3-*O*-Methyldopa Administration Does Not Alter Fluorodopa Transport into the Brain

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To determine if 3-*O*-methyldopa (3OMD) significantly inhibits the transport of 6-[^{18}F]fluorodopa (6-FD) into the brain at the concentration normally encountered during L-dopa administration, we performed 6-FD studies with positron emission tomography in cynomolgus monkeys in the presence and absence of 3OMD. Infusion of 3OMD was designed to produce plasma concentrations equivalent to those seen in patients on chronic L-dopa therapy. Plasma 3OMD levels of 39 ± 4 μM did not alter the blood–brain transfer rate of 6-FD. 6-FD positron emission tomographic studies in parkinsonian patients will therefore not be altered by 3OMD present in the blood in those patients taking L-dopa preparations. These results do not support the hypothesis that transport of L-dopa into the brain is inhibited by 3OMD to cause the declining response seen in patients with advanced Parkinson's disease.

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L-Dopa has dramatic effects in patients with Parkinson's disease and has become the mainstay therapy for this disorder [1]. Despite the success, problems arise with long-term treatment. L-Dopa may be metabolized by two enzymes in the periphery before reaching the brain. The enzyme dopa decarboxylase (DDC) converts L-dopa to dopamine. Inhibition of this enzyme by peripheral DDC inhibitors reduces some of the adverse effects of L-dopa therapy by reducing peripheral formation of dopamine and increasing the amount of L-dopa available for entry into the brain. 3-*O*-Methyldopa (3OMD) is the metabolite of L-dopa formed by catechol-*O*-methyltransferase (COMT). Because most patients taking L-dopa also receive DDC inhibitors, the COMT pathway is the major metabolic pathway for L-dopa in the periphery [2]. 3OMD has a plasma half-life in humans of approximately 15 to 18 hours [1]. Because 3OMD has been shown to use the same facilitated diffusion mechanism as L-dopa to gain access to the brain, it is possible that 3OMD could act as a competitor of L-dopa transport [3].

6-[^{18}F]Fluoro-L-dopa (6-FD) is an analogue of L-dopa and has been used to assess the nigrostriatal dopamine system in vivo with positron emission tomography (PET) [4]. It is unclear if 6-FD scans in patients with Parkinson's disease taking L-dopa are affected by the plasma accumulation of 3OMD. Most 6-FD–PET studies are performed after withdrawal of L-dopa for less than 24 hours to prevent competition of 6-FD with exogenous L-dopa for the large neutral amino acid (LNAA) transport system. 3OMD would not be cleared by that time and scans performed under these conditions may yield invalid results. We performed 6-FD–PET scans in cynomolgus monkeys to assess whether 3OMD infusions, in clinically relevant concentrations, would significantly reduce the blood–brain transfer of 6-FD. Results from these studies also help elucidate the role of 3OMD in the potential inhibition of L-dopa access into the brain in patients in longstanding L-dopa therapy.

Methods

Four male cynomolgus monkeys (*Macacus fascicularis*) weighing between 3 and 6 kg were used in this study. Magnetic resonance (MR) images were obtained with a specially designed head holder for PET–MR correlation, followed by 6-FD–PET studies for control state determinations. Each monkey had two control studies attempted. In one monkey, one of the control scans could not be analyzed due to problems with the high-performance liquid chromatographic

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(HPLC) data. Another monkey had three control studies. Each monkey underwent a separate 6-FD–PET study after the intravenous infusion of 3OMD. All studies were performed with the monkeys fasting from the day before the experiment to reduce risks of general anesthesia and to create similar basal plasma amino acid concentrations during the control and 3OMD studies. Plasma amino acids were measured in the 3OMD infusion experiments to determine the fraction of 3OMD of the total concentration of LNAAs. PET data were analyzed with a tracer kinetic model for 6-FD studies that yields a value for the blood–brain barrier transfer rate (K_1) and DDC activity (k_3) [5].

