PET Studies of Parkinsonian Patients Treated with Autologous Adrenal Implants


ABSTRACT: Transplantation of autologous adrenal medulla tissue into the striatum has recently been proposed as a treatment for Parkinson’s disease. We report the use of positron emission tomography (PET) to evaluate patients who had adrenal implants placed into the right caudate. 6-[18F] fluoro-L-dopa (6-FD) scans were performed to study the integrity and activity of the implant, and the nigrostriatal dopamine system before and six weeks after transplantation surgery. [68Ga] Gallium-ethylenediaminetetraacetate (Ga) scans were also performed to assess the blood brain barrier. The Ga scans performed on two patients showed increased permeability of the blood brain barrier at the surgical site. 6-FD PET scans in five patients did not show a consistent change in striatal uptake following adrenal medullary implantation after six weeks. Further assessment of implant viability with 6-FD PET scans after longer follow up may provide useful information if the blood-brain barrier becomes re-established with the passage of time.


Neuronal grafting has been reported to ameliorate the symptoms of parkinsonism in experimental subjects.1 Since the initial report of clinical improvement following autologous adrenal implantation into the brain of Parkinsonian patients,2 there has been intense interest in the use of this surgical therapy for Parkinson’s disease. The early enthusiastic outlook has been somewhat subdued in the light of subsequent reports of variable success.3,4,5,6,7,8 Positron emission tomography (PET) allows the study of the regional concentration of radioisotopes in the living brain. 6-[18F] fluoro-L-dopa (6-FD) has been used to evaluate the integrity of the nigrostriatal dopamine pathway.9,10 Differences have been found in the striatal uptake of this compound between subjects with parkinsonism and controls.12,13,14 We have employed PET in the assessment of patients before and after autologous adrenal implantation for Parkinson’s disease. In addition to evaluating the nigrostriatal dopamine system with 6-FD, we have utilized [68Ga] gallium-ethylenediaminetetraacetate (EDTA) (Ga) to assess the integrity of the blood brain barrier. Using these in vivo techniques we have identified difficulties in the interpretation of 6-FD PET studies after surgery due to alterations of the blood brain barrier.

METHODS

Patient Population

Five patients were studied at the UBC/TRIUMF PET Centre before and after autologous adrenal implantation. All patients

From the UBC/TRIUMF PET Group, and the Division of Neurology, Department of Medicine, University of British Columbia, Vancouver (M.G., W.R.W.M., R.F.P., M.J.A., T.J.R., D.B.C.) and the Department of Neurosurgery and Neurology, Vanderbilt University, Nashville, Tennessee (R.S.B., G.A., R.A.P., N.B.T.)

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were part of the Vanderbilt University Transplant Project. The patient characteristics are listed in Table 1. After the initial PET study in Vancouver, the patients returned to Nashville for their surgery. All patients had their right adrenal gland removed and had implantation of adrenal medulla tissue into the right caudate nucleus. An Omaya reservoir was placed into the left ventricle in order to sample cerebrospinal fluid post-operatively. Six weeks after surgery the patients returned for repeat PET studies.

**Patient Evaluation**

The initial post-operative clinical characteristics of these patients have been reported. The clinical signs of parkinsonism were assessed using a modified Columbia Rating Scale. Patients were examined before and at 3 month intervals after surgery. In general, three examinations on alternate days during a single week for each patient. In the case of patient 2, only one or two assessments were available. Patients were examined in the morning prior to their first dose of L-dopa for that day. The one patient with on-off reaction, patient 1, was assessed in the off-state. The median total rating scale score at baseline, 3 months and 12 months is presented in Table 1. A formal 6 week score was not performed by the same observers and is therefore not comparable to the other values.

Based on patient variability for visits close together in time, it was estimated that a change of 10 points in the total Columbia Rating Scale score was unlikely to occur by chance and indicated a definite change. A reduction in the total score represents improvement and an increase represents deterioration.

**PET and MRI Studies**

The PET protocol included a 6-FD study pre-operatively, followed by a scan six weeks after surgery with the same compound. Two patients had, in addition, Ga scans at the same time as their post-operative 6-FD study. The PET scans were performed on the UBC/TRIUMF PETT VI tomograph using a previously published protocol. 6-FD was prepared via the fluorination of a mercury-dopa derivative with 18F-acetyl hypofluorite. 17 Ga was prepared from an on-site generator purified by gel filtration and ion exchange techniques. The patients were oriented in the MRI headholder (100 mg orally) one hour before injection of the isotope to prevent peripheral decarboxylation of the compound. Antiparkinsonian medication was withheld from the night before. Patients were positioned in the tomograph using the external markings determined from the MRI study. This technique enabled sampling of the same anatomical structures in all subjects. The head was fixed in place with the use of a thermoplastic mask. An intravenous catheter was placed in the opposite arm for injection of the radiosotope. Post-operatively, patients had the identical protocol of MRI and PET scans using the same positioning techniques.

