DFX was prepared in DMSO, and was diluted in HBS whenever required.

Competition studies with calcein

The affinity of the new chelator for iron can be assessed fluorimetrically via a competition test with the fluorescent chelator calcein (Espósito et al. 2002). Aliquots of 180 µL of calcein (2 µM in HBS, pH 7.4) were placed in flat, transparent 96-well microplates and the fluorescence was recorded at 37 °C in a BMG FluoStar Optima instrument ($\lambda_{exc}/\lambda_{em}$ = 485/520 nm) for 10 min. After that, 10 µL FAS (2 µM in water, final concentration) was added to the wells, and was allowed to react at 37 °C until the fluorescence quenching was stabilized (~10 min). The calcein-Fe (CAFe) solutions which formed in the microplates were treated with 10 µL aliquots of increasing concentrations of the compounds (DFX and conjugate **1**) and fluorescence was further recorded until stabilization (~60 min).

Anti-oxidant activity

The ability of the chelators to halt iron-catalysed formation of ROS under ascorbate at physiological concentrations (Espósito et al. 2003) was assessed. The standard Fe(NTA) complex (1:3 Fe:NTA molar ratio) was prepared by adding FAS to a stock solution of aqueous NTA, and allowed to react for 1 h at 37 °C. In one experiment, 180 μL of a mixture of 40 μM ascorbate and 50 μM DHR in HBS was placed in a flat, transparent 96-well microplate, and treated with 20 μL of solutions of increasing concentrations (0–10 μM, final concentration) of Fe(NTA).

In an alternative experiment, 180 μL of a mixture of 40 μM ascorbate, 50 μM DHR and 20 μM chelators (DFX and conjugate **1**) was placed in a 96-well microplate and treated with 20 μL of either Fe(NTA) at different concentrations or sera from iron overloaded patients. Assays were performed in duplicate. Sera samples were furnished by Dr Nelson Hamerschlak, Albert Einstein Hospital, São Paulo.

Fluorescence was measured on a BMG FluoStar Optima instrument for 60 min at 37 °C (λ_{exc}/λ_{em} = 485/520 nm). The slopes of the oxidation curve (indicating oxidation rate, presented in fluorescence units per minute) were calculated in the time range 15 to 40 min and were plotted against chelator concentration.

Cell permeation studies

Cell cultures

RBE4 cell line was kindly provided by Prof. Michael Aschner (Albert Einstein College of Medicine, USA). RBE4 cells were grown in αMEM/F-10 medium containing 10 % FBS, 1 % antibiotics and 300 μg/mL G418. Cells were incubated at 37 °C in a humidified incubator with a 5 % CO_2 atmosphere and were subcultured three times in a week to prevent overcrowding and cell death (Goswami et al. 2014).

Removal of cytosolic iron by the chelators

RBE4 cells were trypsinized and transferred (3×10^5 cells per well) to the wells of a 6-well microplate and kept for 48 h until complete adherence and confluence. The medium was then removed and the cells were washed with warm HBS buffer and incubated with the cell-permeant iron fluorescent probe acetomethoxycalcein (Cal-AM, 1 μM) for 10 min at 37 °C. Then, the cells were washed and treated with 1 mL of a mixture of 10 μM FAS and 100 μM ascorbic acid in water (iron-overloaded cells) or 1 mL buffer (iron-normal cells) and incubated for 15 min at 37 °C. After washing, iron-overloaded cells were treated with 20 μM chelators (DFX or conjugate**1**) while iron-normal cells were treated with 1 mL buffer for 20 min at 37 °C. The images were obtained in an Axiovert 200 (Carl Zeiss, Germany) microscope under 100× magnification and recorded on digital camera Canon Power Shot G10, and analyzed using ImageJ 1.37c software (Goswami et al. 2014).

*Cell loading with Fe:conjugate **1***

RBE4 cells were trypsinized and plated as described above. Cells were then incubated with 1 mL HBS (control group) or 1 mL of pre-formed $[Fe(DFX)_2]$ or $[Fe(\text{conjugate } \mathbf{1})_2]$([Fe] = 50 μM) in HBS for 30 min at 37 °C. Cells were washed (1 × 1 mL HBS; 2 × 1 mL DFO 100 μM in HBS) to remove any unbound iron left, and were dried under vacuum.

