Functional Study of Blood-Brain Barrier in Neurotransplanted Patients

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Several investigators have studied abnormal free amino acid concentrations in Parkinson's disease (PD) cerebrospinal fluid (CSF). Clearly, the detection of amino acids in CSF and the determination of whether the blood-brain barrier (BBB) is altered in subjects that received transplants of neural tissue by open surgical methods would contribute to our knowledge of the role of these parameters in cerebral transplantation.

Employing automated ion exchange chromatographic techniques, we report in this article measurements of 32 amino compounds in CSF obtained before and after brain transplants in 9 patients (6 males and 3 females) with PD grade III-IV (Hohen and Yahr rating scale), a mean age of 47.2 years, and a mean evolution time of 12.3 years. We also measured CSF and serum concentrations of albumin and IgG by radial immunodiffusion in 4 of these neurotransplanted patients.

The parkinsonian group showed high levels of some amino acids in CSF including leucine, tyrosine (precursor of dopamine), lysine and arginine when compared with the control group.

CSF amino acid levels were elevated through 12 weeks after surgery and some of them remained elevated through 24 weeks of post-operative measurements in five of the six patients. These amino acids were present in both plasma and CSF and the concentration in plasma was 6.5 to 12.3 times greater than that in CSF. These data suggest that there might be a persistent limited defect in the BBB to low-molecular weight molecules following fetal-brain transplantation.

A tendency toward higher albumin quotient values [(CSF albumin)/(serum albumin)/1000] and IgG quotient values [(CSF IgG)/(serum IgG)/1000] was present 4 weeks after transplant in all studied cases. In two of these patients, incremented albumin and IgG quotient values were marked, indicating increased passage of albumin and IgG across the BBB.

To summarize, levels of albumin and IgG quotients post-transplantation as compared with before transplantation showed a tendency to increase for 4 weeks after surgery. Small molecules such as amino acids appeared to cross the BBB more readily 24 weeks following surgery in at least some patients. Physiopathological effects of increased BBB permeability to amino acids are not known. Future studies in animal models are necessary.

Free amino acid concentrations determined in CSF of PD patients before and 24 weeks after transplant. n=5

*Wilcoxon Matched Pair Test. p<0.04
Development and Integration of Intrastriatal Striatal Grafts Implanted Into Long-Term Ibotenate Lesions

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It is well known that fetal striatal tissue grafted into excitotoxically lesioned adult striatum can partially restore the behavioural/1/ and biochemical /3/ deficits, and establish anatomical connections with the host tissue /2,4-6/. Those previous studies were undertaken after grafting in acutely (usually 1 - 4 weeks before) lesioned striatum. Several factors change in the lesioned area after a long time of evolution. However, the capacity of survival and anatomical integration with the host of striatal grafts implanted in chronically lesioned striatum has not been investigated. In this study the development, and afferent and efferent innervation of striatal grafts implanted in long-term lesioned striatum were studied and compared with that observed in our previous studies of grafts implanted in non-lesioned or acutely lesioned host striatum /2,4-6/.

A total of 46 female Sprague-Dawley rats received unilateral intrastriatal injections of ibotenic acid. Three to twenty months later 33 of these rats were grafted with rat or mouse fetal striatal tissue into the same area. The transplanted rats were killed at 7 days, 2 weeks (mouse-to-rat grafts only), 4 - 6 weeks, 8 weeks and 11 weeks (mouse-to-rat grafts only), 7 months and 12 months post grafting (p.g.). Seven rats were lesioned but not transplanted, and sacrificed 6-12 months after the lesion. Finally, 6 acutely (10 days) lesioned rats grafted with the same cell suspension as 8 of the chronically lesioned rats were used as a control group.

DARPP-32-immunocytochemistry was used to differentiate the striatum-like and non-striatum-like tissue-types in the grafts /6/. Tyrosine hydroxylase (TH) and serotonin (5-HT) immunostaining was used to study the afferents from the substantia nigra and the mesencephalic raphe, respectively. Frontal cortical injections of the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L) were made to study the cortico-striatal afferents to the grafts /4/. Finally, a mouse specific neuronal marker (M6) was used in mouse-to-rat grafts to study the efferent projections to the host brain from the striatal grafts /5/.

There was an important shrinkage of the lesioned striatum, and the centre of the lesion was composed of capsula interna bundles close to each other and separated by thin bridges of non-neuronal striatal tissue. The density of the host monoaminergic fibres (TH- and 5-HT-positive fibres) in the lesion-only areas or at the graft-host border was not much reduced, as compared with the acute-lesion controls. In contrast, the cortical innervation was much reduced in long-term lesioned striatum, at the same time that a dense network of PHA-L labelled corticostriatal fibres was observed in the contralateral striatum.

The transplants grew to about 70-80% of the size obtained with control grafts placed into acute lesions, and developed characteristic striatum-like (i.e. DARPP-32-positive) patches. These patches could already be observed in the grafts 7 days after transplantation, i.e. as early as in grafts implanted in acute lesions. The grafts received a dense dopaminergic innervation, which was selective for the striatal-like areas, and began as early as in grafts implanted in acute lesions. The 5-HT-positive fibres also innervated the transplant following a pattern similar to that observed in grafts implanted into acute excitotoxic lesions. As the cortical innervation appeared much degenerated, the