Two patients had Ga scans as part of their post-operative assessment. For these studies, the patients were positioned in the same way as the 6-FD studies. Ga was injected intravenously (4.5 mCi) and ten scans of ten minutes duration were performed with alternating chair positions so that the anatomical levels would be comparable to the 6-FD studies. Summed scans were used for the identification of abnormalities of the blood brain barrier.

**PET Analysis**

Elliptical regions of interest (1.2 cm²) were placed on the striatum and occipito-parietal cortex on each of the four slices containing striatal activity that commenced at 110 minutes and 120 minutes after 6-FD injection. This permitted evaluation of the data from the brain regions acquired from each of the two chair positions. The average striatal radioactivity in excess of background was estimated by subtracting the activity in the background regions of occipito-parietal cerebral cortex from the striatal region. This value was then normalized to the occipito-parietal cortex value. In the background regions, because of the lack of dopa-decarboxylase activity, the only radioactive components are thought to be 6-FD and its metabolite 3-O-methylfluorodopa (3-OMFD). With an intact blood brain barrier, the striatal concentrations of 6-FD and 3-OMFD are thought to be the same as the occipito-parietal cortex concentrations because the transport system is similar. By subtracting the occipito-parietal value from the striatal value, one is left with the striatal dopamine-related metabolites of 6-FD. Each striatum was evaluated for each of the two scan times and the ipsilateral values were then averaged to obtain a left and right striatal measurement. This strategy estimates the functional integrity of the dopamine-synthesizing cells. The same methods were applied to the pre- and post-operative scans. The occipito-parietal cortex was chosen for the reference region rather than the cerebellum because of the previous observations. The activity in the cerebellum has different uptake characteristics compared to areas of cerebral cortex and striatum after the administration of 6-FD, permitting potential errors using the analytic techniques outlined above.

<p>| Table 1: Clinical Characteristics Before Transplantation |
|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>H &amp; Y Stage</th>
<th>Modified Columbia Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>42</td>
<td>M</td>
<td>2 on/4 off</td>
<td>46 0 -6 -3 -2</td>
</tr>
<tr>
<td>2.</td>
<td>42</td>
<td>M</td>
<td>1</td>
<td>18 4 N/E N/E 3</td>
</tr>
<tr>
<td>3.</td>
<td>35</td>
<td>M</td>
<td>2</td>
<td>28 1 -1 -7 -4</td>
</tr>
<tr>
<td>4.</td>
<td>39</td>
<td>M</td>
<td>3</td>
<td>36 8 6 8 6</td>
</tr>
<tr>
<td>5.</td>
<td>46</td>
<td>F</td>
<td>4</td>
<td>55 -22 -22 -13 -14</td>
</tr>
</tbody>
</table>

After the baseline examination the Modified Columbia Scores are expressed as the amount of change time 0.
RESULTS

Patient Characteristics

One out of the five patients, patient 5, showed improvement at 3 months and at 12 months. The other patients were unchanged. None met the criterion for clinical deterioration. In patient 5, improvement was seen in bradykinesia, rigidity, posture, balance and gait at 3 months and the changes were present at 12 months except for rigidity. Although the changes were apparent at 3 months after surgery, they occurred so slowly that the time of onset could not be determined precisely. By design, antiparkinsonian medications were not to be changed during the first year following the transplant, although patients 3, 4, and 5 were taken off medication for 2-14 days perioperatively. Patient 5 was receiving a total daily dose of 500 mg of L-dopa combined with carbidopa during the baseline period and a dose of 350 mg at 3 months and 12 months.

6-FD Scans

Examples of pre- and post-operative 6-FD scans are shown in Figure 1. Results of the analysis of the 6-FD PET studies are shown in Table 2. 6-FD PET scans six weeks after surgery showed an increase in striatal uptake in three patients but this was virtually the same on the left (non-operated) side as on the right side. Two patients showed decreased striatal uptake on the follow up study. The amount of change between the pre- and post-operative scans was not significant using the Wilcoxon Signed Rank test.

Ga Scans

Examples of the Ga scans in two patients are shown in Figure 2. Comparison of 6-FD scans with Ga scans performed in the same patient, in the same positions, is shown in Figure 3. Uptake of Ga was increased in the region of the transplantation site and surgical tract. When 6-FD scans are compared to these scans, there is a similar distribution of altered radioactive uptake in these regions.

DISCUSSION

This study has employed PET scans with 6-FD to evaluate the nigrostriatal dopamine system in patients undergoing autologous adrenal implantation for Parkinson’s disease. 6-FD is an analog of L-dopa and can be employed to evaluate the function of the nigrostriatal dopamine system, and L-dopa cross

Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Side</th>
<th>Pre-operative 6-FD Modified Ratio</th>
<th>Post-operative 6-FD Modified Ratio</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Right</td>
<td>0.280</td>
<td>0.354</td>
<td>+26.4%</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.234</td>
<td>0.286</td>
<td>+19.7%</td>
</tr>
<tr>
<td>2</td>
<td>Right</td>
<td>0.509</td>
<td>0.439</td>
<td>-13.8%</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.413</td>
<td>0.297</td>
<td>-28.1%</td>
</tr>
<tr>
<td>3</td>
<td>Right</td>
<td>0.331</td>
<td>0.357</td>
<td>+7.6%</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.257</td>
<td>0.280</td>
<td>+9.2%</td>
</tr>
<tr>
<td>4</td>
<td>Right</td>
<td>0.278</td>
<td>0.265</td>
<td>-4.5%</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.321</td>
<td>0.306</td>
<td>-4.7%</td>
</tr>
<tr>
<td>5</td>
<td>Right</td>
<td>0.326</td>
<td>0.361</td>
<td>+11.0%</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>0.262</td>
<td>0.264</td>
<td>+1.0%</td>
</tr>
</tbody>
</table>

Difference not significant with Wilcoxon sign rank test.
Modified Ratio = Radioactivity (Striatum - parietal - occipital cortex) / (Parietal - occipital cortex)
the blood brain barrier through the same facilitated transport mechanism. Once past the blood brain barrier, 6-FD is taken up by dopaminergic neurons and converted to 6-fluorodopamine (FDA) by L-aromatic amino acid decarboxylase. Cumming and colleagues have shown that 6-FD and L-dopa have similar $K_m$ values for this enzyme. In regions without dopaminergic neurons, 6-FD remains as a component of the background radioactivity. Tomographic studies in monkeys and in man have demonstrated accumulation of radioactivity in the striatum following 6-FD administration.

The results of the 6-FD studies suggest that a consistent alteration of the striatal radioactivity did not occur in these patients six weeks after adrenal medulla implantation. In the three subjects who demonstrated increases of striatal radioactivity in their PET studies only one showed signs of clinical improvement throughout the year of observation. The clinical improvement in patient 5 was not detected before the 3 month assessment. Her post-operative PET scan with 6-FD showed an increase on the side of the implant but this was smaller in magnitude than another subject who did not improve. The significance of this increase in radioactivity is difficult to interpret. 6-FD PET studies therefore did not provide consistent correlation with the corresponding clinical examinations and did not provide an early indication of clinical improvement that would occur in the future.

It is possible that the striatal dopaminergic function was altered and was not appreciated by the current method of analysis. Small increases in dopaminergic function may occur that are within the error limitations of the semiquantitative analysis. A marginal increase in striatal dopamine content may potentially reverse the symptoms of Parkinson's disease in a patient who is close to the threshold for clinical symptomatology to occur.

Ga PET scans have been employed to evaluate the permeability of the blood brain barrier in two patients. The blood brain barrier normally has a low permeability to Ga. Experiments in rhesus monkeys with this tracer have demonstrated reversible breakdown of this barrier after unilateral carotid administration of hyperosmotic mannitol. Patients with cerebral infarction have been assessed with the same technique. It has been concluded that Ga PET scans were more sensitive to breakdown of the blood brain barrier than CT scans with contrast administration. These studies confirm the usefulness of this technique for the study of the regional integrity of the blood brain barrier using PET technology.

Animal models have been used to assess the permeability of the blood brain barrier after transplantation procedures. The cerebral vasculature has a continuous layer of endothelial cells connected by tight junctions. Adrenal medulla tissue and sympathetic ganglia do not have these junctions. Rosenstein has shown that the blood brain barrier does not form after transplantation of adrenal medulla or autonomic ganglia up to nine months post-operatively. These investigators have demonstrated that labelled dopamine could enter from the blood in the
region of the graft. Furthermore, Rosenstein has reported that rat cerebral cortex grafted into fourth ventricle develops permanent barrier dysfunction regardless of the post-operative time studied. This observation suggests that even tissue with an initially competent blood brain barrier does not maintain this after implantation.

The present study indicates that breakdown of the blood brain barrier is present six weeks after surgery. It is not clear what influence this defect will have on the PET measurements. Increased permeability of the blood brain barrier may allow enhanced passage of 6-FD and 3-OMFD into the brain. This may be incorrectly interpreted as an increase in dopaminergic function at the site of implantation. Conversely, altered permeability may also permit passage of carbidopa into the affected regions, causing inhibition of 6-FD metabolism in that region.

It is possible that the increased regional permeability around the transplant tract and site may resolve at a later date. Long-term studies have not been performed in patients undergoing these procedures and the time course of the alteration is currently unknown. It is not clear if the defect seen on the Ga studies is from surgical alterations causing edema and gliosis or from the ultrastructural differences of the capillary endothelial network in the transplanted tissue.

In this study, the valid comparison of regional differences of radioactive accumulation after 6-FD administration (that may be interpreted as differences of dopaminergic function) are dependent on an intact blood brain interface in all regions. Using current methodology, interpretation of the presence or absence of changes of regional radioactivity uptake is difficult. It is possible that tracer kinetic analysis may provide further observations that will assist in the evaluation of dopaminergic function after neural implantation. This methodology, after suitable development and validation for 6-FD, may be able to independently assess transfer rates across the blood brain barrier and rates of metabolism of the radioligand in various regions. This will enable the differentiation of alterations of dopaminergic function from defects of the blood brain barrier. Unfortunately this analytic technique is not currently available.

ACKNOWLEDGEMENTS

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