Neuropsychopharmacology (2005) 30, 2154–2161

www.neuropsychopharmacology.org

Estimation of Plasma IC_{50} of Donepezil Hydrochloride for Brain Acetylcholinesterase Inhibition in Monkey Using *N*-[^{11}C]methylpiperidin-4-yl Acetate ([^{11}C]MP4A) and PET

Tetsuya Shiraishi[1,2], Tatsuya Kikuchi[1], Kiyoshi Fukushi[1], Hitoshi Shinotoh[1,3], Shin-ichiro Nagatsuka[1,4], Noriko Tanaka[1,5], Tsuneyoshi Ota[1,6], Koichi Sato[1,2], Shigeki Hirano[1,7], Shuji Tanada[1], Masaomi Iyo[2] and Toshiaki Irie*[,1]

[1]Department of Medical Imaging, National Institute of Radiological Sciences, Graduate School of Medicine, Chiba University, Chiba, Japan; [2]Department of Psychiatry, Graduate School of Medicine, Chiba University, Chiba, Japan; [3]Asahi Neurological Hospital, Chiba, Japan; [4]ADME/TOX Research Institute, Daiichi Pure Chemicals, Ibaraki, Japan; [5]Department of Neurosurgery, Tokyo Women's Medical College Daini Hospital, Tokyo, Japan; [6]Department of Psychiatry, Juntendo University School of Medicine, Tokyo, Japan; [7]Department of Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan

Donepezil hydrochloride is a potent and selective inhibitor for brain acetylcholinesterase (AChE) and is currently used worldwide for the treatment of Alzheimer's disease. Until now, there is no *in vivo* study on the relation between the plasma concentration and the brain AChE inhibition. The purpose of this study was to estimate *in vivo* plasma IC_{50} of donepezil in living monkeys by measuring plasma donepezil concentration (LC/MS/MS) and brain AChE activity with positron emission tomography (PET) and *N*-[^{11}C]methylpiperidin-4-yl acetate, which is an acetylcholine analog recently developed by us for quantifying *in vivo* brain AChE activity. PET scans with donepezil at two doses, 100 μg/kg (donepezil-1; $N=5$) or 250 μg/kg (donepezil-2; $N=5$), were performed using the same monkeys at 4-week intervals. Before each PET scan, baseline PET scans ($N=10$ in total) were performed without donepezil. The plasma donepezil concentrations 14 min after intravenous injection were proportional to the doses, 17.2 ± 2.9 ng/ml (donepezil-1) and 44.0 ± 5.0 ng/ml (donepezil-2), and the mean AChE inhibitions in four neocortical regions as evaluated by PET were also dose-dependent, 27% (donepezil-1) and 53% (donepezil-2). In IC_{50} estimation, measured plasma donepezil concentrations were corrected for the change during PET scan. The IC_{50} values (estimate $\pm$ SE) were 42 ± 9.0 (ng/ml; donepezil-1), 34 ± 3.2 (donepezil-2), and 37 ± 4.1 (combined data). The present method may be useful for *in vivo* evaluation of other AChE inhibitors and novel drugs.
Neuropsychopharmacology (2005) **30,** 2154–2161. doi:10.1038/sj.npp.1300759; published online 25 May 2005

Keywords: acetylcholinesterase; Alzheimer's disease; *in vivo* IC_{50}; *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A); positron emission tomography (PET); donepezil hydrochloride

INTRODUCTION

Degeneration of cholinergic basal forebrain neurons innervating the cerebral cortex is believed to contribute substantially to cognitive deficits seen in Alzheimer's disease (AD) (Bartus *et al*, 1982). This discovery triggered development of cholinesterase inhibitors such as donepezil with the aim to raise acetylcholine levels in the brain by blocking acetylcholinesterase (AChE). Donepezil is a reversible inhibitor that exhibits high specificity for centrally active AChE (Yamanishi, 1990; Rho and Lipson, 1997; Rogers *et al*, 1998).

In recent years, *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A) (Irie *et al*, 1994) and *N*-[^{11}C]methylpiperidin-4-yl propionate ([^{11}C]MP4P) (Irie *et al*, 1994; Kilbourn *et al*, 1996) have been developed as radiotracers for brain AChE mapping and applied to quantification of neocortical AChE activity in healthy subjects (Namba *et al*, 1999; Koeppe *et al*, 1999) and in patients with AD (Iyo *et al*, 1997; Kuhl *et al*, 1999). Both tracers were also applied to evaluating the inhibitory effect of donepezil on brain AChE activity in AD patients (Kuhl *et al*, 2000; Shinotoh *et al*, 2001; Kaasinen *et al*, 2002) and in monkeys (Tsukada *et al*, 2004). As for donepezil, however, there has been no report on the quantitative relation between plasma concentration and brain AChE inhibition in the living subjects.

*Correspondence: Dr T Irie, Department of Medical Imaging, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba-shi, Chiba 263-8555, Japan, Tel: +81 43 206 3191, Fax: +81 43 251 7147, E-mail: t_irie@nirs.go.jp
Received 7 December 2004; revised 17 March 2005; accepted 18 March 2005
Online publication: 1 April 2005 at http://www.acnp.org/citations/NPP040105040577/default.pdf

In the case of reversible cholinesterase inhibitors such as tacrine and donepezil, inhibition of enzyme activity can be partially reversed by dilution, because of dissociation of the inhibitor from the enzyme with time (Hunter *et al*, 1989; Dawson, 1990; Kosasa *et al*, 2000). Furthermore, using microdialysis, it is reported that donepezil dose-dependently increases the extracellular acetylcholine concentration in rats (Kawashima *et al*, 1994; Kosasa *et al*, 1999) and in monkey (Tsukada *et al*, 2004), which may cause additional AChE inhibition (substrate inhibition) (Reiner and Radic, 2000). With positron emission tomography (PET), not only can we assess brain AChE inhibition by a reversible inhibitor without tissue-dilution effect, but also we can evaluate the inhibitory effects of increased synaptic acetylcholine levels on AChE *in vivo*.

