Abstract

Despite intense scientific efforts, spinal cord injury (SCI) remains to be a severe neurological condition that has no treatment. Currently, therapy is based on alleviating pressure by surgical spinal cord decompression, administration of methylprednisolone and physical therapy. In this study, therapeutic effects of anti-inflammatory compounds and of three types of stem cells were tested in a balloon compression model of spinal cord injury in rats. Natural compounds epigallocatechin-3-gallate (EGCG) or curcumin were administered *in situ* and then intraperitoneally every day for up to 28 days. Human bone marrow mesenchymal stem cells (MSCs), human spinal neural precursors (SPC-01) and neural precursors derived from human induced pluripotent stem cells (iPS-NPs) were transplanted intrathecally (MSCs) or via spinal injection into immunosuppressed rats 7 days after induction of SCI. To determine effects of therapies, changes in motor function was tested by open field test BBB, flat beam test and score, Plantar test and rotarod. Morphometric analysis was used to assess gray/matter sparing and cavity size. Immunohistochemistry was used to determine survival and differentiation of transplanted cells, activation of classical pathway of NFκB (p65 nuclear translocation), astroglial activation (GFAP) and axonal sprouting (GAP43). To determine levels of produced cytokines and chemokines (MIP1α, IL-4, IL-1β, IL-2, IL-6, IL-12p70, TNFα, RANTES), Luminex custom 8-plex assay was used. After SCI, there are two spikes in NFκB activity in the first month, 3 and 28 days after injury induction. Both anti-inflammatory compounds strongly inhibited NFκB (p65) activation, while MSCs and SPC-01 prevented the second spike of activity. All tested treatments resulted in improved motor function, with the best outcomes observed in animals transplanted with iPS-NPs. Both EGCG and curcumin lowered production of chemokines MIP1α and RANTES, which was also observed after treatment with NPs. All treatments caused downregulation of pro-inflammatory TNFα, IL-2, IL-1β except for EGCG, where initial increase occurred and later subsided. Neuroprotective IL-4 was significantly higher after treatment with EGCG and SPC-01. We found strong upregulation of IL-6 and IL-12p70 in animals treated with curcumin and either type of neural precursors, which correlated with strong enhancement of axonal sprouting and functional recovery, but not with classical NFκB pathway suggesting that these cytokines are regulated by different transcription factor in SCI. All treatments except

for EGCG resulted in lower astrogliosis. Our results demonstrate strong immunomodulatory effects of all treatments resulting in improved tissue preservation and regeneration and locomotor skills of treated rats.

Keywords

Spinal cord injury, neuroinflammation, NFκB (p65) canonical pathway, immunomodulation, EGCG, curcumin, stem cell therapy, neuroregeneration