English Abstract

Parkinson’s disease (PD) is a common movement disorder associated with degeneration of dopaminergic neurons in the substantia nigra and a corresponding loss of dopamine in the striatum. The dopamine precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), is the mainstay of treatment for PD. However, the short duration response and development of dyskinesia partly because of the loss of dopamine storage sites and erratic dopamine delivery limit chronic L-DOPA therapy in PD.

To obtain the capacity to produce and store dopamine in parkinsonian rats, primary fibroblasts were genetically modified with AADC and VMAT-2 genes. Transgene expression in these fibroblasts was confirmed by immunocytochemical staining and enzyme activity assays. Following incubation with L-DOPA in culture, the doubly transduced fibroblast cells (PFVMAA) produced and stored dopamine at a much higher level than the cells with either gene alone ($p < 0.005$). Genetically modified fibroblast cells were grafted in parkinsonian rat striata and time course of biochemical changes in denervated striata after L-DOPA injection were measured by using in vivo microdialysis 3 days after grafting. Higher dopamine levels were sustained for a longer duration in rats grafted with PFVMAA cells than in those grafted with either control cells or cells with AADC alone ($p < 0.01$). To determine whether the prolonged biochemical effects of grafting PFVMAA cells would also increase the duration of improvement in akinesia in parkinsonian rats, we utilized the forepaw adjusting steps as a non-drug-induced behavioral paradigm. PF ($n = 8$), PFAADC ($n = 7$), and PFVMAA ($n = 10$) were grafted into denervated striatum and forepaw adjusting steps were monitored for five hours after administration of L-DOPA on the seventh day after grafting. Compared to PF or PFAADC grafted rats, forepaw adjusting steps at 4 and 5 hours were significantly elevated from the baseline level before L-DOPA injection in PFVMAA grafted rats ($p < 0.05$), consistent with the biochemical data. We demonstrated the beneficial effects of such double gene transduction in the production, storage, and gradual release of dopamine by genetically engineered fibroblast cells both in vitro and in vivo.

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This finding illustrates a novel method of VMAT-2 and AADC gene therapy combined with precursor administration to overcome the major obstacles of PD treatment. Such approach will allow safe clinical application even with limited gene therapy vehicles that are currently available.

**Key words:** Parkinson’s disease, L-DOPA, dopamine, vesicular monoamine transporter, AADC, Forepaw adjusting step

**Student number:** 96801-867