Cell Transplantation, Vol. 26, pp. 000–000, 2017
Printed in the USA.

0963-6897/17 $90.00 + .00
DOI: https://doi.org/10.3727/096368916X694247
E-ISSN 1555-3892
www.cognizantcommunication.com

Neuroprotection of Granulocyte Colony-Stimulating Factor for Early-Stage Parkinson's Disease

Sheng-Tzung Tsai,* Sung-Chao Chu,† Shu-Hsin Liu,‡ Cheng-Yoong Pang,§ Ting-Wen Hou,* Shinn-Zong Lin,* and Shin-Yuan Chen*

*Department of Neurosurgery, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan
†Department of Oncology/Hematology, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan
‡Department of Nuclear Medicine, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan
§Department of Medical Research, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease. Both medical and surgical choices provide symptomatic treatment. Granulocyte colony-stimulating factor (G-CSF), a conventional treatment for hematological diseases, has demonstrated its effectiveness in acute and chronic neurological diseases through its anti-inflammatory and antiapoptosis mechanisms. Based on previous in vitro and in vivo studies, we administered a lower-dose (3.3 μg/kg) G-CSF injection for 5 days and six courses for 1 year in early-stage PD patients as a phase I trial. The four PD patient's mean unified PD rating scale motor scores in medication off status remained stable from 23 before the first G-CSF injection to 22 during the 2-year follow-up. 3,4-Dihydroxy-6-^{18}F-fluoro-L-phenylalanine (^{18}F-DOPA) positron emission tomography (PET) studies also revealed an annual 3.5% decrease in radiotracer uptake over the caudate nucleus and 7% in the putamen, both slower than those of previous reports of PD. Adverse effects included transient muscular–skeletal pain, nausea, vomiting, and elevated liver enzymes. Based on this preliminary report, G-CSF seems to alleviate disease deterioration for early-stage PD patients. The effectiveness of G-CSF was possibly due to its amelioration of progressive dopaminergic neuron degeneration.

Key words: Parkinson's disease (PD); Granulocyte colony-stimulating factor (G-CSF); Neuroprotection

INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease[1]. With the characteristics of slow progression of clinical symptoms and degeneration of dopaminergic cells within the substantia nigra in PD, several disease-modifying modalities have been tested for neuroprotection, including medication or even surgery[2–4]. Apoptosis has been considered to result in the pathogenesis of neurodegenerative disorders, including PD[5]. Preventing programmed and progressive neuronal cell death by interfering with apoptotic pathways has been suggested to lead to neuroprotection from these dreadful neurodegenerative diseases[6,7].

Granulocyte colony-stimulating factor (G-CSF), one of the hematopoietic growth factors, regulates the production of circulating blood cells by the bone marrow and is usually used for the treatment of neutropenia in humans. G-CSF was shown to possess trophic and anti-inflammatory effects[8]. To activate multiple survival pathways and enhance neurogenesis, G-CSF has been applied to animal models of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) or Alzheimer's disease[9,10]. In our previous in vitro evidence, G-CSF protects dopaminergic neurons from 6-hydroxydopamine (6-OHDA)-induced toxicity via the extracellular-regulated kinase pathway[11]. G-CSF has also shown neuroprotection in in vivo models of PD through the G-CSF receptor and downstream antiapoptotic pathway[12–14]. The evidence of increased dopaminergic neuronal fibers in the striatum with G-CSF injection was also demonstrated, which significantly ameliorated the behavioral impairment of the PD rat model[15].

Following growing evidence of the repair of cell injury in vitro and in vivo, G-CSF has been tested in several clinical trials in humans to confirm its safety and efficacy[16,17]. With the similar idea of mobilizing bone marrow stem cells or hematopoietic stem cells to the ischemic area through a short period of serum elevation

Received October 12, 2016; final acceptance January 31, 2017. Online prepub date: December 7, 2016.
Address correspondence to Dr. Shin-Yuan Chen, Department of Neurosurgery, Tzu Chi General Hospital, 707, Sec. 3, Chung-Yang Road, Hualien 970, Taiwan. Tel: +886-3-8561825, ext. 2151; Fax: +886-3-8463164; E-mail: william.sychen@msa.hinet.net

of signaling factor, previous phase I trials for human ischemic stroke have demonstrated G-CSF administration to be safe and well tolerated[18,19]. Based on the foregoing rationale and evidence, the present clinical trial was designed to assess the efficacy of the neuroprotective effect of repeated and low doses of G-CSF for early-stage PD patients.

