High occupancy of $\sigma_1$ receptors in the human brain after single oral administration of donepezil: a positron emission tomography study using $[^{11}\text{C}]SA4503$

Masatomo Ishikawa$^{1,2,3}$, Muneyuki Sakata$^{2}$, Kenji Ishii$^{4}$, Yuichi Kimura$^{4}$, Keiichi Oda$^{2}$, Jun Toyohara$^{1,2}$, Jin Wu$^{1,2}$, Kiichi Ishiwata$^{2}$, Masaomi Iyo$^{3}$ and Kenji Hashimoto$^{1}$

$^1$ Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan
$^2$ Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
$^3$ Department of Psychiatry, Chiba University Graduate School of Medicine, Chiba, Japan
$^4$ Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan

Abstract

The acetylcholinesterase (AChE) inhibitor donepezil is also a $\sigma_1$ receptor agonist. We examined whether donepezil binds to $\sigma_1$ receptors in the living human brain after a single oral administration. Dynamic positron emission tomography (PET) data acquisition using the selective $\sigma_1$ receptor ligand $[^{11}\text{C}]SA4503$ was performed to evaluate quantitatively the binding of $[^{11}\text{C}]SA4503$ to $\sigma_1$ receptors in eight healthy male volunteers. Each subject had a PET scan before and after receiving a single dose of donepezil (5 or 10 mg). The binding potential of $[^{11}\text{C}]SA4503$ was calculated. Doses of 5 mg and 10 mg donepezil bound to $\sigma_1$ receptors in the human brain with occupancies of $\sim 60\%$ and $\sim 75\%$, respectively, in a dose-dependent manner. This study demonstrated that donepezil binds to $\sigma_1$ receptors in the living human brain at therapeutic doses. Therefore, $\sigma_1$ receptors may be implicated in the pharmacological mechanism of donepezil in the human brain.

Key words: Acetylcholinesterase inhibitor, donepezil, PET, $\sigma_1$ receptor, $[^{11}\text{C}]SA4503$.

Introduction

Donepezil, an acetylcholinesterase (AChE) inhibitor, is the most widely prescribed drug for Alzheimer’s disease (Blennow et al. 2006). In addition to AChE inhibition, donepezil also binds to sigma ($\sigma$) receptors with high affinity (IC$_{50}$ = 14.6 nM), although the radioligand $[^{3}H]$DTG (for $\sigma_1$ and $\sigma_2$ receptors) used in this study is not selective for $\sigma_1$ receptors (Kato et al. 1999). Accumulating evidence suggest that $\sigma_1$ receptors play a role in the pathophysiology of neuropsychiatric diseases, including Alzheimer’s disease, schizophrenia, and major depression (Hashimoto & Ishiwata, 2006; Hayashi & Su, 2004). Interestingly, it has been demonstrated that $\sigma_1$ receptors are implicated in the anti-amnesic and neuroprotective effects of donepezil against learning impairments induced by the N-methyl-D-aspartate receptor antagonist dizocilpine (Maurice et al. 2006) and against amyloid $\beta_{25-35}$ peptide-induced neurotoxicity (Meunier et al. 2006) in mice. Recently, we reported that donepezil, but not physostigmine, significantly potentiated the nerve growth factor-induced neurite outgrowth in PC12 cells, and that potentiation of nerve growth factor-induced neurite outgrowth by donepezil was significantly blocked by co-administration of the selective $\sigma_1$ receptor antagonist NE-100 (Ishima et al. 2008). Taken together, these results suggest that the pharmacological actions of donepezil as both an AChE inhibitor and $\sigma_1$ receptor agonist probably contribute to the efficacy of this drug in patients with Alzheimer’s disease (Ishima et al. 2008; Maurice et al. 2006; Meunier et al. 2006).
Positron emission tomography (PET) is the most effective technique to estimate the receptor occupancy by drugs in the human brain. SA4503 has an affinity of $\sim 17.4$ nM ($K_d$) for the $\alpha_1$ receptor, which is about 100 times higher than those for $\alpha_2$, $\alpha_5$, adrenergic, dopamine $D_2$, serotonin (5-HT)$_2$, histamine $H_1$, muscarinic $M_1$, and muscarinic $M_3$ receptors, and has no affinity for other 29 receptors, ion channels, and second-messenger systems (Matsuno et al. 1996). Using PET and $[^{11}C]$SA4503 (Kawamura et al. 2000), Ishiwata et al. (2006) reported a high occupancy by the typical antipsychotic drug haloperidol (3 mg) for $\alpha_1$ receptors ($\sim 80\%$) as well as dopamine $D_2$ receptors ($\sim 60\%$) in the human brain after a single oral administration. Furthermore, we reported a high occupancy of $\alpha_1$ receptors ($\sim 60\%$) in the human brain after a single oral administration of fluvoxamine, a selective serotonin reuptake inhibitor, using $[^{11}C]$SA4503 PET, suggesting that $\alpha_1$ receptors may be implicated in the mechanisms of action of fluvoxamine (Ishikawa et al. 2007). These studies demonstrate that $[^{11}C]$SA4503 PET is useful for evaluation of $\alpha_1$ receptor occupancy by therapeutic drugs in the human brain (Ishikawa et al. 2007; Ishiwata et al. 2006).

