NeuroToxicology 34 (2013) 118–127

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Manganese transport via the transferrin mechanism

Thomas E. Gunter [a,*], Brent Gerstner [a], Karlene K. Gunter [a], Jon Malecki [a], Robert Gelein [b], William M. Valentine [c], Michael Aschner [d], David I. Yule [e]

[a] Department of Biochemistry and Biophysics, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
[b] Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
[c] Department of Pathology and Microbiology, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[d] Department of Pediatrics and the Kennedy Center for Research on Human Development, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[e] Department of Physiology & Pharmacology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA

ARTICLE INFO

Article history:
Received 14 August 2012
Accepted 30 October 2012
Available online 9 November 2012

Keywords:
Manganese transport
Transferrin
Endosomal transport
Mitochondria
Manganese toxicity

ABSTRACT

Excessive manganese (Mn) uptake by brain cells, particularly in regions like the basal ganglia, can lead to toxicity. Mn^{2+} is transported into cells via a number of mechanisms, while Mn^{3+} is believed to be transported similarly to iron (Fe) via the transferrin (Tf) mechanism. Cellular Mn uptake is therefore determined by the activity of the mechanisms transporting Mn into each type of cell and by the amounts of Mn^{2+}, Mn^{3+} and their complexes to which these cells are exposed; this complicates understanding the contributions of each transporter to Mn toxicity. While uptake of Fe^{3+} via the Tf mechanism is well understood, uptake of Mn^{3+} via this mechanism has not been systematically studied. The stability of the Mn^{3+}Tf complex allowed us to form and purify this complex and label it with a fluorescent (Alexa green) tag. Using purified and labeled Mn^{3+}Tf and biophysical tools, we have developed a novel approach to study Mn^{3+}Tf transport independently of other Mn transport mechanisms. This approach was used to compare the uptake of Mn^{3+}Tf into neuronal cell lines with published descriptions of Fe^{3+} uptake via the Tf mechanism, and to obtain quantitative information on Mn uptake via the Tf mechanism. Results confirm that in these cell lines significant Mn^{3+} is transported by the Tf mechanism similarly to Fe^{3+}Tf transport; although Mn^{3+}Tf transport is markedly slower than other Mn transport mechanisms. This novel approach may prove useful for studying Mn toxicity in other systems and cell types.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

While manganese (Mn) is an essential biological element and a necessary cofactor in a number of important enzymatic reactions, excessive brain Mn accumulation particularly in the globus pallidus and striatum can lead to neurotoxicity with symptoms and signs resembling those of Parkinson's disease. The evidence indicates that in the 2+ oxidation state (Mn^{2+}), Mn enters cells via a number of transport mechanisms, including the divalent metal transporter 1 (DMT1) (Au et al., 2008), a Mn citrate transporter (Crossgrove et al., 2003; Crossgrove and Yokel, 2004), a store activated Ca^{2+} channel (Yokel and Crossgrove, 2004), the ZIP8 mechanism (He et al., 2006; Liu et al., 2008), and the ZIP14 mechanism (Fujishiro et al., 2012; Girijashanker et al., 2008). In the 3+ oxidation state (Mn^{3+}), the evidence suggests that Mn is transported via the transferrin (Tf) mechanism (Aschner and Aschner, 1990; Aschner and Gannon, 1994). Mn uptake into a specific cell type is thus determined by the activity of each type of uptake mechanism expressed in that cell type and the oxidation state of the Mn reaching the cell. Once inside the cell, most of the Mn is found in the mitochondrial and nuclear fractions (Maynard and Cotzias, 1955).

A study of Mn speciation in animal cells using X-ray absorption near edge structure (XANES) spectroscopy identified only Mn^{2+} and a trace amount of Mn^{3+} having the spectrum of the enzyme Mn superoxide dismutase (Gunter et al., 2004, 2005, 2006a). This is undoubtedly because Mn^{2+} is by far the more stable species of these two oxidation states (Latimer and Hildebrand, 1956). The concentration of free Mn^{3+} is not zero, but exists in a steady state in which its concentration is much lower than that of free Mn^{2+} and below the XANES detection limit. A number of factors influence this steady state, but generally free Mn^{3+} is more stable at low pH

Abbreviations: Tf, transferrin; Mn^{3+}Tf, Mn^{3+} bound to transferrin; DMT1, divalent metal transporter 1; XANES, X-ray absorption near edge structure spectroscopy; TfRs, transferrin receptors; EXAFS, extended X-ray absorption fine structure; EPR, electron paramagnetic resonance; Apo-Tf, apotransferrin; AA, atomic absorption; AG, Alexa green; AGMn^{3+}Tf, Alexa green manganese transferrin.