MR Scans

The MR images were obtained with a Philips Gyroscan S15 1.5 Tesla (Philips Medical Systems, NL-5680DA Best, Netherlands). The monkeys were initially anesthetized with ketamine hydrochloride (10 mg/kg IM) and atropine (0.05 mg/kg IM). After endotracheal intubation and intravenous catheter insertion, the monkeys received pentobarbital (7.5–10 mg/kg IV) as a loading dose and were maintained with 15 to 20% of the induction dose as required to suppress responses to toe pinch. When the monkeys were stable, they were transported to the MR facility and placed in the stereotactic head holder. This device was designed to produce a five-point bony fixation in a manner similar to the Kopf stereotactic frame. The monkey assumed a supine position with the ear bars and eye fixation in place. Three series of fiducial markers containing copper sulfate were used to compare the MR images with the PET images for interimage volume alignment. The Z-shaped markers were oriented along the axial plane vertically on each side of the head and horizontally underneath the head. The copper sulfate–filled tubes were fitted into grooves in the leucite frame for exact repositioning and correspondence with the lead bar placement for PET studies that fit in the same groove. T1-weighted 2-mm transverse images were acquired for anatomical correlation.

PET Studies

All studies were performed with the Scanditronix PC2048-15B PET scanner (Scanditronix AB, Uppsala, Sweden) [6]. The monkeys were anesthetized with the same protocol used for the MR imaging studies and transported to the PET unit. Carbidopa (5 mg/kg) was administered intravenously over 30 minutes between 90 and 120 minutes before the injection of 6-FD. A femoral or tibial arterial line was placed for rapid blood sampling and for invasive blood pressure monitoring. Respiratory rate, temperature, and heart rate were also monitored.

Tracer 6-FD was prepared using an adaptation of the method of Luxen and Barrio [7] that has been published previously [5]. A dose of 1.5 to 3 mCi was injected for each study with an approximate specific activity of 4×10^{-4} μCi/pmol at the end of the synthesis. The estimated total mass of 6-FD injected was approximately 1 to 2 mg per scan. The 27 frames were acquired in 90 minutes. Frames of 30 seconds were followed by frames of 1, 2, 5, and 10 minutes. During the experiment, 23 blood samples were taken to determine radioactivity in plasma. Of these, between 9 and 12 samples were submitted for HPLC separation with a radioactivity detector to assess the proportion of the plasma radioactivity due to 6-FD or 3-*O*-methylfluorodopa.

3OMD Infusions

For the 3OMD infusion experiments, each monkey received an intravenous infusion of 15 mg/kg of 3OMD over 15 minutes approximately 2 hours before the injection of 6-FD. Nine blood samples were drawn to assess the plasma 3OMD concentration during the PET studies. These samples were assayed for 3OMD by the HPLC method of Cedarbaum and colleagues [8]. In one experiment, the samples drawn for 3OMD determination were not accurately timed and a second experiment was performed to simulate the 3OMD concentration on the day of the PET study, without 6-FD administration.

Measurement of Plasma LNAAs

Plasma LNAAs were measured by an HPLC procedure in the same samples used for quantitation of 3OMD [9].

PET Scan Analysis

Plasma radioactivity concentrations were normalized to allow comparisons between experiments, by calculating the pharmacokinetic circulation time, Θ:

$$\Theta = \frac{\int_0^T C_a(t)dt}{C_a(T)}$$

where $C_a(t)$ is the radioactivity concentration in plasma as a function of time and $C_a(T)$ is the concentration at a specific time (T). The quantity Θ may be calculated for a specific compound, such as 6-FD, or for total plasma radioactivity. The pharmacokinetic circulation time is an indication of the time required to circulate the substance to obtain the same availability achieved by maintaining the plasma concentration constant [$C_a(T)$].

PET images were analyzed using a three-compartment tracer kinetic model that yields values for the unidirectional blood–brain clearance of 6-FD (K_1) and the fractional rate of decarboxylation of 6-FD (k_3) [10].

Regions of Interest

Regions of interest (ROIs) were generated based on MR images matched to transmission images (used for the reconstruction of PET data) using three fiducial markers ($CuSO_4$ for MR and lead for transmission scans). Homologous points were identified from both image methods and brought to correspondence using an affine transformation. MR volumes were then "resliced" so direct correspondence with PET slices could be achieved. From the resulting MR images, ROIs were drawn corresponding to striatum (1.7 cm^2 × 2) and cortical structures (1.0 cm^2 × 8). These ROIs were then used for PET image analysis.