The purpose of this study was to determine whether donepezil binds to $\alpha_1$ receptors in the human brain by using $[^{11}C]$SA4503 PET.

**Methods**

This study was approved by the Ethical Committee of the Tokyo Metropolitan Institute of Gerontology and the Ethics Committee of the Chiba University Graduate School of Medicine. Eight healthy Japanese male volunteers participated in the study (mean age $= 33.0\, \text{yr}$, s.d. $= 8.6\, \text{yr}$, range $= 24–46\, \text{yr}$) after providing written informed consent. None of the subjects had any neurological or psychological findings, or showed any abnormalities in the brain magnetic resonance imaging scan taken between the two PET scans. None had been receiving any medications of any kind. None had a history of alcoholism. Each volunteer participated in two $[^{11}C]$SA4503 PET scans, one before and one after a single oral administration of donepezil (Aricept^® 5 mg or 10 mg tablet; Eisai Co. Ltd, Japan). Twenty-six arterial blood samples were collected during each PET scan and were used as the input function. Either a 5 mg or a 10 mg donepezil tablet was administered randomly within 5 min of the end of the first (baseline) PET scan. The second (donepezil-loading) PET scan took place 3–3.5 h after taking donepezil to coincide with the peak plasma level ($T_{\max} = \sim 3.5\, \text{h}$) in healthy male Japanese subjects (Ohnishi et al. 1993). Blood samples were collected just before the tracer injection of the second PET scan to determine the plasma concentration of donepezil. The plasma concentration of donepezil was measured by high-performance liquid chromatography.

$[^{11}C]$SA4503 was prepared as described previously (Kawamura et al. 2000). Ninety-minute PET scans were carried out using previously described methods (Ishikawa et al. 2007; Ishiwata et al. 2006). Regions of interest (ROIs) were defined over the frontal, temporal, parietal, occipital and anterior cingulate cortices, head of the caudate nucleus, putamen, thalamus, hippocampus, and cerebellum with reference to the co-registered magnetic resonance images.

Binding of $[^{11}C]$SA4503 to $\alpha_1$ receptors was calculated as the binding potential ($BP_{ND}$) (Innis et al. 2007) with methods described elsewhere (Sakata et al. 2007) with slight modifications. Briefly, the $BP_{ND}$ was determined using a two-tissue three-compartment model (Mintun et al. 1984). The modifications were employment of two constraints for more stable estimations: the rate $K_1/K_2$ in the model is constant in each scan and this rate is not affected by donepezil. The $\alpha_1$ receptor occupancy (%) by donepezil was calculated for each ROI as

$$100 \times \frac{BP_{ND} \text{ at baseline} - BP_{ND} \text{ at donepezil loading}}{BP_{ND} \text{ at baseline}}.$$

Images of the total volume of distribution ($V_T$) of $[^{11}C]$SA4503 were calculated using the Logan plot method (Logan et al. 1996; Sakata et al. 2007). $V_T$ is a good reflection of the $BP_{ND}$ in the case of $[^{11}C]$SA4503 (Kimura et al. 2007).

The equation

$$\text {Occ} = \text {Occ}_{\max}(F/(F + ED_{50})),$$

where Occ refers to occupancy, $F$ refers to blood level of donepezil, $\text {Occ}_{\max}$ is the maximal receptor occupancy and $ED_{50}$ is the blood donepezil level resulting in 50% maximal receptor occupancy, was used to evaluate the relationship between $\alpha_1$ receptor occupancy and the blood concentration of donepezil.

The data are presented as means $\pm$ S.D. Statistical analysis was performed with SPSS software package version 12.0 J (SPSS Inc., Japan). Concentration-dependent relationships were evaluated by nonlinear regression analysis.

**Results**

Representative parametric images of $V_T$ of $[^{11}C]$SA4503 before and after donepezil (10 mg) loading are shown in Fig. 1. A single administration of
donepezil (10 mg) markedly decreased $V_T$ of $[^{11}C]SA4503$ in the brain (Fig. 1). Figure 2 shows the occupancies of $\sigma_1$ receptors in each ROI by donepezil (5 or 10 mg). The mean occupancies by 5 mg and 10 mg donepezil were $58 \pm 11\%$ and $76 \pm 5.3\%$, respectively (Fig. 2).