MATERIALS AND METHODS

Eligibility of Subjects

This study was designed as a pilot phase I trial to explore the safety, risks, and effectiveness of G-CSF for PD patients. Our inclusion criteria were as follows: (1) patients with idiopathic PD who met the UK Parkinson's Disease Society Brain Bank diagnostic criteria and had a good response to levodopa [>30% improvement in the unified PD rating scale (UPDRS)]; (2) the onset of PD symptoms must have occurred after 40 years of age [to exclude young-onset PD (YOPD)]; (3) patients may have had symptoms of levodopa-induced dyskinesia, but patients must have rated between Hoehn and Yahr stages 1 and 2 when in an off-medication state; and (4) patients must have been in their optimal medication treatment state and would not change their medications within 3 months before and after enrollment.

Our exclusion criteria included the following: (1) patients who had YOPD and/or were genetically related; (2) women of childbearing potential or who were pregnant or lactating; (3) patients who exhibited tumor growth and/or malignancy; (4) patients with a past (within 1 year) or present history of psychotic symptoms who required antipsychotic treatment; (5) patients with active symptoms of major depression with suicidal ideation or suicide attempt; (6) patients with previous brain surgery (including pallidotomy and deep brain stimulation); and (7) patients with significant cognitive impairment [Mini-Mental State Examination (MMSE) <24]. This study was approved by the institutional review board (IRB) of Tzu Chi General Hospital (IRB 097-54) and registered on ClinicalTrials.gov (NCT01227681). All patients provided written informed consent.

Administration of Treatments

G-CSF (Cat. No. 000670; Filgrastim Injection® M300; Kirin, Tokyo, Japan), a commercial product, is available as a colorless fluid (polysorbate 0.028 mg, D-mannitol 35 mg, acetic acid 0.42 mg with sodium hydroxide) in a small glass bottle. The route of G-CSF administration was through subcutaneous injection. The patients received 3.3 μg/kg/day of G-CSF in a course of five daily doses. Patients had 2 months of rest before the next course of G-CSF injection. There were six courses of injections (Fig. 1).

Follow-Up Schedules and Markers

Before starting each G-CSF injection course, patients were evaluated with a complete UPDRS examination. To specify how G-CSF influences the evolution of PD, patients were evaluated in both "medication off" (Med off) and "medication on" (Med on) status during the visits. Med off was defined as a patient who had not taken antiparkinsonian medication for at least 12 h. Acute levodopa drug test measures the effect of levodopa with the UPDRS in both the on and off states. Prior and current medications, including levodopa equivalent daily dosage (LEDD) measurements, were recorded. Neuropsychological assessments and quality of life assessments included the MMSE, Cognitive Assessment Inventory (CASI), Beck Depression Inventory (BDI), Parkinson's Disease Quality-39 (PDQ-39), and the Schwab and England Score of Activity of Daily Living (SEADL).

Laboratory measures included red blood cell count, hemoglobin, hematocrit, red blood cell indices, leukocyte count (with differential count), and platelet count. Liver and renal functions, blood glucose, and electrolytes were

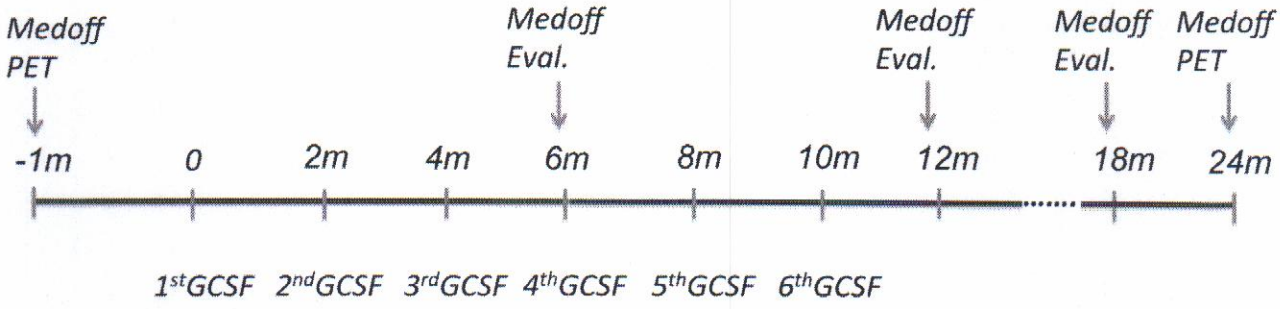


Figure 1. Schematic diagram illustrating the experimental protocols in the present study. Medoff Eval., antiparkinsonian "medication off" to evaluate disease with the unified Parkinson's disease rating scale; PET, positron emission tomography; G-CSF, granulocyte colony-stimulating factor.

checked during each injection and follow-up course. C-reactive protein and $CD34^+$-expressing cells were also examined.

