CHAPTER 16

Carotid Body Transplants as a Therapy for Parkinson's Disease

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16.1 Cell Therapy in Parkinson's Disease

Parkinson's disease (PD) is characterized by the progressive degeneration of specific neuronal populations, particularly the dopaminergic neurons in the *substantia nigra* (SN) projecting to the *striatum*. Loss of these neurons leads to a lack of striatal dopamine, which is responsible for the principal motor symptoms characteristic of the disease (tremor, rigidity, slowness of movement and postural instability).[1–3] Current PD pharmacological therapies are based on the administration of pro-dopaminergic drugs, such as levodopa (a dopamine precursor), agonists of dopamine receptors, or inhibitors of dopamine degradation. However, none of these therapeutic strategies can stop disease progression. Moreover, they become less effective with time and can eventually produce motor complications as dyskinesias.[4]

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During recent decades intrastriatal transplantation of dopamine-secreting cells has been intensely investigated as a possible treatment to re-establish striatal dopamine levels in PD patients.[5,6] Among the different cell types and transplantation protocols assayed, the intrastriatal graft of fetal mesencephalic neurons has provided the best clinical results.[7–10] However, the clinical efficacy of this procedure has been questioned, since in two double-blind controlled trials[11,12] it showed little clinical benefit and in some patients it induced the appearance of disabling dyskinesias. In addition to these 'neuroreparative' dopamine cell transplants, neurotrophic factors have been shown to have beneficial effects in several preclinical models of PD.[13–17] Based on these promising results cell therapy protocols that deliver trophic factors in the *striatum* have also been applied to protect the nigrostriatal neurons affected by the ongoing neurodegenerative process. This 'neuroprotective' cell therapy aims to diminish the progression of PD and even induce a partial reversal. Therefore, the availability of dopaminergic and/or neurotrophic-factor-producing cells is a major limitation in the search for effective novel cell therapies for PD.

For over a decade, our group has studied the anti-Parkinsonian benefits of intrastriatal carotid body (CB) transplants. In this chapter we review the effects and mechanisms of the action of CB grafting on different preclinical models of PD as well as in two open, Phase I/II, clinical trials performed with Parkinsonian patients.

16.2 Anatomical and Physiological Features of the Carotid Body

The CB is a small, paired, organ located at the carotid bifurcation [Figure 16.1(a)]. It is a highly irrigated organ that receives blood through a vessel branch originating from the external carotid artery. The CB is composed of neural-crest-derived parenchyma, formed by the migration of sympathoadrenal progenitors from the superior cervical ganglion during fetal development, and afferent sensory nerve fibers joining the glossofaringeal nerve.[18] The adult CB parenchyma is organized in clusters of cells, called "glomeruli", which are in close contact with capillaries and nerve fibers [Figure 16.1(b)–(d)]. These glomeruli contain two main cell types: the neuron-like glomus, or Type I, cells, surrounded by the glial-like sustentacular, or Type II, cells. Type I cells are highly dopaminergic and can be easily identified by the expression of tyrosine hydroxylase (TH; see Figure 16.1), the limiting enzyme in dopamine biosynthesis. In contrast, Type II cells, which are TH-negative, express classical glial markers such as the glial fibrillary acidic protein [GFAP; see Figure 16.1(d)].

The CB, the principal arterial chemoreceptor, mediates cardiorespiratory homeostatic reflexes in response to changes in the chemical composition of the blood. Hypoxemia, the main stimulus for CB,[18,19] triggers hyperventilation and sympathetic activation. Besides acute hypoxia, other parameters in arterial blood, such as hypercapnia, acidosis or hypoglycemia, can also activate CB

