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BRAIN RESEARCH

Research Report

# Control of dopamine-secretion by Tet-Off system in an in vivo model of parkinsonian rat

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ABSTRACT

We established a PC12 cell line (PC12TH Tet-Off) in which human tyrosine hydroxylase (TH) expression can be negatively controlled by Doxycycline (Dox). First, dopamine (DA)-secretion from PC12TH Tet-Off cells was controlled by Dox-administration in a dose-responsive manner ranging from 0 to 100 ng/ml for 70 days in vitro. Furthermore, Parkinson's disease model of rats receiving encapsulated PC12TH Tet-Off cells displayed a significant decrease of dopamine concentration in the cerebrospinal fluid (CSF) and increase of the number of apomorphine-induced rotations by Dox-administration, as compared to transplanted rats without Dox-administration, although the significant decrease of the reduction ratio of DA concentration in the CSF with Dox-administration was recognized over time. At 2 months post-implantation, concentration of dopamine in the implanted striatum and from the retrieved capsules demonstrated that the control of DA-secretion could be partially achieved for 2 months in vivo. Our results support both the value of cell therapy using Tet-Off system and the technique of encapsulation might be a feasible option for Parkinson's disease especially in resolving the problem of dopamine oversupply in the future, although a more efficient way to control DA-secretion with quicker regulation and much titration of dose should be explored before clinical application.



## 1. Introduction

The major pathology of Parkinson's disease (PD) is neurodegeneration of the dopamine-producing nigrostriatal system (Dawson and Dawson, 2002). Dopamine replacement therapy (DRT) was first established as a therapeutic modality for PD. However, patients with DRT for a long time often develop both dyskinesia and wearing-off (Nutt et al., 2002) with impairment of the synaptic dopamine (DA) metabolism in the putamen (Rajput et al., 2004). In recent years, cell therapy using fetal nigral cells (Freed et al., 2001; Olanow et al., 2003) was also reported to display a certain therapeutic effects on PD patients with significant effectiveness in the younger but sometimes induce dyskinesia in some severely staged PD patients. Positron emission tomography revealed that $^{18}$F fluorodopa uptake in the putamen significantly increased in the patients developing persistent dyskinesia (Ma et al., 2002). Thus, dyskinesia induced by DRT or fetal nigral cell transplantation might be certainly associated with oversupply of dopamine in a localized area. In this study, we demonstrated doxycycline

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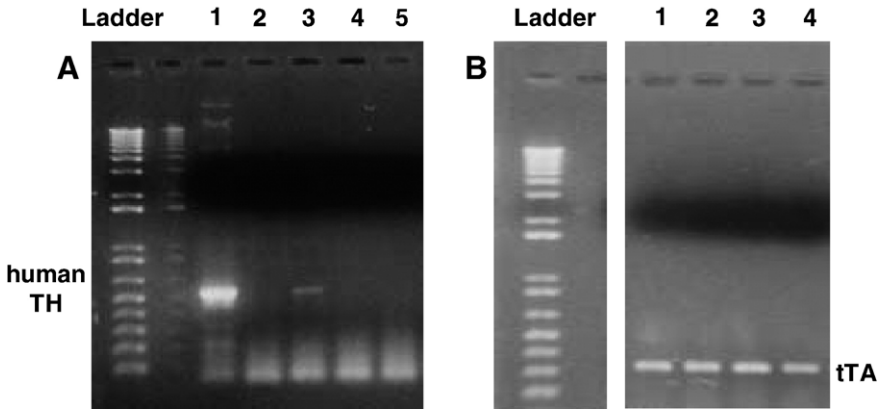


**Fig. 1 – RT-PCR revealed the reduction of human TH-expression of PC12TH Tet-Off cells by Dox-administration in vitro. (A) RT-PCR of hTH revealed the expression of hTH RNA from the PC12TH Tet-Off cells without Dox-administration (lane 3) and the reduced expression from cells with Dox-administration (lane 2). The expression of hTH was not recognized in PC12 Tet-Off Control cells with or without Dox-administration (lanes 4 and 5). Lane 1 was shown as a positive control using pTRE2hygTH1, plasmid carrying hTH gene. (B) Expression of Tet transactivator (tTA) RNA from PC12TH Tet-Off and PC12 Tet-Off Control cells with or without Dox-administration was recognized equally likely. Lanes 1 and 2: PC12TH Tet-Off cells with and without Dox-administration. Lanes 3 and 4: PC12 Tet-Off Control cells with and without Dox-administration.**