To collect more markers to better illustrate the disease progression, imaging analysis with 3,4-dihydroxy-6-^{18}F-fluoro-L-phenylalanine (^{18}F-DOPA)-positron emission tomography (PET) was evaluated before G-CSF treatment and 2 years after the first course of G-CSF injections. The rater marked four regions of interest (ROIs), which included the bilateral head of the caudate nucleus and putamen; they were included in serial axial ^{18}F-DOPA images. The individual occipital cortex was set as the reference region. We used a previously published method of striatal uptake quantification with the striatal-to-occipital ratio (caudate/occipital or putamen/occipital) method[20]. PET series with ^{18}F-DOPA uptake were plotted, and the calculated ratios were defined as one of the indicators of disease progression. Adverse events were recorded during or after the treatment follow-up period.

Statistical Analysis

Student's *t*-test was used for comparing clinical scores and lab values between pretreatment and posttreatment stages (Excel; Microsoft, Redmond, WA, USA). Significance was set at $p<0.05$ for all tests.

RESULTS

From January 2009 to June 2012, we included four PD patients who fulfilled the inclusion criteria (Table 1). They were all males with mean age of 49.25 years at the beginning of G-CSF treatment. Their mean disease duration was 4.75 years (mean ages of disease onset at 44.5 years). They all completed six courses of G-CSF treatment and 2 years of follow-up after injection. Their mean score on the UPDRS part III motor subscale in the Med off state was 23 before the first injection course and remained at 22, 1 year after the last injection course (24 months after the first injection course) (Fig. 2). In addition, all other sub-item analyses of UPDRS remained stable and did not show significant deterioration 2 years after the first injection of G-CSF (Table 1). Neuropsychological assessments and quality of life status, assessed by MMSE, CASI, BDI-II, and PDQ-39, remained stable 2 years after G-CSF treatment (Table 1). In terms of medication use, LEDD also did not show a significant increase.

Lab analysis revealed that the $CD34^+$ count remarkably increased during G-CSF injection (Table 2). Leukocyte counts also increased significantly (more than five times above baseline) during treatment. Two patients had slightly elevated fasting blood glucose levels during the two courses of G-CSF, while one patient, who had glucose intolerance, had elevated fasting blood glucose levels during all six treatments. A mild increase in the liver enzyme alanine transaminase to 51 IU/L was found during one course of treatment for one patient. For renal function, G-CSF injection did not result in any abnormality.

^{18}F-DOPA-PET showed that the right putamen did progress after 2 years of follow-up compared with the pretreatment baseline ^{18}F-DOPA-PET ($p<0.05$) (Table 3). For the left caudate nucleus and putamen, the uptake of ^{18}F-DOPA was at a similar level. When we pooled the striatal-to-occipital ratio of the bilateral hemispheres, the uptake of ^{18}F-DOPA in the putamen significantly decreased by 14% 2 years after G-CSF treatment ($p=0.02$). Although radiotracer uptake in the caudate nucleus revealed a 7% decrease, it did not reveal a significant decrease. PET results from one illustrative case are shown in Figure 3. PD patient 3 (PD03; Table 1) has nearly comparable radiotracer uptake ratio at 2 years after G-CSF (Fig. 3B) with level before G-CSF treatment (Fig. 3A).

The most common adverse effects after G-CSF injection were lower back pain (16% per treatment day) and muscular–skeletal pain in the four limbs (6.7%). Mild chest tightness (5.8%), dizziness (2.5%), and nausea (2.5%) were also noted during the treatment period (G-CSF injection 5-day course). One of four patients, who had a history of gouty arthritis, had recurrent acute gouty arthritis pain during three courses of treatment. Most symptoms only persisted transiently after injection, and muscular–skeletal pain could be quickly ameliorated with nonsteroidal anti-inflammatory drugs.