* Corresponding author. Tel.: +1 585 275 3129; fax: +1 585 275 6007.
E-mail addresses: thomas_gunter@urmc.rochester.edu (T.E. Gunter), brentgerstner@gmail.com (B. Gerstner), karlene_gunter@urmc.rochester.edu (K.K. Gunter), jon_malecki@urmc.rochester.edu (J. Malecki), bob_gelein@urmc.rochester.edu (R. Gelein), bill.valentine@Vanderbilt.edu (W.M. Valentine), michael.aschner@Vanderbilt.edu (M. Aschner), david_yule@urmc.rochester.edu (D.I. Yule).

0161-813X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuro.2012.10.018

than at neutral pH (Latimer and Hildebrand, 1956). Mn^{3+} can also enter aqueous solution when stabilized by formation of stable complexes (Gunter et al., 2006b), including some formed with common organic anions usually found in cells and tissue, such as citrate or Tf.

The Tf system is known to transport iron (Fe) primarily into mitochondria for incorporation into hemes and other Fe-containing proteins (Sheftel et al., 2007). Fe^{3+} transport via the Tf mechanism involves the following steps: binding of the Fe^{3+} to extracellular Tf, binding of the Fe^{3+}Tf complex to Tf receptors (TfRs) and movement of the Tf-bound TfR into clathrin-coated pits, invagination of these pits into endosomes bringing the Fe^{3+}Tf inside the cell, movement of the endosomes into the region of the cytosol in which the mitochondrial network is found, release of Fe^{3+} from binding to the Tf complex by acidification of the endosomal interior, reduction of the Fe^{3+} to Fe^{2+} by Steap proteins in the endosomes, transport of the Fe^{2+} out of the endosomes by DMT1, and uptake of the Fe^{2+} by mitochondria where it can be incorporated into hemes and other iron-containing proteins (Hentze et al., 2004; Ohgami et al., 2005, 2006; Richardson and Ponka, 1997; Sheftel et al., 2007).

Mn and Fe are elements with atomic numbers 25 and 26, respectively, within the transition metal series and have many similar characteristics. Mn uptake via the Tf mechanism has usually been assumed to be analogous to that of Fe; nevertheless, there are differences in their chemistry, which could cause Mn^{3+} transport via Tf to differ from transport of Fe^{3+}. First, the oxidation state in which Fe binds most strongly to Tf, Fe^{3+}, is also its most stable oxidation state at physiological pH; in contrast, while Mn also binds Tf as Mn^{3+}, it is most stable at physiological pH as Mn^{2+}. Since the concentration of free blood Mn^{3+} is very low at physiological pH, even when Mn is present in excess, mass action would predict that Mn^{3+} would bind to Tf very slowly as Mn^{3+} transferrin (Mn^{3+}Tf). This is supported by the observation that the procedure set up by Aisen et al. (1969) for preparing Mn^{3+}Tf requires a week for the accumulation of the stable Mn^{3+}Tf complex to approach completion. We have studied this slow conversion of Mn^{2+} to Mn^{3+} more closely under somewhat more physiological conditions in a XANES experiment described below. Studies of transport of Fe^{3+} via the Tf mechanism show that the Fe^{3+} is reduced to Fe^{2+} by Steap proteins inside endosomes and transported out of the endosomes via DMT1 (Hentze et al., 2004; Ohgami et al., 2005, 2006; Richardson and Ponka, 1997). Mn^{2+}, like Fe^{2+} is transported by DMT1 (Au et al., 2008) and hence should be released from the endosomes similarly to Fe^{2+}. Furthermore, the Tf transport mechanism for Fe is known to deliver Fe^{2+} to the vicinity of the mitochondrial network, where it is sequestered into mitochondria for incorporation into hemes (Sheftel et al., 2007). If it also delivers Mn^{2+} to mitochondria, it could contribute to mitochondrial Mn toxicity associated with deficits in energy production (Brouillet et al., 1993; Galvani et al., 1995; Gavin et al., 1992; Malecki, 2001; Malthankar et al., 2004; Roth et al., 2000, 2002; Zwingmann et al., 2003).