Results

3OMD Administration

The plasma concentrations of 3OMD were 38.84 ± 3.81 (SEM) μM at 2 hours and 33.23 ± 1.56 μM at

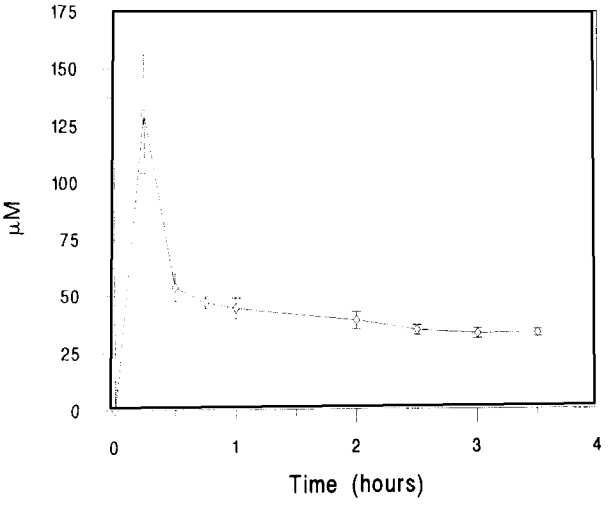


Fig 1. Plasma 3-O-methyldopa concentration versus time after intravenous administration of 15 mg/kg in cynomolgus monkeys. Positron emission tomographic studies were performed between 2 and 3.5 hours.

3.5 hours after infusion (n = 4; Fig 1; p = NS, paired t test). Because the 6-FD infusion occurred at approximately 2 hours after the 3OMD infusion for the PET experiments, the plasma concentration of 3OMD was constant during the period of 6-FD transport into the brain. The pharmacokinetics of 3OMD in cynomolgus monkeys has been reported previously and is similar to that in humans [11].

Plasma Amino Acid Concentrations

Plasma LNAAs were measured at the time of 6-FD administration in the 3OMD infusion experiments. The purpose was to identify the fraction that 3OMD represented of LNAA competing for transport at the time of 6-FD administration. Plasma concentrations of valine, leucine, isoleucine, phenylalanine, and tyrosine were summed to calculate the total plasma dietary LNAA at the time of the 6-FD administration. The average total LNAA concentration in plasma was 477 ± 32 μM (n = 4). The analysis of plasma samples taken before 3OMD infusion in selected monkeys showed consistent fasting LNAA concentrations.

PET Studies

Values of Θ, K_1, and k_3 are listed in the Table. Infusion of 3OMD did not significantly affect the plasma pharmacokinetics of 6-FD as indicated by unchanged tracer pharmacokinetic circulation times (Θ) in the infusion experiments. As expected, neither was the blood–brain transfer rate altered significantly by infusion of 3OMD. With 10 to 20% coefficients of variation, a small decline of K_1 is not statistically demonstrable with our technique. Finally, the 3OMD infusion did not significantly alter the metabolism of 6-FD in the striatal regions studied as indicated by the absence of a change in k_3. Figure 2 shows PET images that include the striatal slices in a control study and a 3OMD infusion study.

Blood–Brain Barrier Clearance (K_1) *and Decarboxylation* (k_3) *Coefficients Before and After 3OMD Infusion*

Variable	Control (n = 4)	3OMD Infusion (n = 4)
$\Theta_{(6\text{-FD})}$ (min)	252 ± 32	233 ± 62[a]
Striatal K_1 (ml cm^{-3} h^{-1})	3.0 ± 0.4	3.2 ± 0.7[a]
Striatal k_3 (h^{-1})	3.9 ± 0.8	3.1 ± 0.6[a]