cells.[20] It is well established that Type I, or glomus, cells are electrically excitable and function as the chemoreceptive elements of the CB. These cells contain secretory vesicles with, among other neurotransmitters, high dopamine content.[21] They behave as presynaptic-like elements that upon stimulation release neurotransmitters to activate afferent sensory nerve fibers of the IX cranial nerve. Besides its role in sensing acute hypoxia, the CB is also special among other adult neural or paraneural tissues because it can grow in conditions of chronic hypoxemia. It is well known that sustained hypoxia lasting several days stimulates CB cell proliferation and excitability,[18,22] as well as the synthesis of dopamine, due to TH induction.[23] This special sensitivity to hypoxia makes the CB particularly well-suited for intracerebral transplantation due to its particular durability in low oxygen tensions, a normal environmental condition in brain tissue,[24] which is probably accentuated inside intracerebral grafts. Recently, our laboratory has shown that, besides proliferation of TH-positive cells, hypoxic CB growth is produced by the activation of a population of resident stem cells in the adult organ. CB stems cells are the Type II, sustentacular cells (or a subpopulation of them), that under physiological hypoxia can proliferate and differentiate into new CB glomus cells.[25] Type II cells are non-excitable[26,27] and comprise around 15–20% of the cells within the CB. Classically, they had been considered to belong to peripheral glia with a supportive function, but the recent identification of Type II cells as peripheral neural progenitors has generated interest in unraveling the interactions between Type I and Type II cells in the CB.

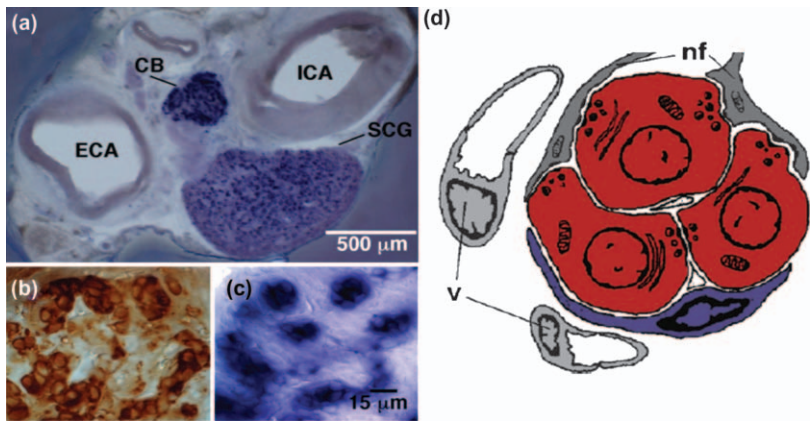

Figure 16.1 Structural organization of the carotid body (CB). (a) Histological section of the rat carotid bifurcation stained by *in situ* hybridization with a probe against tyrosine hydroxylase mRNA (TH; blue). Note the localization of the CB between the internal (ICA) and external (ECA) carotid arteries and near to the superior cervical ganglion (SCG). (b) and (c) Clusters, glomeruli, of TH^+ glomus cells in the CB, revealed by immunohistochemistry of TH [(b); brown] and TH *in situ* hybridization (c). (d) Schematic representation of a CB glomerulus indicating Type I (red) and Type II (purple) cells, blood vessels (V) and sensory nerve fibers (nf). Adapted from refs. 24 and 39.

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Another interesting physiological feature of the CB, of special value for its use in neural protection and repair, is that it contains high levels of several neurotrophic factors (brain-derived neurotrophic factor (BDNF), glial cell line neurotrophic factor (GDNF) and artemin, among others).[28–32] During recent years we have shown that the CB is among the tissues with the highest levels of GDNF in the adult rodent nervous system.[30]

16.3 Carotid Body Cell Therapy for Parkinson's Disease

16.3.1 Initial Preclinical Studies: The Carotid Body as a Source of Dopamine Cells

The use of CB grafts in PD animal models was initially proposed as a dopamine cell replacement therapy, based on the high content in dopamine of CB glomus cells.[33,34] The first study describing the use of CB glomus cells implants in PD models was reported by Gash and colleges in the 1980s.[35] The authors performed grafts of enzymatically dispersed CB glomus cells in the hemi-Parkinsonian 6-hydroxydopamine (6-OHDA) rat model, showing a slight behavioral recovery after implantation. However, in this study only a small number of CB glomus cells remained viable four weeks after grafting. In the late 1990s our group performed CB grafts in the same hemi-Parkinsonian rat model but using a different experimental methodology, which consisted of the use of CB cell aggregates instead of isolated glomus cells. CB cell aggregates were used instead of dispersed cell since enzymatically treated glomus cells are known to lose some of their physiological properties.[36] Using this graft procedure, the CB transplants produced a notable behavioral recovery three months after implantation, and subsequent histological and functional analysis revealed numerous clusters of TH-positive CB glomus cells and the improvement of striatal dopamine release.[37] During recent years several groups have confirmed that intrastriatal CB cell transplantation induces a marked recovery of hemi-Parkinsonian rats, as determined by behavioral, histological and neurochemical analyses.[38–41]

