

In Vivo PET Imaging of Gene Expression in Parkinsonian Monkeys

To the Editor:

The ability to determine the level and duration of gene expression *in vivo* is critical for the clinical use of gene therapy. Positron emission tomography (PET) has been used to monitor gene expression both directly, using tracers that image the spatial distribution of therapeutic transgene products, and indirectly, using tracers that image the spatial distribution of “reporter genes” as markers of gene expression. The indirect imaging technique requires the assumption that expression of the reporter gene reflects the expression of the therapeutic gene. This method has been used frequently because there are no radiotracers available to image most transgene products and the development and validation of new imaging agents would be costly and time consuming [1]. However, while the indirect method is more feasible in some respects, it is also more limited in its potential application to human clinical studies [1]. A key question for both direct and indirect imaging techniques is how well do the PET images correspond to the expression of the therapeutic gene? We used a direct PET imaging approach to monitor gene expression in an MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) primate model of Parkinson’s disease (PD). We found that PET measures of gene expression were proportional to immunocytochemical measures of gene transduction. These findings demonstrate a direct *in vivo* measure of gene transfer in the primate brain that should be useful for both clinical and research applications.

Our therapeutic strategy was aimed at increasing striatal aromatic L-amino acid decarboxylase (AADC) levels. Striatal neurons infected with the AADC gene by an adeno-associated viral (AAV) vector can convert low doses of systemically administered L-dopa/carbidopa to dopamine, resulting in clinical improvement without the side effects typically associated with higher doses of L-dopa [2]. We previously reported that PET measures of striatal uptake of the AADC tracer 6-^[18F]fluoro-L-*m*-tyrosine (FMT) were substantially increased following AAV-mediated delivery of the AADC gene (AAV-AADC) in hemiparkinsonian monkeys [2]. Here, we extend these findings by evaluating the relationship between striatal FMT uptake and histological measures of the extent of gene expression in MPTP-treated monkeys following intrastriatal infusion of AAV-AADC.

We studied seven *Macaca mulatta* (3–5 kg). Four monkeys received unilateral intracarotid artery (ICA) infusions (4 ml/min) of 60 ml of saline containing 2.5–3.5 mg of

MPTP-HCl, producing a near-complete lesion on the side of infusion (ipsilateral) and mild to moderate damage in the other (contralateral) hemisphere [3]. One of these animals received follow-up doses of MPTP-HCl (0.3 mg/kg, iv) until a stable parkinsonian syndrome was achieved [4]. The remaining three animals were subcutaneously injected with 2 to 3 mg of MPTP-HCl at 2- to 7-day intervals until stable bilateral parkinsonian features were established.

We infused a 33- μ l volume of AAV-AADC into the target sites in the caudate and putamen. Approximately 1×10^{12} total vector genomes (or “full capsids”) of AAV-AADC was administered into each monkey. The vector preparation also contained empty AAV capsid particles at a ratio of 4:1 over full capsids; therefore, total AAV particles administered to each animal was 5×10^{12} .

We performed PET scans using a Siemens-CTI ECAT EXACT (Model 951) 31-slice scanner following MPTP administration, when the animals were clinically stable, and again following AAV-AADC. We analyzed the PET data by determining the radioactivity counts for the striatum and cerebellum and creating radioactivity count ratios using the cerebellum as a reference tissue. The cerebellum was selected as a reference region because FMT uptake is negligible and should not change between baseline and post-treatment studies. Change scores for the count ratios were calculated representing the percentage change from baseline to post-AAV-AADC. As expected, animals that received unilateral ICA MPTP infusions showed negligible FMT uptake on the lesioned side of the brain at baseline, with striatal-to-cerebellar ratios ranging from 1.2 to 1.8, while the contralateral striatum was relatively spared. Three animals that received subcutaneous MPTP showed negligible FMT uptake bilaterally, with striatal to cerebellar ratios ranging from 1.3 to 1.9. Following bilateral AAV-AADC infusion, all animals showed increased striatal FMT uptake bilaterally. Paired *t* tests revealed significant increases in striatal to cerebellar ratios in both hemispheres ($P < 0.01$). These data are shown in Fig. 1.

