

Diazinon and Diazoxon Impair Astrocyte Production of Fibronectin & Laminin



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Abstract

Mounting evidence suggests organophosphorous insecticides (OPs) are developmental neurotoxicants. Astrocytes have been shown to be integral in fostering neurite outgrowth, and that oxidants impair their ability to do so. The widely used OP diazinon (DZ) and its active metabolite, diazoxon (DZO), are thought to indirectly impair neuronal development by increasing oxidative stress in astrocytes and disrupting normal glial-neural interactions. This project focuses on the effect of DZ and DZO on expression of the proteins fibronectin and laminin. Fibronectin and laminin are extracellular matrix proteins, and are recognized as important in neurite outgrowth. Primary cortical astrocyte cell cultures were exposed to 10 uM DZ and DZO, and protein levels of fibronectin and laminin were measured in the cell lysate using Western blots. Exposure of primary cortical astrocytes to DZ or DZO (10 uM) appears to impair expression of fibronectin and laminin. Pre-treatment with the antioxidants melatonin and PBN seems to prevent the decreases in these protein levels. These results support the hypothesis that DZ and DZO impair astrocytes' ability to promote neurite outgrowth by increasing oxidative stress on astrocytes and limiting their production of fibronectin and laminin.

Introduction

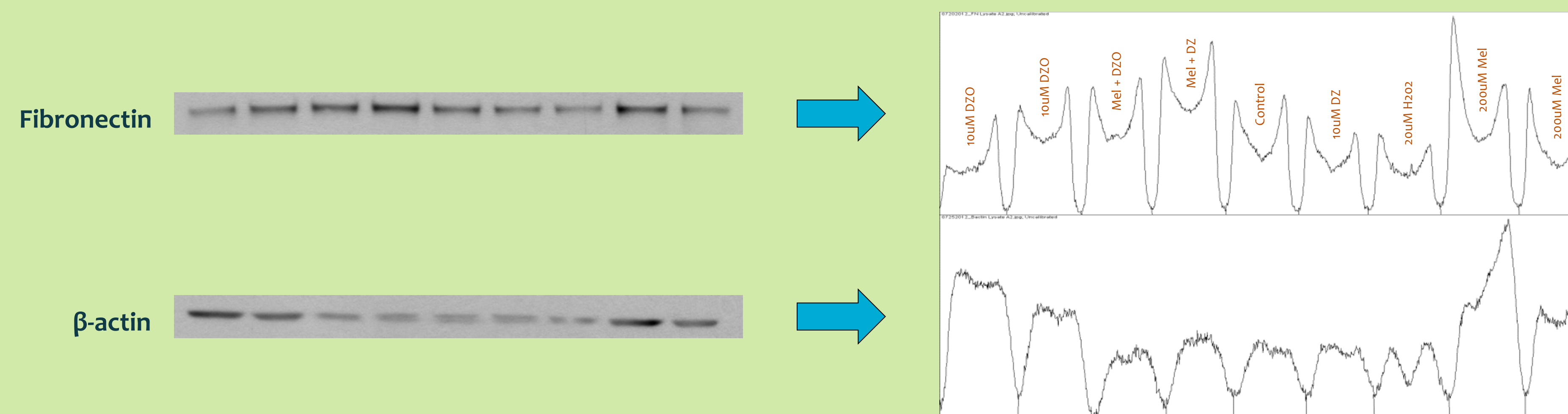
- ◆ **Astrocytes** are cells in the brain whose functions include supporting neurons and their ability to form connections in the brain
 - Their function is important in normal brain development
 - They have many other important roles in the brain as well
- ◆ **Fibronectin** and **laminin** are proteins that are important in promoting neurite outgrowth
- ◆ **Organophosphorus Pesticides (OPs)** are the most widely used pesticides today, and came into use as replacements for the phased out pesticides like DDT
 - **Diazinon** is an OP insecticide that is commonly used in commercial agriculture
 - **Diazoxon** is diazinon's major active metabolite

