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REVIEW ARTICLE

Restoration of Nigrostriatal Pathway in Parkinson's Animals by the Bridge Transplantation Technique

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Laboratory and clinical studies have indicated that fetal ventral mesencephalic tissue transplantation into the lesioned striatum improves the behavioral and biochemical symptoms of Parkinsonian animals and patients. Restoration of tyrosine hydroxylase immunoreactivity and dopamine release is limited to the transplantation site. Unfortunately, clinical studies using this approach have demonstrated severe dyskinetic side effects. This may be because afferent fibers normally providing input to the nigra are not available at the heterotypic striatal site. In this review, we discuss a bridge transplantation technique that has been shown to be effective in restoring the nigrostriatal dopaminergic pathway of Parkinsonian animals, and that may have translational applications to humans.

1. Introduction

The transplantation of dopaminergic (DA-ergic) cells to lesioned striatum of Parkinsonian animals or patients has been found to improve behavioral deficits.[1–4] These grafted DA-ergic neurons can survive in hosts and provide sources of dopamine (DA) in the striatum.[5,6] Unfortunately, several reports of human transplantation have indicated development of significant dyskinesias, hence limiting therapeutic potential.[7] While the causes of these dyskinesias are unclear, they may be related to the fact that cell bodies of DA-ergic neurons are normally located in the ventral mesencephalic (VM) area, which receives inputs from different sites, such as the globus pallidus, raphe nucleus, and striatum.[8] Intrastriatal grafting does not re-establish the nigrostriatal DA-ergic pathway and DA modulatory mechanisms in the nigral area.

Different approaches have been used to accurately reconstruct nigrostriatal circuitry. It has been reported that DA neurons grafted to both nigra and striatum reconstitute tyrosine hydroxylase (TH) immunoreactivity between two transplants in 6-hydroxydopamine (6-OHDA)-lesioned rats. Animals that received double grafting showed a reduction in amphetamine-induced rotation.[9] Since amphetamine-induced rotation is reduced by intrastriatal (but not by intranigral) transplantation,[10] it is unclear if the behavioral changes in the double-grafted rats were mediated through the nigra or striatum transplant. The improvement in DA function may thus not necessarily correspond to the regeneration of the nigrostriatal TH pathway in double-grafted rats. Over the past 10 years, several approaches have been used to regenerate the nigrostriatal DA-ergic pathway in Parkinsonian animals using a bridge transplantation technique. In this review, we discuss

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the techniques, implications, and limitations of bridge transplantation in Parkinsonian animals. We hypothesize that the bridge technique has potentially useful applications in Parkinsonian patients.

2. Methods Used for Bridge Transplantation in Adult Hemi-Parkinsonian Rats

In several studies, grafted brain tissues (i.e., fetal VM tissue) were obtained from the VM region of fetuses at 15 days of gestation. Either a one- or a two-step procedure was used for intranigral transplantation and intracranial peptide injection.[11,12] In the first part of the two-step procedure, the fetal VM tissues (two pieces, approximately 1 mm^3 each) were vertically implanted by a 20-gauge implantation cannula into the lesioned nigral area of rats during chloral hydrate (400 mg/kg i.p.) anesthesia. Over a 30-minute interval, bridge material [such as glial cell line derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), fetal kidney tissue; Table 1[11–16]] was subsequently administrated between graft and striatum through a Hamilton needle (Hamilton Co., Reno, NV, USA). In animals receiving the one-step procedure, the fetal VM tissues and bridge material were injected through the same needle. The transplant was placed at the tip of the needle and first injected into the lesioned nigral area. The bridge material was later delivered from the lesioned nigra to the striatum as the needle was retracted over 30 minutes. A histological example of bridge transplantation using GDNF as bridge material in a hemi-Parkinsonian rat is demonstrated in Figure 1. Based on the different bridge materials used, five different bridge transplantation strategies have been reported. A comparison of these techniques is summarized as follows and in Table 1.[11–16]

3. Comparison of Bridge Transplantation Techniques

3.1. Excitatory amino acid + DA-ergic cell transplants

Bridge transplantation in Parkinsonian animals using excitatory amino acid was first reported by Zhou et al.[11,17] Kainic acid, laid down in a track between nigra and striatum, generates a trophic environment that effectively guides the growth of a TH immunoreactivity fiber tract from VM transplants in the lesioned nigra to the

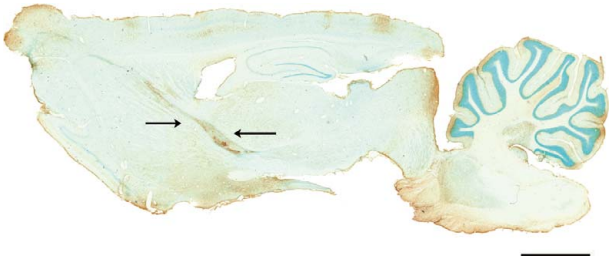

Figure 1 Regeneration of tyrosine hydroxylase (TH) immunoreactive tract from nigra to striatum in a unilaterally 6-hydroxydopamine-lesioned brain after ventral mesencephalic/glial cell line derived neurotrophic factor bridging in rat. TH immunoreactivity was examined 3 months post-transplantation. In this sagittal section, TH-positive fiber tracts (arrows) can be found in the lesioned striatum and nigra. Calibration bar: 2000 μm.