We confirmed the effects of MPTP on the dopaminergic system histologically. Immunocytochemical examination of brain sections by anti-tyrosine hydroxylase (TH) and anti-AADC antibody staining revealed almost complete bilateral absence of TH-immunoreactive (IR) fibers in the caudate and putamen in systemically treated monkeys and ipsilateral to MPTP administration in the ICA-treated monkeys. TH-IR examination of midbrain sections

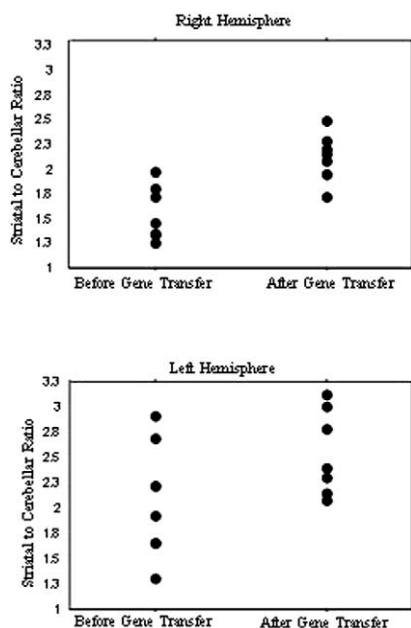


FIG. 1. Striatal-to-cerebellar ratios for the right and left hemispheres before and after gene transfer.

corresponded to the pattern of the striatal lesion with a dramatic reduction in TH-IR cells in the substantia nigra pars compacta (SNc), with some cells surviving in the dorsal aspect of the SNc and in the ventral tegmental area. AADC-IR examination was identical in the midbrain regions; however, in the striatal regions we detected strong but variable AADC-IR in the striatal neurons surrounding areas of AAV/AADC gene administration (see Fig. 2). We quantified transgene expression for each brain by scanning brain sections containing AADC-positive cell regions using a UMAX scanner. The images were downloaded into Adobe PhotoShop and selected regions of interest were outlined using NIH Image 1.63 (Bethesda, MD) software. We determined the distribution volume of AADC-IR regions and targeted regions (i.e., striatum) for each hemisphere.

As shown in Fig. 3, the percentage increase in the FMT uptake ratios from baseline corresponded closely to the area of gene expression as measured by AADC-IR, represented as a percentage of the total striatum. These measures were highly correlated for both right ($R = 0.78$) and left hemispheres ($R = 0.88$), although the correlation for the left hemisphere appeared to be partially driven by a single outlier. When we excluded the outlier, the correlation was weak ($R = 0.35$, $P = 0.5$). This is probably due to a ceiling effect because the left hemisphere was partially spared in the ICA-treated animals. In fact, the two animals with the highest FMT ratios at baseline showed the least change after AAV-AADC infusion, while the animals with the lowest baseline ratios showed the greatest change. PET images with corresponding histological sec-

tions for four of the animals are shown in Fig. 2. The monkeys with the most observable increase in striatal FMT uptake (M1 and M2) also stained strongly for AADC transgene expression.

The use of PET to monitor transgene expression *in vivo* is likely to play a critical role in the future implementation of a variety of gene therapy approaches. We found that PET and FMT can be used to monitor AADC gene expression using a ratio method that does not require arterial blood sampling and involves imaging times of only 30 min. While a number of PET studies, primarily in rodents, have shown evidence of transgene expression, many of these approaches rely on quantitative methods that typically involve arterial blood sampling. A simpler approach that does not involve arterial blood sampling would be much more easily implemented in humans, especially since patients will typically receive multiple PET scans to monitor gene expression over time. One alternative is to use radioactivity in the blood pool of the heart as the input function [5,6]. While this approach avoids arterial sampling, it still requires longer dynamic imaging and would not be possible for human studies that image only the brain. Approaches that use another brain region as a reference tissue to model the input function are more feasible but still require dynamic scanning and fairly long imaging times. The ratio method used in the current study requires emission acquisition times of only 30 min and is likely to be well tolerated by patients.