(Dox)-controlled expression of human tyrosine hydroxylase (hTH) with encapsulated cell transplantation with which we can use safely genetically engineered xenogeneic graft in an in vivo model of PD. These technologies can be used for dose control of neurotransmitter like dopamine or growth factor like vascular endothelial growth factor (VEGF), both of which might cause deteriorated outcomes with inappropriate dose administration (Yasuhara et al., 2005b). Furthermore, critical problems like tumorigenesis by transplanted cells might be prevented using cells encoded suicide gene in advance (Bondanza et al., 2005). The control of DA-secretion from the outside like this study might reduce the side effects of DRT upon PD patients and diminish the burden of PD patients suffering from severe dyskinesia induced by DRT.

## 2. Results

### 2.1. *Dox-controlled TH-expression in vitro*

The expressions of TH and tTA of PC12TH Tet-Off cells without Dox-administration were recognized by RT-PCR, and the TH-expression of PC12TH Tet-Off cells cultured with 1-week Dox-administration reduced remarkably (Fig. 1). The amount of LD and DA-secretion from cultured PC12TH Tet-Off, PC12 Tet-Off Control, PC12, and BHK cells were as follows: LD-secretion were 470 ± 8.4, 102 ± 4.0, 96 ± 4.2, and 0 ng/$10^6$ cells/day and DA-secretion was 56 ± 2.5, 16 ± 0.8, 9.5 ± 0.6, and 0 ng/$10^6$ cells/day, respectively. In addition, the amount of LD and DA-secretion from the 4 kinds of capsule at 2 weeks post-encapsulation was almost the same as each non-encapsulated $10^6$ cells (LD: 481 ± 13.8, 102 ± 6.8, 91.5 ± 5.7, and 0, DA: 57.3 ± 3.9, 15.6 ± 2.1, 9.2 ± 1.1, and 0 ng/$10^6$ cells/day, respectively). Dox-administration for 1 week (10–1000 ng/ml) was considered to reduce TH-expression of encapsulated PC12TH Tet-Off cells, and subsequently LD and DA-secretion significantly decreased in a dose-responsive manner from 0 to

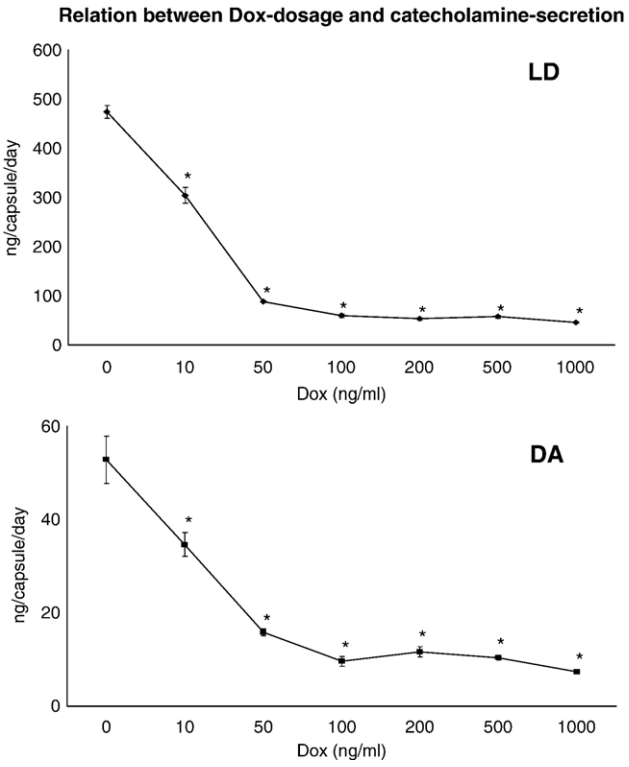


**Fig. 2 – Relation between the dose of Dox-administration and catecholamine-secretion from PC12TH Tet-Off cells in vitro. DA and LD-secretion from PC12TH Tet-Off cells was reduced by Dox-administration for 1 week in a dose-responsive manner ranging from 0 to 100 ng/ml. The degree of reduction of DA and LD-secretion with 100 ng/ml of Dox-administration was almost the same as with 1000 ng/ml. Data are shown as mean values ± SE expressed as ng/capsule/day. $n = 4$ in each group. *$P$'s < 0.01 vs. DA and LD-secretion from PC12TH Tet-Off capsule without Dox-administration by post hoc Scheffe's test.**