1. Introduction

Fragile X syndrome (FXS) is an X-linked genetic disorder resulting from over-expansion of CGG trinucleotide repeats within the Fragile X Mental Retardation 1 (FMR1) gene, inhibiting the activity of its transcript, Fragile X Mental Retardation protein (FMRP) (Gaub et al. 2008). FMRP has been shown to regulate translation by binding a subset of mRNAs important in synaptic plasticity, and therefore plays an important role in cognitive development (Barber et al. 2008). Thus, those with FXS suffer cognitive disabilities, including learning and memory loss (Teresacono et al. 2005).

Minocycline is a drug traditionally used in bacterial infections (Broden et al. 1975). In recent medical literature, minocycline has been found to possess neuroprotective ability in common neurological diseases (Plante et al. 2010) such as Parkinson’s and Huntington’s diseases, as well as FXS. Furthermore, our own behavioral tests using minocycline-treated FXS mice show improved learning and memory compared to controls. However, the methodology behind these improved cognitive functions in minocycline treated FXS model mice (Fmr1 KO mice) is lacking in literature and requires further studies.

In this experiment, we investigate neurogenesis, the growth and development of nervous tissue, via cell proliferation and neuronal differentiation in the dentate gyrus (DG) of minocycline treated Fmr1 KO mice as a possible mechanism for the improved cognitive functions. We hypothesize that minocycline can up-regulate cell proliferation and neuronal differentiation in the DG, and we investigate this by staining KI67+, PCNA+, and DCX+ cells using immunohistochemistry and counting these in a manual sample-blinded manner. Cell counts for minocycline treated Fmr1 KO mice are compared to water-treated/ wild-type littermate controls.

References


Acknowledgements

We would like to thank Erica Truandsell and Tansi Potluri for their assistance with minocycline treatment, as well as Alicia Meconi and Christine Fontaine for their assistance for perseverance. This work is supported by a CIHR operating grant to B.R.C. Fragile X Research Foundation of Canada Fellowship to S.Y., and a James Cassels Research Award to Y.Y.

2. Materials and Methods

In this experiment, we investigate the effects of chronic minocycline treatment on neurogenesis via cell proliferation (KI67+, PCNA+) and neuronal differentiation (DCX) in the dentate gyrus of Fmr1 KO mice, as behavioral data in the literature has shown minocycline rescues many of the cognitive deficits shown in FXS, in both mouse models (Bilboesier et al. 2009) as well as human subjects (Leigh et al. 2013). We found that there were no statistically significant differences in the number of KI67+ (Figure 2A) or PCNA+ (Figure 2B) cells across all experimental groups (Fmr1 KO/WT) in the dorsal and ventral portions of the DG. The total number of cells was calculated as the average KI67+/PCNA+/DCX+ cells in one 35µm slice multiplied by the number of 35µm slices of the dorsal or ventral portions of the DG. KI67+ is typically used to identify the cell of origin in the dentate gyrus. In addition, we found that there were no statistically significant differences in the number of DCX+ (Figure 2C) cells across all experimental groups (Fmr1 KO/WT) in the dorsal and ventral portions of the DG. However, we found that the number of DCX+ cells showed a decreasing trend (Figure 2C) in the minocycline treated group for Fmr1 KO mice compared to the water treated group, but no difference between the WT groups. This suggests neuronal differentiation may play a role in improved cognitive functions in Fmr1 KO mice, but not cellular proliferation. In comparison to other studies, Matti et al. 2014 found that minocycline normalizes neurogenesis in a schizophrenia model, and Ekdahl et al. 2005 found that minocycline restored impaired neurogenesis in inflammation. Thus, it is possible that minocycline may also play a role in increasing neurogenesis in Fmr1 KO mice given behavioral data, as well as comparative studies.

Conclusion:

The improved cognitive functions of Fmr1 KO mice given minocycline treatment (30mg/ kg) could be in part due to increased neurogenesis, specifically increased neuronal differentiation in the dentate gyrus.

4. Discussion & Conclusion

In this experiment, we investigated the effects of chronic minocycline treatment on neurogenesis via cell proliferation (KI67+, PCNA+) and neuronal differentiation (DCX) in the dentate gyrus of Fmr1 KO mice, as behavioral data in the literature has shown minocycline rescues many of the cognitive deficits shown in FXS, in both mouse models (Bilboesier et al. 2009) as well as human subjects (Leigh et al. 2013). We found that there were no statistically significant differences in the number of KI67+ (Figure 2A) or PCNA+ (Figure 2B) cells across all experimental groups (Fmr1 KO/WT) in the dorsal and ventral portions of the DG. The total number of cells was calculated as the average KI67+/PCNA+/DCX+ cells in one 35µm slice multiplied by the number of 35µm slices of the dorsal or ventral portions of the DG. KI67+ is typically used to identify the cell of origin in the dentate gyrus. In addition, we found that there were no statistically significant differences in the number of DCX+ cells showed a decreasing trend (Figure 2C) in the minocycline treated group for Fmr1 KO mice compared to the water treated group, but no difference between the WT groups. This suggests neuronal differentiation may play a role in improved cognitive functions in Fmr1 KO mice, but not cellular proliferation. In comparison to other studies, Matti et al. 2014 found that minocycline normalizes neurogenesis in a schizophrenia model, and Ekdahl et al. 2005 found that minocycline restored impaired neurogenesis in inflammation. Thus, it is possible that minocycline may also play a role in increasing neurogenesis in Fmr1 KO mice given behavioral data, as well as comparative studies.

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Figure 2. Effects of minocycline on neurogenesis staining for KI67, PCNA, and DCX. Total number of KI67+ (Figure 2A) or PCNA+ (Figure 2B) cells counted in the dentate gyrus (DG) of water or minocycline (30mg/kg) treated Fmr1 KO/WT. Error bars represent standard error of the mean. * indicates p-value < 0.01. WT (water) and WT (Fmr1 KO) minocycline treated KO mice n=10, n=12, n=10, n=8, respectively.