Determination of Fe concentration in each well was performed with a ZEEnit® 60, Analytikjena AG (Jena, Germany) graphite furnace atomic absorption spectrometer equipped with a two-field mode Zeeman effect background corrector, integrated platform graphite tube atomizer, and a hollow cathode lamp (wavelength = 248.3 nm, slit width = 0.8 nm and lamp current = 4.0 mA). Calibration curves were obtained by using aqueous standard solutions. Cells samples were digested by adding 300 μLof HNO_3 (65 % v/v) and 100 μL H_2O_2 (30 % v/v) to 0.4 mg of cell samples which were heated to 100 °C for 5 min. The volume was adjusted to 2 mL using deionized water. The heating program used for iron measurement was [Step: Temperature, °C/Ramp (°C/s)/Hold (s)]: (Drying 1: 100, 10, 15); (Drying 2: 130, 10, 20); (Pyrolysis: 1000, 100, 20); (Atomization: 2200, 2600, 5) and (Cleaning: 2500, 1200, 5).

Results and discussion

Synthesis and characterization of conjugate **1**

Conjugate **1** was obtained at a 15 % yield with >95 % purity. After purification, the final target was evaluated

by analytical RP-HPLC analysis and LC-ESI/MS analysis (Fig. 1) ($[MH]^+$ calcd: 1915.2 g/mol; found: 1915.6 g/mol). Full acid hydrolysis/amino acid analysis of the hydrolyzates showed 49 % of peptide content. This information is important in order to prepare standards with accurate peptide concentration. The synthesis was straightforward and was achieved without any practical surprises.

The calculated $\log P_{OW}$ (octanol/water) values for deferasirox and conjugate **1** were found to be 6.43 ± -0.93 and -4.47 ± 1.50 respectively. The reported $\log P_{OW}$ for DFX (6.3 at pH 7.40) is similar to our calculated value (Druglead 2015). These values clearly indicate that, as expected, conjugate **1** is highly soluble in water. Experimentally, the solubility of conjugate **1** in water (pH 7.4, >10 mg/mL) was found to be much higher than that of deferasirox (<1 mg/mL). This was in accordance with the calculated $\log P_{OW}$ values. Therefore, this conjugate may be seen as a better candidate for oral administration than DFX.

Competitive iron binding studies

Calcein, a fluorophore, is routinely used as a chemosensor for iron. Rapid and stoichiometric chelation of Fe(III) by calcein quenches its fluorescence, which is recovered in presence of a strong iron chelator (Espósito et al. 2002). A competition study with calcein is important for determining the iron binding ability of the conjugate **1** and to compare that with DFX. FAS, which is promptly oxidised to Fe(III) upon coordination with calcein, was used as a source of iron. Addition of FAS to calcein quenched the fluorescence, which was recovered by the exchange of the ferric ion from calcein to the added chelators at increasing concentrations. The iron-binding ability of the conjugate **1** at physiological pH was compared to that of DFX (Fig. 2). Plot of fluorescence recovery versus chelator concentration indicated conjugate **1** was able to compete with calcein, indicating the high thermodynamic stability of their iron derivatives, and potential usefulness in chelation therapy. Also, these data indicate that conjugation of DFX to the peptide did not affect the iron binding moiety of the chelator. The same conjugation strategy preserved the iron sequestering ability DFO-CPP conjugates (Goswami et al. 2014).

Anti-oxidant activity

Under physiological conditions, generation of ROS by labile iron in presence of ascorbate can lead to an unregulated oxidative stress. Hence, to behave as an effective anti-oxidant, the candidate chelator should be able to halt this reaction (Espósito et al. 2003). This was monitored by the oxidation rate of DHR caused by ROS produced by labile iron and ascorbate. The rate of oxidation is proportional to the concentration of labile iron. However, in presence of an iron chelator, the rate of DHR oxidation diminishes. As evident from Fig. 3a, the oxidation rate of DHR decreases to zero in presence of DFX or conjugate **1**. Conjugate **1** (and DFX, to a similar extent) was also able to decrease the oxidation rate of DHR resulting from sera of iron-overloaded patients (Fig. 3b).

