Blood–Brain Barrier Dysfunction in Parkinsonian Midbrain In Vivo

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Parkinson’s disease (PD) is associated with a loss of neurons from the midbrain. The cause of PD is unknown, but it is established that certain neurotoxins can cause similar syndromes. The brain is normally protected from these noxious blood-borne chemicals by the blood–brain barrier which includes specialized proteins on the inside of blood vessels in the brain. These act as molecular efflux pumps and P-glycoprotein (P-gp) is an abundant representative. Vulnerability to PD appears codetermined by the genotype for the P-gp gene. We hypothesized that PD patients have reduced P-gp function in the blood–brain barrier. We used positron emission tomography to measure brain uptake of [11C]-verapamil, which is normally extruded from the brain by P-gp. Here, we show significantly elevated uptake of [11C]-verapamil (18%) in the midbrain of PD patients relative to controls. This is the first evidence supporting a dysfunctional blood–brain barrier as a causative mechanism in PD.

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Parkinson’s disease is associated with neuronal death in several cell groups in the midbrain that normally produce large amounts of neurotransmitters such as dopamine (substantia nigra pars compacta, ventral tegmental area, retrorubral field), noradrenaline (locus coeruleus), serotonin (dorsal raphe nucleus), and acetylcholine (Edinger–Westphal nucleus).1 Clinical symptoms include rigidity, hypokinesia, and tremor. Despite a huge research effort during the last decades, the cause of PD remains unknown.

The observation that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a parkinsonian syndrome similar to Parkinson’s disease (PD) has led to the hypothesis that environmental toxins similar to MPTP could play an important role in the pathogenesis of PD.2 Many pesticides fit the profile in that they induce oxidative stress, mitochondrial damage, and apoptosis3,4 and cause damage to the brain’s dopaminergic cell groups.5,6 Also, the chemical structure of the widely used pesticide parathion is strikingly similar to that of MPTP. Indeed, exposure to pesticides is a risk factor for PD,7 and the association between PD and rural residence and well-water drinking8 might also be mediated by pesticides. A postmortem study found detectable levels of pesticides in the substantia nigra of PD patients that were higher than in controls,9 suggesting that these toxins are able to gain access to the brain.

Because not everyone who is exposed to pesticides develops PD, there is likely to also be a genetic component that determines vulnerability. The blood–brain barrier (BBB) gene MDR1 encodes P-glycoprotein (P-gp), a 170kDa peptide with ATPase activity that actively transports a wide range of molecules from the brain side to the blood side. A recent study found that PD patients exposed to pesticides were five times more likely to carry the 3435T allele of the MDR1 gene,10 which presumably results in reduced pump function.

In 1996, we reported the synthesis and initial in vivo evaluation of [11C]-verapamil,11 showing that it can be used as a positron emission tomography (PET) probe for P-gp function in vivo. We further characterized its distribution behavior in rats,12,13 normal and MDR1 knockout mice,14 and humans,15 confirming that in vivo uptake and distribution of [11C]-verapamil is a sensitive measure of P-gp function.

On the basis of the above, we hypothesized that PD patients would have reduced P-gp function in the brain.

Subjects and Methods

Subjects

Subjects were five nondemented PD patients (mean age ± standard deviation [SD], 64.8 ± 7.4; one woman, four men) who were undergoing treatment at the Movement Disorders Unit of the Neurology Clinic of the Groningen University Hospital, Hanzeplein 1, 9700 RB, Groningen, The Netherlands.

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Hospital. They met the Gelb criteria for “probable PD” and had a mean (± SD) disease history of 5.1 (± 1.9) years. Four of the five were taking antiparkinsonian medication (two were taking a dopamine agonist, one was taking a dopamine agonist plus amantadine, and one was taking l-dopa plus amantadine). Five healthy age-matched drug-free controls (mean age ± SD, 63.6 ± 13.1; two women, three men) were recruited through an advertisement. All subjects underwent a general physical and a neurological examination and were rated according to the Unified Parkinson Disease Rating Scale and the Mini-Mental State Examination by the same neurologist. No clinical indication of central nervous system disease was present in the control subjects. Mean Unified Parkinson Disease Rating Scale–III scores (±SD) in patients were 20.2 (± 7.9). Subjects gave written informed consent and the protocol was approved by the internal medical-ethical committee of the Groningen University Hospital.

