# CHONDROITINASE ABC TREATMENT AND THE PHENOTYPE OF NEURAL PROGENITOR CELLS ISOLATED FROM INJURED RAT SPINAL CORD

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## Summary

The aim of the present study was to investigate whether enzyme chondroitinase ABC-ChABC treatment influences the phenotype of neural progenitor cells (NPCs) derived from injured rat spinal cord. Adult, as well as fetal spinal cord contain a pool of endogenous neural progenitors cells, which play the key role in the neuroregenerative processes following spinal cord injury (SCI) and hold particular promise for therapeutic approaches in CNS injury or neurodegenerative disorders. In our study we performed in vitro model demonstrating the differentiation potential of NPCs isolated from adult rat spinal cord after SCI, treated with ChABC. The intrathecal application delivery of ChABC (10 U/ml) was performed at day 1 and 2 after SCI. The present findings indicate, that the impact of SCI resulted in a decrease of all NPCs phenotypes and  the ChABC treatment, on the contrary, caused an opposite effect.

## Keywords

spinal cord injury, enzyme chondroitinase ABC- ChABC, neural progenitors cells

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Disorders of the central nervous system are a major concern in modern human society. Spinal cord injury (SCI) is a major cause of paralysis. Currently, there are no effective therapies to reverse this disabling condition. In the mammals, the adult neural tissues have limited regenerative capability. Neural progenitor cells (NPCs) refer to the multipotent cells that give rise to the cells of the nervous system. They are found in both embryonic and adult tissues, in mammalian brain and spinal cord (Horner *et al.* 2000, Alvarez-Buylla *et al*. 2001), differentiating into neurons, astrocytes, oligodendrocytes. When NPCs are cultured in the presence of growth factors, they form neurospheres which are free-floating colonies of cells primarily composed of progenitor cells and <1% stem cells (Morshead *et al.* 1994). NPCs play an important role in the neuroregenerative processes following spinal cord injury (SCI) and NPCs have been explored as a potential therapy for SCI (Willerth and Sakiyama-Elbert 2008). It is well docomented that SCI initiates a chain of events that lead to cell death, scarring and the loss of function. The initial trauma injures cells, plasma endothelin-1 levels are elevated (Guo *et al.* 2010), the damaged cells release toxins that cause necrosis of the cells above and below the injury site. Subsequent events include the formation of a cystic cavity at the injury site, which becomes surrounded by a glial scar, composed of mainly reactive astrocytes (Fawcet and Asher 1999). The main class of inhibitory molecules produced by reactive astrocytes after SCI are chondroitin sulfate proteoglycans (CSPGs) (Fok-Seang *et al.* 1995). CSPGs are inhibitory molecules enriched in the extracellular matrix in the CNS that are upregulated at the injury site in vivo and their manipulation may be useful for treatment of human spinal injuries (Fitch and Silver 1997, Fawcet and Asher 1999, Tang *et al*. 2003). Furthermore, ChABC application promotes regeneration and restores function after SCI (Bradbury *et al.* 2002, Yick *et al.* 2003, Matsui and Oohira 2004, Sandvig *et al*. 2004, Huang *et al.* 2006) Therefore, degradation of CSPG using enzyme chondroitinase ABC-ChABC might impact the NPCs phenotype development at the lesion site.

Spinal progenitors were harvested from spinal cords of adult male Wistar rats weighting 290-320g. All experiments conformed to the Slovak Law for Animal Protection No.23/2009, which is transposed from the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes and were approved by the Institutional Ethical Committee for animal research. Trauma was performed by modified balloon-compression technique (Vanicky *et al.* 2001) under isofluorane vapor inhalation anesthesia (1.5-3%). A rectal probe was inserted, and body temperature was maintained at 37–38°C using a heating pad. Animals were divided into 3 groups: i) naive rats, (n=5); ii) rats after SCI with IT application of saline (SCI+saline), (n=5); and iii) rats after SCI with intrathecal application (IT) of ChABC (10 U/ml, protease free, C3667, Sigma-Aldrich) (SCI+ChABC), (n=5). The IT delivery of ChABC or saline was performed at day 1 and 2 after SCI, according to IT application previously described (Cizkova *et al.* 2010). At the fifth day after SCI, NPCs were isolated from spinal cord. The dissected tissue of spinal cords was cut into small pieces and transferred to the papain dissociation system according to the Worthington kit protocol, to isolate neural stem cells. Harvested single cells were cultivated in Nunc T25 culture flasks, grown in proliferation culture medium composed of Dulbecco’s Modified Eagle Medium (DMEM) and Ham’s F12 (1/1 v/v) supplemented with 5 mg/ml streptomycin, 5 IU/ml penicillin, B27, N2 and growth factors FGF-2, bEGF (both 20 ng/ml) to allow for the formation of neurospheres (37ºC, 5% CO2). The arisen neurospheres that were formed within one week of in vitro cultivation, were dissociated by mechanical trituration and differentiated in growth factors free differentiation medium containing fetal bovine serum. The cultures were grown for additional 10 days to induce differentiation and then fixed for immunocytochemical detection in 15 wells of the 24-well plate per each group, 5 individual wells per each antibody, altogether 45 wells were analysed. Immunocytochemistry was performed by applying primary antibodies for detection of astrocytes/ anti-mouse GFAP (1:500), oligodendrocytes /anti-mouse RIP (1:1000) and neurons/ anti-rabbit MAP2 (1:1000) (Tab. 1) followed with corresponding secondary fluorescence (FITC, CY3) antibodies.