Thus, the anti-oxidant ability and efficiency of conjugate **1** was similar to that of DFX. Again, we could demonstrate that conjugate **1** and DFX have

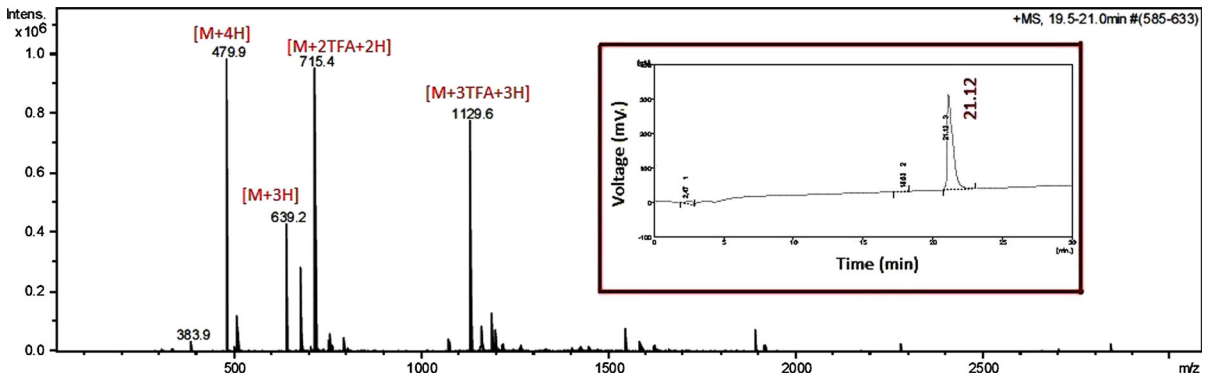

Fig. 1 Full mass spectrum and HPLC chromatogram (*inset*) of purified DFX-TAT(47–57) (Conjugate **1**)

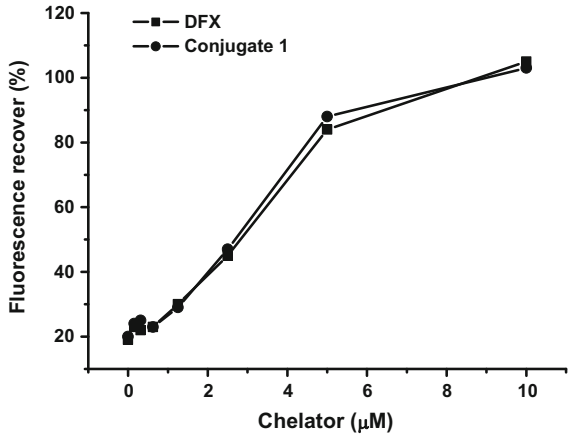

Fig. 2 Fluorescence recovery (%) of 2 μM calcein as a function of chelator (DFX or conjugate **1**) concentration. Results are the average of duplicate experiments and representative of at least two separated experiments

similarly high thermodynamic affinity for iron, and form stable, redox-inactive complexes suited for chelation therapy.

Removal of cytosolic iron by the chelators

Although DFX has been very successful in removing intracellular iron, its poor efficiency to bind brain iron has led several researchers to pursue alternative ways of delivery. The conjugation of a BBB permeable moiety can represent a solution to this. Along the years, CPPs have emerged as promising tools for cargo delivery within the cells (Brooks et al. 2005). Although the exact mechanism of the localization of CPPs into the cells is still debated, a recent study (Mishra et al. 2011) proposed internalization of TAT and several TAT conjugates e.g. TAT coupled to Cy5-tagged PLA nanoparticles, TAMRA-labelled TAT etc. via normal endocytosis after binding to heparan sulfate proteoglycans (HSPG).

To evaluate the BBB permeability of conjugate **A** compared to DFX, RBE4 cell line was chosen. RBE4 has been accepted as an in vitro model of the blood–brain barrier (BBB) (Wilhelm et al. 2011; Reichel et al. 2002). When the cells were loaded with the probe calcein-AM, they showed fluorescence. After treatment with iron, cell fluorescence was quenched, and was regenerated after incubating with both DFX and conjugate **1** (Fig. 4). This indicated that both the compounds crossed the biological membrane.