The favorable results obtained in the hemi-Parkinsonian rat model prompted us to evaluate the beneficial effects of CB transplantation in a non-human primate PD model. CB grafts were performed in Parkinsonian monkeys, that were previously injected with the neurotoxin 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP). CB transplantation induced, in this PD model, a long-term (five month) amelioration of the Parkinsonian symptoms that were accompanied by survival of CB glomus cells in the grafted *striata*.[42]

16.3.2 Recent Preclinical Studies: The Carotid Body as a Biological Pump Releasing Dopaminotrophic Factors

As indicated above, the first studies attempting transplantation of CB cells in PD animal models were originally thought of as a dopamine cell replacement

therapy. However, they unexpectedly revealed that the CB grafted *striata* showed clear signs of re-innervation, in both rat and monkey PD models, with a high density of striatal TH immunoreactive fibers.[35,37,42] These results posed the question of whether the recovery induced by the CB grafts was due to dopamine release by the transplanted glomus cells or because the transplants secreted trophic substances that induced the re-generation of the host dopaminergic striatal fibers. Thus, we analyzed the long-term recovery after CB grafting (between 5 and 15 months after transplantation) of hemi-Parkinsonian rats with a degree of SN lesions higher than the animals analyzed in the previous studies.[35,37] Interestingly, CB-grafted animals could be clearly differentiated in two distinct groups on the basis of their behavioral and histological characteristics. One group of rats showed a significant and stable behavioral recovery [Figure 16.2(a)] that correlated with an important re-innervation of the grafted *striatum* [Figure 16.2(b)]. The origin of the fibers re-innervating the *striatum* was studied by retrograde labeling experiments, showing that these fibers originated from the remaining ipsilateral dopaminergic SN neurons [Figure 16.2(c)]. In contrast, the other group of rats did not show behavioral recovery despite the fact that they presented a large CB graft, well located in the *striatum* and with numerous highly dopaminergic glomus cells [Figure 16.2(d)]. The histological examination of the nigrostriatal pathway of these animals revealed a complete denervation of the transplanted *striatum* [Figure 16.2(e)] and a total destruction of the ipsilateral SN [Figure 16.2(f)]. Altogether, the behavioral and histological analyses of these animals suggested that the beneficial effect of CB grafts on Parkinsonian animals was due to a trophic effect on dopaminergic nigrostriatal neurons, rather than to the local release of dopamine by the transplant.[39]

The trophic action of CB grafts, suggested by these experiments, could be explained by the fact that the adult CB produces high amounts of the dopaminotrophic factor GDNF.[29,30] GDNF has been shown to induce neuroprotection and fiber outgrowth in several animal models of PD.[13–16] Using different methodologies, including RT-PCR, genetically modified animals (GDNF/LacZ) and ELISA ~~assay~~, we have shown that the CB is one of the few areas in the nervous system expressing high levels of GDNF in adult life.[30,39] Interestingly, CB GDNF is produced selectively in the dopaminergic glomus, or Type I, cells and GDNF expression is maintained after intrastriatal transplantation (Figure 16.3).[30]

The preliminary evidence supporting the trophic action of CB implants on the nigrostriatal pathway was obtained using 6-OHDA hemi-Parkinsonian rats.[39] However, this model presents significant limitations to identify and study a trophic effect. Firstly, it lacks an internal control to normalize the variability of the lesion. Moreover, the acute lesion can mask the slow and progressive protective effects of the transplant on the nigrostriatal pathway. Finally, the lesion of the nigrostriatal pathway can be non-uniform, thus it is uncertain if the graft is placed in a region of the *striatum* that preserves the dopaminergic axon terminals necessary for the uptake of the trophic factors released by the transplanted cells. To further investigate if CB implants

