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Different Responses of Divalent Metal Transporter 1 (DMT1) Isoforms to Iron Chelation

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Abstract

DMT1 is a metal transporter within the body which functions both to transport metal directly into the cytoplasm of cells and to export metal that has been moved into an endosome via the transferrin cycle. There are four isoforms of DMT1.The immediate aim of this project was to learn how iron deprivation, induced by deferoxamine (dfo) in this experiment, affects the function of two of the isoforms of DMT1. To do so, we used HEK293 cells engineered to over-express DMT1 when exposed to doxycycline (doxy).

Two cell lines containing two different isoforms of DMT1 were used; one has an iron responsive element and an extended N-terminal (referred to as 1A+) and one does not have either (referred to as 2-). Dfo is a metal chelator, so addition of dfo to cell medium simulates a low iron environment, probably causing cells with an iron responsive element (1A+ cells) to upregulate their DMT1 expression and increase manganese uptake. Since 2- cells do not contain an IRE, addition of dfo to wells containing these cells might not affect the amount of manganese uptake.

Introduction

This project is a part of a much larger exploration of metal homeostasis. Metal uptake, toxicity, transport, and function are all explored via different projects completed by fellow students. In particular, the metal transporter DMT1 is the focus of many of our projects. What DMT1 does and how it functions, conditions that defects in DMT1 can lead to, how the isoforms differ from each other in function, and why DMT1 is important are all areas of study within the lab.

This project specifically focuses in on the isoforms of DMT1. It is looking to determine whether or not the isoforms differ in regulation of metal uptake. Jiang et al showed that 1A+ IRE DMT1 mRNA increased many fold when dfo was added to cells, so we expected manganese uptake to increase as a result. 2- cells exhibited a small effect on DMT1 mRNA so we expected little or no effect on manganese uptake. Determining such differences between the two isoforms will unlock information about why multiple isoforms exist and what each is designed to do.

This project has particularly interesting implications regarding manganism, an overdose of manganese. This condition presents with neurological symptoms like those of Parkinson's disease. High expression of DMT1 has been suggested in the basal ganglia area of the brain, which may help explain the neurological symptoms of manganism (Au, Benedetto, and Aschner 569-70).

Materials and Methods

Preparation of Cells:

Both the 1A+ and the 2- cell lines were cultured throughout the semester. Each week, one of the cell lines was passaged into two six well plates. The cells were allowed to grow in these wells for two days. Doxycycline was added to all twelve wells and dfo was added to every other well. The cells were allowed to incubate for about 24 hours. Then each well was washed twice with phosphate buffered saline and the cells from each well were collected into 2mL Ependorf tubes. The cells from each well were then split into two tubes.

Manganese Uptake:

The tubes were then separated into three groups; a 15 minute incubation group, a 10 minute incubation group, and a 5 minute incubation group. The tubes were centrifuged and the supernatant was discarded. Warm incubation buffer was added to each tube, followed by Mn^{54} (radioactive manganese). Each tube was then incubated for either 15, 10, or 5 minutes in a warm water bath with a shaker. After the incubation, each tube was centrifuged and the supernatant was discarded. The cells in each tube were then washed with cold incubation buffer twice.

Assessment of Results:

A gamma counter was used to assess the amount of Mn^{54} that had been taken up into each group of cells. Then sodium dodecyl sulfate (SDS), a detergent, was added to each tube of cells in order to solubilize them. At least 24 hours later, each tube of cells was assessed for total protein concentration using a BCA assay; this was done in order to correct for varying amounts of cells being present in each well. Stata was used to convert the results into graph form and to assess each experiment for statistical significance.

Results

Figure 1: Uptake of 1A+ cells with and without the addition of dfo, after correction for total protein; referred to as specific activity (SA). This data was collected from an experiment completed on 10/24/14.

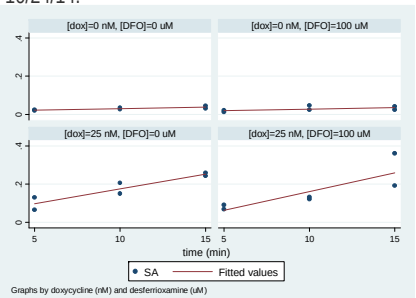
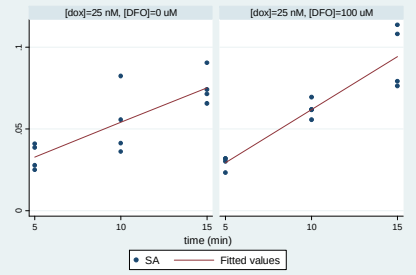
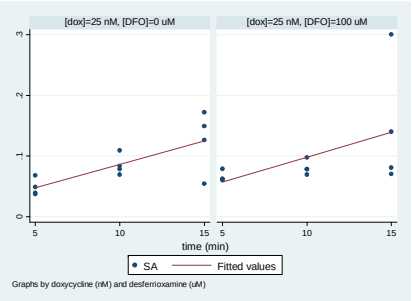


Figure 2: Uptake of 1A+ cells with and without the addition of dfo, after correction for total protein; referred to as specific activity (SA). This data was collected from an experiment completed on 11/7/14.



Results

Figure 3: Uptake of 2- cells with and without the addition of dfo, after correction for total protein; referred to as specific activity (SA). This data was collected from an experiment completed on 11/14/13.



Conclusion

Not all experiments gave the expected results, and not all results were statistically significant. However, a clear trend in the data supports the hypothesis that the 1A+ cells increase their DMT1 expression in a low manganese environment and that the 2- cells are less affected.

Literature Cited

1. Jiang, L., M. D. Garrick, L. M. Garrick, L. Zhao, and J. F. Collins. "Divalent Metal Transporter 1 (Dmt1) Mediates Copper Transport in the Duodenum of Iron-Deficient Rats and When Overexpressed in Iron-Deprived HEK-293 Cells." *Journal of Nutrition* 143.12 (2013): 1927-933. Print.

2. Au, Catherine, Alexandre Benedetto, and Michael Aschner. "Manganese Transport in Eukaryotes: The Role of DMT1." *NeuroToxicology* 29.4 (2008): 569-76.*ScienceDirect*. July 2008. Web. 18 Apr. 2013. <http://www.sciencedirect.com/science/article/pii/S0161813X08000685>.

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