Table 1 Comparison of bridge transplantation

Bridge material	Animal	Outcome	References
Kainic acid	Rat	Behavior recovery (rotation) Restoration of striatal dopamine release Nigrostriatal TH immunoreactive pathway	11
GDNF	Rat	Behavior recovery (rotation) Restoration of striatal dopamine release Nigrostriatal TH immunoreactive pathway	12, 13
BDNF	Rat	No behavioral recovery No regeneration of the nigrostriatal TH immunoreactive pathway	12
Fetal kidney cells	Rat	Recovery of behavior (rotation) Nigrostriatal TH immunoreactive pathway	14
GDNF-secreting Schwann cell line	Rat	Recovery of behavior (rotation) Nigrostriatal TH immunoreactive pathway	15
bFGF + peripheral nerve	Rat	Recovery of behavior (rotation) Nigrostriatal TH immunoreactive pathway	16

TH = tyrosine hydroxylase; GDNF = glial cell line derived neurotrophic factor; BDNF = brain derived neurotrophic factor; bFGF = basic fibroblast growth factor.

target striatum. After bridge transplantation, amphetamine-induced rotation is significantly attenuated. An *in vivo* electrochemical chronoamperometric recording revealed that kainate/VM bridging transplantation restored KCl-evoked DA release and clearance in lesioned striatum. The release of DA after kainate/VM bridging transplantation can be achieved in a much broader area in the striatum compared to intrastriatal VM grafting, in which the restoration of DA release is limited to within 1 mm of the grafted area.[18] These data suggest that kainate/VM bridging transplantation can anatomically, neurochemically, and functionally reinstate an artificial nigrostriatal DA-ergic pathway in 6-OHDA-lesioned animals. Moreover, it further provides evidence that the bridged transplantation technique is a potential approach for the repair of a completely damaged neuronal pathway.

One major limitation to this method is that kainate is an excitatory neurotoxin; the application of kainate can produce seizures[13] or destroy neurons.[16] This toxic effect of kainate may limit its therapeutic use in Parkinsonian patients.

3.2. GDNF + DA-ergic cell transplants

GDNF is a potent trophic factor for DA-ergic neurons. This molecule: (1) is trophic to DA neurons *in vitro* and *in vivo*; (2) is protective against 6-OHDA or MPTP lesioning;[19] and (3) promotes the differentiation and survival of VM grafts. Furthermore, GDNF has been found to be upregulated by kainite.[20] It is possible that the kainite-induced trophic response seen in Zhou et al's[11,17] study is secondary to the upregulation of GDNF.

Wang et al reported that transplantation of fetal VM tissues into the 6-OHDA-lesioned nigral area followed by an injection of 100 μg GDNF along a tract from the nigra to striatum restores the nigrostriatal DA pathway.[12] Animals receiving transplantation and GDNF injection, but not BDNF or saline, showed a significant decrease in rotation 1–3 months post-grafting. Meanwhile, immunocytochemical studies indicated that TH-positive neurons and fibers were respectively present in the nigra and striatum post-grafting. No effects of similarly injected BDNF were seen. These results indicate that fetal nigral transplantation and GDNF injection can restore the nigrostriatal DA-ergic pathway in Parkinsonian animals, and support the hypothesis of trophic activity of GDNF on midbrain DA-ergic neurons. This response is selective for GDNF because the administration of BDNF or saline did not restore this DA-ergic pathway, although other dopaminotrophic factors have not been tested.

Using high-speed chronoamperometric recording techniques and Nafion-coated carbon fiber electrodes to record DA signals *in vivo*, Tang et al demonstrated that GDNF-bridged grafting restores KCl-induced DA release both in nigra and striatum at 3 months after transplantation.[15] The KCl-evoked DA release area was limited to the GDNF-induced bridging tract in the striatum. Conversely, KCl did not induce DA release in BDNF- or saline-bridged grafts. Taken together, these data suggest that fetal nigral transplantation and GDNF injection can restore the nigrostriatal DA pathway and DA release in hemi-Parkinsonian animals, thus supporting the hypothesis of trophic activity of GDNF on fiber outgrowth from midbrain DA neurons.

3.3. Fibroblast growth factor-primed peripheral nerves + DA-ergic cell transplants

It has been reported that transplantation of Schwann cells overexpressing fibroblast growth factor (FGF) promotes peripheral nerve regeneration.[21] Furthermore, severed axons in the injured spinal cord regenerate through grafted peripheral nerves treated with FGF.[22] These data raise the possibility that peripheral nerve tissue in the presence of FGF may facilitate regeneration of central fiber tracts.