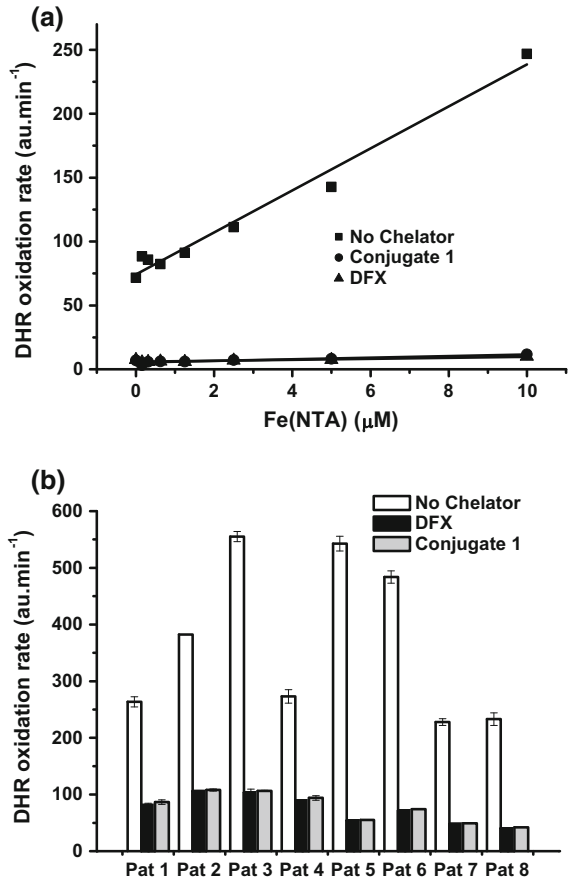

Fig. 3 **a** Effect of the chelators (20 μM) on the rate of DHR oxidation catalyzed by iron/ascorbate in HBS/Chelex buffer (pH 7.4). **b** Sera from iron-overloaded patients (Pat#) treated with chelators (20 μM)

When compared to DFX-treated cells, x_c (the cell population with maximum fluorescence) for cells treated with **1** was marginally higher, and closer to the non-iron-overloaded cells, indicating that the conjugation to DFX does not hamper cell permeability of **1**.

Cell loading with Fe:conjugate **1**

Finally, permeation of the complex between iron and conjugate **1** to RBE4 cells was investigated as it could indicate the ability of the conjugate to ferry iron out of the cell. RBE4 cells were incubated with pre-formed iron complex ([Fe] = 50 μM), and the metal level in cells were quantified by GF-AAS (Table 1). Not only conjugate **1** is permeable to RBE4 cells, but its iron-loaded complex also is able to cross this biological