DISCUSSION

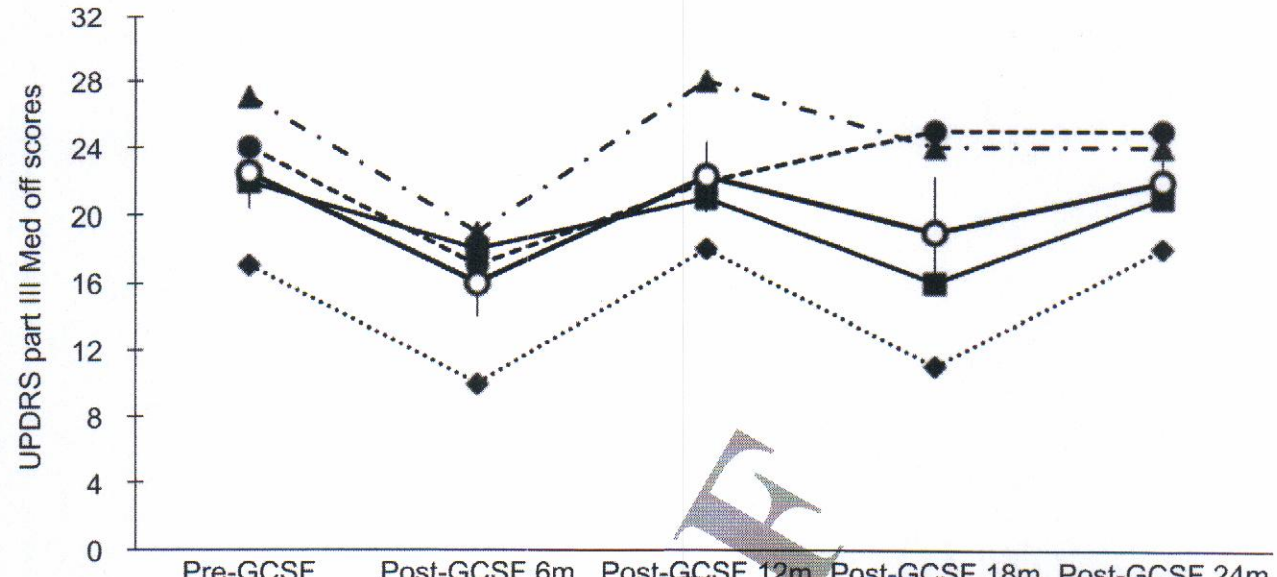
Based on our knowledge, this is the first trial to use low-dose G-CSF as a repeated and chronic (during a 1-year period) treatment for early-stage PD. Two years after the first G-CSF injection course, UPDRS part III scores (Med off status) did not progress, which may suggest the possibility of neuroprotection by G-CSF for a persistent deteriorating neurodegenerative disease in PD. PD has been estimated with an annual score progression of 3–5 in UPDRS part III[21]. Along with stable motor scores (including sub-items of motor symptoms), other neuropsychological follow-ups all did not reveal significant deterioration over 2 years of follow-up. In terms of safety for G-CSF treatment, all adverse effects were mild and transient.

Most in vivo evidence has shown that the acute administration of a large dose (40–50 μg/kg body weight of rodents) provided neuroprotection in a murine model of PD[15]. This might be due to the pathology underlying the current animal model, originating from the acute intoxication of dopaminergic substantia nigra neurons by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-OHDA[14,22]. However, the inherent slow deterioration

Table 1. Clinical Demographics of Parkinson's Disease Patients From Before and After G-CSF Treatment Course

	PD01			PD02			PD03			PD04			*p*-value		
Sex	M			M			M			M					
Age	53			42			46			56					
Disease onset	47			40			41			50					
Disease duration	6			2			5			6					
UPDRS Med Off	Pre	1 Year	2 Years	Pre	1 Year	2 Years	Pre	1 Year	2 Years	Pre	1 Year	2 Years	Pre Versus 1 Year	1 Year Versus 2 Years	Pre Versus 2 Years
Weight	61.8	63.0	59.0	65.1	66.7	65.7	66.7	63.9	65.7	67.0	66.5	67.9	0.9085	0.7583	0.5476
MMSE	29		28	29		28	30		30	29		27			0.0917
CASI-II	96.9		94.5	97.0		95.0	95.0		95.0	95.4		93.0			0.0596
BDI-II	4	1	1	10	4	12	14	5	5	12	5	12	0.0154*	0.1831	0.3738
PDQ-39	8	4	5	34	18	41	13	4	15	37	44	33	0.3383	0.4677	0.8561
LEDD	895	895	895	220	220	330	630	630	630	840	895	895	0.3910	0.3910	0.2152
Part I	3	1	2	3	0	1	2	0	0	3	3	3	0.0689	0.1817	0.0796
Part II	10	4	9	9	6	11	8	7	8	14	8	10	0.0469*	0.0511	0.5908
Part III	22	16	21	17	11	18	24	25	25	27	24	24	0.1253	0.1904	0.6376
Brady	12	9	11	10	9	9	12	15	14	14	9	12	0.4444	0.3534	0.6042
Tremor	0	1	0	0	1	2	1	0	0	1	4	2	0.3081	0.4950	0.4950
Rigidity	7	5	6	4	1	5	5	6	7	3	4	3	0.5195	0.3120	0.4950
Posture & Gait	2	0	2	1	0	0	2	2	2	4	4	4	0.2152	0.3910	0.3910
Axial	3	1	4	2	0	1	4	3	3	7	6	6	0.0138*	0.2522	0.3910
Part IV	2	5	2	0	2	2	2	3	2	1	4	2	0.0182*	0.1027	0.2152
Total	37	26	34	29	19	32	36	35	35	45	39	39	0.0542	0.1992	0.4222
Hoehn & Yahr Stage	2	2	2	1.5	2	2	2	2	2	2.5	2.5	2.5	0.3910		0.3910
SEADL Score (%)	90	90	90	100	100	90	100	90	90	100	90	90	0.1817	0.3910	0.0577

Pre, pre-GCSF treatment; UPDRS, unified Parkinson's disease rating scales; MMSE, Mini-mental status exam; CASI, Cognitive assessment inventory; BDI, Beck depression inventory; PDQ-39, Parkinson's disease quality-39; LEDD, Levodopa equivalent daily dose; SEADL, Schwab and England score of activity daily living; G-CSF, granulocyte - colony stimulating factor. *$p<0.05$