In this study, we estimated *in vivo* plasma IC_{50} of donepezil for brain AChE inhibition in monkeys from measurement of plasma donepezil concentration and cerebral cortical AChE inhibition as evaluated with [^{11}C]MP4A-PET. We also discussed the difference between *in vivo* IC_{50} and *in vitro* IC_{50} for donepezil.

MATERIALS AND METHODS

Principle of the [^{11}C]MP4A Method

[^{11}C]MP4A is a lipophilic acetylcholine analog with high AChE specificity (95% in monkey brain; unpublished data). Figure 1 represents a three-compartment model for [^{11}C]MP4A, consisting of an arterial blood compartment and two tissue compartments. The analog readily enters the brain by diffusion, and then a portion of incorporated [^{11}C]MP4A diffuses back into blood, whereas the remainder is specifically hydrolyzed by AChE into the hydrophilic metabolite, [^{11}C]methylpiperidinol ([^{11}C]MP4OH), which is trapped at the site of metabolic reaction within the brain. The entire process is described by three functional parameters: K_1, representing the rate constant for transport from blood to brain, k_2, representing the rate constant for transport from brain to blood, and k_3, representing the rate constant for hydrolysis by AChE. The k_3 value is used as an index of regional AChE activity.

[^{11}C]MP4A was synthesized by the reaction of its demethyl precursor with [^{11}C]methyl iodide as described previously (Namba *et al*, 1999).

Animals

Five male rhesus monkeys (Macacca mulatta, body weight 4–6.5 kg) were used for the study with PET. Monkeys were maintained and handled in accordance with the recommendations of the US National Institute of Health and the guidelines of the Central Research Laboratory, Hamamatsu Photonics. They were trained to sit on a chair twice a week for more than 3 months. Magnetic resonance images (MRI) of all monkeys were obtained with a Toshiba MRT-50A/II (0.5 T) under anesthesia with pentobarbital. The stereotactic coordinates of PET and MRI were adjusted based on the orbitomeatal (OM) line with a specially designed head holder. At least 1 month before the PET study, an acrylic plate, with which the monkey was fixed to the monkey chair, was attached to the head under pentobarbital anesthesia as described previously (Onoe *et al*, 1994).

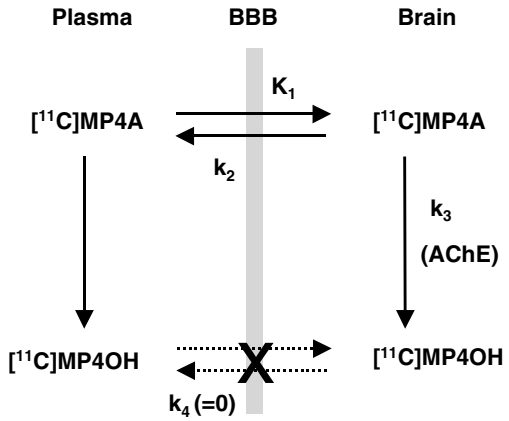

Figure 1 Schematic representation of the three-compartment model of [^{11}C]MP4A in the brain.

Measurement of Time Courses of Donepezil in Plasma

In preliminary studies using another three monkeys, to estimate the time when distribution volumes of donepezil between plasma and tissues reach the steady state, time courses of plasma donepezil concentration were measured after intravenous injection of donepezil at a dose of 250 μg/kg. A cannula was implanted into the posterior tibial vein for administration of donepezil. Another cannula was put into the femoral artery of the other leg to obtain arterial blood samples. Donepezil was intravenously administered to awake monkey by single bolus injection in a volume of 0.5 ml/kg. Arterial blood samples were collected (2 ml/tube) at 5, 10, 15, 30, 60, and 90 min after injection and the concentration was analyzed by liquid chromatography/two-mass spectrometry (LC/MS/MS) as described below. Donepezil hydrochloride was kindly supplied by Eisai Chemicals (Ibaraki, Japan).

Protocols for Donepezil Administration and PET Scan

PET scans with donepezil were performed at a dose of 100 μg/kg (donepezil-1; $N=5$) and 250 μg/kg (donepezil-2; $N=5$) using the same five monkeys at 4-week intervals. Before each PET scan with donepezil, baseline PET scans (Baseline-1 or Baseline-2; $N=5$ for each) were performed. Donepezil was intravenously administered by single bolus injection 15 min prior to the start of PET scanning. The experimental design is illustrated in Figure 2.

The PET scan was carried out with a high-resolution PET scanner (SHR-7000, Hamamatsu Photonics K.K., Hamamatsu, Japan) with transaxial resolution of 2.6 mm full-width at half-maximum (FWHM) and a center-to-center distance of 3.6 mm. PET images were reconstructed by the filtered-back projection with a Hunning 4.5 mm filter.

In practice, a monkey was fixed on the monkey chair with stereotactic coordinate aligned parallel to the OM line in the gantry of PET scanner after overnight fasting. The monkeys were fully awake during the whole PET procedures. A cannula was implanted into the posterior tibial vein for administration of donepezil and [^{11}C]MP4A. Another

