PAPER

Carotid body autotransplantation in Parkinson disease: a clinical and positron emission tomography study

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Background: Carotid body (CB) glomus cells are highly dopaminergic and express the glial cell line derived neurotrophic factor. The intrastriatal grafting of CB cell aggregates exerts neurotrophic actions on nigrostriatal neurons in animal models of Parkinson disease (PD).
Objective: We conducted a phase I–II clinical study to assess the feasibility, long term safety, clinical and neurochemical effects of intrastriatal CB autotransplantation in patients with PD.
Methods: Thirteen patients with advanced PD underwent bilateral stereotactic implantation of CB cell aggregates into the striatum. They were assessed before surgery and up to 1–3 years after surgery according to CAPIT (Core Assessment Programme for Intracerebral Transplantation) and CAPSIT-PD (Core Assessment Programme for Surgical Interventional Therapies in Parkinson's Disease) protocols. The primary outcome measure was the change in video blinded Unified Parkinson's Disease Rating Scale III score in the off-medication state. Seven patients had ^{18}F-dopa positron emission tomography scans before and 1 year after transplantation.
Results: Clinical amelioration in the primary outcome measure was observed in 10 of 12 blindly analysed patients, which was maximal at 6–12 months after transplantation (5–74%). Overall, mean improvement at 6 months was 23%. In the long term (3 years), 3 of 6 patients still maintained improvement (15–48%). None of the patients developed off-period dyskinesias. The main predictive factors for motor improvement were the histological integrity of the CB and a milder disease severity. We observed a non-significant 5% increase in mean putaminal ^{18}F-dopa uptake but there was an inverse relationship between clinical amelioration and annual decline in putaminal ^{18}F-dopa uptake ($r = -0.829$; $p = 0.042$).
Conclusions: CB autotransplantation may induce clinical effects in patients with advanced PD which seem partly related to the biological properties of the implanted glomus cells.

Parkinson disease (PD) is a progressive neurodegenerative disorder of unknown aetiology. Its main pathological hallmark is the degeneration of midbrain dopaminergic neurons projecting to the striatum, although other neuronal systems are also affected.[1] Current pharmacological and surgical therapies are symptomatically effective but their long term utility is limited because of disease progression.[2 3] Therefore, there is a need for neuroprotective and/or neurorestorative therapies capable of arresting or reversing the neurodegenerative process.

Over the past two decades, cell replacement therapies have been tested in PD patients with the objective of restoring the striatal dopaminergic deficit.[4] Transplantation of fetal mesencephalic neurons, the most frequently used technique, can increase the striatal dopamine storage, but does not always produce the expected clinical benefit and may induce disabling off-medication dyskinesias.[5 6] Thus it appears that the ectopic placement of dopamine secreting cells in the striatum is not the ideal approach to compensate for progressive nigrostriatal neuronal loss.[7] Given this scenario, the clinical applicability of other transplantation procedures based on a similar rationale (eg, intrastriatal grafting of porcine mesencephalic neurons, retinal pigment epithelial cells or stem cell derived dopaminergic neurons) is, for the moment, uncertain.

More recently, other strategies aiming to protect or restore the nigrostriatal pathway have emerged. Glial cell line derived neurotrophic factor (GDNF) has been shown to exert neuroprotective and neurorestorative actions in animal models of PD.[8–10] The clinical efficacy of GDNF has been assayed in clinical trials, but the method of delivery is a critical issue. Whereas intraventricular administration failed to induce clinical benefit,[11] intraputaminal infusion showed promising results,[12 13] although a placebo controlled trial using this route has been halted because of lack of efficacy and safety concerns about recombinant human GDNF administration.[14] Other alternative methods being tested experimentally in parkinsonian animals include in vivo gene therapy using GDNF encoding viral vectors[15–17] and the intrastriatal grafting of recombinant GDNF producing cell lines.[18–21] Carotid body (CB) glomus cells are neural crest derived dopaminergic cells that express high levels of GDNF. Glomus cell GDNF production is resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration, and maintained in aged rodents or after intrastriatal grafting.[22 23] The survival rate of these cells after transplantation (>70%) is particularly high as hypoxia stimulates their growth and function. Moreover, CB grafts performed in young rats remain active for the entire animal lifespan.[22 23] Transplantation of CB cell aggregates has been shown to induce