	Pre-GCSF	Post-GCSF 6m	Post-GCSF 12m	Post-GCSF 18m	Post-GCSF 24m
PD01	22	18	21	16	21
PD02	17	10	18	11	18
PD03	24	17	22	25	25
PD04	27	19	28	24	24
Mean	23	16	22	19	22

Figure 2. The evolution of the unified Parkinson's disease rating scale (UPDRS) of individual Parkinson's disease (PD) patients (with mean scores) from before granulocyte colony-stimulating factor (G-CSF) injection to 24 months after the first G-CSF injection. Med off, a patient who had not taken antiparkinsonian medication for at least 12 h (medication off).

of a PD patient is associated with progressive neuronal death in the substantia nigra. This suggests that treatment for PD is tailored to the disease pathophysiology. Therefore, we designed a lower dose (3.3 μg/kg of body weight) compared with the standard treatment to boost autologous hematopoietic stem cells for donor and repeated (5 days per course and six courses per trial) the injection of G-CSF in our PD patients. This type of design for G-CSF has been shown to be safely administered and resulted in partial improvement in secondary parkinsonism patients[23].

For the four patients, we compared ^{18}F-DOPA-PET imaging between baseline and 2 years after the first G-CSF treatment, and the uptake of ^{18}F-DOPA over the right putamen showed a significantly progressive decline. This was in agreement with the fact that three of four patients had symptoms in the left limbs on disease onset. Most neuroimaging studies using single-photon emission computed tomography (SPECT) and PET revealed an annual 4%–12.5% decrease in uptake in the caudate nucleus and 8%–13.1% in the putamen[24–28]. Morrish et al. analyzed 32 PD patients with a mean age of 58 years and followed disease evolution with ^{18}F-DOPA-PET for 18 months[26]. The results showed that the annual deterioration of baseline mean radiotracer uptake in the putamen was 8.9%. Another study, which included early-stage PD that were followed for 2 years, revealed a higher decline rate at 13.1% per year[25]. The wide variation on the estimated progression resulted from sensitivity of the imaging methods and the patient's disease status. There is growing evidence that shows inconsistencies in the rate between clinical progression in PD and nigrostriatal degeneration examined by imaging[29,30]. These results postulated that dopaminergic degeneration might follow a negative exponential pattern with the fastest rate of decline in the early disease stage, even though younger PD patients have a slower clinical progression rate at the early stage[29]. Our annual 3.5% decrease in the caudate nucleus and 7% in the putamen in radiotracer binding

Table 2. Lab Evolution Before and After G-CSF

	Day 1	Day 5
WBC (*1000/mm3)	5.98±0.67	33.77±6.65*
CD^{34+} (%)	0.027±0.013	0.041±0.018*
Absolute CD^{34+} count	1.556±0.674	14.587±8.143*

Data presented as mean±SD.
G-CSF, granulocyte-colony stimulating factor. *$p<0.05$

Table 3. Striatal to Occipital Ratio Values Before and 2 Years After First G-CSF Treatment Course

		PD01		PD02		PD03		PD04		
Brain		Pre	2 years	Pre	2 years	Pre	2 years	Pre	2 years	*p*-value
Right	Caudate/occipital	2.58	2.44	2.40	2.06	2.21	2.34	2.38	2.06	0.2216
	Putamen/occipital	2.04	1.87	2.42	1.90	1.86	1.36	2.09	1.75	0.0183*
Left	Caudate/occipital	2.40	2.32	2.40	1.96	2.43	2.58	2.28	2.02	0.3212
	Putamen/occipital	1.78	1.64	1.78	1.81	2.16	1.67	1.87	1.86	0.1070

PD, Parkinson's disease patient; Pre, pre-GCSF treatment; G-CSF, granulocyte-colony stimulating factor. *$p<0.05$

of ^{18}F-DOPA-PET, which were close to the slower rate of deterioration from a previous report[31], indicated that G-CSF might still possibly provide neuroprotection. The rate of disease progression in PD varied according to age at disease onset, age of patients, and treatment choices. The results from ^{18}F-DOPA showed low interindividual variability, which might have been due to the strict inclusion criteria of our study and the early stages (I–II) of disease in our patients.

Given the continuous progression seen in PD, much effort has been made to design disease-modifying drugs. Rasagiline, a medication for symptomatic treatment, has been shown to have promising neuroprotective properties in a variety of in vitro and in vivo PD animal models[32]. Rasagiline induces the regeneration of substantia nigra dopaminergic neurons in post-MPTP-induced parkinsonism via the activation of the tyrosine kinase receptor signaling pathway and an antiapoptotic mechanism[32,33]. By sharing similar mechanisms of treatment, G-CSF might halt the progression of PD through an anti-apoptotic mechanism and even restore the loss of dopaminergic neurons[11,14]. In addition, none of the patients took any potential disease-modifying drugs during the trial period, which may have led to bias in the interpretation of the course of disease.