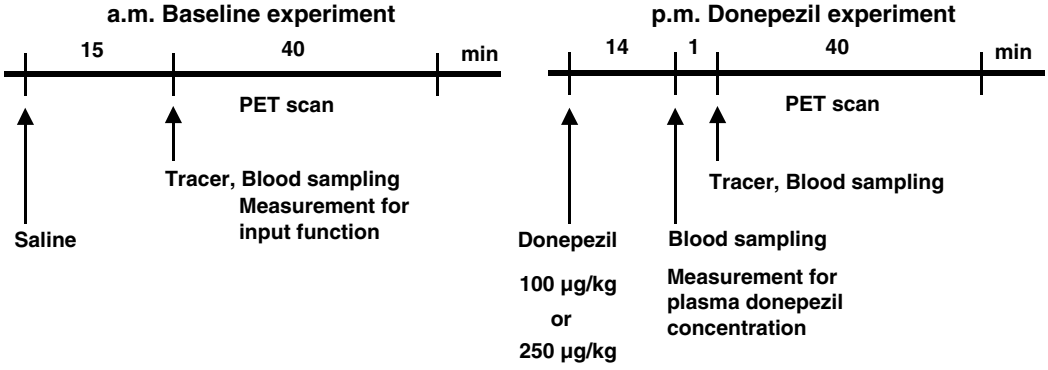

Figure 2 Illustrating of a time schedule for baseline experiment and donepezil experiment.

cannula was put into the femoral artery of the other leg to obtain arterial blood samples. PET scan was performed under dim light. After 30 min of transmission scanning using ^{68}Ge–^{68}Ga external standard, a monkey received an intravenous infusion of [^{11}C]MP4A (100–350 MBq/kg) in 3 ml of saline for 30 s. PET scan started simultaneously and 16 sequential frames were acquired dynamically over a period of 40 min. The image data were reconstructed, and regions of interest (ROIs) were placed in the frontal, temporal, parietal and occipital cortices on the PET image of the brain with reference to the corresponding MRI of each monkey brain and then the time–activity curve in ROIs were obtained.

Measurement of Arterial Input Function for [^{11}C]MP4A

Seventeen arterial blood samples were collected (0.5 ml/tube) in a 1.5 ml microtube containing 0.1 mg of physostigmine, an inhibitor of cholinesterases, in 0.01 ml of heparinized saline. Blood sampling was started at PET scan start time and continued for 30 min at appropriate time intervals. The total radioactivity in the plasma and the ratio of authentic [^{11}C]MP4A to the metabolite [^{11}C]MP4OH were measured, and then the time–activity curve of authentic [^{11}C]MP4A in plasma (input function; Ca(t)) was calculated as reported previously (Namba *et al*, 1999).

Kinetic Analysis for [^{11}C]MP4A

The regional K_1–k_3 parameters of [^{11}C]MP4A were estimated from PET data based on a three-compartment model as described below (Namba *et al*, 1999), where $C_S(t)$ represents the theoretical function for authentic tracer, $C_M(t)$ the metabolite, $C_T(t)$ the total radioactivity and $\otimes$ the mathematical operation of convolution.

$$C_S(t) = \int_0^t K_1 \mathrm{Ca}(\theta) e^{-(k_2+k_3)(t-\theta)} d\theta \quad (1)$$

$$C_M(t) = \int_0^t k_3 C_S(\theta) d\theta \quad (2)$$

$$\begin{aligned} C_T(t) &= C_S(t) + C_M(t) \\ &= \frac{K_1}{k_2+k_3}[k_3 + k_2 e^{-(k_2+k_3)t}] \otimes \mathrm{Ca}(t) \end{aligned} \quad (3)$$

The K_1-k_3 values were estimated by fitting $C_T(t_i)$ ($i=$ 1–16) to the observed PET data using an iterative nonlinear least-squares optimization.

Measurement of Plasma Donepezil Concentration

An arterial blood sample was obtained 1 min before the start of PET scan (14 min after injection of donepezil) and analyzed for donepezil by LC/MS/MS as follows (Kosasa *et al*, 2000). The analysis was entrusted to ADME/TOX Research Institute, Daiichi Pure Chemicals, Ibaraki, Japan. Blood samples were centrifuged at 3000 rpm for 15 min, and obtained plasma samples were stored at -20°C. Electrospray ionization-MS/MS was carried out on an API 3000 (Applied Biosystems, Tokyo, Japan) mass spectrometer equipped with a LC system, Agilent 1100 (Agilent Technologies, Tokyo, Japan). The spectrometer was set to admit the protonated molecules $[M+H]^+$ at m/z 380 (donepezil) and m/z 394 (internal standard; (*R*,*S*)-1-benzyl-4-[2-[(5,6-dimethoxy-1-indanon)-2-yl]-ethyl]piperidine hydrochloride), with monitoring of the product ions at m/z 91 (donepezil) and m/z 91 (internal standard). The limit of quantification for donepezil was set at 5 ng/ml.

The Correction of Plasma Donepezil Concentration

Even after reaching the steady state following intravenous bolus injection of donepezil in monkeys, plasma donepezil concentration decreases gradually, and consequently brain AChE activity may be changed during 40-min PET scan. We actually measure brain AChE activity as an average value during the PET scan, more correctly, during the residence time of authentic [^{11}C]MP4A in the brain. By this reason, in the estimation of plasma IC_{50}, we used the corrected value of plasma donepezil concentration, that is, the concentration at the mean residence time (MRT) of authentic [^{11}C]MP4A in the brain. The correction of plasma donepezil concentration was made as follows.

First, based on the results of measurements of plasma donepezil concentration in three monkeys after intravenous injection of donepezil at a dose of 250 μg/kg, the standard curve (Cp(t) in Figure 3) was obtained by averaging the results from three monkeys, followed by curve fitting with bi-exponential function as $\mathrm{Cp}(t) = 84.9e^{-0.058t} + 46.6e^{-0.011t}$. Of each monkey in donepezil-1 and donepezil-2 studies, we measured the plasma concentration only once, 14 min after

injection of donepezil (open triangle in Figure 3). The correction curve, $Cp'(t)$, was defined as the mono-exponential curve which has the same slope as the second term of $Cp(t)$ and pass through the measured point. Second, based on the result of nonlinear least-square analysis of [^{11}C]MP4A data in the occipital cortex, MRT (min) for authentic [^{11}C]MP4A in the brain was calculated for each monkey as follows:

$$\mathrm{MRT} = \frac{\int_0^{40} t\mathrm{Cs}(t)\mathrm{d}t}{\int_0^{40} \mathrm{Cs}(t)\mathrm{d}t}$$

where $Cs(t)$ represents a theoretical curve for authentic [^{11}C]MP4A in the brain. Finally, because PET scan was performed after 15 min donepezil injection, the corrected value for plasma donepezil concentration was obtained as $Cp'(15 + \mathrm{MRT})$ from the correction curve, $Cp'(t)$, at the time $15 + \mathrm{MRT}$ min (open square in Figure 3).