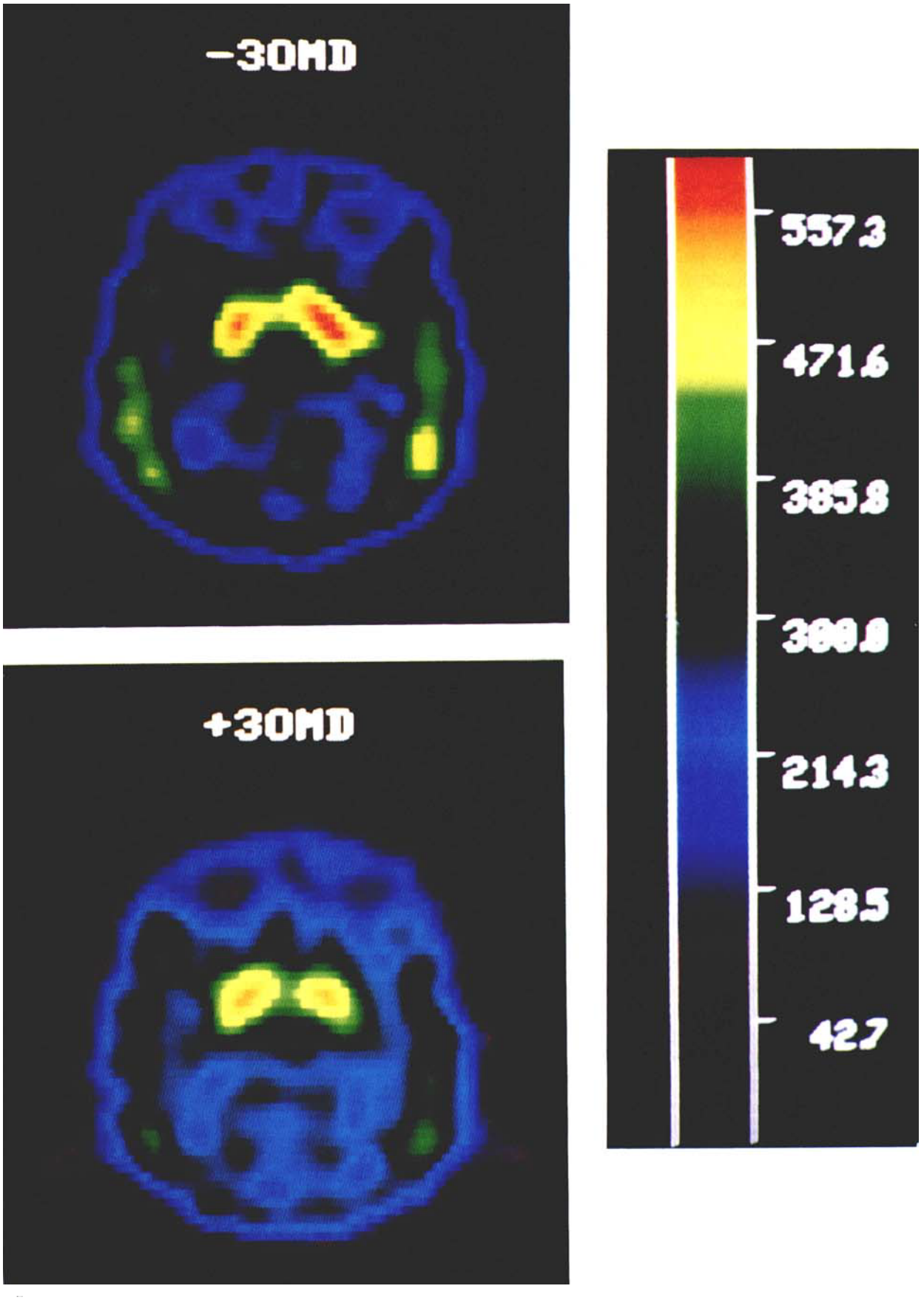
Values are the mean ± SD.
[a]Values are not significantly different from control by paired t test compared with control.
3OMD = 3-*O*-methyldopa; 6-FD = 6-[^{18}F]fluorodopa.

