INTRODUCTION

Brain transplantation of neurons may act by two different mechanisms: 1) by a drug delivery system: e.g. dopaminergic (DA) neurons in Parkinson’s disease (PD) or indirect gene therapy in Huntington’s disease (HD), and 2) by organotypic reconstruction: e.g. striatal neurons in HD. Parkinson’s disease is not a lethal disease, and surgery has to be safe, and effective for many years. Today, surgery is considered only in severe PD patients at stages IV and V of the Hoehn and Yahr scale, after optimization of drug therapy. Several preclinical phases are mandatory: 1) a biotechnological one, e.g. transfection of cells, vector design for gene therapy; 2) preclinical trials: in vitro, in vivo rodents, then large animal (primate) studies; finally, 3) clinical trials, classifiable into three categories: phase 1 or pilot, phase 2, often controlled, including dose-response assessment, and phase 3, which involve large cohorts of patients who are randomized to receive the study treatment or a control treatment. In this paper, we do not consider adrenal autografts, which have been abandoned, but concentrate on the heterotopic allografting of fetal mesencephalic DA neurons (1).
LONG-TERM REPORTED RESULTS

Most published reports are phase 1, pilot trials that have included few patients, and assessed uni- or bilateral transplant; the patient serves as his own control, and Positron Emission Tomography (PET) using radiolabelled Levodopa is mandatory to evaluate the results. Restricting ourselves to published trials with a long-term follow up and with PET performed in all patients, we find that only few are available (2-5), amounting to a total of fewer than 30 patients (Table I).

The results can be summarized as follows: not all patients are responders, grafting is followed by a 30% decrease in daily L-dopa intake, with an improvement in duration and severity of defined “off” periods and a decrease in the UPDRS score measured in “off” periods. The trials also demonstrated good safety. Since the first pilot trials began more than 10 years ago, one question must be asked: why, with this treatment, has there been no progression to phase 2-3 trials or clinical practice, as in the case of deep brain stimulation (DBS)? Technical and ethical concerns may be the main reasons.

Only one phase 2 trial has been completed (Freed et al., as yet unpublished): 36 PD patients were randomized, and a control group receiving sham surgery (general anesthesia, incomplete craniotomy) allowed a double blind follow up by self-reporting, neurological evaluation, and PET scanning. The design was very costly, but gave “statistically significant results”. A moderate improvement of motor symptoms was observed along with a decrease in daily antiparkinsonian drug intake. The patients’ own answers constituted the major endpoint of the trial, and statistically more patients in the treatment group reported, after one year, that they believed themselves to have been grafted, whereas no difference emerged between the groups after 3 months. Ethical concerns relating to sham surgery have been discussed elsewhere (6).

CAN THE BRAIN ALLOGRAFT BE A LONG-TERM TREATMENT FOR PD PATIENTS?

The answer to this question must be based on long-term clinical results, as well as on long-term PET follow-up of grafted areas and evolution of non-grafted striatum. We look at the results of a case with a long follow up: this male, 49 years old, stage V of the Hoehn and Yahr scale, was unable to walk in “off” state and complained of severe drug induced dyskinesias in “on”; two unilateral transplantations were performed at an 18-month interval (1993 and 1994). Three months after the first graft, the percentage of daily “on” periods increased from 40 to 70%, and the patient was able to suspend the subcutaneous injections of apomorphine. This percentage rose to 80% after the second graft, while “off” phases became less severe (his Hoehn and Yahr score dropped from 5 to 4 in “off”), and daily drug intake decreased, over 5 years, from 1200 to 250 mg, with concomitant reduction in dyskinesias. Immunosuppression was reduced 6 months after, and stopped one year after, the second graft. Two severe adverse events occurred: a confusional state with excitation and hypersexuality immediately after the first graft, a transient frontal syndrome with minor bleeding along the needle tract after the second session. Both were transient. No major cognitive change was measured at the 1-, 2- and 3-year follow ups. No improvement was noticed in vegetative symptoms, and the patient had to be administered drugs for orthostatic hypoten-

Table I - Long-term brain fetal allograft studies with clinical and PET follow up

<table>
<thead>
<tr>
<th>Study</th>
<th>Author</th>
<th>Year</th>
<th>Number of Patients</th>
<th>Side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defer et al., 1996</td>
<td>(ref. 2)</td>
<td>5</td>
<td>Unilateral</td>
<td></td>
</tr>
<tr>
<td>Wenning et al., 1997</td>
<td>(ref. 3)</td>
<td>10</td>
<td>Unilateral</td>
<td></td>
</tr>
<tr>
<td>Hauser et al., 1999</td>
<td>(ref. 4)</td>
<td>6</td>
<td>Bilateral</td>
<td></td>
</tr>
<tr>
<td>Hagell et al., 1999</td>
<td>(ref. 5)</td>
<td>5</td>
<td>Bilateral</td>
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sion. On the basis of “favourable results” of this kind, which cannot be reproduced in all cases, transplantation may be viewed as a palliative treatment for motor symptoms in PD, and has been shown to improve quality of life (7).

