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BRAIN RESEARCH

Research Report

Effects of *ex vivo* transduction of mesencephalic reaggregates with bcl-2 on grafted dopamine neuron survival

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ABSTRACT

Survival rates of dopamine (DA) neurons grafted to the denervated striatum are extremely poor (5–20%). Gene transfer of survival promoting factors, such as the anti-apoptotic protein bcl-2, to mesencephalic DA neurons prior to transplantation (*ex vivo* transduction) offers a novel approach to increase graft survival. However, specific criteria to assess the efficacy of various vectors must be adhered to in order to reasonably predict successful gene transfer with appropriate timing and levels of protein expression. Cell culture results utilizing three different herpes simplex virus (HSV) vectors to deliver the reporter β-galactosidase gene (lacZ) indicate that transduction of mesencephalic cells with a helper virus-free HSV amplicon (HF HSV-TH9lac) that harbors the 9-kb tyrosine hydroxylase (TH) promoter to drive *lacZ* gene expression elicits the transduction of the highest percentage (≈50%) of TH-immunoreactive (THir) neurons without significant cytotoxic effects. This transduction efficiency and limited cytotoxicity was superior to that observed following transduction with helper virus-containing HSV (HC HSVlac) and helper virus-free HSV amplicons (HF HSVlac) expressing lacZ under the transcriptional control of the HSV immediate-early 4/5 gene promoter. Subsequently, we assessed the ability of HSV-TH9lac and the bcl-2 expressing HSV-TH9bcl-2 amplicon to transduce mesencephalic reaggregates. Although an increase in bcl-2 and β-galactosidase protein was induced by transduction, amplicon-mediated overexpression of bcl-2 did not lead to an increase in grafted THir neuron number. Even with highly efficient viral vector-mediated transduction, our results demonstrate that *ex vivo* gene transfer of bcl-2 to mesencephalic reaggregates is ineffective in increasing grafted DA neuron survival.

1. Introduction

Parkinson's disease (PD) is a chronic, neurodegenerative disorder that affects approximately 1–2% of the population over the age of 65 (Mouradian, 2002). The disease is caused by the specific degeneration of the dopaminergic neurons of the substantia nigra pars compacta, which is likely due to cumulative effects of genetic and environ-

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mental factors. Replacement strategies for PD, such as transplantation of primary embryonic dopamine (DA) neurons, are directed at restoring lost DA neurochemistry, returning brain function to the state that existed prior to the onset of symptoms of PD. Many years of successful research on neural grafting in animal models of PD (Brundin et al., 1987; Yurek and Sladek, 1990) have led to several clinical trials worldwide (Olanow et al., 1996; Piccini et al., 1999).

While the field of transplantation continues to advance at a great pace, several challenges remain. In contrast to what might have been anticipated, double-blind clinical trials with grafting of DA neurons failed to provide clinical benefits for all patient groups and yielded dyskinetic behaviors (Freed et al., 2001; Olanow et al., 2003). It was originally hypothesized that postoperative exacerbation of dyskinesias was due to graft overgrowth in the striatum and a generalized hyperdopaminergic effect. However, data from both clinical trials (Ma et al., 2002) and animal studies (Maries et al., 2006; Steece-Collier et al., 2003) suggest that non-homogeneous DA fiber reinnervation is more likely causative. The percentage of grafted fetal DA neurons that survive transplantation is extremely low (5–20%), further limiting the ability of these grafted cells to provide innervation. The reported negative impact of grafting on dyskinesias does not imply that neural grafting has failed to fulfil its clinical promise, but rather that it remains critical to the field of cell replacement, whether the source is embryonic, stem or other, to understand the means by which enhanced survival, and therefore enhanced and homogeneous DA fiber reinnervation, can be attained.