Abbreviations: CAPIT, Core Assessment Programme for Intracerebral Transplantation; CAPSIT-PD, Core Assessment Programme for Surgical Interventional Therapies in Parkinson's Disease; CB, carotid body; GDNF, glial cell line derived neurotrophic factor; PD, Parkinson disease; PET, positron emission tomography; TH, tyrosine hydroxylase; UPDRS, Unified Parkinson's Disease Rating Scale

a neurotrophic mediated recovery in animal models of PD[22–27] and stroke.[28 29]

We conducted a phase I–II video blinded clinical study to assess the long term safety, clinical and neurochemical effects of intrastriatal CB autotransplantation in patients with advanced PD. In a pilot report of our first six patients, we showed this procedure to be feasible.[30] Here we report the clinical outcomes and prognostic factors in the whole study (n = 13), as well as ^{18}F-dopa positron emission tomography (PET) outcomes in a subgroup of patients (n = 7).

METHODS

Patients

We recruited 14 consecutive patients with PD and motor complications (henceforth designated by their order number). The inclusion criteria were: (1) diagnosis of PD according to the London Brain Bank criteria; (2) age younger than 65 years; (3) a history of the disease for more than 5 years; (4) presence of motor fluctuations; (5) Hoehn and Yahr stage 3 or above in the off-medication state, (6) functional disability despite optimal pharmacological treatment; and (7) the ability to provide freely given informed consent and follow the study protocol. The exclusion criteria were: (1) the presence of other diseases that may increase surgical risk or interfere with study outcomes; (2) previous cranial or carotid surgery; (3) women in fertile age unless adequate birth control methods were being used; (4) quality of life limited for reasons other than PD; and (5) major cognitive or psychiatric comorbidity.

The study was approved by the Ethics Review Committee of Virgen de las Nieves University Hospital, Granada, Spain (patient selection, surgery and clinical follow-up) and those of Hammersmith, Queen Charlotte's and Chelsea and Acton Hospitals, London, UK (^{18}F-dopa PET studies). All patients gave their written informed consent.

Surgical procedure

The transplantation procedure was completed in 13 of 14 selected patients (see Results: procedure feasibility). The surgical technique has been described in detail elsewhere.[30] In brief, a single surgical intervention was performed under general anaesthesia involving three steps: (1) the right CB was removed; (2) it was divided into approximately 100–200 pieces; CB cell aggregates from patient Nos 1–5 were additionally subjected to mild enzymatic treatment (1 mg/ml trypsin, 1 mg/ml collagenase and 0.2 mg/ml deoxyribonuclease for 20 min); and (3) these aggregates were implanted stereotactically into the striatum: three targets in each putamen (posterior, middle and anterior); patient Nos 5 and 6 underwent further implants in the head of each caudate nucleus. A 0.5 T MRI was obtained before surgery and during the first postoperative week to assess the localisation of surgical tracks and possible structural complications.

Table 1 Presurgical characteristics of the 13 transplanted patients (see also table 3)

Baseline characteristic (n = 13)	
Sex (M/F)	7/6
Age (y)	52 (5) (43–61)
Duration of disease (y)	11 (4) (7–18)
Levodopa response (%)	70 (12) (51–93)
Hoehn and Yahr stage in ''off'' (3/4)	3/10
CB histological integrity (0/1/2/3/4)	1/3/3/4/2

CB, carotid body; Levodopa response, percentage of improvement in Unified Parkinson's Disease Rating Scale III scores when passing from ''off'' to ''on'' states.
For quantitative variables, values are means (SD) and ranges in brackets.
For qualitative variables, values are absolute frequencies of each category in the order expressed in each case.

Clinical evaluations

Patients were assessed before surgery and every 3 months after surgery in accordance with the Core Assessment Programme for Intracerebral Transplantation (CAPIT)[31] and Core Assessment Programme for Surgical Interventional Therapies in Parkinson's Disease (CAPSIT-PD)[32] protocols, including extensive clinical evaluation in both off-medication and on-medication states. The main variables recorded at each assessment were: Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn and Yahr staging, Schwab and England scale, time spent in ''off'' obtained from a diary of fluctuations for the preceding week (categorised as indicated in item 39 of the UPDRS) and CAPIT Dyskinesia Rating Scale.

The primary outcome variable was the UPDRS III (motor subscale) score in the off-medication state evaluated by an independent neurologist in a blinded fashion from masked and randomly presented video sequences. Rigidity subscore (item 22) cannot be evaluated from video images and therefore was added from open assessments.

The antiparkinsonian medication was optimised in all patients during the months before the surgery and was only modified after surgery when major clinical changes occurred. Medication was reduced when we observed amelioration of the off-medication periods or if there was an intensification of the adverse effects (such as on-period dyskinesias). The appropriate increase was ordered if there was a rise in the severity or duration of off-medication periods.

