Amiloride Promotes Oligodendrocyte Survival And Remyelination After Spinal Cord Injury In Rats

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Introduction: After spinal cord injury (SCI), secondary injury results in an expanding area of glial cell apoptosis. Oligodendrocyte precursor cells (OPC) that are involved in remyelination and regeneration of the lateral tract have been shown to actively proliferate within this expanding lesion, but a majority of these cells succumb to apoptosis instead of differentiating into functional oligodendrocytes. One of the factors that exacerbate secondary injury is endoplasmic reticulum (ER) stress, which arises from the accumulation of misfolded proteins in the ER, and leads to cell death. We previously reported that amiloride, an FDA-approved diuretic used to treat hypertension, enhances the ER stress response and suppresses apoptosis of glial cells in SCI rats. The aim of this study is to show that amiloride administration improves OPC survival and differentiation, enhances remyelination, and improves hind limb motor and sensory dysfunction.

Methods: A moderate contusive SCI was induced in Sprague-Dawley rats using an IH-impactor (200 Kdyne). Beginning 24 hours after SCI, 10 mg/kg/day of amiloride (A group) or PBS (C group) was intraperitoneally administered daily for 14 days. In order to trace cell division after SCI, BrdU was intraperitoneally administered to both groups every 12 hours for 3 days after SCI. Hind limb motor function was assessed daily using the BBB scale (n=14 for each group) and sensory function was assessed weekly using the von Frey test (n=5 for each group). At 7, 14, 28, and 56 days after SCI, animals were killed and the injured spinal cords were resected for analysis. Immunostaining was performed on sections taken 5 mm rostral to the injury epicenter, and cells in the dorsal funiculus that were double positive for BrdU and NG2 or APC were counted to examine the survival and differentiation of OPCs (n=5 for each group). Western blot analysis was conducted on a 5 mm section of the spinal cord (2.5 mm rostral and caudal to the epicenter) to examine myelin basic protein (MBP) expression (n=5 for each group). Using samples resected 28 days after SCI, sections taken 5 mm rostral to the injury epicenter were examined with an electron microscope to confirm myelination by new mature oligodendrocytes.

Results: The BBB scores revealed significantly higher motor scores in the A group from day 17 (p <0.05) to day 56 after injury (p <0.01), and the von Frey tests revealed a significant improvement of allodynia (an increase in the stimulus reaction load powers) in the A group on day 14 after SCI (p <0.05). Immunohistochemistry showed that the number of BrdU-positive, NG2-positive OPCs increased for 14 days after SCI and gradually decreased after that in both groups, but a significantly greater number of proliferated OPCs were observed in the A group at day 14 (p <0.05). The number of BrdU-positive, APC-positive oligodendrocytes gradually increased until day 56 after SCI in both groups, but a significantly greater number of new oligodendrocytes were observed in the A group at day 56 (p <0.05). The western blotting showed that MBP expression was significantly higher in the A group at days 28 and 56 after SCI (p <0.05), suggesting that improved remyelination may be taking place in the A group. Electron...
microscopy confirmed the presence of oligodendrocytes and neurons with newly formed myelin sheaths in the tissue surrounding the lesion epicenter.

**Discussion:** ER stress has been shown to exacerbate the secondary injury following traumatic SCI, leading to expansion of demyelination and reduced remyelination due to OPC apoptosis. We previously reported that amiloride ameliorates ER stress in the injured spinal cord and improves hind limb function after SCI by inhibiting cell death, leading to OPC survival. In this study, we labeled proliferating OPCs and demonstrated that amiloride treatment led to greater numbers of OPCs and also oligodendrocytes in the injured spinal cord. The increased MBP expression revealed through western blotting and confirmed by electron microscopy suggest that the increased numbers of mature oligodendrocytes led to improved remyelination, which significantly improved motor function recovery.

**Significance:** Our results show that amiloride administered after SCI inhibited apoptosis of OPCs and increased differentiation OPCs to oligodendrocytes, which led to an increase in remyelination and improved hind limb function.