Gene therapy for the treatment of PD has mainly been directed at the delivery of trophic factors and dopaminergic enzymes to cells of the striatum (*in vivo* gene therapy) (Sortwell and Kordower, 2006) or to non-neuronal cells that are subsequently grafted (*ex vivo*) (Bankiewicz et al., 1997; Fisher et al., 1991; Freed et al., 1990; Horellou et al., 1990; Lundberg et al., 1996; Tseng et al., 1997; Wolff et al., 1989). Very little research has investigated the feasibility of *ex vivo* gene delivery to mesencephalic DA neurons in an effort to increase their survival rate after grafting. In our laboratory we have observed a peak of apoptotic nuclear profiles in mesencephalic grafts immediately after implantation (1–4 days) (Sortwell et al., 2000b, 2001). These studies, along with previous findings (Barker et al., 1996; Duan et al., 1995; Emgard et al., 1999), underscore the fact that the critical interval during which grafted DA neurons are dying in grafts to rats is during the first 4 days following implantation. Therefore, neuroprotection of DA neurons to be implanted will most effectively be accomplished via *ex vivo* transduction that generates optimal protein expression at the time of implantation.

In the present study, we examine the *ex vivo* transduction of primary mesencephalic DA neurons and reaggregate cultures with different herpes simplex viral (HSV) vectors to derive an optimal set of transduction conditions and to assess the effects of amplicon-mediated delivery of the gene encoding the anti-apoptotic factor bcl-2 on graft survival and efficacy *in vivo*.

2. Results

2.1. Cytotoxicity of vectors

Three HSV amplicon vectors were assessed for cytotoxic effects on transduced mesencephalic cultures in general (lactate dehydrogenase, LDH, assay) and on THir neurons specifically (counts of THir neurons). On DIV 4, all vectors displayed significantly higher LDH levels at an MOI of 2.0 when compared to their respective LDH values at MOI levels of 0.5 and 1.0 [$F(8,36)=114.525$; $P\leq0.0001$]. Across vectors, both the HC HSVlac and the HF HSVlac amplicons displayed significantly higher LDH levels than HF HSV-TH9lac-transduced cultures at all three MOI levels examined ($P\leq0.0003$). These results are depicted in Fig. 1A.

Only HC HSVlac transduction yielded a dose-dependent decrease in THir neurons observed in culture at PID 4 [$F(11,49)=27.342$; $P\leq0.0001$]. There were no significant differences in THir neuron number due to increasing MOI levels of either HF virus examined (HF HSVlac or HF HSV-TH9lac, $P\geq0.5$). Across vectors, infection with HF HSV-TH9lac yielded significantly fewer THir neurons in culture than HF HSVlac when examined on post infection day 4 (PID 4, $P\leq0.0001$). However, there were no significant differences in THir neuron number between HF HSVlac-transduced cells at PID 4 and HF HSV-TH9lac at PID 7 for MOI levels of 1.0 and 2.0 ($P\geq0.5$). These results are depicted in Fig. 1B.

2.2. General transduction efficiency

Individual mesencephalic cultures transduced with the three amplicon constructs were examined on PID 4 and PID 7 for total number of β-gal positive cells (Figs. 2A and B). Cells infected at the time of plating with either HC HSVlac or HF HSVlac exhibited numerous transduced cells at the PID 4 and PID 7, while cultures incubated with HF HSV-TH9lac at the time of plating were completely devoid of transduced cells at either timepoint (data not shown). Therefore, subsequent culture studies utilizing HF HSV-TH9lac were transduced 24 h after plating.

Within vector treatment groups, at PID 4, only HF HSV-TH9lac-transduced cells displayed significantly higher numbers of β-gal positive cells when infected at an MOI of 2.0, compared to MOI 0.5 [$F(17,36)=15.078$; $P\leq0.0001$]. Neither HC HSVlac nor HF HSVlac-transduced cells displayed significant differences in β-gal positive cell number with increasing MOI levels when examined at PID 4 (Fig. 2A, $P\geq0.5$). Across vector treatment groups, there were no significant differences in the number of β-gal positive cells observed at PID 4 ($P\geq0.5$).

Three days later (Fig. 2B), at PID 7, HC HSVlac-transduced cells at MOI levels of 1.0 and 2.0 displayed significantly higher numbers of β-gal positive cells compared to MOI level of 0.5 ($P\leq0.0001$). There were no significant differences in β-gal positive cell numbers between HC HSVlac-transduced cells infected at MOI 1.0 and 2.0 ($P\geq0.5$). Similarly, at PID 7 mesencephalic cells infected with HF TH9HSVlac at an MOI level of 2.0 displayed significantly more β-gal positive cells than those infected at MOI 0.5 ($P\leq0.0001$). There were no significant differences with increasing MOI levels of HF