Estimation of Plasma IC_{50}

The value of plasma IC_{50} of donepezil, that is, the concentration of donepezil in plasma that inhibits brain AChE activity by 50% in the steady-state conditions of distribution volumes between plasma and brain, was calculated using a simple one-parameter model as follows:

$$\text{AChE inhibition} = \frac{[\mathrm{I}]}{\text{plasma } \mathrm{IC}_{50} + [\mathrm{I}]}$$

where AChE inhibition represents mean k_3 reduction in the four regions and [I] represents the corrected donepezil concentration in plasma. The plasma IC_{50} values were estimated in three ways, that is, for each dose experiment, separately, and from the combined data. The standard errors of plasma IC_{50} estimates were calculated with the variance–covariance matrix (Veng, 1977; Carson, 1986).

Statistically, the following null hypothesis was used to compare the plasma IC_{50} values between each dose experiment. The null hypothesis was that the plasma IC_{50} values obtained from the two-doses experiments would be sampled from the same normal distribution and the null hypothesis was to be rejected at the 0.05 level probability.

RESULTS

Time Courses of Donepezil Concentration in Plasma

After intravenous injection of donepezil at a dose of 250 μg/kg in three monkeys, the shape of concentration curve plotted in semilogarithmic scale, $Cp(t)$, showed a biphasic pattern (Figure 3). Nonlinear regression analysis was used to determine the standard concentration curve as $Cp(t) = 84.9e^{-0.058t} + 46.6e^{-0.011t}$, which is composed of the early phase (half-life; 12 min) and the later phase (63 min). From the shape of this curve, it was assumed that the steady-state conditions of distribution volumes between plasma and brain had been established 15 min after intravenous injection of donepezil in monkeys.

Baseline Study

In baseline studies, the regional k_3 values of [^{11}C]MP4A were measured using the same monkeys ($N = 5$) before the two doses of donepezil studies at 4-week intervals, once before (Baseline-1) and once (Baseline-2) after donepezil administration (100 μg/kg). The baseline k_3 values (mean ± SD; min^{-1}) in the four cerebral cortical regions were as follows: (Baseline-1) temporal, 0.174 ± 0.014; frontal, 0.173 ± 0.016; occipital, 0.145 ± 0.015; parietal, 0.142 ± 0.036. (Baseline-2) temporal, 0.186 ± 0.040; frontal, 0.171 ± 0.026; occipital, 0.154 ± 0.032; parietal, 0.144 ± 0.031. Figure 4 shows the change in cerebral cortical mean k_3 for five monkeys between the two baseline studies. The mean and SD values of the cerebral cortical mean k_3 were 0.158 ± 0.019 in Baseline-1 (○) and 0.164 ± 0.030 in Baseline-2 (□). Though the mean value in Baseline-2 was higher by 3.5% compared with Baseline-1, the difference was statistically not significant ($P = 0.55$ using a paired t-test). Actually, two of the five monkeys showed reductions (−8.7 and −7.0%) and three showed increases (2.2, 6.4, and 23.8%). As for the reproducibility of baseline k_3 values, mean absolute difference in cerebral cortical k_3 values between two experiments was 9.2%.

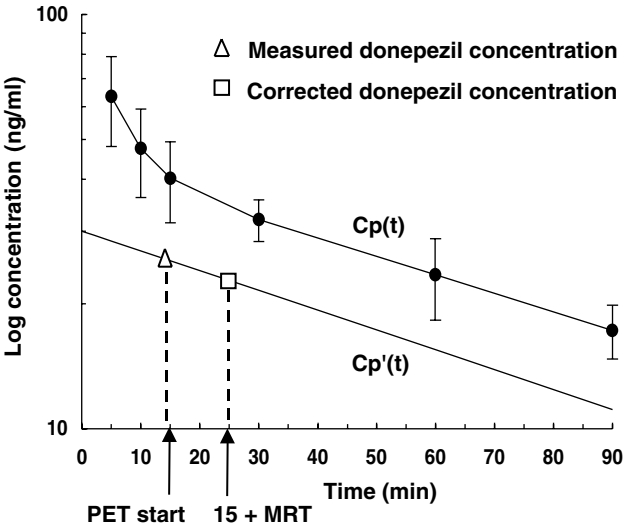

Figure 3 (Upper curve) A time course of plasma donepezil concentration after intravenous injection of 250 μg/kg donepezil in three monkeys: The curve showed a biphasic pattern which was fitted with bi-exponential function as $Cp(t) = 84.9e^{-0.058t} + 46.6e^{-0.011t}$. (Lower curve) The $Cp'(t)$ represents a correction curve, derived from the slower component of $Cp(t)$ and the measured value (△) in each monkey. The corrected value (□) was calculated as the $Cp'(15 + \mathrm{MRT})$ value, where MRT represents the estimated value of mean residence time of authentic [^{11}C]MP4A in the brain for each monkey.

Donepezil Study

Figure 5 shows the dose dependency of regional AChE inhibition as calculated by k_3 change between baseline experiments and donepezil experiments. In both doses of donepezil, the AChE reductions were almost the same across the four regions examined, 27 ± 0.6% in donepezil-1 (100 μg/kg) and 53 ± 0.7% in donepezil-2 (250 μg/kg). In the estimation of plasma IC_{50}, therefore, the mean value for the four regions was used as AChE inhibition for each monkey.