Methods

Primary Cortical Astrocyte Cell Culture and Western Blot Analysis



Results



Lysate Fibronectin Levels

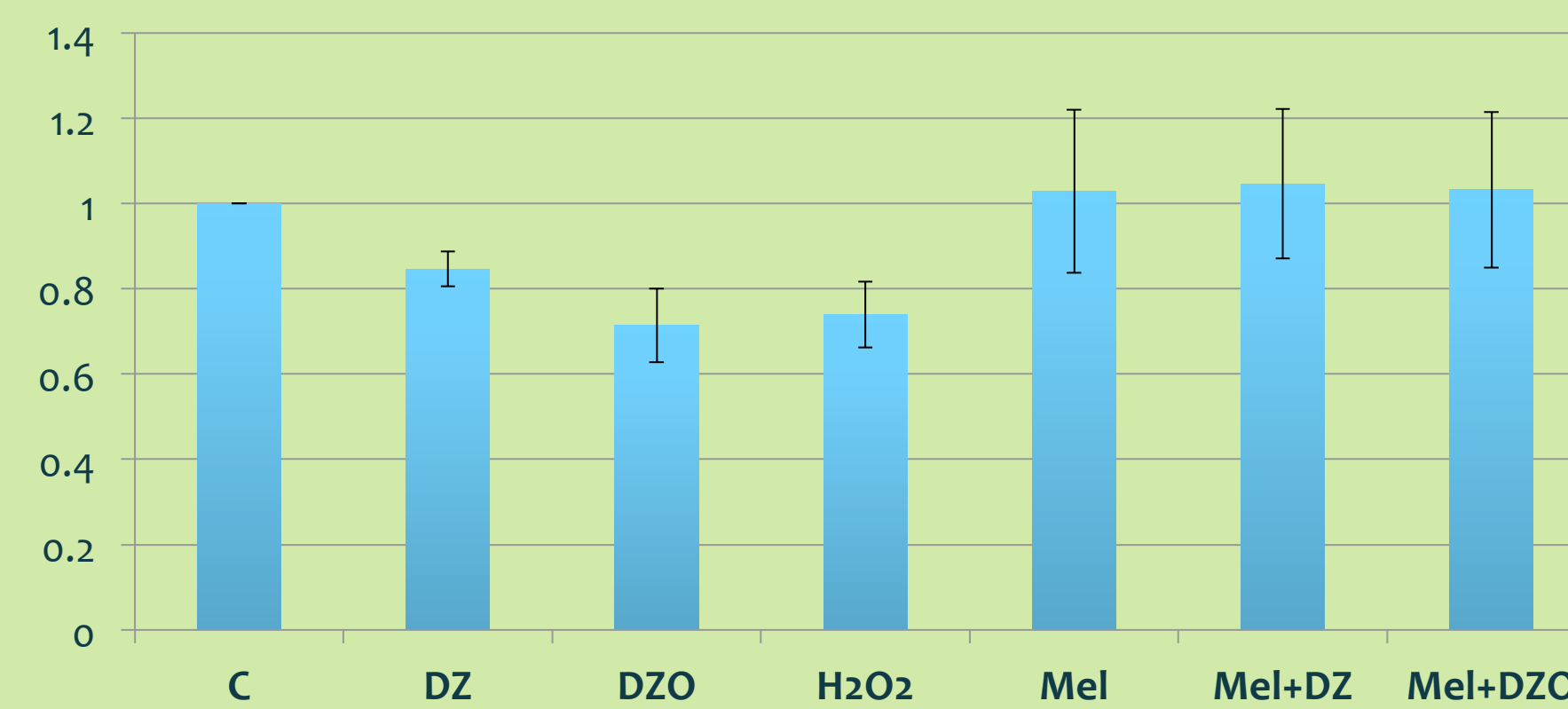
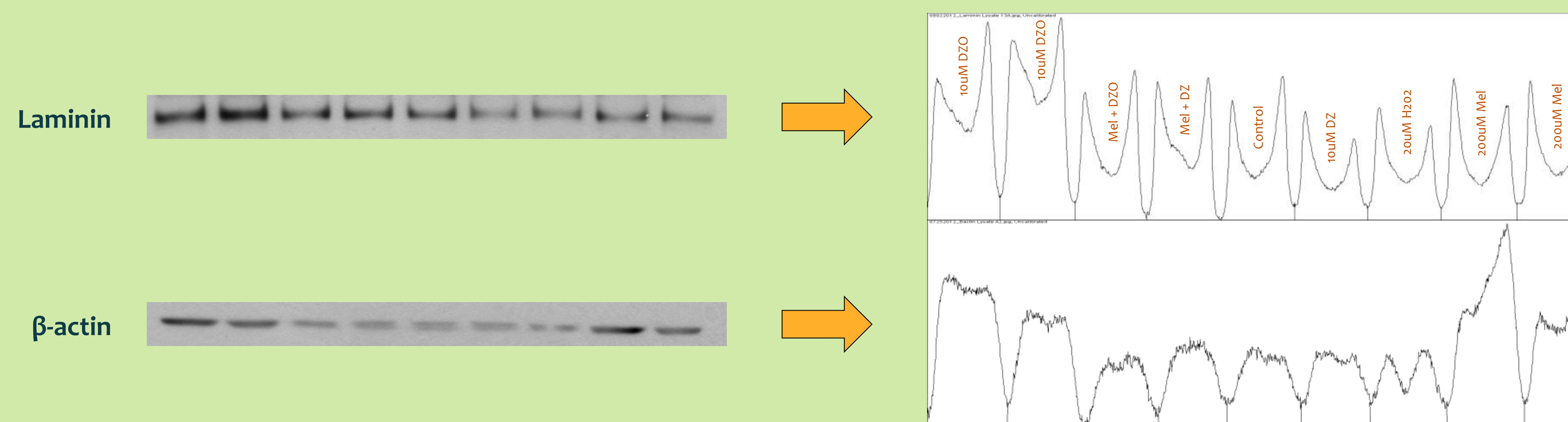


Figure 1. Levels of fibronectin protein in lysates of treated astrocytes as compared to an untreated control. These data are the result of 3 separate experiments where each treatment is completed in duplicate.