**Positron Emission Tomography Procedure**

One cannula was inserted into an arm vein for administration of tracers, while a second, sampling cannula was inserted into the radial artery. After a bolus injection of [15O]-H2O, radioactivity was measured over a period of 4 minutes. [11C]-Verapamil was synthesized as described previously and after the [11C]-verapamil injection (111–430MBq), ten 30-second frames, three 5-minute frames, and three 10-minute frames were acquired with the PET camera. We used racemic [11C]-verapamil to avoid chiral purification, thus maximizing specific activity (P-gp has no stereoselectivity for [11C]-verapamil26). PET data were reconstructed to a 128 x 128 x 63 matrix with a plane separation of 2.425 mm and a bin size of 2.250 mm. During the [11C]-verapamil PET scan, 17 arterial blood samples were drawn at 0:15, 0:30, 0:45, 1:00, 1:15, 1:30, 1:45, 2:15, 2:45, 3:45, 4:45, 7:30, 12:30, 17:30, 25:00, 35:00, and 45:00 min. Radioactivity was counted in total blood, plasma, and in high-performance liquid chromatography–fractionated plasma. The latter method allows for identification of labeled metabolites, and for calculation of their contribution to the total signal.

**Data Analysis**

Using Matlab (MathWorks, Natick, MA) and a linearization according to Logan and colleagues27 starting at t = 5 minutes, parametric images for distribution volume (DV images) were constructed from the [11C]-verapamil scans. This pharmacokinetic model–independent analysis calculates the influx (Kv) and distribution volume (DV) of a reversibly binding radiotracer. For all subjects, the [11C]-verapamil scans were summated across frames using Clinical Applications Programming Package 5 (Siemens, Erlangen, Germany). The [15O]-H2O image was spatially normalized by Statistical Parametric Mapping (SPM) 99 to match the SPM [15O]-H2O template, and the normalization parameters were saved. These were used to normalize the DV images, which were then summed to form a [11C]-verapamil DV template. To further reduce interindividual differences in normalization, we normalized individual DV images again to this [11C]-verapamil DV template. After spatial smoothing by convolution with a Gaussian kernel of 20mm full-width at half-maximum, a two-sample t test was done with proportional scaling at an analysis threshold of 0.8, global calculation: mean voxel value. The t contrasts that were calculated were patients minus controls and vice versa, without masking at a threshold of an uncorrected p value less than 0.001. The individual values for the significant cluster were calculated with SPM99.

**Results and Discussion**

As shown previously,15 uptake of [11C]-verapamil was determined by the degree of vasculization and the presence or absence of a BBB: very high in the pituitary, high in the ventricles and skin, moderate in gray matter, and low in white matter and bone. Visual inspection of the uptake images resulted in no obvious differences in [11C]-verapamil uptake between patients and controls. A representative image of [11C]-verapamil uptake is given in Figure 1.

Although visual inspection of the uptake images themselves showed no differences, a pixel-by-pixel t test on the two groups showed significantly increased [11C]-verapamil uptake in the Parkinson’s disease patients. Strikingly, this increased uptake was restricted to one area that covered most of the midbrain and part of the dorsal pons (Fig 2). There were no brain areas in which patients had lower uptake than controls. The magnitude of the effect was 18%, and there was complete separation of the groups; that is, all the patients showed higher uptake than all the controls (Fig 3).

The location of the BBB impairment found in this study overlaps to a considerable extent the region known to show neuronal damage in postmortem PD brains. Of all cell groups known to be damaged,1 a
Reduced activity or expression of molecular efflux pump P-gp is likely to mediate the increased DV because $[^{11}C]$-verapamil is a substrate for P-gp. Although increased mesencephalic binding of $[^{11}C]$-verapamil in brain tissue past the endothelial cells theoretically could also cause an increased DV, we consider it unlikely that this is the case here. No evidence is available in the literature to suggest such altered binding in PD. With the Logan analysis, we could also exclude an increased influx ($K_i$) of $[^{11}C]$-verapamil as the cause of the increased DV. $K_i$ (± SD) was of the same magnitude in both groups: 0.89 (± 0.068) in controls and 0.95 (± 0.068) in PD patients ($t = 1.21$; not significant). Unaltered $K_i$ in the PD group is also evidence against endothelial damage and perfusion differences as the cause of the increased DV confirming previous results.\textsuperscript{12,13} We believe based on these considerations that the difference in DV between the groups is caused by reduced P-gp mediated efflux in the patients.

Any effects of antiparkinsonian medication on the uptake of $[^{11}C]$-verapamil are likely to be of a global nature. Because our data analysis includes an intrasubject numerical normalization it is insensitive to global differences. It is unlikely that PD medication would preferentially affect P-gp function in the midbrain. Also, there was one unmedicated patient who showed the same increase in DV in the midbrain.

In view of our findings, stimulation of P-gp in the...
BBB should be considered as a novel neuroprotective strategy. Many studies have described stimulation of P-gp activity in the gut or in cell lines in vitro by a wide range of prescription drugs, grapefruit juice, and St. John’s wort. There are also endogenous factors that stimulate P-gp expression, such as progesterone and HSP90β. Antagonists for the σ₂ receptor could also be promising because agonists reduce transcription of the MDR1 gene which encodes P-gp.

In summary, PD patients have reduced P-gp function in the midbrain. This suggests that P-gp dysfunction is part of PD pathogenesis.

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