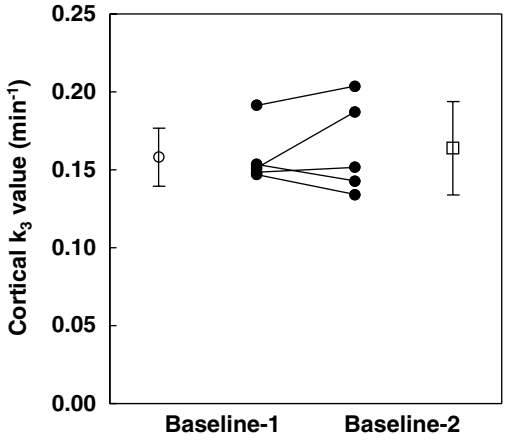

Figure 4 Reproducibility of cerebral cortical k_3 values between two baseline experiments performed at 4-week intervals after single intravenous injection of donepezil in five monkeys at a dose of 100 μg/kg. The open circle and open square represent the mean of cortical k_3 values in five monkeys before and after donepezil injection, respectively. The closed circles represent cerebral cortical k_3 values for repeated scans of each monkey.

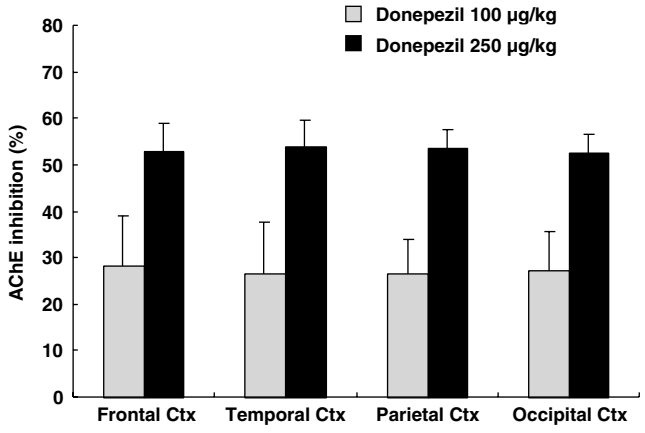

Figure 5 Dose effects of donepezil on AChE activity as measured by [^{11}C]MP4A in the cerebral cortical regions of monkeys. Data are expressed as a percent inhibition for five monkeys per treatment condition. The difference in AChE inhibition between the two doses was statistically significant in all regions ($P<0.05$ using a paired *t*-test).

Donepezil Concentration in Plasma

The plasma donepezil concentrations 14 min after intravenous injection were 17.2 ± 2.9 ng/ml (mean $\pm$ SD) in donepezil-1, and 44.0 ± 5.0 ng/ml in donepezil-2 experiment. The ratio of the measured donepezil concentration in plasma between donepezil-1 and donepezil-2 experiment (2.6-fold) was almost the same as the ratio of administered dosage of donepezil (2.5-fold).

Since the concentration of donepezil in plasma gradually decreased during PET scan, in the estimation of plasma IC_{50} of donepezil, we used the corrected value of plasma donepezil concentration, which was calculated as follows. Representative time–radioactivity curve of authentic tracer in the brain calculated by nonlinear least-squares analysis is shown in Figure 6. From the time–activity curve of authentic tracer in the brain, MRT (min) of authentic tracer was calculated in each dose experiment, which was 7.7 ± 1.5 (mean $\pm$ SD) in donepezil-1, and 10.1 ± 1.2 in donepezil-2 experiment. Using the methods as described (Figure 3), the corrected donepezil concentration (ng/ml) at 15 + MRT (open square in Figure 3) after injection of donepezil was estimated to be 15.8 ± 2.5 (mean $\pm$ SD) in donepezil-1 and 39.3 ± 4.0 in donepezil-2 experiment.

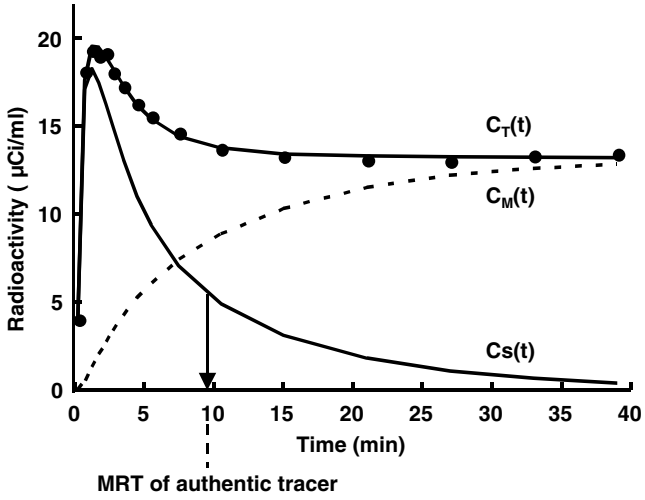

Figure 6 Time–radioactivity data in the occipital cortex of one subject given with donepezil at a dose of 250 μg/kg and the fitted curves by nonlinear least-squares analysis: $C_T(t)$, representing the theoretical curve for the total radioactivity; $C_M(t)$, the metabolite; Cs(*t*), the authentic tracer. MRT represents the mean residence time of the authentic tracer in the brain.

Plasma IC_{50}

The plasma IC_{50} values were estimated from the values of corrected plasma donepezil concentration and cerebral cortical mean AChE inhibition as measured by PET. The plasma IC_{50} values (ng/ml) were 42 ± 9.0 (estimate $\pm$ SE) and 34 ± 3.2 in donepezil-1 and donepezil-2 experiments, respectively. Though the IC_{50} in donepezil-2 experiment was about 20% lower than that in donepezil-1 experiment, the difference was not statistically significant ($P = 0.35$). Therefore, we have estimated plasma IC_{50} from combined data, which was 37 ± 4.1 ng/ml. Figure 7 shows the concentration-inhibition curve for the case of IC_{50} of 37 ng/ml.

