

Minocycline Increases Injury But Reduces Gliosis Following Neonatal Hypoxia-Ischemia in Mice

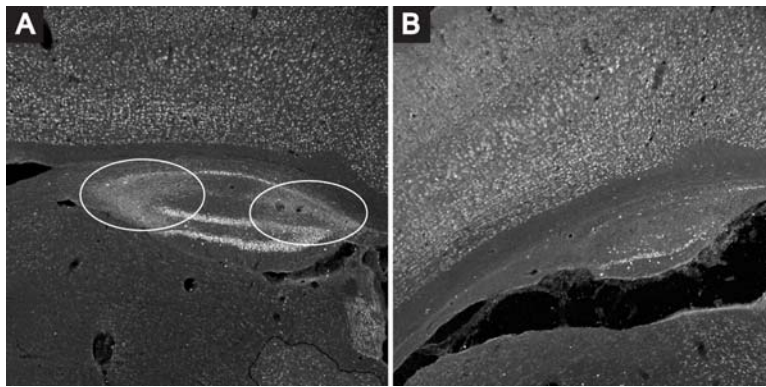
Author(s): JJ McAuliffe, B Joseph

Affiliation(s): Cincinnati Children's Hospital and University of Cincinnati, Cincinnati, OH

Introduction: Minocycline is a tetracycline derived antibiotic that has been found to have anti-inflammatory properties. It has been shown to reduce injury following neonatal hypoxia-ischemia (HI) in rats but one study found injury in mice to be increased with minocycline (1). Astroglial activation and proliferation are seen following neonatal HI. The signals for astrocyte proliferation include inflammatory cytokines (e.g. IL-6) and bone morphogenetic protein-2/4 (2). Minocycline has been found to suppress microglia activation and production of IL-6 (3). We tested the ability of minocycline to protect against inflammatory injury in a neonatal mouse model of HI and hypothesized that glial response should be attenuated.

Methods: A total of 12 (2 groups, 6 per group) C57Bl/6 X 129T2 F1 hybrids received the thymidine analogue BrdU 50 mcg/g twice on P9 and again 1 hour before surgery on P10 in order to label proliferating cells. The mice were then randomized to either no treatment or minocycline treatment and 45 minutes of HI on postnatal day 10 (P10). HI insult is accomplished by ligating the right common carotid artery and 2 hours later exposing the mice to 10% oxygen in nitrogen while maintaining normothermia (4). The minocycline treatment protocol consisted of minocycline 45 mcg/g IP 12 hours pre injury and again immediately after HI, 24, 48 and 72 hours after HI. The surviving mice were attained the age of 45 days (P45) at which time they were killed under anesthesia and perfused with 4% paraformaldehyde. The brains were removed and post-fixed in paraformaldehyde, cross-sectioned and immunostained for BrdU, NeuN (a mature neuron nuclear envelope protein) and GFAP (a marker for astrocytes). Alexa-fluor conjugated secondary antibodies were used such that BrdU is marked by a 488nm fluor (green), NeuN with a 555 nm fluor (red) and GFAP with a 633 nm fluor (pseudo-blue). Images were acquired using a three laser scanning confocal Leica microscope in the sequential scanning mode to minimize cross-talk between channels. Sections of dorsal hippocampus were evaluated at low power for presence of intense GFAP signal in areas of neuronal loss. Additionally, the area of hippocampus remaining on the injured side relative to the contra-lateral side was determined.

Results: All of the untreated mice survived to P45 while 3 of 6 minocycline treated mice died ($\chi^2 = 4.00$, $p = 0.048$). The 3 surviving minocycline treated mice all had less remaining hippocampus than the 6 untreated mice (Kolmogorov-Smirnov $Z = 1.414$, $p = 0.037$). The average area for the minocycline mice was 42% of the contralateral side vs. 65% for the untreated mice. There was no statistical difference between groups in the number of BrdU labeled cells in the dentate gyrus. There was a trend toward fewer of the BrdU labeled cells showing colocalization with either NeuN or GFAP positive cells in the minocycline treated group but the difference was not significant, possibly due to the small number of surviving minocycline treated animals. There was a striking difference in the intensity of GFAP staining in the regions in which neuronal cell loss had occurred between the minocycline treated and untreated groups. This is shown in Figure 1. Panel A is a untreated animal exposed to 45 minutes of HI on P10. The circled areas are regions of neuronal loss and the bright background is due to intense GFAP staining. Similarly intense GFAP staining is not seen in Panel B in the areas of neuronal loss.



Discussion: Minocycline treatment resulted in reduced survival and greater neuronal loss in the affected hippocampus. The failure of minocycline to protect murine brain from neonatal HI is consistent with a previous study (1). No overt effect on neurogenesis from precursors that were dividing (in S-phase) on P9-P10 was noted in the minocycline treated mice. However, the astroglial proliferation noted in areas of neuronal loss in the untreated mice (Fig 1A) was not seen in any of the sections of the minocycline treated mice (Fig 1B). The anti-inflammatory actions of minocycline appear to manifest by suppression of astrocyte proliferation following neonatal hypoxia-ischemia.

- References:**
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