There were significant correlations between the blood concentration of donepezil and occupancy in all regions (frontal cortex: $ED_{50} = 5.49$, $r = 0.87$, $p < 0.01$; temporal cortex: $ED_{50} = 5.40$, $r = 0.80$, $p < 0.05$; parietal cortex: $ED_{50} = 4.78$, $r = 0.76$, $p < 0.05$; occipital cortex: $ED_{50} = 5.40$, $r = 0.75$, $p < 0.05$; anterior cingulate gyrus: $ED_{50} = 5.18$, $r = 0.82$, $p < 0.05$; head of the caudate nucleus: $ED_{50} = 5.91$, $r = 0.80$, $p < 0.05$; putamen: $ED_{50} = 7.03$, $r = 0.85$, $p < 0.01$; thalamus: $ED_{50} = 5.88$, $r = 0.91$, $p < 0.002$; cerebellum: $ED_{50} = 4.99$, $r = 0.88$, $p < 0.01$) except the hippocampus ($ED_{50} = 5.05$, $r = -0.09$). Supplementary Fig. S1 (available online) shows representative data of four regions.

Discussion

The major finding of this study is that, after a single oral administration, donepezil bound to $\sigma_1$ receptors in the living human brain, in a blood-concentration-dependent manner. To our knowledge, this is the first report demonstrating that donepezil binds to $\sigma_1$ receptors in the living human brain at therapeutic doses. This finding is consistent with the previous $in$-vivo receptor-binding data from guinea-pig brain membranes and mouse brain (Kato et al. 1999; Kunitachi et al. 2009). There are several reports demonstrating a moderate inhibition ($\sim 20–40\%$) of AChE in the brains of Alzheimer’s disease patients after oral administration of donepezil (3, 5 or 10 mg) (Bohnen et al. 2005; Kaasinen et al. 2002; Shinotoh et al. 2001). Taken together, these findings suggest that, at therapeutic doses, donepezil not only inhibits AChE, but also binds to $\sigma_1$ receptors in the human brain. Therefore, it is possible that $\sigma_1$ receptors are involved in the mechanism of the pharmacological action of donepezil in the human brain.

Maurice et al. (2006) reported that dizocilpine-induced learning impairments were attenuated by donepezil, and that this effect was blocked by the $\sigma_1$ receptor antagonist BD1047. Furthermore, Meunier et al. (2006) reported that donepezil, the $\sigma_1$ receptor agonist PRE-084, and the AChE inhibitors (tacrine, rivastigmine, galantamine) showed anti-amnestic effects against intracerebroventricular injection of amyloid $\beta_{25–35}$ peptide, but only the effects of donepezil and PRE-084 were blocked by BD1047. Very recently, we found that repeated administration of the $N$-methyl-$\alpha$-aspartate receptor antagonist phencyclidine significantly decreased the density of $\sigma_1$ receptors in the mouse brain (Ishima et al. 2009; Kunitachi et al. 2009), and that, via $\sigma_1$ receptors, donepezil significantly ameliorated phencyclidine-induced cognitive impairments in mice (Kunitachi et al. 2009). In addition, we found that donepezil inhibited $[^{3}H](+)$-pentazocine (a selective $\sigma_1$ receptor ligand) binding with high affinity ($IC_{50} = 29.12$ nm, Kunitachi et al. 2009). These findings suggest that the effect of donepezil is not only based on its AChE inhibition but also its action through $\sigma_1$ receptors.

Recently, Hayashi & Su (2007) reported that the endoplasmic reticulum (ER) protein $\sigma_1$ receptor, which has also been implicated in neuroprotection and neuroplasticity, is a $Ca^{2+}$-sensitive and ligand-operated receptor chaperone at the mitochondrion-associated ER
membrane. This suggests that \( \sigma_1 \) receptors play important roles in ER mitochondrial interorganelle \( Ca^{2+} \) signalling and in cell survival. Considering the role of ER and mitochondria in the pathophysiology of neuropsychiatric diseases such as Alzheimer’s disease, the \( \sigma_1 \) receptor agonist donepezil may exhibit unique actions relevant to the treatment of these diseases.

In conclusion, we demonstrated that donepezil binds to \( \sigma_1 \) receptors in the living human brain at therapeutic doses. Therefore, it is likely that \( \sigma_1 \) receptors may be involved in the mechanism of the pharmacological action of donepezil in the human brain.

Note
Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

Acknowledgements
This work was supported by a Grant-in-Aid for Scientific Research (no. 19390301 to K.H. and no. 20390334 to K.I.) from the Japan Society for the Promotion of Science. We thank Drs Masaya Hashimoto, Kenji Ishibashi, and Takahiro Saito, Mr Kunpei Hayashi and Ms. Hiroko Tsukinari for technical assistance.

Statement of Interest
None.

References


Hayashi T, Su TP (2007). Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate \( Ca^{2+} \) signaling and cell survival. *Cell* 131, 596–610.


Meunier J, Ieni J, Maurice T (2006). The anti-amnesic and neuroprotective effects of donepezil against amyloid β25–35 peptide-induced toxicity in mice involve an interaction with the σ1 receptor. British Journal of Pharmacology 149, 998–1012.