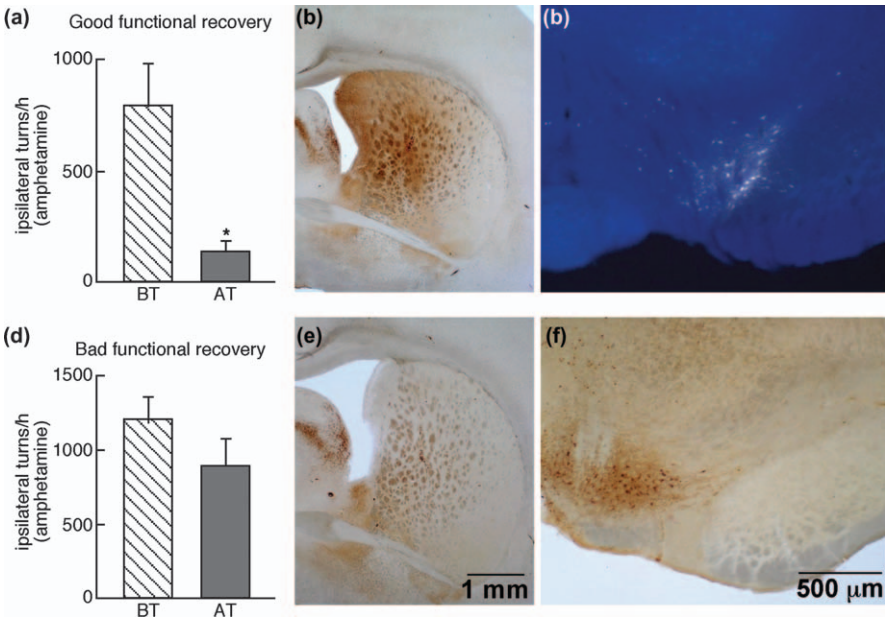

Figure 16.2 Functional and histological analysis of long-term CB-grafted hemi-Parkinsonian rats. (a) Rotational behavior of hemi-Parkinsonian rats with optimal functional recovery, before (BT) and after (AT) CB transplantation. (b) and (c) Histological sections of the *striatum* [(b), after TH immunohistochemistry] and SN [(c) labeled with the fluorescent retrograde tracer fluorogold] of a rat with optimal functional recovery, showing a significant re-innervation of the grafted *striatum* (b) arising from the remaining ipsilateral SN neurons (c). (d) Rotational behavior of hemi-Parkinsonian rats with bad functional recovery, before (BT) and after (AT) CB transplantation. (e) and (f) The histological examination, after TH immunohistochemistry, of the nigrostriatal pathway of these rats showed a complete denervation of the transplanted *striatum* (e) and a total destruction of the ipsilateral SN (f). *t*-Test $*p < 0.05$.
Adapted from ref. 37.

can trophically protect the nigrostriatal pathway, and thus amielorate Parkinsonism, we performed CB grafts in a novel systemic and chronic MPTP mouse model. This chronic MPTP model recapitulates better the slow and progressive death of dopaminergic neurons in PD and allowed us to test for the trophic effect of unilateral CB grafts on the nigrostriatal pathway, using for comparison the contralateral sham-grafted *striatum* as a robust internal control. With this experimental procedure we have recently shown that intrastriatal CB grafts demonstrate a marked protective action on ipsilateral SN neurons projecting to the area of the transplant [Figure 16.4(a) and (b)], and produce fiber outgrowth in the *striatum*. In fair consistency with a classical trophic effect, the trophic protection exerted by the CB graft on the nigrostriatal dopaminergic neurons showed dose–response dependence in relation to the size of the CB transplant [Figure 16.4(c)]. Moreover, the dose-dependent