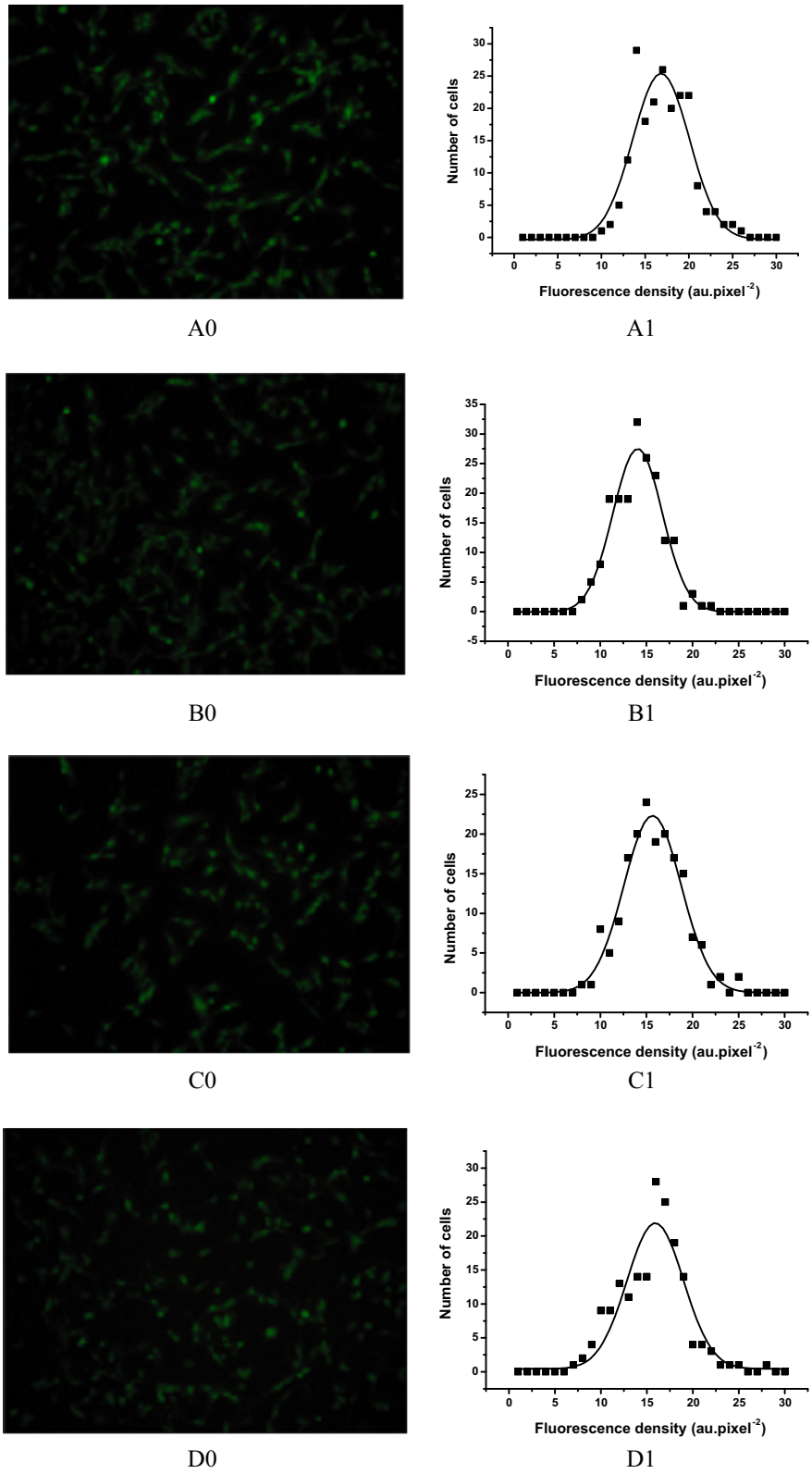

Fig. 4 Effect of DFX and conjugate **1** on the fluorescence of calcein in RBE4 cells. Cells were treated with buffer (A0), FAS/ascorbic acid (excess iron, B0), and excess iron plus DFX (C0) or conjugate **1** (D0) (see "Materials and methods" section). The histograms show cell population against fluorescence density for cells treated with buffer (normal iron, A1; $x_c = 16.82 \pm 0.24$ f.u./pixel^2), after treatment with FAS/ascorbic acid (excess iron, B1, $x_c = 14.06 \pm 0.14$ f.u./pixel^2), and excess iron plus DFX (C1, $x_c = 15.66 \pm 0.13$ f.u./pixel^2) or conjugate **1** (D1, $x_c = 15.91 \pm 0.25$ f.u./pixel^2). (f.u. = fluorescence units)

Table 1 Loading of iron by $[Fe(DFX)_2]$ or $[Fe(TAT\text{-}DFX)_2]$ complexes in RBE4 cells, as measured by GF-AAS

Treatment	Iron concentration (mg/g cells)
Control	0.22 ± 0.04
$[Fe(DFX)_2]$	0.90 ± 0.01
$[Fe(\text{conjugate } \mathbf{1})_2]$	0.65 ± 0.02

model of BBB. Conjugates between other siderophore (DFO) and CPP also display a similar ability to traverse cell membranes (Goswami et al. 2014).

Conclusions

In an endeavour to find water soluble alternatives to DFX and better efficacy to chelate brain iron, we synthesized the DFX-TAT(47–57) conjugate (**1**). Conjugate **1** showed excellent water solubility, retained the iron chelation and antioxidant abilities of DFX, and also kept good permeability in the RBE4 model both in a free and in a iron-laden form.

Acknowledgments DG received a postdoctoral fellowship from São Paulo Research Foundation (FAPESP 11/18958-0). FAPESP and CNPq funded these investigations. The authors thank Dr. Cleber Wanderlei Liria for discussions and technical assistance.

References

Andersen JK (2004) Oxidative stress in neurodegeneration: cause or consequence? Nat Rev Neurosci 5(Suppl):S18–S25

Batista-Nascimento L, Pimentel C, Menezes RA, Rodrigues-Pousada C (2012) Iron and neurodegeneration: from cellular homeostasis to disease. Oxid Med Cell Longev. doi:10.1155/2012/128647

Brooks H, Lebleu B, Vives E (2005) Tat peptide-mediated cellular delivery: back to basics. Adv Drug Deliv Rev 57(4):559–577

Bruin GJM, Faller T, Wiegand H, Schweitzer A, Nick H, Schneider J, Boernsen K-O, Waldmeier F (2008) Pharmacokinetics, distribution, metabolism, and excretion of Deferasirox and its iron complex in rats. Drug Metab Dispos 36(12):2523–2538

Choudhury VP, Naithani R (2007) Current status of iron overload and chelation with deferasirox. Indian J Pediatr 74(8):759–764

Druglead (2015) http://www.druglead.com/cds/deferasirox.html. Accessed 12th June 2015

Espósito BP, Breuer W, Sirankapracha P, Pootrakul P, Hershko C, Cabantchik ZI (2003) Labile plasma iron in iron overload: redox activity and susceptibility to chelation. Blood 102(7):2670–2677