Larger doses (15 μg/ kg) in 5 days of subcutaneous G-CSF injection have been associated with transient headache, bone pain, and abnormal liver function in a previous clinical trial for stroke treatment[34]. In using G-CSF for progressive neurodegenerative diseases, most studies choose smaller doses and longer duration of treatment. Treatment of atypical parkinsonism patients with lower doses (5 μg/kg) but for a longer period (three 6-day courses) revealed stable or better outcomes in 5 out of

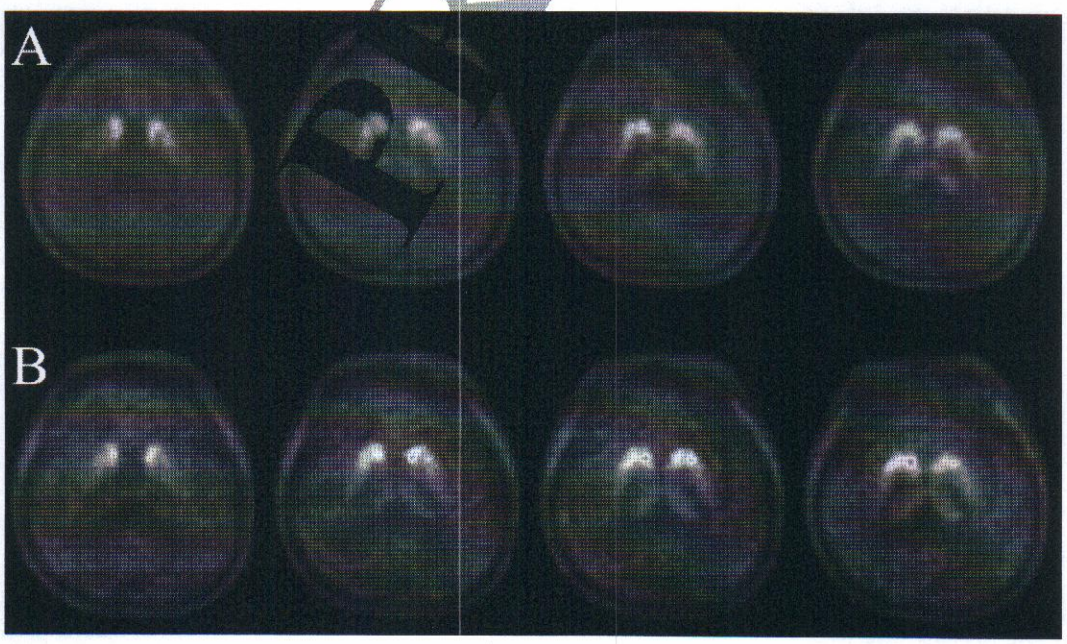


Figure 3. Illustrative images of 3,4-dihydroxy-6-^{18}F-fluoro-L-phenylalanine positron emission tomography (^{18}F-DOPA-PET) from PD03 (Parkinson's disease patient 3) in Table 1 were arranged before granulocyte colony-stimulating factor (G-CSF) injection (A) and 24 months after G-CSF (B). Both showed a similar striatal-to-occipital ratio of uptake.

10 patients; they tolerate the administration of G-CSF well, and treatment was safe[23]. Another study that used the same dose for 4 days every 3 months in 1 year for ALS also showed safe treatment and no major side effects[35]. To meet with the progressive characteristics of PD, we adjusted the injection schedules with similar doses of G-CSF in six courses for up to 1 year. All adverse effects were temporary during injection, and tolerability was good. Indeed, the small sample sizes with open-label designs would undermine interpretation of results. The lower scores of UPDRS in the middle of the trial might suggest a placebo effect, which needs to be considered in the design of neuroprotection studies for neurodegenerative diseases, such as PD[36,37].

In this preliminary study, we show that G-CSF might halt disease-related deterioration in the early stages of PD. The results from ^{18}F-DOPA-PET suggest that G-CSF may slow down nigrostriatal degeneration as well. The low dose and repeated-manner design of G-CSF treatment for PD is safe. Most adverse effects were mild musculoskeletal pains during drug injection, and patients' tolerance of G-CSF was high.