Discussion

The PET studies directly measured the blood–brain transport of 6-FD in cynomolgus monkeys. The results show that 3OMD, in clinically relevant concentrations, does not significantly inhibit the blood–brain transfer of 6-FD. 6-FD studies of patients with Parkinson's disease, who take L-dopa, therefore need not be preceded by a prolonged drug holiday to avoid interference with 6-FD's access into the brain by 3OMD. By analogy, we can conclude that 3OMD, in the concentration found in our experiments, does not inhibit L-dopa transport into the brain because 6-FD is an analogue of L-dopa with similar kinetic properties [12]. The total mass of 6-FD used in these experiments was extremely low when compared with the usual dose of L-dopa given to patients. Because inhibition of blood–brain transport was not observed with the tracer doses of 6-FD, it is probable that 3OMD would not inhibit L-dopa transport given in clinical doses.

6-FD and 3OMD share the facilitated diffusion mechanism of LNAA for entry into the brain [13]. Competition for this transporter is determined by the plasma concentrations of all competing LNAAs relative to the transporter affinities of the individual amino acids [14]. The competing LNAAs are mostly the dietary amino acids that saturate the transporter at their normal plasma concentrations [15]. Plasma concentrations of eight LNAAs in humans during fasting are approximately 20-fold higher than the plasma concen-

Fig 2. 6-Fluorodopa–positron emission tomographic scans in the same monkey before (top) and after infusion of 3-O-methyldopa (bottom). No changes were observed in radioactive uptake and retention. Images are displayed on a common scale and are normalized against total plasma radioactivity.


-3OMD
+3OMD
5573
4716
3858
3000
2143
1285
427

tration of 3OMD found in the present experiments [16]. In the monkeys, the sum of the concentrations of five LNAAs is approximately 15-fold higher than that of 6-FD infusion. Also the LNAA transporter has a higher affinity for most dietary LNAAs than L-dopa [17] and 3OMD [3]. In rats, the apparent K_1 of the combined LNAAs in plasma is low, 15 μM [18] (relative to phenylalanine), whereas the total concentration of amino acids (also relative to phenylalanine) is close to 500 μM, indicating 97% saturation of the blood–brain barrier LNAA transporters. If LNAA concentrations and individual blood–brain transfer affinities are similar in monkeys, addition of 35 μM 3OMD with a transport affinity of at least 25 μM will inhibit LNAA extraction, including 6-FD, by less than 0.5%. The concentration of 3OMD at the time of 6-FD infusion was approximately 35 μM, or less than 7% of all LNAAs in the fasting state in these monkeys. Thus, it is not surprising that the addition of a relatively small amount of 3OMD resulted in no changes of the transport of 6-FD into the brain. A similarly insignificant change would be expected in humans.

By increasing the concentrations of eight dietary LNAAs by a factor of 2.4 with an intravenous infusion, Leenders and colleagues [16] demonstrated a profound decrease in the cerebral uptake of 6-FD in a subject with PET. Further analysis of this data with a tracer kinetic model for 6-FD showed that K_1 in the striatum was reduced by a factor of 3.6 with the amino acid infusion [18], in approximate agreement with the theoretical prediction of a 2.5-fold reduction. The reduction is in keeping with the clinical deterioration that is experienced by some parkinsonian patients taking L-dopa during an increased intake of dietary amino acids [9].

Earlier studies in rats revealed that 3OMD infusion (250 and 1,000 μM) reduced the blood–brain transport of L-dopa [3]. Further studies showed that 3OMD infusion blocks striatal dopa metabolism in a dose-dependent fashion [19] and inhibits the behavioral response to L-dopa in lesioned rats [20]. Gervas and colleagues [21] also reported that striatal [^{14}C]dopa retention in rats was decreased with 3OMD infusions. The doses of 3OMD used in these studies ranged from 50 to 800 mg/kg, with significant changes occurring with doses of 200 mg/kg or greater. This was much larger than the 15 mg/kg dose that we used. Plasma 3OMD concentrations were not measured in these experiments. It is possible that significant changes occurred in total plasma LNAA with the large doses of 3OMD that were administered. The plasma 3OMD concentrations established in the present PET studies were approximately fourfold as high as those found in patients on chronic L-dopa therapy and twofold as high as found in patients taking Sinemet CR-3 [22].

Our findings are compatible with prior reports that fluctuations in 3OMD plasma concentrations do not play an important role in the adverse effects of antiparkinsonian therapy or the clinical fluctuations found in patients with Parkinson's disease [23–25]. Other mechanisms must therefore be advanced to explain these important problems.

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