A neuropsychological evaluation was performed before surgery and at 6–12 months after transplantation, including several tests to assess visuomotor coordination, perception, attention, verbal and visual memory, language and executive functions. The core battery included: Rey Complex Figure Test, logical memory sub-test of the Barcelona Test, Boston Naming Test, Stroop Colour–Word Test, phonemic and semantic verbal fluency tests and similarities subtest of the Wechsler Adult Intelligence Scale.

^{18}F-dopa positron emission tomography

A subgroup of seven patients (Nos 7–9 and 11–14) had preoperative and 1 year postoperative ^{18}F-dopa PET scans using an ECAT EXACT HR++ camera (CTI/Siemens 966; Knoxville, Tennessee, USA). After withdrawal of medication for at least 12 h, patients received 150 mg of carbidopa and 400 mg of entacapone. ^{18}F-dopa (111 MBq) in normal saline was administered 1 h later as an intravenous bolus at the start of scanning. Images were acquired in three dimensional mode as 26 time frames over 94.5 min. After motion correction, parametric influx constant (Ki) images were generated from time frames 25.5–94.5 min after injection using inhouse software based on the multiple time graphical analysis approach of Patlak and Blasberg.[33] The images were then interrogated using regions of interest and statistical parametric mapping techniques. PET studies and subsequent analysis were carried out by an independent research team unaware of clinical outcomes (Cyclotron Unit, Hammersmith Hospital, London, UK).

Carotid body histology

During the surgical procedure, a small marginal piece of CB was kept for subsequent histological analysis. Tyrosine hydroxylase (TH) immunohistochemistry was performed (further details are given elsewhere[30]) and a qualitative classification of the CB histology was established by investigators unaware of clinical outcomes. This variable was called ''histological integrity'' ranging from ''0'' (almost complete absence of glomeruli

Table 2 Adverse events in the 13 transplanted patients*

Adverse event	Time from surgery	Patients (No. order)	Sequelae
Immediate			
Mild oedema of cervical soft tissues	1st day	6; 12	No
Lacunar infarct	1st day	13	Hemiplegia
Cortical haemorrhage/seizure	1st day	14	No
Nosocomial pneumonia	3rd day	3	No
1st postoperative year			
Worsening of previous hypertension	1st month	12	Treated hypertension
Transient cervical radiculopathy	4th month	8	No
Humerus fracture (fall)	7th month	13	No
Wrist fracture (fall)	8th month	7	No
Scapula fracture (fall)	10th month	3	No
Hip fracture (fall)	12th month	4	No
2nd and 3rd postoperative years*			
Worsening of previous ''on'' dyskinesia	2nd year	4	''On'' dyskinesia
Shoulder dislocation (fall)	30th month	5	No

*The 2nd and 3rd years refers only to the six patients with longer follow-up. The patient's number refers to the order of recruitment (1–14).

containing TH positive cells) to ''4'' (uniform distribution of glomeruli with abundant TH positive cells). Considering that a human grade ''4'' CB contains approximately 50 000 glomus cells, this histological grading may be used as an estimation of the amount of glomus cells transplanted to each patient. However, it must be taken into account that the true CB integrity might have been underestimated, because to maximise the amount of tissue used for transplantation, the piece kept for histological analysis was selected from peripheral, more fibrous, parts of the organ.

Statistical analysis

For quantitative variables, means (SDs) were calculated and the one sample Komolgorov–Smirnov test of normality was applied. The magnitude of the change at 6 and 12 months after surgery was calculated, and pre and postsurgical values were compared by Wilcoxon's signed rank test. When appropriate, a general linear model for repeated measures (analysis of variance, ANOVA) was also applied. Association between clinical and neurochemical outcomes was evaluated by non-parametric Spearman correlation coefficients. Predictive factors for clinical efficacy were analysed by paired Spearman coefficients and multivariate linear regression. All tests applied were two tailed, and $p<0.05$ was considered significant. The SPSS 12.0 programme (SPSS Inc, Chicago, Illinois, USA) was used for the statistical analysis.

RESULTS

Baseline clinical characteristics

The presurgical characteristics of the 13 transplanted patients are given in table 1 (see also baseline clinical values in table 3).

Most of the patients had early onset PD with a mean age at the beginning of motor symptoms of 41 years (range 35–48). At the time of surgery, mean patient age was 52 years. All patients had motor fluctuations with ''off'' periods of variable duration, with bradykinesia and rigidity as predominant features (only patient No 1 had more severe tremor). Their levodopa response was good, with a mean 70% improvement in UPDRS III when patients passed from the ''off'' to the ''on'' motor state. Most of the patients had slight to mild ''on'' dyskinesia and only patient No 4 had moderate dyskinesia.