REFERENCES

1. Sherer TB, Chowdhury S, Peabody K, Brooks DW. Overcoming obstacles in Parkinson's disease. Mov Disord. 2012;27(13):1606–11.
2. Wallace BA, Ashkan K, Heise CE, Foote KD, Torres N, Mitrofanis J, Benabid AL. Survival of midbrain dopaminergic cells after lesion or deep brain stimulation of the subthalamic nucleus in MPTP-treated monkeys. Brain 2007;130(Pt 8):2129–45.
3. Hung AY, Schwarzschild MA. Clinical trials for neuroprotection in Parkinson's disease: Overcoming angst and futility? Curr Opin Neurol. 2007;20(4):477–83.
4. Pålhagen S, Heinonen E, Hägglund J, Kaugesaar T, Mäki-Ikola O, Palm R, Swedish Parkinson Study Group. Selegiline slows the progression of the symptoms of Parkinson disease. Neurology 2006;66(8):1200–6.
5. Mattson MP. Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol. 2000;1(2):120–9.
6. Vila M, Przedborski S. Targeting programmed cell death in neurodegenerative diseases. Nat Rev Neurosci. 2003;4(5):365–75.
7. Fan H-C, Chen S-J, Harn H-J, Lin S-Z. Parkinson's disease: From genetics to treatments. Cell Transplant. 2013;22(4):639–52.
8. Frank T, Schlachetzki JCM, Göricke B, Meuer K, Rohde G, Dietz GPH, Bähr M, Schneider A, Weishaupt JH. Both systemic and local application of granulocyte-colony stimulating factor (G-CSF) is neuroprotective after retinal ganglion cell axotomy. BMC Neurosci. 2009;10(1):49.
9. Tsai K-J, Tsai Y-C, Shen C-KJ. G-CSF rescues the memory impairment of animal models of Alzheimer's disease. J Exp Med. 2007;204(6):1273–80.
10. Yamasaki R, Tanaka M, Fukunaga M, Tateishi T, Kikuchi H, Motomura K, Matsushita T, Ohyagi Y, Kira J-I. Restoration of microglial function by granulocyte-colony stimulating factor in ALS model mice. J Neuroimmunol. 2010;229(1–2):51–62.
11. Huang H-Y, Lin S-Z, Kuo J-S, Chen W-F, Wang M-J. G-CSF protects dopaminergic neurons from 6-OHDA-induced toxicity via the ERK pathway. Neurobiol Aging 2007;28(8):1258–69.
12. Cao X-Q, Arai H, Ren Y-R, Oizumi H, Zhang N, Seike S, Furuya T, Yasuda T, Mizuno Y, Mochizuki H. Recombinant human granulocyte colony-stimulating factor protects against MPTP-induced dopaminergic cell death in mice by altering Bcl-2/Bax expression levels. J Neurochem. 2006;99(3):861–7.
13. Frank T, Klinker F, Falkenburger BH, Laage R, Lühder F, Göricke B, Schneider A, Neurath H, Desel H, Liebetanz D, Bahr M, Weishaupt JH. Pegylated granulocyte colony-stimulating factor conveys long-term neuroprotection and improves functional outcome in a model of Parkinson's disease. Brain 2012;135(Pt 6):1914–25.
14. Meuer K, Pitzer C, Teismann P, Krüger C, Göricke B, Laage R, Lingor P, Peters K, Schlachetzki JCM, Kobayashi K, Dietz GP, Weber D, Ferger B, Schabitz WR. Granulocyte-colony stimulating factor is neuroprotective in a model of Parkinson's disease. J Neurochem. 2006;97(3):675–86.
15. Liao C-H, Chen S-Y, Kuo J-S, Pang C-Y. Reduction of motor disorder in 6-OHDA-induced severe parkinsonism rats by post treatment with granulocyte-colony stimulating factor. Chin J Physiol. 2013;56(3):147–54.
16. Kang H-J, Lee H-Y, Na S-H, Chang S-A, Park K-W, Kim H-K, Kim S-Y, Chang H-J, Lee W, Kang WJ, Koo BK, Kim YJ, Lee DS, Sohn DW, Han KS, Oh BH, Park YB, Kim HS. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: The MAGIC Cell-3-DES randomized, controlled trial. Circulation 2006;114 (1 Suppl):I145–51.
17. Derakhshanrad N, Saberi H, Yekaninejad MS, Eskandari G, Mardani A, Rahdari F, Meybodi KT. Safety of granulocyte colony-stimulating factor (G-CSF) administration for postrehabilitated motor complete spinal cord injury patients: An open-label, phase I study. Cell Transplant. 2013;22 Suppl 1:S139–46.
18. Shyu WC. Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells. Circulation 2004;110(13):1847–54.
19. Sprigg N, Bath PM, Zhao L, Willmot MR, Gray LJ, Walker MF, Dennis MS, Russell N. Granulocyte-colony-stimulating factor mobilizes bone marrow stem cells in patients with subacute ischemic stroke: The Stem cell Trial of recovery EnhanceMent after Stroke (STEMS) pilot randomized, controlled trial (ISRCTN 16784092). Stroke 2006;37(12):2979–83.
20. Shyu W-C, Lin S-Z, Chiang M-F, Pang C-Y, Chen S-Y, Hsin Y-L, Thajeb P, Lee Y-J, Li H. Early-onset Parkinson's disease in a Chinese population: 99mTc-TRODAT-1 SPECT, Parkin gene analysis and clinical study. Parkinsonism Relat Disord. 2005;11(3):173–80.
21. Alves G, Wentzel-Larsen T, Aarsland D, Larsen JP. Progression of motor impairment and disability in Parkinson disease: A population-based study. Neurology 2005;65 (9):1436–41.