To determine the number of differentiated progeny (identified by specific cell phenotype) generated, the positive cells were counted as a percentage of total DAPI+ nuclei in 10 random fields. Data are presented as mean ± SEM. Statistical differences between groups were evaluated with paired Student´s t-test.

Using immunocytochemistry by applying specific antibodies, the population of neurons-MAP 2, astrocytes-GFAP and oligodendrocytes-RIP were analyzed (Fig. 1). The number of differentiated cells were correlated in SCI+saline vs. SCI+ChABC: neurons 21.59% vs. 24.57%, astrocytes 11.10% vs. 21.23%, oligodendrocytes 26.45% vs. 34.81% and. In naive rats we observed following values: neurons 28.47%, astrocytes 15.1% and oligodendrocytes 37.38%.

In present study we performed in vitro model demonstrating the differentiation potential of NPCs isolated from adult rat spinal cord after SCI, treated with ChABC. Based on cultivation strategies and immunocytochemical analyses for cell markers (MAP 2, GFAP, RIP) we were able to characterize the occurence and representation of different cell types: neurons, astrocytes and oligodendrocytes derived from injured adult rat spinal cord tretaed with ChABC. These findings indicate, that the impact of SCI resulted in a decrease of NPCs phenotypes in general. On the contrary, the ChABC treatment caused an opposite effect, elevated numbers of surviving neurons and oligodendroglial cells, reaching almost control values, with acceleration of astrocytes. However, no significant differences between experimental and control groups were detected. These data partially corresponds with results of Sirko *et al.* (2007) that systematically addressed the question of whether ChABC affects stem cell behavior. They showed that the selective elimination of CSPGs with ChABC, both in vivo and in vitro, reduces NSCs proliferation and the differentiation of radial glia to neurons, whereas it favors the maturation of astrocytes. Removal of CSPGs severely impairs neurospheres formation, self-renewal and the generation of their neuronal progeny. This implies a role of CSPGs in the regulation of growth and differentiation factors for NPCs. Although, our data confirm that ChABC delivery may enhance NPCs, particularly astrocytes and oligodendroglia, we did not detect differences in the ability of neurospheres formation between both groups. This may be due to the more limited capacity of adult spinal cord tissue for neurosphers formation when  compared with embryonic tissue (Davis and Temple 1994).

## Conflict of Interest

There is no conflict of interest.

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**Tab. 1.** *Markers used in immunocytochemical analyses*

| Marker | Specifity |
|---|---|
| MAP 2 | *microtubule associated protein 2*, detects mature neurons |
| GFAP | *glial fibrillary acidic protein*, marker for astrocytes |
| RIP | *receptor interacting protein*, oligodendrocytes marker |

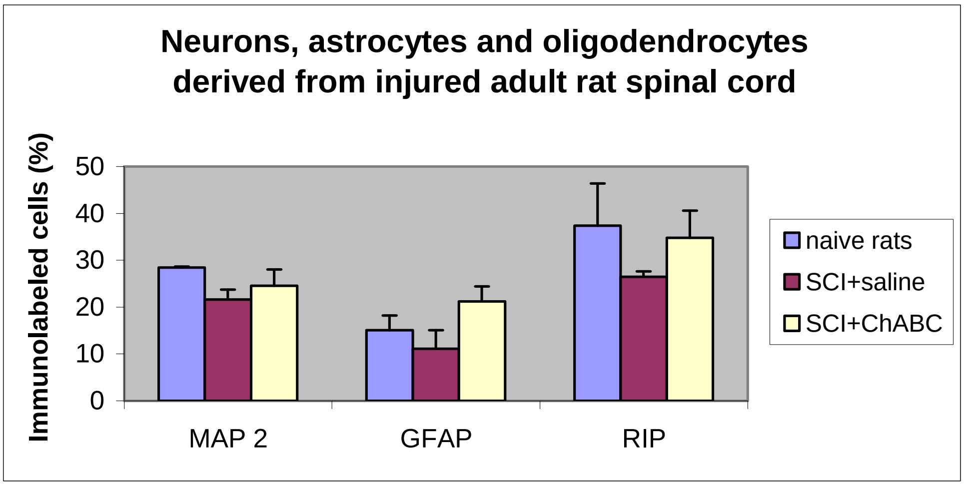

**Fig. 1.** *Comparison of the percentage of immunopositive cells derived from control-naive (blue bars), SCI with IT application of saline (SCI+saline) (purple bars) and SCI with IT application of ChABC (10 U/ml) (SCI+ChABC), (yellow bars) animals. The impact of SCI resulted in decrease of all NPCs phenotypes. On the other side, application of the ChABC caused increase of all NPCs phenotypes.*