Table 3 Clinical outcomes after transplantation (n = 12)

Clinical variable (n = 12)	Baseline value	6 months			1 year		
		Value	Change†	p Value‡	Value	Change†	p Value‡
UPDRS total score in ''off'' (0–199)	71.4 (24.3)	55.8 (21.7)	−22% (19.7)	0.006**	60.8 (23.4)	−15% (21.5)	0.034*
UPDRS II (ADL) score in ''off'' (0–52)	20.7 (6.9)	17.3 (5.9)	−16% (15.9)	0.009**	16.8 (7.0)	−20% (14.4)	0.005**
UPDRS III (motor) score in ''off'' (0–108)	41.7 (15.7)	32.3 (13.9)	−23% (22.3)	0.006**	37.0 (14.1)	−9% (28.4)	0.120
Tremor subscale (blinded) (0–28)	5.2 (5.2)	3.1 (3.8)	−2.1 (2.6)	0.016*	4.7 (4.5)	−0.5 (1.9)	0.256
Rigidity subscale (0–20)	9.6 (2.8)	7.9 (3.6)	−1.7 (3.3)	0.067	8.4 (3.4)	−1.2 (2.5)	0.138
Bradykinesia subscale (blinded) (0–32)	14.6 (4.4)	11.7 (5.4)	−2.9 (3.2)	0.011*	13.6 (5.3)	−1.0 (5.7)	0.348
Posture/gait subscale (blinded) (0–12)	5.2 (2.7)	3.9 (1.6)	−1.3 (1.6)	0.016*	4.0 (2.3)	−1.2 (1.5)	0.019*
UPDRS IV score (0–23)	8.3 (3.1)	5.3 (2.8)	−32% (36.1)	0.010**	5.8 (2.3)	−22% (37.2)	0.026*
Hoehn and Yahr stage in ''off'' (1–5)	3.8 (0.5)	3.3 (0.8)	−0.5 (0.6)	0.034*	3.4 (0.8)	−0.3 (0.5)	0.066
Schwab and England scale in ''off'' (0-100)	51.7 (23.7)	67.5 (18.2)	16.0 (13.1)	0.006**	62.9 (21.2)	11.0 (7.4)	0.004**
Time spent in ''off'' (0–4)	1.7 (0.7)	1.7 (0.8)	0.0 (0.9)	1	1.7 (0.7)	0.0 (0.6)	1
CAPIT Dyskinesia Rating Scale (0–5)	1.3 (1.0)	1.0 (0.9)	−0.3 (0.5)	0.098	0.9 (1.1)	−0.4 (0.5)	0.026*
Levodopa equivalent dose (mg/day)	1072 (452)	1033 (459)	−4% (10.5)	0.173	969 (483)	−10% (22.4)	0.059

ADL, activities of daily living; CAPIT, Core Assessment Programme for Intracerebral Transplantation; UPDRS, Unified Parkinson's Disease Rating Scale.
†Change refers to the mean (SD) of the distribution of individual changes (paired comparisons with baseline values). Minus sign denotes reduction (improvement for all clinical scales except Schwab and England scale) and plus sign increment.
‡p Value was calculated from paired comparisons with baseline (Wilcoxon's signed rank test): *⩽0.05; **⩽0.01.
Values are mean (SD).
The range of values is expressed in parentheses. ''Posture/gait subscale'' is the sum of UPDRS items 28, 29 and 30. ''Time spent in off'' refers to UPDRS item 39. A higher value indicates greater severity in all clinical scales, except in the Schwab and England scale.
The daily levodopa equivalent dose was calculated based on the following equivalences: 100 mg standard levodopa = 140 mg controlled release levodopa = 1 mg pergolide = 1 mg pramipexole = 5 mg ropinirole = 10 mg bromocriptine = 1.5 mg cabergoline = 10 mg selegiline = 100 mg amantadine.