The systems transporting Mn^{2+} into cells are complex and difficult to functionally isolate experimentally. Furthermore, the expression of each type of transporter varies with cell type. However, we have been able to purify the Mn^{3+}Tf complex and also to covalently bind a fluorescent label (Alexa green) to Mn^{3+}Tf because of the stability of this complex. This has permitted us to study the transport of Mn via the Tf mechanism independently of the other cellular Mn transport mechanisms, to follow the transport of Mn^{3+}Tf into neuronal cells and into the region of the mitochondrial network using confocal microscopy, and to confirm that Mn transport via the Tf system functions analogously to the transport of ferric iron (Fe^{3+}) by this mechanism. Using the purified Mn^{3+}Tf and atomic absorption spectroscopy (AA), we have also been able to measure the accumulation of Mn via Mn^{3+}Tf independently of the other Mn transport mechanisms using atomic absorption and to compare it with the accumulation from a similar concentration of Mn^{2+}. The primary goal of the work reported here is to introduce this novel approach to the study of transport of Mn^{3+} via the Tf system to those interested in its role in Mn neurotoxicity so that it may be applied to studies of Mn^{3+}Tf transport in additional systems and cell types and to more complete studies of its uptake kinetics.

The uptake of Mn^{3+} via the Tf mechanism in the two neuronal cell types used in the current study is slower than that of Mn^{2+}, but is not negligible and therefore could contribute to Mn toxicity, along with Mn transported into cells by the other transport mechanisms. However, this does not necessarily mean that transport of Mn^{3+} via the Tf system is so small in all cell types, and hence other cell types should be studied. Furthermore, Mn^{3+} is also a strong oxidizing agent, which might be expected to oxidize many components of the endosome in addition to the Steap proteins. Thus, transport of Mn via the Tf mechanism exposes an additional set of cell components to Mn, particularly within the endosomal system, which could be damaged by this transport process, but not by other processes of Mn transport into the cell.

2. Materials and methods

2.1. Neuronal cells

Mn uptake via the Tf mechanism was studied in cultured mouse hippocampal (HT22) and striatal neurons (STHdhQ7/Q7) (Trettel et al., 2000). The latter were chosen because the striatum is an area where Mn preferentially accumulates, and one of the areas most affected by Mn toxicity (Finkelstein et al., 2007). HT22 cells were grown at 37 °C in an incubator with 5% CO_2 in Gibco F-12 mixture (HAM) containing 5% FBS, 5% HS, 20 mM glucose, and Pen Strep. The STHdhQ7/Q7 cells were grown at 33 °C with 5% CO_2 in Gibco F-12 containing 10% FBS, 5% HS and Pen Strep. Both cell types were passed with 0.05% trypsin and 0.53 mM EDTA about every 3 days. For long-term storage, each type of cell was frozen in 90% fetal calf serum/10% DMSO and stored in a liquid nitrogen refrigerator at 77° K. The cells were used between passages 3 and 7.

2.2. XANES spectroscopy

XANES spectroscopy is a technique similar to extended X-ray absorption fine structure (EXAFS) in which an energy shift is observed between the absorption edges of different oxidation states of an element. Furthermore, unlike some spectroscopies, such as electron paramagnetic resonance (EPR), where some spectra may be relaxation broadened to the point where they are no longer observable, XANES spectroscopy shows the spectra of all compounds and complexes containing the element of interest weighted by their abundance, with spectra of each oxidation state found near its appropriate absorption edge. These absorption edges are separated by several electron volts, a difference which is easily measurable. This makes XANES spectroscopy the "gold standard" for determining the oxidation state or states of an unknown biological compound or complex containing the element of interest. The details of the techniques used and methods of handling the XANES sample described here are outlined in Supplemental material and more completely in earlier publications (Gunter et al., 2004, 2005, 2006a,b).

A sample of Mn^{2+} (0.2 mM) with Tf (2 mM) in HEPES (10 mM) buffered saline (160 mM Na^+ and Cl^- pH 7.2) was allowed to incubate at room temperature (22 °C) for 24 h. Polymerized dextran (10%, w/v final concentration) was mixed into the sample, which was rapidly frozen using liquid nitrogen and maintained at

Download English Version:

https://daneshyari.com/en/article/5855143

Download Persian Version:

https://daneshyari.com/article/5855143

Daneshyari.com