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Can Digestion of Chondroitin Sulphate Proteoglycans Aid Functional Recovery in the 6-Hydroxydopamine Lesion Mouse Model of Parkinson's Disease?

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Parkinson's disease (PD) is characterised by dopaminergic cell loss within the substantia nigra pars compacta (SNc) which, in turn, reduces striatal dopamine innervation leading to motor deficits. Remaining dopaminergic neurones of the SNc possess limited capabilities for axonal regrowth and rewiring, potentially due to surrounding inhibitory extracellular matrix proteins, notably chondroitin sulphate proteoglycans (CSPGs). Previous work has shown that digestion of chondroitin sulphates using the bacterial enzyme chondroitinase ABC (ChABC) permits axonal sprouting after axotomy injury to the rodent nigrostriatal tract (1). This led us to hypothesise that CSPG digestion can aid functional recovery in animal models of PD. This hypothesis was addressed in the present study using the unilateral 6-hydroxydopamine (6-OHDA) lesion mouse model.

All animal procedures were conducted in accordance with the Animal (Scientific Procedures) Act, 1986. Mice (C57Bl/6, 8 weeks, 25g) were anaesthetised with 5% isofluorane in 95% oxygen and maintained with 3% isofluorane/oxygen mixture thereafter. To induce the unilateral lesion, mice (n=25) received a single 1 µl injection of 8 µg 6-OHDA-hydrobromide in 0.2% ascorbate/saline at a rate of 0.5 µl/min into the SNc (coordinates AP: -3.0, ML: +1.2 DV: -4.5; relative to skull surface at Bregma). The injection needle was left in place for a further 5 min to avoid toxin reflux. Immediately following lesioning, ChABC (10U/ml in 1 µl saline; n=12) or saline (n=13) was injected as a single 1 µl bolus into two sites of the 6-OHDA affected nigrostriatal tract (rostral SNc AP: -2.3; ML: +1.0; DV: -4.2; caudal striatum AP: +0.02; ML: +2.2; DV: -3.5). After suturing of the surgical site, animals were administered 0.1 ml buprenorphine (0.1 mg/kg; s.c.) and 1 ml Hartmann’s solution (Aqupharm 11; s.c.) for analgesia and rehydration purposes, respectively. A battery of motor behavioural tests, including cylinder reaching and beam walking was performed prior to and up to four weeks post lesion to monitor functional outcomes of ChABC treatment. Amphetamine (5 mg/kg; i.p.)-induced rotations were also assessed at the end of the behavioural period. At the end of the treatment period, 4 weeks post-lesion, animals were terminally anaesthetised and their brains removed, fixed, paraffin-embedded and sectioned (7 µm) through the nigrostriatal tract. Tyrosine hydroxylase (TH) positive cell counts in the SNc and striatal TH immunoreactivity were quantified as a measure of nigrostriatal tract integrity while the ability of ChABC to digest CSPGs was examined using detection of antibodies specific for CSPG digestion products, notably CS4 stub antibodies.

ChABC was shown to produce marked digestion of CSPGs along the nigrostriatal tract, as confirmed by the presence of C4S stub immunoreactivity at intervals along the tract. Despite the successful CSPG digestion, ChABC treatment did not provide any beneficial effects on motor function, nor did it elicit any neuroprotection: TH

positive cell loss within the SNc reached 93.3% ± 3.7 and 93.8% ± 3.1 for ChABC- and vehicle-treated groups, respectively.

In conclusion, these data indicate that digestion of CSPGs does not aid functional recovery or offer neuroprotection or regeneration in the 6-OHDA mouse model of PD. Examination of the efficacy of ChABC in less severe lesion models and in combination with growth promoting factors is currently underway.

(1) Moon LD *et al.* (2001). *Nat Neurosci* **4**: 465-466.