BRAIN IMAGING

MRI shows the tracts and a hypersignal in the region of the graft during the first 12 months. After several years, this turns into a T2 hyposignal, with a minimal growth of the size of the image (12). PET imaging with labelled fluorodopa typically shows an increase in Ki, meaning an increase of uptake in the striatum as compared to the cortex. We reported a statistical correlation between PET data and finger dexterity measurements, or daily “on”, self-reported by the patients (8). This supported the physiological function of the transplanted neurons, recently demonstrated by Piccini et al. (9). In one patient, who had received a transplant in the right putamen 10 years earlier, grafts had restored both basal and drug-induced dopamine release to normal levels. This was associated with sustained, marked clinical benefit and normalized levels of dopamine storage in the grafted putamen. This elegant clinical experiment confirms that the brain allograft can become a long-term therapy for severe PD patients.

MECHANISMS

Several clinical observations give insights into mechanisms; the increase of endogenous release of dopamine may be explained by the reduction in daily “off”, by the improvement in motor “off” UPDRS, and also by the improvement in movement speed in “off”. Alteration of exogenous L-dopa metabolism is illustrated by the increase in the duration of the “on” period induced by a single dose of L-dopa, and also by an increase in contralateral peak-dose dyskinesias. This confirms the PET results, and points to some functional integration of the graft. Dyskinesias seen in some patients suggest either a lesion effect in the striatum, or indicate that the heterotopy of the grafted neurons may be partially harmful to the motor pathways (2).

MINIMAL AMOUNT OF GRAFTED TISSUE

Few post mortem studies have been performed that include a full histochemical analysis of the grafted striatum. Kordower et al. reported well-documented data indicating that 80,000 and 130,000 tryosine hydroxylase (TH) positive neurons remained alive in the brains of grafted patients who died more than one year after surgery and after discontinuation of immunosuppressive treatment, respectively (10). On the contrary, xenografts with porcine neurons led to poor survival (less than 1,000 per grafted hemisphere) as compared to grafts with human neurons (11).

In our series (unpublished), some patients received one mesencephalic area in one hemisphere, whereas others had 2-4 per side. There was a striking difference between the two groups, with positive results in “on-off” self-report, “off” UPDRS, motor speed and drug intake. It is generally accepted that at least 80,000 neurons per side are the minimal amount (around 20% of normal values) needed to obtain positive results. In these double blind trials, the number of surviving neurons was lower than 80,000 in one patient, which is perhaps explained by a long time elapsing between abortion and transplant (more than 1 week), with a low survival rate as a consequence.

Placement of tissue

A systematic striatal somatotopic study has been performed by our group (12), showing that different targets can be defined according to the
clinical goal of the graft. Further studies are needed to document the usefulness of a strategy aiming at graft placement tailored to the major symptoms of the patient in accordance with the known data on striatal somatotopy. It has been claimed that a complete filling of the putamen, or caudate and putamen, should be reached. In this regard, a single injection seems to fill a striatal area included in a sphere of 5 mm diameter. Injection tracks should thus be separated by 1 cm (4,13). The heterotopic placement of a graft may well prevent a physiological reorganization of striatal DA innervation. Other targets have been proposed: the ventral tegmental area and substantia nigra itself. Currently, we are not able to promote the elongation of DA axons from the nigra to the striatum; it has been shown that this is age dependent (14). Further studies are needed to ascertain whether co-grafting in both the striatum and the nigra is clinically useful.

Dissection and selection of tissue and number and age of transplanted neurons

Several methods have been used for fetal DA transplantation: solid pieces of tissue, cell suspensions, or both. We use a mix of cell suspension and micropieces obtained following mechanical dissociation of tissue. We do not know what is the best method at this time. Conservation of fetal mesencephalic areas should not exceed 48h because of reduced viability of neurons and increase of contamination by germs. Cryopreservation is also a major factor in reduced viability. Post mitotic cells are used today; stem cells or progenitor cells need an ex vivo differentiation prior to transplantation. At this time, preclinical data are not convincing enough to launch trials with these cells (13). Present data on xeno transplantation are not convincing, with a lack of congruence between improvement and graft development assessed by PET.