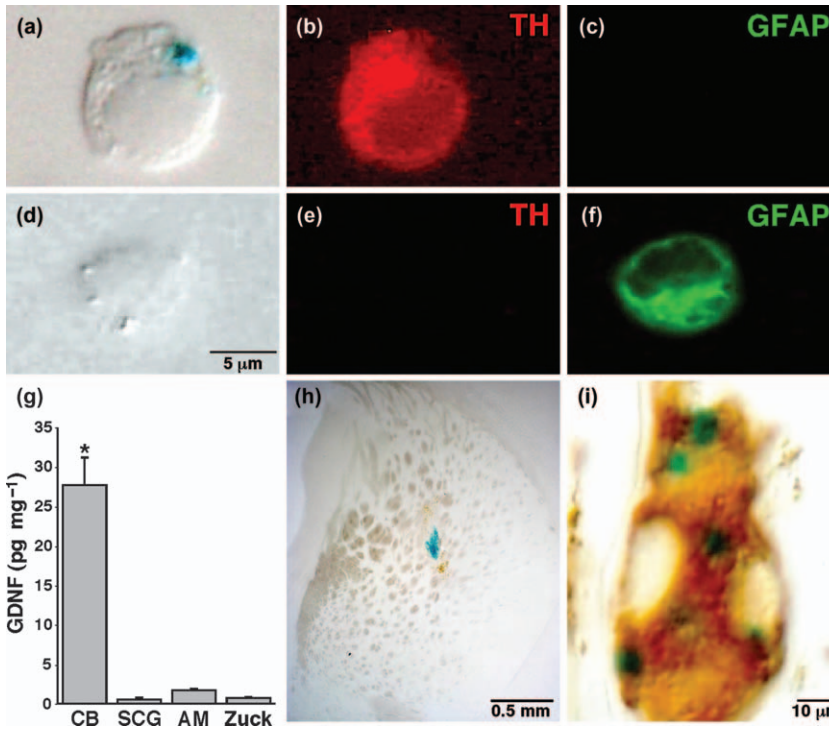

Figure 16.3 GDNF production in CB glomus cells *in situ* and after intrastriatal transplantation. (a)–(c) GDNF expression [blue precipitate, (a)] in a dispersed TH-positive [(b), red fluorescence] and GFAP-negative (c) CB Type I or glomus cell. (d)–(f) Lack of GDNF expression in a Type II or sustentacular CB cell, showing the characteristic GFAP expression [(f), green fluorescence] and absence of TH (e). The cells are representative examples obtained from primary cultures of heterozygous GDNF/LacZ CB, which were stained for Xgal and immunofluorescence (TH and GFAP). (g) GDNF protein content measured by ELISA in CB and other neural or paraneural tissues (SCG = superior cervical ganglion, AM = adrenal medulla, Zuck = Zuckerland's organ). (h) and (i) GDNF expression in intrastriatally grafted CB glomus cells. Note the GDNF expression (blue stain) in an intrastriatal implant of a heterozygous GDNF/lacZ CB (h), counterstaining with TH antibodies the blue GDNF-lacZ dots clearly appeared in the transplanted glomus CB cells (i). *t*-Test $*p < 0.05$.
AQ:4 Adapted from ref. 29.

trophic action of CB transplants was also analyzed by performing CB grafts from heterozygous GDNF/lacZ ($GDNF^{+/-}$) mice, which contained less GDNF than wild-type controls.[43,44] Interestingly, intrastriatal grafting of $GDNF^{+/-}$ CB showed a reduced, non-significant, protection of nigral neurons, compared with wild-type ($GDNF^{+/+}$) CB grafts that, as indicated before, AQ:5 produced a strong preservation of SN [Figure 16.4(d)].[44] Altogether, these results strongly support the view that the beneficial action produced by CB