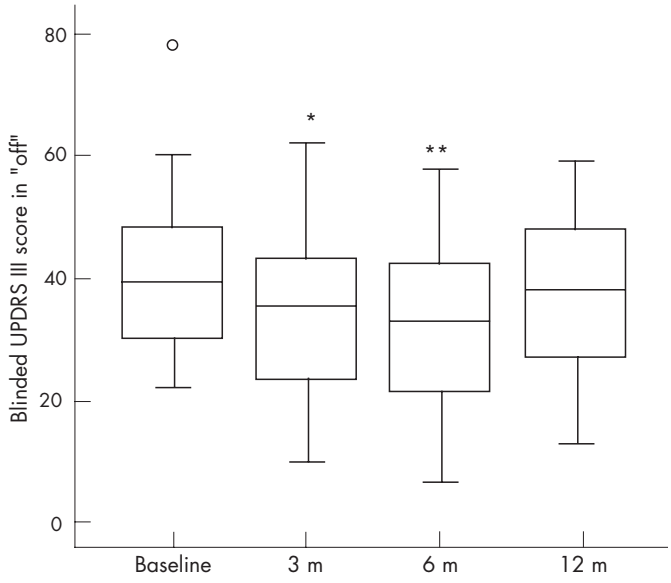

Figure 1 Box plots showing the evolution of blinded Unified Parkinson's Disease Rating Scale (UPDRS) III scores in the off-medication state (''off'') in the first year after transplantation (n = 12). The baseline value indicated as a circle represents an ''outlier'' (patient No 1).*$p \leqslant 0.05$; **$p \leqslant 0.01$ (comparison with baseline; Wilcoxon's signed rank test).

Carotid body histology

The histological integrity of the peripheral CB piece was classified as ''3'' or ''4'' in six transplanted patients (46%) and only patient No 3 had no TH positive cells in the studied fragment (table 1). A trend was observed towards a higher integrity in younger patients ($p = 0.127$).

Procedure feasibility

Transplantation was achieved in 13 of the 14 selected patients. Patient No 10 did not undergo cranial surgery after the finding of a highly fibrous CB. The decision not to proceed to implantation was based on the observation that patient No 3, who also had a fibrotic CB, had not obtained benefit after transplantation.[30] Because of technical problems in patient No 5, the amount of CB cell aggregates deposited in the left hemisphere was less than 30% of that on the contralateral side.[30] In the rest of the patients the procedure was completed without major incident. Postsurgical MRI showed cannula tracks to the intended targets in all cases, although the image resolution (voxel size ~3 mm^3) was not high enough to provide an objective evaluation of the size and localisation of the implants.

Adverse events

The adverse events that required medical intervention are listed in table 2.

Two intracranial complications arose: (i) patient No 13 suffered a lacunar infarct located in the right internal capsule during the immediate postoperative period, with motor sequelae; (ii) patient No 14 had a small cortical haemorrhage adjacent to the left burr hole, responsible for an epileptic seizure in the first postoperative day, which resolved without sequelae. During the follow-up period, five patients had a bone fracture or dislocation secondary to accidental falls (the affected patients were older than the non-affected ones: 57 (3) vs 49 (4) years; $p = 0.013$). Patient No 4 experienced a progressive worsening of her moderate presurgical on-period

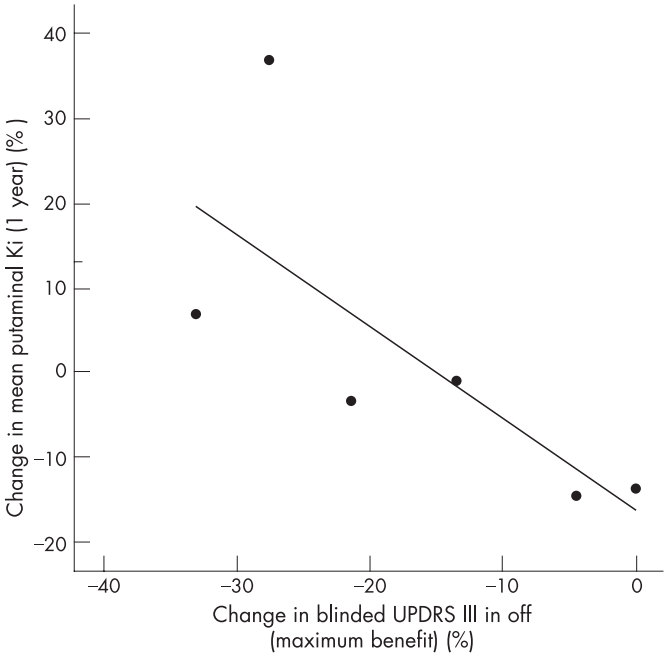

Figure 2 Correlation between clinical and neurochemical outcomes (n = 6). Values are percentage change with respect to baseline. Minus sign (reduction) denotes improvement for Unified Parkinson's Disease Rating Scale (UPDRS) III scores and worsening for parametric influx constant (Ki) values. A linear regression fit is superimposed on the data points ($r = -0.829$, $p = 0.042$; non-parametric Spearman coefficient).