DISCUSSION

This is the first report on the estimation of *in vivo* plasma IC_{50} of donepezil using [^{11}C]MP4A-PET and monkeys. Based on the preliminary studies on pharmacokinetics of donepezil in plasma following intravenous injection in three monkeys, we have designed the experimental protocol based on single intravenous injection of donepezil. We have estimated the plasma IC_{50} at two different doses of donepezil, 100 and 250 μg/kg, to examine the possible inhibitory effects of enhanced synaptic acetylcholine levels

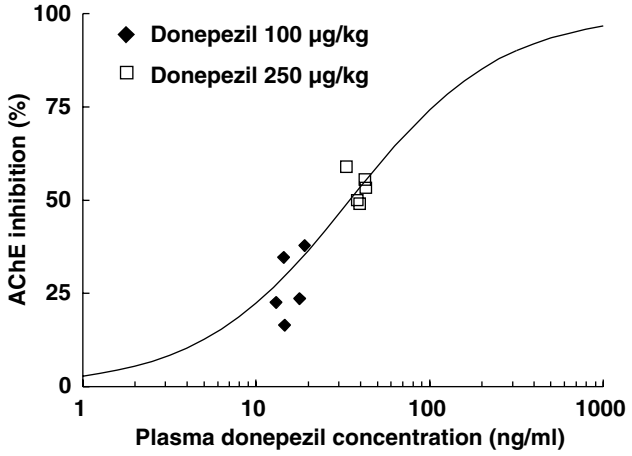

Figure 7 Relation between the plasma donepezil concentration and the percent inhibition of brain AChE activity. The curve represents the concentration–inhibition curve for the estimated IC_{50} value of 37 ± 4.1 ng/ml (estimate ± SE) from the combined data.

resulting from AChE inhibition by donepezil on the hydrolysis rate (k_3) of [^{11}C]MP4A.

In order to determine the experimental protocol such as the sampling time for measurement of plasma donepezil concentration and the start time of [^{11}C]MP4A-PET scan, we have performed preliminary studies on the time courses of donepezil concentration in plasma after intravenous bolus injection of donepezil at a dose of 250 μg/kg in three monkeys. With a bolus injection, since the plasma curve is simple in itself, that is, representing the response to an impulsive input, it would be much easier to determine the time of reaching the steady state with regard to the distribution volume of donepezil between plasma and brain by analysis of the shape of the curve, compared with infusion. Another merit of intravenous injection, compared with oral administration, is to achieve high drug concentration in the brain from minimal dose, which is important from ethical point of view. The plasma donepezil concentration showed a bi-exponential curve (Figure 3), composed of the rapid component ($T_{1/2}$; 12 min) representing the distribution of the drug from blood to tissues and the slow component ($T_{1/2}$; 63 min) corresponding to the redistribution and the elimination of the drug from body. The result suggests that the distribution of donepezil to all tissues was very rapid and that the steady state had been established within 15 min after intravenous injection. In mice experiments using [^{11}C]donepezil, it is reported that the ratio of donepezil concentration between blood and brain became constant within 5 min after intravenous injection (De Vos *et al*, 2000). Furthermore, in monkey experiments, the maximal increase in intracerebral acetylcholine levels resulting from AChE inhibition by donepezil occurred as early as 14 min after intravenous injection of donepezil as measured by microdialysis (Tsukada *et al*, 2004). These results support our assumption that distribution volume of donepezil between plasma and brain reaches the steady state rapidly enough, before the start of PET scan (15 min after intravenous injection of donepezil).

In general, nonlinear least-squares analysis using measured input function data is known to be the most reliable method for k_3 estimation, though arterial blood sampling and metabolite analysis are required. Even with this analysis, due to higher hydrolysis rate of [^{11}C]MP4A compared with [^{11}C]MP4P and also due to higher AChE activity in the cortical regions of monkey compared with human, [^{11}C]MP4A is limited to measurement of k_3 in brain regions with relatively low AChE activity such as cerebral cortical regions. By this reason, we have measured cerebral cortical k_3 change to evaluate the inhibitory effect of donepezil on brain AChE activity. In donepezil studies, the values of regional AChE inhibition showed a very small variability (1–2%, Figure 5) for both donepezil doses, which validates the reliability of the present method.

It is reported that when living brain slices from mice were exposed to AChE inhibitors such as physostigmine and pyridostigmine, AChE mRNA levels were markedly increased 30 min after treatment, followed by enhancement of AChE in cerebral cortical regions (Kaufer *et al*, 1998). By this reason, we have performed two baseline experiments, once (baseline-1) before and once (baseline-2) after the donepezil administration. The beseline-2 experiment (after exposure of 100 μg/kg donepezil) showed only a slightly higher k_3 (3.5%; statistically not significant) compared with baseline-1 experiment, indicating that the possibility of AChE induction by donepezil is low at least under the present condition, that is, 1 month after single intravenous injection of donepezil at a dose of 100 μg/kg.

We observed a good correlation between the dose of donepezil and the reduction of cerebral cortical k_3 value, about 27 and 53% reductions at 100 and 250 μg/kg, respectively (Figure 5). The values of k_3 reduction were almost the same among four cerebral cortical regions. Therefore, in the estimation of plasma IC_{50}, we used the cortical mean as the value of AChE inhibition for each monkey. As for donepezil plasma concentration at steady state in human, the value of 26.4 ± 3.9 ng/ml (mean ± SD) was reported from measurements in healthy subjects after oral doses of 5 mg donepezil for 28 days (Tiseo *et al*, 1998). Using this value of plasma donepezil concentration in human and the IC_{50} value (37 ng/ml) obtained in the present monkey study, brain AChE inhibition in human is calculated as 41%, which is compatible with the reported AChE inhibition (27–40%) as measured with PET in patients with AD under treatment by donepezil at a dose of 3–10 mg/day using [^{11}C]MP4A (Shinotoh *et al*, 2001; Kaasinen *et al*, 2002) and [^{11}C]MP4P (Kuhl *et al*, 1999), supporting the validity of the present method for estimation of plasma IC_{50} of donepezil in monkeys.