It is generally accepted that neuroprotection may improve graft survival and thus, therapeutic efficacy. Preclinical trials or pilot trials have pointed to tirilazad or flunarizine (15). The use of neurons after in vitro preservation with glial cell line-derived neurotrophic factor (GDNF) has also been published (16). However, we still do not have standardized protocols that might allow graft survival and the magnitude of the expected clinical improvement to be predicted with reasonable certainty.

Selection of patients

Today, severe PD patients (Hoehn and Yahr stages IV and V) are included in graft trials. Preference should be given to patients under the age of 60. There is now evidence that multiple system atrophy (MSA) and other degenerative diseases do not benefit from DA neuron grafting. Long-term follow up of cohorts of grafted patients must indicate whether patients will be protected against deterioration of motor signs. A long-term comparison with results of subthalamic deep brain chronic stimulation (STN DBS) is also warranted. Available data indicate that over a period of 5 years, STN DBS allows better control of motor symptoms in most patients (17). If long-term results indicate no major deterioration after graft, and no major side effect, grafts could be attempted in younger patients at stage III, before the occurrence of severe on-off fluctuations, dystonia and dyskinesias. The same considerations apply to STN DBS. Table II compares the effects of grafting and DBS, findings obtained following comparison of cohorts included in different trials. No comparative trial has been performed at the present time.

It must also be borne in mind that whereas no major cognitive or psychiatric side effects follow striatal DA grafts, vegetative, axial, cognitive as well as psychiatric disturbances are not improved by grafting, with the exception of the partial benefit obtained by reduction of DA drug intake.

It can be concluded, in short, that grafting remains a palliative treatment for motor impairment in PD.
SIDE EFFECTS

In two patients in our series, we observed a drastic motor improvement in the 8 to 15 days following the graft. Because of severe dyskinesia, the patients had to suspend, transiently, their DA treatments. In patient 5 this occurred after the first graft, with confusional status, and in patient 11 after the 2 grafts, without any confusional symptom. The mechanism of their improvement remains unclear, but the suggestion is that there was an acute DA release either by the grafted neurons or by the remaining DA striatal terminals.

Immediate side effects in our series were in line with known risks of stereotaxy: 1 symptomatic frontal hematoma among 20 surgical sessions; transient frontal symptoms (4/20); transient psychiatric events: confusion, aggressiveness, schizophrenia: (6/20); and one severe postoperative infectious status in one patient.

Long-term side effects are, in most cases, explained by the disease or concomitant pathology: e.g. recurrence of previously experienced events (delusions in one patient) or sural phlebitis (one patient). In patients with a long interval between the first engraftment and bilateralization, asymmetry of motor signs was common, but it could also be observed in other patients with shorter intervals. In the event of asymmetry of motor signs, PET must be used to check for graft growth asymmetry (2-4). Dyskinesias usually improve in those patients who can reduce their DA drug intake. In two patients, “spontaneous” dyskinesias, observed in defined “off” state, i.e., 12 hours after cessation of drugs, occurred on the most affected side. This indicates a spontaneous release of dopamine by the graft itself, and suggests a possible deleterious effect of the heterotopic grafted neurons: long-term follow up of patients is also warranted to assess such effects.

CONCLUDING REMARKS

It is now more than ten years since the first results in PD patients were published, and while long-term results where there is improvement of motor symptoms and, in some cases, reduction or cessation of DA drugs may be reached in severe patients, transplantation continues to be a matter of technical and ethical concern that remains within the domain of medical research (1). At the same time, chronic DBS in the STN (first trial in humans in 1993) has been performed in more

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>GRAFT</th>
<th>STN DBS</th>
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<tbody>
<tr>
<td>Akinesia</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Rigidity</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tremor</td>
<td>+/-</td>
<td>+++</td>
</tr>
<tr>
<td>Speech</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Writing</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Autonomic impairment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gait</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Axial impairment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-dopa intake</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
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Abbreviations: DA = dopaminergic; STN DBS = subthalamic deep brain stimulation.

Table II - Comparison of improvement following DA graft and STN DBS (literature findings)
than 2,000 PD patients worldwide. However, many questions remain unanswered, and these concern, namely: the best location and size of transplantation; the quantity of grafted neurons; the need for and duration of immunosuppressive treatments; the best source of neurons for transplantation and the long-term effect as compared to other medical or surgical treatments. Nevertheless, we can be sure that this method is not harmful to patients and thus deserves new clinical trials aimed at giving logical and documented answers to the many questions raised above. It may be that a better understanding of neural repair can promote neurotrophic treatments without transplantation. Furthermore, results obtained in PD may promote research into transplantation therapy for other diseases of the central nervous system (13). No trial of striatal transplantation in Huntington’s disease could have been launched without the information provided by trials completed in PD patients.

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16. Mendez I, Dagher A, Hong M et al. En-