22. Kim NK, Choi BH, Huang X, Snyder BJ, Bukhari S, Kong T-H, Park H, Park HC, Park SR, Ha Y. Granulocyte-macrophage colony-stimulating factor promotes survival of dopaminergic neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced murine Parkinson's disease model. Eur J Neurosci. 2009;29(5):891–900.
23. Pezzoli G, Tesei S, Canesi M, Sacilotto G, Vittorio M, Mizuno Y, Mochizuki H, Antonini A. The effect of repeated administrations of granulocyte colony stimulating factor for blood stem cells mobilization in patients with progressive supranuclear palsy, corticobasal degeneration and multiple system atrophy. Clin Neurol Neurosurg. 2010;112(1):65–7.
24. Hilker R, Portman AT, Voges J, Staal MJ, Burghaus L, van Laar T, Koulousakis A, Maguire RP, Pruim J, de Jong BM, Herholz K, Sturm V, Heiss WD, Leenders KL. Disease progression continues in patients with advanced Parkinson's disease and effective subthalamic nucleus stimulation. J Neurol Neurosurg Psychiatry 2005;76(9):1217–21.
25. Nurmi E, Ruottinen HM, Kaasinen V, Bergman J, Haaparanta M, Solin O, Rinne JO. Progression in Parkinson's disease: A positron emission tomography study with a dopamine transporter ligand [18F]CFT. Ann Neurol. 2000;47(6):804–8.
26. Morrish PK, Rakshi JS, Bailey DL, Sawle GV, Brooks DJ. Measuring the rate of progression and estimating the preclinical period of Parkinson's disease with [18F]dopa PET. J Neurol Neurosurg Psychiatry 1998;64(3):314–9.
27. la Fuente-Fernández de R, Schulzer M, Kuramoto L, Cragg J, Ramachandiran N, Au WL, Mak E, McKenzie J, McCormick S, Sossi V, Ruth TJ, Lee CS, Calne DB, Stoessl AJ. Age-specific progression of nigrostriatal dysfunction in Parkinson's disease. Ann Neurol. 2011;69(5):803–10.
28. Pavese N, Rivero-Bosch M, Lewis SJ, Whone AL, Brooks DJ. Progression of monoaminergic dysfunction in Parkinson's disease: A longitudinal 18F-dopa PET study. NeuroImage 2011;56(3):1463–8.
29. Kuriakose R, Stoessl AJ. Imaging the nigrostriatal system to monitor disease progression and treatment-induced complications. Prog Brain Res. 2010;184:177–92.
30. Badoud S, Nicastro N, Garibotto V, Burkhard PR, Haller S. Distinct spatiotemporal patterns for disease duration and stage in Parkinson's disease. Eur J Nucl Med Mol Imaging 2016;43(3):509–16.
31. Pavese N, Rivero-Bosch M, Lewis SJ, Whone AL, Brooks DJ. Progression of monoaminergic dysfunction in Parkinson's disease: A longitudinal 18F-dopa PET study. NeuroImage 2011;56(3):1463–8.
32. Mandel SA, Sagi Y, Amit T. Rasagiline promotes regeneration of substantia nigra dopaminergic neurons in post-MPTP-induced Parkinsonism via activation of tyrosine kinase receptor signaling pathway. Neurochem Res. 2007; 32(10):1694–9.
33. Weinreb O, Amit T, Bar-Am O, Youdim MBH. Rasagiline: A novel anti-Parkinsonian monoamine oxidase-B inhibitor with neuroprotective activity. Prog Neurobiol. 2010; 92(3):330–44.
34. Shyu W-C, Lin S-Z, Lee C-C, Liu DD, Li H. Granulocyte colony-stimulating factor for acute ischemic stroke: A randomized controlled trial. CMAJ 2006;174(7):927–33.
35. Nefussy B, Artamonov I, Deutsch V, Naparstek E, Nagler A, Drory VE. Recombinant human granulocyte-colony stimulating factor administration for treating amyotrophic lateral sclerosis: A pilot study. Amyotroph Lateral Scler. 2010;11(1–2):187–93.
36. Murray D, Stoessl AJ. Mechanisms and therapeutic implications of the placebo effect in neurological and psychiatric conditions. Pharmacol Ther. 2013;140(3):306–18.
37. Udupa K, Fox SH. Placebo effect in Parkinson's disease: Harnessing the mind in the treatment of PD. Mov Disord. 2015;30(6):786.