Table 4 Evolution of ^{18}F-dopa positron emission tomography Ki values at 1 year after transplantation (n = 7)

		1 year			
Neurochemical variable ^{18}F-dopa PET (n = 7)	**Baseline value (mean (SD))**	**Value (mean (SD))**	**Change† (min; max)**	**Change† (mean (SD))**	**p Value‡**
More damaged putamen Ki	0.0043 (0.0009)	0.0047 (0.0010)	(−28%; 73%)	12% (32.1)	0.397
Less damaged putamen Ki	0.0056 (0.0013)	0.0056 (0.0010)	(−31%; 49%)	4% (23.5)	0.596
Mean putamen Ki	0.0049 (0.0009)	0.0051 (0.0010)	(−15%; 37%)	5% (19.7)	0.735
More damaged caudate Ki	0.0085 (0.0018)	0.0087 (0.0016)	(−17%; 31%)	3% (15.6)	0.586
Less damaged caudate Ki	0.0104 (0.0016)	0.0102 (0.0020)	(−22%; 23%)	−1% (14.4)	0.917
Mean caudate Ki	0.0095 (0.0017)	0.0094 (0.0018)	(−20%; 16%)	1% (13.3)	0.917

Ki, parametric influx constant; PET, positron emission tomography.
†Change refers to the distribution of individual changes (paired comparisons with baseline expressed in percentage): lower and higher values are displayed in the first column and the mean (SD) in the second column. Minus sign denotes reduction (worsening) and plus sign increment (improvement).
‡p value, see footnote to table 3.
The ''more damaged'' putamen or caudate (lower Ki value) was the left in all but two patients. ''Mean putamen or caudate Ki'' denotes the averaged right and left values.

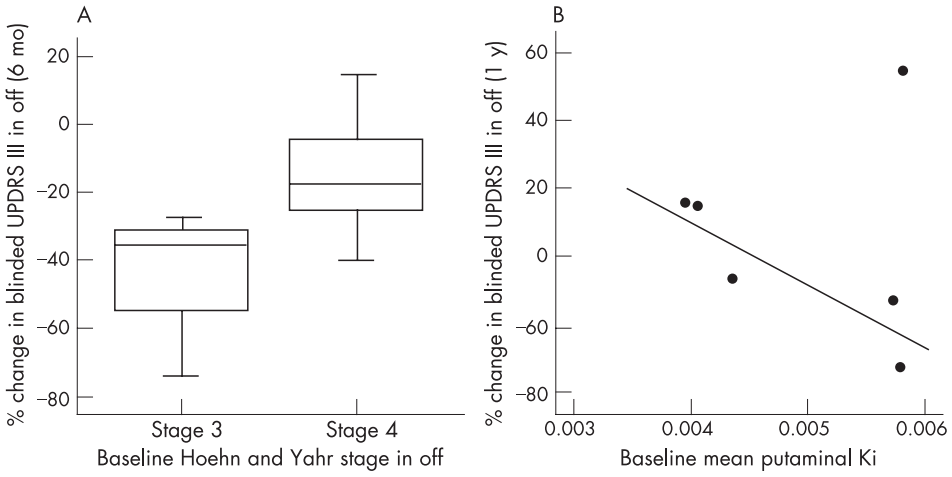

Figure 3 Severity of disease as a prognostic factor for carotid body autotransplantation. (A) Box plots showing the relationship between baseline Hoenh and Yahr stage and clinical outcome (n = 12; p = 0.033, Mann–Whitney U test). (B) Correlation between baseline putaminal parametric influx constant (Ki) values and clinical outcome (n = 6). For baseline putaminal Ki, a lower value denotes more severe disease. For Unified Parkinson's Disease Rating Scale (UPDRS) III percentage change, a minus sign (reduction) denotes improvement. A linear regression fit is superimposed on data points excluding the outlier value of patient No 12 ($r = -1$, $p < 0.0001$; non-parametric Spearman coefficient).

dyskinesias during the second postoperative year. None of the patients developed off-period dyskinesias.

Study outcomes after 1 year of follow-up

Clinical outcomes

Clinical variables at 6 and 12 months after transplantation and the magnitude of the changes in the 12 evaluated patients are displayed in table 3 (patient No 13 was excluded from the overall efficacy analysis because of motor sequelae which specifically prevented video blinded assessments).