Espósito BP, Epsztejn S, Breuer W, Cabantchik ZI (2002) A review of fluorescence methods for assessing labile iron in cells and biological fluids. Anal Biochem 304(1):1–18

Finkenstedt A, Wolf E, Höfner E, Gasser BI, Bösch S, Bakry R, Creus M, Kremser C, Schocke M, Theurl M, Moser P, Schranz M, Bonn G, Poewe W, Vogel W, Janecke AR, Zoller H (2010) Hepatic but not brain iron is rapidly chelated by deferasirox in aceruloplasminemia due to a novel gene mutation. J Hepatol 53(6):1101–1107

Food and Drug Administration (2015) Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system: guidance for industry. Food and Drug Administation Rockville, MD. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070246.pdf. Accessed 12th June 2015

Gabathuler R (2010) Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. Neurobiol Dis 37(1):48–57

Goswami D, Machini MT, Silvestre DM, Nomura CS, Espósito BP (2014) Cell penetrating peptide (CPP)-conjugated Desferrioxamine for enhanced neuroprotection: synthesis and in vitro evaluation. Bioconjugate Chem 25(11):2067–2080

Kamalinia G, Khodagholi F, Atyabi F, Amini M, Shaerzadeh F, Sharifzadeh M, Dinarvand R (2013) Enhanced brain delivery of Deferasirox-Lactoferrin conjugates for iron chelation therapy in neurodegenerative disorders: in vitro and in vivo studies. Mol Pharm 10(12):4418–4431

Kurrikoff K, Suhorut J, Langel Ü (2011) Chapter 37: cell-penetrating peptides in cancer targeting. In: Kratz F, Senter P, Steinhagen H (eds) Drug delivery in oncology: from basic research to cancer therapy. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 1187–1217

Langel Ü (2002) Cell-penetrating peptides: processes and applications. CRC Press, Boca Raton, p 257

Lee S-M, Yoon M-Y, Park H-R (2008) Protective effects of *Paeonia lactiflora* pall on hydrogen peroxide-induced apoptosis in PC12 cells. Biosci Biotechnol Biochem 72(5):1272–1277

Mazel M, Clair P, Rousselle C, Vidal P, Scherrmann J-M, Mathieu D, Temsamani J (2001) Doxorubicin-peptide conjugates overcome multidrug resistance. Anti-cancer Drug 12(2):107–116

Mishra A, Lai GH, Schmidt NW, Sun VZ, Rodriguez AR, Tong R, Tang L, Cheng J, Deming TJ, Kamei DT, Wong GCL (2011) Translocation of HIV TAT peptide and analogues induced by multiplexed membrane and cytoskeletal interactions. Proc Natl Acad Sci USA 108(41):16883–16888

Mounsey RB, Teismann P (2012) Chelators in the treatment of iron accumulation in Parkinson's disease. Int J Cell Biol. doi:10.1155/2012/983245

Novartis (2015) https://www.pharma.us.novartis.com/product/pi/pdf/exjade.pdf. Accessed 12th June 2015

Reichel A, Abbot NJ, Begley DJ (2002) Evaluation of the RBE4 cell line to explore carrier-mediated drug delivery to the

CNS via the L-system amino acid transporter at the blood-brain barrier. J Drug Target 10(4):277–283

Rousselle C, Clair P, Lefauconnier JM, Kaczorek M, Scherrmann JM, Temsamani J (2000) New advances in the transport of Doxorubicin through the blood-brain barrier by a peptide vector-mediated strategy. J Mol Pharmacol 57(4):679–686

Sripetchwandee J, Pipatpiboon N, Chattipakorn N, Chattipakorn S (2014) Combined therapy of iron chelator and antioxidant completely restores brain dysfunction induced by iron toxicity. PLoS ONE 9(1):e85115

Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF (1999) In vivo protein transduction: delivery of a biologically active protein into the mouse. Science 285(5433): 1569–1572

Tai M, Matsuhashi N, Ichii O, Suzuki T, Ejiri Y, Kono S, Terada T, Miyajima H, Harada M (2014) Case of presymptomatic aceruloplasminemia treated with deferasirox. Hepatol Res 44(12):1253–1258

Wilhelm I, Fazakas C, Krizbai IA (2011) In vitro models of the blood-brain barrier. Acta Neurobiol Exp 71(1):113–128, and the references cited therein

Yang LP, Keam SJ, Keating GM (2007) Deferasirox: a review of its use in the management of transfusional chronic iron overload. Drugs 67(15):2211–2230