Lysate Laminin Levels

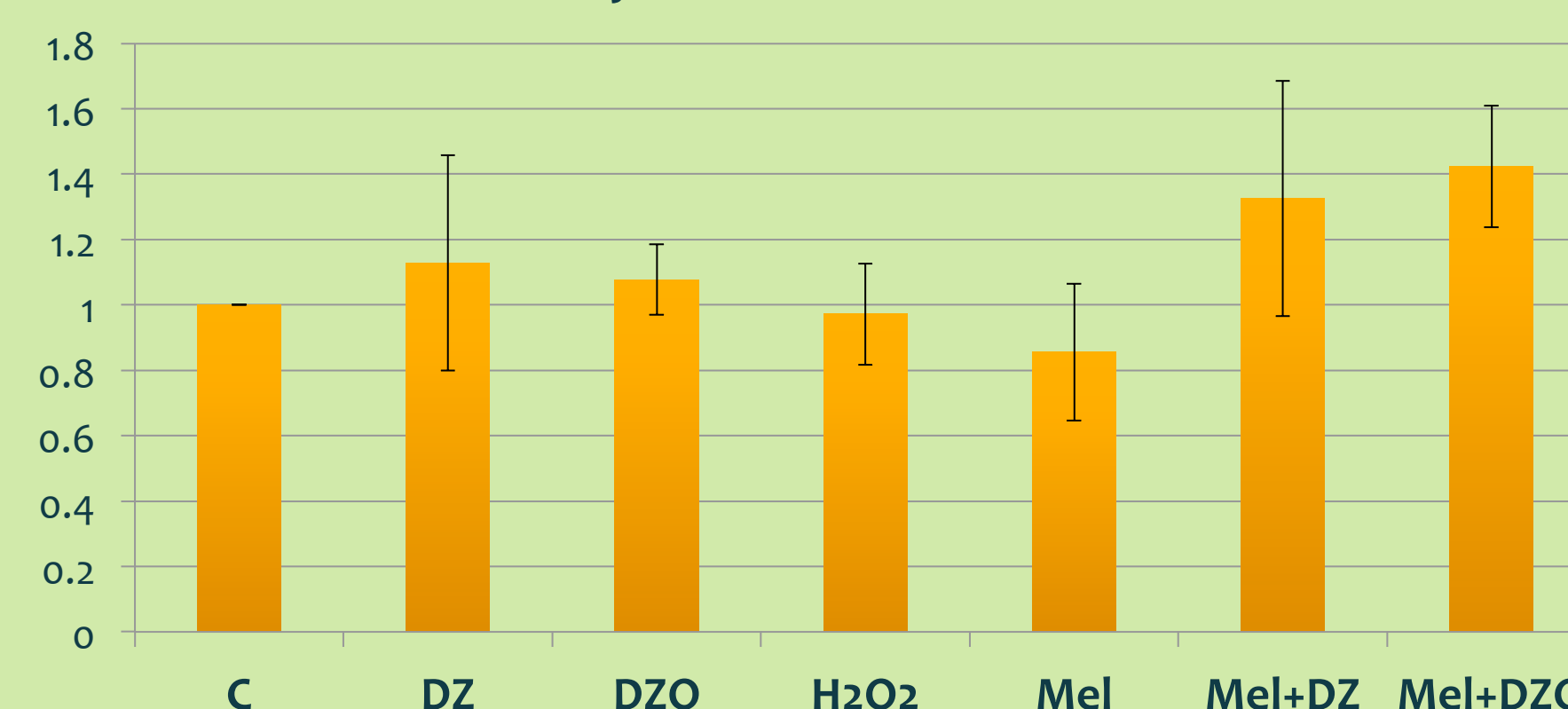


Figure 2. Levels of laminin protein in lysates of treated astrocytes as compared to an untreated control. These data are the result of 2 separate experiments where each treatment is completed in duplicate.

Conclusions

- ◆ Diazinon and diazoxon impair fibronectin production
- ◆ To a lesser extent, diazinon and diazoxon impair laminin production
 - Further experiments are needed to adequately determine diazinon and diazoxon's effect on laminin production
- ◆ Pre-treatment with the antioxidant melatonin prevents diazinon and diazoxon's effect on fibronectin and laminin production
- ◆ These results support the hypothesis that diazinon and diazoxon indirectly impair normal neuronal development by increasing oxidative stress in astrocytes.

References

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