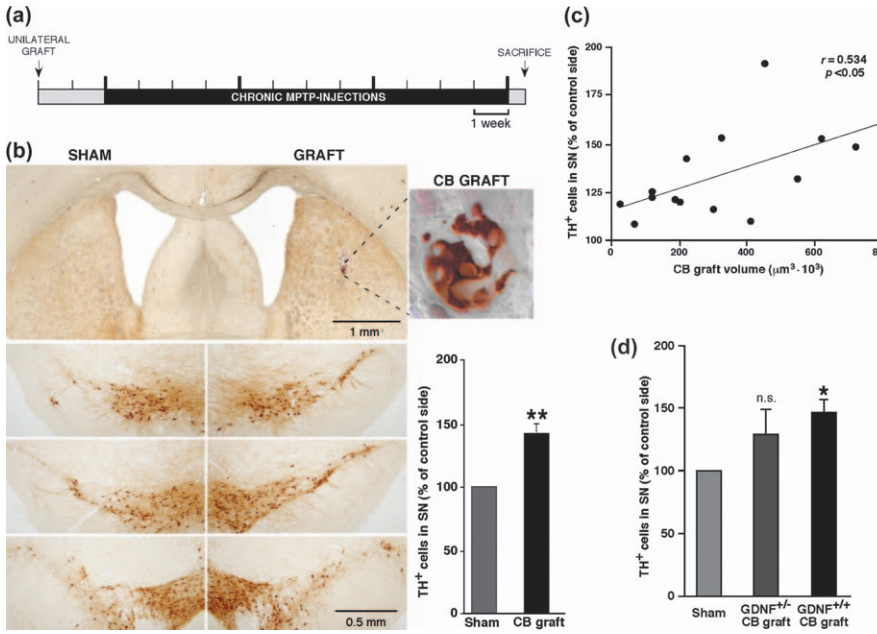

Figure 16.4 Trophic protection of SN neurons by CB grafts and dose-dependence regarding transplant size and GDNF expression. (a) Scheme of the experimental protocol. Briefly, unilateral striatal CB grafts were performed with a sham graft in the contralateral hemisphere, afterwards mice were chronically ($20\,mg\,kg^{-1}$, three times per week over three months) treated with MPTP. (b) Brain coronal sections after immunochemistry of TH showing the trophic protection exerted by CB graft (inset on right panel) on the nigrostriatal pathway. Note the stereological quantification, expressed as the percentage of sham-grafted side, of TH^+ nigral neurons on the right-bottom plot. (c) Graph illustrating the linear regression ($r = 0.534$, $p < 0.05$) established between the nigral protection exerted by the CB transplant (ordinate) and the graft volume (abscissa). (d) Stereological quantification of dopaminergic SN neurons of MPTP-treated mice grafted with $GDNF^{+/-}$ or $GDNF^{+/+}$ CBs. $GDNF^{+/-}$ CB-grafted mice showed a reduced and not significant (n.s.) protection of SN neurons compared with wild type ($GDNF^{+/+}$), which induces a notable protection of these dopaminergic neurons. *t*-Test $*p < 0.05$, $**p < 0.001$. Adapted from ref. 42.

grafting is compatible with a retrograde trophic action of the grafted tissue on the nigrostriatal pathway, and positively correlates with the size and the GDNF expression level of the CB implant.

Based on the high content of trophic factors, especially GDNF, encountered in the adult CB, different authors have proposed the cografting of CB and ventral mesencephalic neurons to improve the survival and the anti-Parkinsonian effects of the grafted fetal dopaminergic neurons.[40,45] Moreover, it have been shown that CB grafts can promote an increase in the number of striatal dopaminergic cells in Parkinsonian monkeys.[46] This effect of CB

grafting has been attributed to the release of GDNF by the transplant, because the administration of neurotrophic factors can produce similar effects.[47] In addition, a neuroprotective effect of CB grafts has been also suggested in experimental stroke.[48,49]

16.4 Clinical Studies of Carotid Body Autotransplantation on Parkinson's Disease Patients

The significant improvement induced by the CB graft in the different PD animal models encouraged the evaluation of the efficacy of CB transplantation in PD patients. In addition to their dopaminergic nature and their high content of neurotrophic factors, especially GDNF, the CB presents an important clinical advantage because its unilateral surgical re-section has no significant side-effects.[50] Two pilot Phase I/II open trials were performed to test the feasibility, safety and clinical efficacy of CB autotransplantation in Parkinsonian patients.[51,52] The experimental procedure in these trials consisted of unilateral removal of the CB and subsequent preparation of CB cell aggregates with fine scissors that were bilaterally placed in each putamen. Thirteen patients with advanced PD assessed before and up to 1–3 years after surgery were included in the two clinical trials. The primary outcome measure was change in motor ability [motor subscale (Part III) of the Unified Parkinson's Disease Rating Scale (UPDRS)] in the "off" medication state evaluated by an independent neurologist in a blinded fashion from masked and randomly presented video sequences.