Primary clinical outcome variable

The blinded UPDRS III off-medication score improved progressively during the first months after surgery (fig 1), reaching a mean reduction of 23% at 6 months ($p = 0.006$). The improvement was dependent on statistically significant reductions in scores for tremor, bradykinesia and posture/gait subscales (see table 3). At 1 year, only the first subgroup of six patients still maintained a significant improvement ($p = 0.018$, ANOVA for repeated measures).

Individually, two patients never improved their blinded UPDRS III score, and the remaining 10 patients improved by 5–74% at 6 months and by 7–52% at 12 months. The improvement was higher than 20% of the presurgical value in seven patients at 6 months, and in four patients at 12 months. ''Double-blind'' assessments of patient No 5, successfully transplanted only in the right hemisphere (see above), showed an UPDRS III improvement only in the left hemi-body.[30]

Secondary clinical outcome variables (see table 3)

The total UPDRS score in the off-medication state, as well as the UPDRS II (activities of daily living) and UPDRS IV (complications) subscale scores, significantly improved at 6 and 12 months. The Schwab and England scale score in ''off'' also significantly improved during the first year after transplantation. There were no significant changes in UPDRS scores in the best on-medication state (data not shown) or in the time spent in ''off'' (item 39 of UPDRS). The CAPIT Dyskinesia Rating Scale progressively improved, reaching a statistically significant mean reduction of half a point at 1 year ($p = 0.026$). The levodopa equivalent dose was reduced by a mean of 10% at 1 year ($p = 0.059$), this reduction being higher than 25% in one-third of patients.

Neuropsychological variables

Overall, no statistically significant changes were observed in neuropsychological tests at 6–12 months after transplantation. The only exception was an improvement in visual memory measured by the delayed recall score of the Rey Complex Figure Test ($p = 0.033$), although it could be related to a learning effect.[34]

Neurochemical outcomes

In the seven patients studied by ^{18}F-dopa PET (a subgroup with modest clinical outcome), changes in Ki values at 1 year were highly variable (table 4) and, overall, no significant difference was found. Statistical parametric mapping 99 technique failed to localise any cluster of voxels of significantly increased ^{18}F-dopa uptake. However, instead of the expected yearly decrement in putaminal ^{18}F-dopa uptake characteristic of advanced PD patients (estimated to be approximately 10%[35]), we observed a trend towards a 5% increment. Moreover, there was a significant inverse relationship between clinical amelioration and annual decline in putaminal uptake ($r = -0.829$; $p = 0.042$) (fig 2). Thus those patients who obtained a greater motor improvement exhibited a slower disease progression on PET 1 year after transplantation. When this clinical PET correlation was analysed separately in the two hemibodies-contralateral hemispheres of each patient (n = 6), the association was clearly stronger in the less damaged hemispheres ($r = -0.771$) than in the most affected ones ($r = -0.086$).

Long term follow-up

Patient Nos 1–6 were evaluated for 3 years after transplantation. Patient No 3, who did not obtain clinical benefit, was subjected to bilateral deep brain stimulation 2 years after transplantation. For the other five patients, maximum reductions in blinded UPDRS III in off-medication (from 26% to 74%) were achieved 6–12 months after transplantation. In spite of a progressive trend towards presurgical values, after 2 years of follow-up these five patients maintained improvements of 14–33%. At 3 years, the scores of three patients were still above their presurgical values (improvements from 15% to 48%). Patient No 2 had an improvement in the primary outcome variable higher than one-third of the presurgical value during the 3 years of follow-up, which was accompanied by a sustained reduction in ''off'' time and meaningful improvement in functional scales.

Prognostic factors

Prognostic factors for clinical efficacy were analysed considering the percentage change in blinded UPDRS III off-medication score as the main dependent variable (n = 12). The following three major factors were found.

Histological integrity of CB

This was the main predictive factor for the magnitude of clinical efficacy at 6 months in the multivariate lineal regression analysis ($r^2 = 0.82$; $p = 0.002$). Greater motor improvement was significantly associated with higher CB integrity, even when other sociodemographic or clinical variables were equal. However, the influence of this biological factor was significantly stronger in the first subgroup of six patients ($p = 0.027$), and this statistical interaction was included in the overall multivariate model.

Severity of disease

The improvement in the off-medication motor score at 6 months was significantly greater in patients in presurgical Hoehn and Yahr stage 3 with respect to those in stage 4 ($p = 0.033$) (fig 3A). In the subgroup of patients studied by ^{18}F-dopa PET, we also observed a trend towards a better clinical outcome in those with higher baseline putamen Ki values (fig 3B).