Donepezil is thought to exert its therapeutic effect by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by AChE, thereby enhancing cholinergic functions. This mechanism of action has been experimentally confirmed in rats (Kawashima *et al*, 1994; Kosasa *et al*, 1999) and monkeys (Tsukada *et al*, 2004), as measured qualitatively by microdialysis. Since [^{11}C]MP4A is a substrate-type radiotracer, the increase in synaptic acetylcholine levels would affect k_3 of [^{11}C]MP4A through two mechanisms as follows. First, acetylcholine by itself acts as a competitive inhibitor for [^{11}C]MP4A. Second, acetylcholine in high concentration may cause substrate inhibition through binding to a regulatory site on AChE (Reiner and Radic, 2000). To examine whether such indirect

inhibitory effects of donepezil through the change in synaptic acetylcholine levels might be detectable using [^{11}C]MP4A-PET, we carried out donepezil-1 and donepezil-2 experiments and compared plasma IC_{50} values between different doses. We expected that the IC_{50} obtained at higher dose would become lower if such indirect inhibitory effects of donepezil are large. Though donepezil-2 experiment showed about 20% lower plasma IC_{50} (34 ng/ml) than donepezil-1 experiment (42 ng/ml), the difference was not significant statistically. It is unclear whether the change in synaptic acetylcholine levels can be detectable *in vivo* using [^{11}C]MP4A-PET. Further studies are needed in this respect. In the discussions below, we have used plasma IC_{50} estimated from the combined data, which was 37 ± 4.1 ng/ml.

In this study, the plasma IC_{50} of donepezil was obtained on a basis of the total concentration as 37 ng/ml (89 nM). Using the reported protein-bound fraction (92.6%) of donepezil in human plasma (Mihara *et al*, 1993), we obtain plasma IC_{50} of donepezil on a basis of free form as 6.6 nM (89 nM $\times$ (100−92.6) %). At the steady state, free donepezil concentration in brain is also 6.6 nM. This value is almost the same as *in vitro* IC_{50} of donepezil (6.7 nM), as measured using rat brain tissue homogenate at a highly diluted condition (600-fold), where protein binding is considered to be negligible (Ogura *et al*, 2000). A close relation between *in vivo* plasma IC_{50} on a basis of free donepezil (6.7 nM) obtained in this study and the reported *in vitro* IC_{50} (6.6 nM) implies that we can estimate the value of brain AChE inhibition from measurement of plasma total concentration of the drug in each subject based on the plasma IC_{50}, which is obtainable from *in vitro* IC_{50} and information on the plasma protein binding.

Based on the plasma IC_{50} estimated in this study (89 nM on a basis of total donepezil concentration) and distribution volume of donepezil in rats brain (6–8 ml/g) reported by Kosasa *et al* (2000), the total donepezil concentration in brain at 50% AChE inhibition (*in vivo* brain IC_{50}) is estimated to be in the range of 500–700 nM, which is almost two orders larger than *in vitro* IC_{50} (6.7 nM). Such a large difference in IC_{50} values between *in vivo* and *in vitro* experiments may be due to strong tissue binding of donepezil in the brain. Of the total concentration of donepezil in the brain, the free fraction may be less than 1%, which provides the inhibitory effect on brain AChE activity in clinical environment.

In conclusion, this study provides information on the quantitative relation between plasma concentration of donepezil and brain AChE inhibition measured *in vivo* using PET and [^{11}C]MP4A. The major difference of *in vivo* experiment from *in vitro* and *ex vivo* experiments is the ability to evaluate the effects of intrinsic acetylcholine as an inhibitor and the strong tissue-binding effects of donepezil. Therefore, PET evaluation would provide unique information on the *in vivo* pharmacology of AChE inhibitors and novel drugs.

ACKNOWLEDGEMENTS

We thank the staff of the Central Research Laboratory, Hamamatsu Photonics KK, for performing PET studies and especially Dr Hideo Tsukada, for supplying the monkey cortex samples.

REFERENCES

Bartus RT, Dean III RL, Beer B, Lippa AS (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**: 408–414.

Carson RE (1986). Parameter estimation in positron emission tomography. In: Phelps M, Mazziotta J, Schelbert H (eds). *Positron Emission Tomography and Autoradiography: Principles and Applications for the Brain and Heart*. Raven Press: New York. pp 347–390.

Dawson RM (1990). Reversibility of the inhibition of acetylcholinesterase by tacrine. *Neurosci Lett* **118**: 85–87.

De Vos F, Santens P, Vermeirsch H, Dewolf I, Dumont F, Slegers G (2000). Pharmacological evaluation of [^{11}C]donepezil as a tracer for visualization of acetylcholinesterase by PET. *Nucl Med Biol* **27**: 745–747.

Hunter AJ, Murray TK, Jones JA, Cross AJ, Green AR (1989). The cholinergic pharmacology of tetrahydroaminoacridine *in vivo* and *in vitro*. *Br J Pharmacol* **98**: 79–86.

Irie T, Fukushi K, Akimoto Y, Tamagami H, Nozaki T (1994). Design and evaluation of radioactive acetylcholine analogs for mapping brain acetylcholinesterase (AchE) *in vivo*. *Nucl Med Biol* **21**: 801–808.