Chiang et al demonstrated that the nigrostriatal pathway in hemi-Parkinsonian rats can be regenerated by bridge transplantation with FGF-primed peripheral nervous tissue after placement of fetal VM cells into the nigral area.[23] At 1 month after unilateral 6-OHDA-lesioning, fetal VM cells were grafted into the lesioned nigral region followed by nigral–striatal transplantation of sciatic or intercostal nerves as a bridge. These bridging nerves were pretreated with basic FGF (bFGF) or saline. Animals receiving bFGF-primed peripheral nerve bridge transplantation had reduced rotational behavior and reinnervation of TH-positive fibers into the lesioned striatum. By itself, bFGF did not increase outgrowth of TH-positive fibers from the VM transplants. Animals receiving saline/nerve bridge experienced only partial rotational improvement. No TH-immunoreactive fibers in the lesioned striatum or reductions in rotational behavior were found in animals receiving VM only, or VM plus bFGF. These results suggest that peripheral nerve tissue, along with the aid of bFGF, facilitates the reconstitution of the TH pathway from nigra to striatum and improves motor function in hemi-Parkinsonian rats.

3.4. GDNF-secreting Schwann cells + DA-ergic cell transplants

Wilby et al transfected a Schwann cell line, SCTM41, derived from neonatal rat sciatic nerve cultures, with a rat GDNF construct through lipofectamine.[24] *In vitro* studies indicated that these cells can increase GDNF level in the medium after 48-hour incubation. Co-culture with these GDNF-secreting cells enhanced survival and fiber growth of embryonic DA-ergic neurons. Untransfected SCTM41 cells had no effect on DA-ergic neuronal survival. Using the bridge grafts of GDNF-secreting SCTM41 cells, Wilby et al demonstrated that these cells promote

the growth of axons to the lesioned striatal targets from DA-ergic neurons implanted into the 6-OHDA-lesioned substantia nigra. These data suggest that bridge grafting with GDNF-secreting Schwann cells increases the survival of implanted embryonic DA-ergic neurons and promotes the growth of axons through the grafts to the lesioned striatum.

3.5. Fetal kidney tissue+DA-ergic cell transplants

GDNF has been shown to be important during kidney development. The outer mesenchyme of developing metanephric kidney contains a particularly strong GDNF mRNA signal, peaking at a gestational age of 16–21 days in rats. In the developing kidney, GDNF family receptors, such as GDNF receptor alpha-1 (GFR-α1), are also highly expressed and coexpressed with Ret mRNA. GDNF and GFR-α1 in fetal kidney are critically involved in the development and segmentation of the renal cortex. Null mutated mice, which are deficient in GDNF, are born with no kidneys. Animals that lack GFR-α1 also have complete bilateral renal agenesis and ureteral deficits.[25] Since fetal kidney tissue has a high level of GDNF protein and GDNF mRNA, and protein remains elevated in the graft site post-transplantation of fetal kidney tissue to adult rat brain,[26,27] it is possible that fetal kidney tissue may be an endogenous source of GDNF that can be substituted for high-dose GDNF injections previously used during bridge transplantation. Moreover, other trophic factors in the transforming growth factor-beta (TGF-β) superfamily, such as bone morphogenetic proteins,[14] neurturin, and TGF-β itself, are all highly expressed in fetal kidneys. These proteins have been shown to be neuroprotective and may synergistically interact with other trophic factors in the brain, optimizing conditions for survival and fiber outgrowth from fetal VM grafts.

Based on these findings, Chiang et al demonstrated that intranigral transplantation of fetal VM tissue and nigrostriatal fetal kidney bridge grafting restore TH-positive input to the lesioned striatum of hemi-Parkinsonian rats.[28] The lesioned animals that received no VM/kidney grafts or VM transplants alone did not display this TH-positive pathway. Similarly, only animals that received VM/kidney bridge grafts demonstrated recovery of amphetamine-induced rotation and body asymmetry. These data suggest that fetal VM/kidney bridge transplantation normalizes behavioral deficits and restores the DA pathway in hemi-Parkinsonian rats. As fetal kidneys contain a variety of trophic proteins, transplantation may provide a synergistic admixture that optimally promotes DA fiber outgrowth.

4. Conclusions

Bridge transplantation regenerates a new nigrostriatal pathway in Parkinsonian animals. The bridge materials used in these studies are largely trophic factors for DA-ergic neurons, including GDNF, GDNF-producing cells, and pharmacological agents that upregulate GDNF expression. A recent study from Redmond et al has also demonstrated in adult non-lesioned St. Kitts green monkeys that overexpression of GDNF by administration of adeno-associated virus serotype 2 (AAV2)-GDNF in striatum induces neurite outgrowth from fetal VM grafts in the nigra to the target striatum.[29] These data suggest that GDNF has a potent trophic effort on DA-ergic transplants enabling outgrowth of axons/fibers of TH-positive cells for long distances, such as from nigra to striatum, in the primate. The reconstruction of this nigrostriatal DA-ergic pathway not only provides a source of DA, but may also rebuild the regulatory connections from other brain regions in animals with Parkinson's. It will be of interest to examine the difference in recovery time of locomotor and cognitive functions post-bridge and intrastriatal transplantation in Parkinsonian animals. If future technique refinements are achieved, this bridge transplantation technique may become useful in treating human Parkinson's disease.

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