Patient's age

In the first six patients, younger age was associated with greater improvement at 1 year[30]; however, this trend was not significant in the analysis of the whole series.

DISCUSSION

Results shown here indicate that CB autotransplantation may induce a variable, generally modest, degree of clinical amelioration in patients with advanced PD associated with a slower rate of neurochemical decline. An open trial is a reasonable first approach to evaluate a new therapy when the optimal methodological issues are not precisely known, although clinical results must be interpreted cautiously because they are subject to patient and observer biases. In our study, observer biases were carefully minimised, but the magnitude of the placebo effect could not be formally addressed.

Despite these major limitations, analysis of our data suggests the existence of true biological effects derived from CB grafting. Firstly, the time evolution and magnitude of blinded UPDRS III changes in our study closely resemble the results of the most favourably responding subgroups in double blind placebo controlled trials on fetal mesencephalic transplantation, whose outcomes were significantly different to placebo.[5 6] Furthermore, the placebo effect on blinded UPDRS III was absent in these studies: the control group in the trial by Olanow *et al* experienced about 8% worsening at 6 months and 22% at 24 months (approximate data obtained from published figures).[6] In the trial by Freed *et al*,[5] the placebo effect was found to be strong on quality of life measures when patients thought they received the transplant, which also resulted in better motor scores, as rated by medical staff.[36] In this respect, the possibility of an indirect influence of the patient over the examiner was prevented in our study by well designed video blind ratings of motor scores. Although rigidity subscores were added from open assessments, statistical significant changes in UPDRS did not depend on this subscale, but on the other three blinded subscales (see table 3). Secondly, predictive factors for motor improvement in our study were the integrity of donor tissue (an estimation of the amount of dopamine/GDNF producing cells) and a milder disease severity, in agreement with experimental evidence[22 23] and with one of the above mentioned double blind trials.[6] Thirdly, double blind assessment of one patient who was successfully transplanted in only one hemisphere (although received needle tracks bilaterally) further suggests, as in animal experiments,[22–25] that the effects are mediated by CB cells rather than by surgical lesions in the striatum. Finally, correlation between clinical and neurochemical outcomes, especially in the less damaged hemispheres, might reflect a neuroprotective effect on nigrostriatal neurons. This finding is in accordance with previous laboratory evidence, suggesting that the biological effects of intrastriatal CB transplants depend on neurotrophic actions rather than on dopamine cell replacement alone.[22 23] Of note, our patients, unlike those subjected to fetal transplantation,[5 6] did not significantly increase their ^{18}F-dopa uptake or develop off-period dyskinesias, and they obtained comparable clinical outcomes when grafted with much lower number of cells (tens of thousands vs millions).

The safety of CB autotransplantation was mainly determined, as in other stereotactic procedures, by the risk of intracranial complication. The incidence rate of these complications in large series of patients that underwent either fetal transplantation[37] or deep brain stimulation[38] has been estimated at approximately 5%. In our study, two patients suffered an intracranial complication, although because of the small sample size this proportion was not significantly higher than a maximal incidence rate of 5% ($p = 0.184$; Fisher exact test). Because resection of CB tumours may induce a baroreflex failure due to carotid sinus denervation,[39] we systematically monitored cardiovascular function in our patients. One patient, with previously treated hypertension, experienced increased blood pressure after the first postoperative month, although other features suggestive of baroreflex failure were absent. Only one patient (who presented moderate presurgical dyskinesia) experienced a slowly progressive worsening of on-period dyskinesia after the first postoperative year, probably related to the progression of PD. It is worth mentioning that the older patients in our cohort had a relatively high incidence of complications related to falls, although a control group to establish comparisons is lacking. Whereas a direct relationship to transplantation seems unlikely, we cannot rule out an indirect relationship as trial participation could have forced the patients to increase their physical activity[30].

It must be acknowledged that, from a clinical standpoint, the magnitude and duration of clinical changes in most of our advanced, relatively young, PD patients did not greatly reverse their disability. Therefore, at the current stage, CB autotransplantation cannot be considered as a realistic therapeutic option. Nevertheless, the possibility of GDNF mediated neuroprotection on nigrostriatal neurons should encourage additional research. In this respect, several methodological issues should be addressed before considering further development of the procedure: (i) the characteristics of suitable candidates and the objectives of therapy should be clearly defined; (ii) the origin and quantity of donor tissue should be determined (although autotransplantation is conceptually attractive, several limitations deserve consideration); and (iii) methodological aspects relative to cell processing and implantation should be further refined.

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