Iyo M, Namba H, Fukushi K, Shinotoh H, Nagatsuka S, Suhara T, Sudo T, Suzuki K, Irie T (1997). Measurement of acetylcholinesterase by positron emission tomography in the brains of healthy controls and patients with Alzheimer's disease. *Lancet* **349**: 1805–1809.

Kaasinen V, Nagren K, Jarvenpaa T, Roivainen A, Yu M, Oikonen V (2002). Regional effects of donepezil and rivastigmine on cortical acetylcholinesterase activity in Alzheimer's disease. *J Clin Psychopharmacol* **22**: 615–620.

Kaufer D, Friedman A, Seidman S, Soreq H (1998). Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature* **393**: 373–377.

Kawashima K, Sato A, Yoshizawa M, Fujii T, Fujimoto K, Suzuki T (1994). Effects of the centrally acting cholinesterase inhibitors tetrahydroaminoacridine and E2020 on the basal concentration of extracellular acetylcholine in the hippocampus of freely moving rats. *Naunyn-Schmiedebergs Arch Pharmacol* **350**: 523–528.

Kilbourn MR, Snyder SE, Sherman PS, Kuhl DE (1996). *In vivo* studies of acetylcholinesterase activity using a labeled substrate, *N*-[^{11}C]methylpiperdin-4-yl propionate ([^{11}C]PMP). *Synapse* **22**: 123–131.

Koeppe RA, Frey KA, Snyder SE, Meyer P, Kilbourn MR, Kuhl DE (1999). Kinetic modeling of *N*-[^{11}C]methylpiperidin-4-yl propionate: alternatives for analysis of an irreversible positron emission tomography trace for measurement of acetylcholinesterase activity in human brain. *J Cereb Blood Flow Metab* **19**: 1150–1163.

Kosasa T, Kuriya Y, Matsui K, Yamanishi Y (2000). Inhibitory effect of orally administered donepezil hydrochloride (E2020), a novel treatment for Alzheimer's disease, on cholinesterase activity in rats. *Eur J Pharmacol* **389**: 173–179.

Kosasa T, Kuriya Y, Yamanishi Y (1999). Effect of donepezil hydrochloride (E2020) on extracellular acetylcholine concentration in the cerebral cortex of rats. *Jpn J Pharmacol* **81**: 216–222.

Kuhl DE, Koeppe RA, Minoshima S, Snyder SE, Ficaro EP, Foster NL *et al* (1999). *In vivo* mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease. *Neurology* **52**: 691–699.

Kuhl DE, Minoshima S, Frey KA, Foster NL, Kilbourn MR, Koeppe RA (2000). Limited donepezil inhibition of acetylcholinesterase

measured with positron emission tomography in living Alzheimer cerebral cortex. *Ann Neurol* **48**: 391–395.

Mihara M, Ohnishi A, Tomono Y, Hasegawa J, Shimamura Y, Yamazaki K *et al* (1993). Pharmacokinetics of E2020, a new compound for Alzheimer's disease, in healthy male volunteers. *Int J Clin Pharmacol Ther Toxicol* **31**: 223–229.

Namba H, Iyo M, Fukushi K, Shinotoh H, Nagatsuka S, Suhara T *et al* (1999). Human cerebral acetylcholinesterase activity measured with positron emission tomography: procedure, normal values and effect of age. *Eur J Nucl Med* **26**: 135–143.

Ogura H, Kosasa T, Kuriya Y, Yamanishi Y (2000). Comparison of inhibitory activities of donepezil and other cholinesterase inhibitors on acetylcholinesterase and butyrylcholinesterase *in vitro*. *Methods Find Exp Clin Pharmacol* **22**: 609–613.

Onoe H, Inoue O, Suzuki K, Tsukada H, Itoh T, Mataga N *et al* (1994). Ketamine increases the striatal *N*-[^{11}C]methylspiperone binding *in vivo*: positron emission tomography study using conscious rhesus monkey. *Brain Res* **663**: 191–198.

Reiner E, Radic Z (2000). Mechanism of action of cholinesterase inhibitors. In: Giacobini E (ed). *Cholinesterases and cholinesterase inhibitors*. Martin Dunitz: London. pp 103–119.

Rho JP, Lipson LG (1997). Focus on donepezil: a reversible acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Formulary* **32**: 677–678.

Rogers SL, Doody RS, Mohs RC, Friedhoff LT (1998). Donepezil improves cognition and global function in Alzheimer disease: a 15-week, double-blind, placebo-controlled study. Donepezil Study Group. *Arch Intern Med* **158**: 1021–1031.

Shinotoh H, Aotsuka A, Fukushi K, Nagatsuka S, Tanaka N, Ota T *et al* (2001). Effect of donepezil on brain acetylcholinesterase activity in patients with AD measured by PET. *Neurology* **56**: 408–410.

Tiseo PJ, Rogers SL, Friedhoff LT (1998). Pharmacokinetic and pharmacodynamic profile of donepezil HCl following evening administration. *Br J Clin Pharmacol* **46**(Suppl 1): 13–18.

Tsukada H, Nishiyama S, Fukumoto D, Ohba H, Sato K, Kakiuchi T (2004). Effects of acute acetylcholinesterase inhibition on the cerebral cholinergic neuronal system and cognitive function: functional imaging of the conscious monkey brain using animal PET in combination with microdialysis. *Synapse* **52**: 1–10.

Veng PP (1977). Curve fitting and modeling in pharmacokinetics and some practical experiences with NONLIN and a new program FUNFIT. *J Pharmacokinet Biopharm* **5**: 513–531.

Yamanishi Y (1990). Inhibitory action of E2020, a novel acetylcholinesterase inhibitor, on cholinesterase: comparison with other inhibitors. In: Nagatsu T, Fisher A, Yoshida M (eds). *Basic, Clinical, and Therapeutic Aspects of Alzheimer's and Parkinson's Diseases*. Plenum Press: London. pp 409–413.