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Erik D. Marchant
Brigham Young University
Chad Hancock
Brigham Young University

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NF κB as a Mediator in Iron Regulation

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Erik D Marchant and Chad Hancock, Nutrition, Dietetics & Food Science

Introduction

Doxorubicin (DOX) is a commonly used chemotherapy drug. Although it is very effective in treating many different types of cancer, it has also been shown to induce oxidative stress in multiple tissues, partially due to severe iron-dysregulation. The effects of DOX have mostly been studied in heart tissue, because DOX has been shown to increase the risk of cardiomyopathy and other heart diseases. Besides causing damage to the heart, DOX has also been shown to contribute to muscle wasting. For this purpose, we decided to investigate how DOX causes iron-dysregulation in C2C12 myotubes, which are immortalized cells from mouse skeletal muscle.

We also investigated the ability of metformin (MET) to protect cells from the harmful effects of DOX. MET is an anti-diabetic drug, commonly prescribed to type II diabetic patients. MET has been shown to protect the heart from some of the negative side effects of DOX but has not been studied in skeletal muscle. This study focuses on the effects of MET and DOX on skeletal muscle. It has yet to be shown by what mechanism MET exerts its protective effects against DOX treatment. For this purpose, iron dysregulation was measured in both normal C2C12 cells and C2C12-IκBα-SR cells, in which classical NF-κB signaling has been blocked.

We looked at NF-κB because it is involved in both inflammatory and apoptosis pathways and is a possible upstream regulator of ferritin heavy chain (FHC) and transferrin receptor (TfR), which are proteins involved in iron regulation. FHC acts to sequester potentially reactive free iron in the cell in order to prevent it from interacting with reactive oxygen species (ROS) and inducing stress in the cell. The purpose of TfR is to allow extracellular iron to enter the cell. This allows for the possibility of more iron entering the cell, increasing the potential for ROS production. ROS production is an important contributor to muscle wasting. Thus, this project aimed to see what role NF-κB plays in the DOX-induced and MET-ameliorated iron dysregulation.

Methods

• Cell Culture
  o C2C12 cells were cultured in DMEM and allowed to differentiate into myotubes. Upon differentiation, cells were treated with 5 micromolar DOX and/or 4 mM MET for 15 hours.

• Western Blotting
  o Total protein content was quantified using a standard BSA protein assay. All samples were then normalized to ensure equal amounts of protein being loaded into gels. Proteins in each sample were separated using SDS-PAGE and blotted onto nitrocellulose membrane. Corresponding fluorescent antibodies were applied to the membranes and then FHC and TfR levels were quantified using the Li-Cor CLx imaging software.
Results

In C2C12 cells, DOX caused a significant increase in FHC and a decrease in TfR (p<0.05). MET appeared to partially blunt the changes in FHC and TfR expression, but results were not significant. When NF-κB was inactivated, results were similar, except that there was no significant decrease in TfR induced by DOX. There were no changes in FHC compared to the control. MET still had no effect when used as a co-treatment with DOX.

Discussion

It appears that the iron dysregulation caused by DOX led to a higher expression of FHC and a lower expression of TfR in C2C12 cells. This is what we expected to see, because an increase in FHC is expected to serve as a protective mechanism against DOX-induced ROS. This appears to be an attempt of the cell to sequester iron and prevent it from unwanted reactions in the cell. The decrease in TfR expression also makes sense, since this decrease would theoretically lead to a decrease in the amount of iron that would be allowed into the cell. Again, less free iron in the cell would decrease the chances of iron reacting in harmful ways within the cell.

In the cells where NF-κB was inactive, we observed that the decrease in TfR was no longer seen following DOX treatment. This implies that NF-κB may be a possible upstream regulator of TfR levels following DOX treatment. It is possible that, in the absence of NF-κB, the cell was unable to correctly regulate TfR levels to protect against DOX-induced oxidative stress. Thus, it is possible, that the cell relies on the NF-κB pathway to regulate how much iron enters the cell in a stressed environment.

MET did not appear to have a significant effect in ameliorating symptoms induced by DOX. Though MET did not prove effective in this model, studies in our lab have been very focused on the potential for another compound, namely curcumin (CUR), to prevent oxidative stress in C2C12 cells. It appears that iron-dysregulation and decreased respiratory capacity of cells may be prevented when pre-treated with CUR. This has led us to begin experimenting to see whether CUR may be a suitable co-treatment to prevent DOX-induced iron-dysregulation and restore impaired mitochondrial function in C2C12 cells.

Conclusion

NF-κB appeared to be partially responsible for the decrease in TfR induced by DOX treatment, and MET had no effect in preventing iron-dysregulation in this model. Though MET was unable to protect against iron-dysregulation in this model, this project improved my understanding of research methods and how to ask important questions. This will aid me in my future studies as I pursue a PhD. Future research in our lab, which is already underway, will focus on the potential for CUR to prevent DOX-induced oxidative stress. Other research that will be performed in our lab will investigate the role of NF-κB in CUR treatment because CUR has been shown to downregulate NF-κB, bind iron, increase cellular respiration, and kill tumor cells. We are excited to continue to investigate the properties of CUR.

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