Clinical improvement in the primary outcome measure was observed in 10 of 12 patients, being maximal at 6–12 months after transplantation (5–74%; 23% of mean improvement). Although a long-term trend towards presurgical clinical status was generally observed, a sustained improvement was detected in three of the six patients evaluated three years after grafting. In seven patients ^{18}F-DOPA positron emission tomography (PET) scans were performed before and one year after transplantation.[52] In these patients we observed a trend towards a 5% increment in intraputaminal ^{18}F-DOPA uptake instead of the expected yearly decrement, characteristic of advanced PD patients, estimated in approximately 10% of patients.[53] Because of technical problems one patient was successfully transplanted in only one hemisphere and received a needle track in the contralateral hemisphere. Interestingly, this patient only showed motor improvement in the contralateral hemi-body.[51] The results obtained in this patient indicated that the clinical effect of CB transplant is mediated by a specific action of the grafted cells, as it occurs in the different animal models of PD, rather than by placebo or some unspecific effect of the surgical procedure.

The clinical outcome obtained by autotransplantation of CB is similar to the effects reported by grafting fetal dopaminergic neurons.[11,12] However, CB-grafted patients did not develop dyskinesias unlike those patients subjected to fetal neuron transplantation. This could be related to the fact that the number of dopaminergic CB cells transplanted was markedly smaller than in the case of

fetal grafts. Hence, it seems that the main action of CB-grafted cells is a neuroprotective effect on nigrostriatal neurons rather than solely dopamine cell replacement. Additionally, the most significant predictive factors for motor improvement in the patients analyzed were the histological integrity of the CB (an estimation of the number of dopaminergic-GDNF-secreting cells) and a milder disease severity, which further indicated that the main action of the CB transplant is a trophic neuroprotective effect.

16.5 Conclusions and Perspectives

During recent years several studies have demonstrated that intrastriatal transplantation of CB cells produces a significant histological and functional recovery in various preclinical models of PD.[37,39,42,44] These beneficial actions of CB grafting have been independently confirmed by several authors.[38,40,46] Detailed analyses of the mechanism underlying the anti-Parkinsonian action of CB transplants have revealed that their effect is mainly due to a trophic stimulation of the nigrostriatal pathway, producing both striatal fiber outgrowth and protection of the SN neurons.[39,44] This trophic action is mediated, at least in part, by the release of GDNF by the CB graft.[30,44] Thus, dopaminergic glomus CB cells appear to be ideally suited for the endogenous delivery of neurotrophic factors, especially GDNF, in PD and probably in others neurological disorders.

Two pilot Phase I/II open trials have shown that CB autotransplantation is a safe and feasible procedure with potential clinical applicability to treat PD, producing a clinical improvement[51,52] similar to that obtained after fetal mesencephalic transplantation.[11,12] However, the effectiveness of CB cell therapy observed in clinical trials is considerably lower than in experimental models. This differential efficacy had led us to revaluate experimentally putative limitations that can affect the clinical outcome of CB transplantation, such as the severity of the disease, patient age and the amount (and integrity) of CB tissue grafted. The influence of these variables on anti-Parkinsonian CB cell therapy is currently under investigation. We are analyzing the effect of the donor and receptor age on the trophic protection exerted by the CB graft on the nigrostriatal pathway. Moreover, we are testing the putative efficacy of *in vitro* expanded CB cells, based on the recent identification of adult CB stem cells that can proliferate and differentiate into new dopaminergic and GDNF-producing CB glomus cells.[25] The results obtained in these on-going scientific projects would help to design new clinical approaches that could eventually improve the outcome of CB transplantation.

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