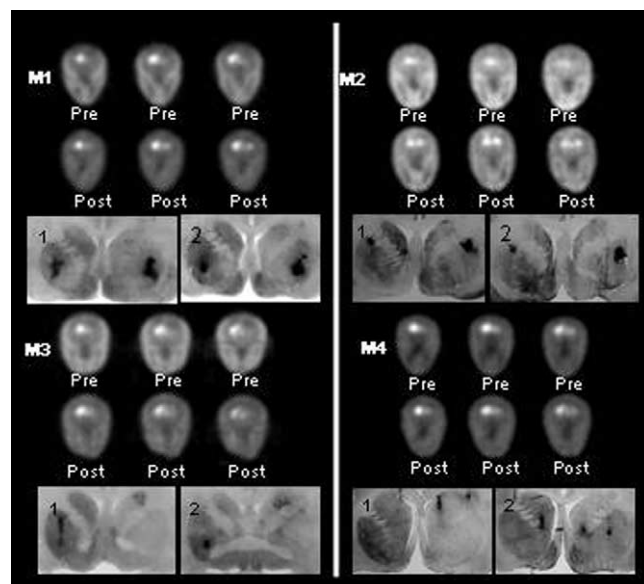


FIG. 2. Representative FMT PET images before and after AADC gene transfer and AADC immunoreactivity for the same animals. PET images for each animal show three levels through the striatum rostrally to caudally. Representative histological sections taken at two rostrocaudal levels demonstrate variable AADC expression in the striatum.

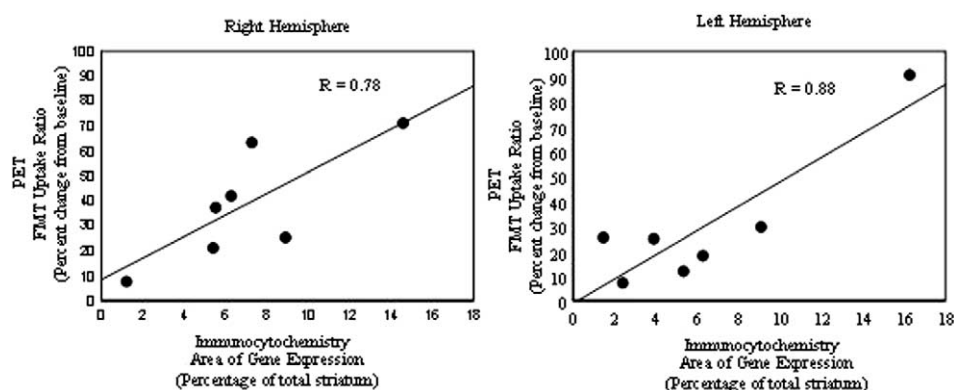


FIG. 3. Regression plots showing the relationship between PET measures of gene expression and immunocytochemical measures of the area of gene expression. PET measures are expressed as the percentage change in the striatal-to-cerebellum radioactivity ratios from baseline to after gene therapy. The immunocytochemical measures of gene expression are expressed as the percentage of the total striatum. The correlation for the left hemisphere dropped from 0.88 to 0.35 when the outlier was excluded.

To our knowledge, this is the first report showing a correlation between a direct *in vivo* measure of gene transfer with an immunocytochemical measure of gene transfer in the primate brain, although Kordower *et al.* previously reported similar findings following the lentiviral delivery of glial cell line-derived neurotrophic factor using an indirect imaging approach [7]. Our approach should be useful for the *in vivo* monitoring of gene transfer for the clinical use of gene therapy, especially for PD.

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