The response of the autonomic nervous system to the cholinesterase inhibitor, donepezil

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Key words: donepezil; acetylcholine; acetylcholinesterase; heart rate variability; autonomic nervous system

Abstract

OBJECTIVE: Donepezil, a cholinesterase inhibitor (ChEI) that is widely used to treat Alzheimer’s disease, is thought to act by increasing extracellular acetylcholine (ACh) in the central nervous system. The effects of the administration of ChEIs on the autonomic nervous system, however, are complex and controversial.

METHODS: In this study we observed the effects of donepezil at a dose of 3 mg/kg intraperitoneally (i.p.) on the autonomic nervous system for two weeks using heart rate variability (HRV). In HRV, the high frequency (HF) band is influenced by cardiac parasympathetic tone, and the low frequency (LF) band is influenced by both sympathetic and parasympathetic activity. The ratio of LF and HF (LF/HF) is used for the assessment of the sympathetic nervous system.

RESULTS: In the current study the LF/HF was found to be significantly increased by day 1, but the magnitude of the response gradually decreased. The absolute power of HF increased after an i.p of donepezil of 3 mg daily in groups treated for 1 week and for 2 weeks compared to the single-dose groups.

CONCLUSION: Our results suggested that ChEI administration induced sympathetic nervous activation acutely, but chronic administration induced parasympathetic activation.

Abbreviations:
ACh - acetylcholine
ChEI - cholinesterase inhibitor
HRV - heart rate variability
HF - high frequency
LF - low frequency
LF/HF - low frequency to high frequency ratio

INTRODUCTION

Acetylcholine is one of the main neurotransmitters in the brain and is distributed in many brain regions including the hippocampus and cortex (Schliebs & Arendt 2006). A wide range of cognitive functions is modulated by the cholinergic system (Bentley et al. 2011), and the functions of memory and learning have been shown to be associated with the cholinergic system (Zeisel 1981). Alzheimer’s disease (AD) is the most prevalent type of dementia world-wide. The degeneration in the central cholinergic system is supposed to be one of main underlying mechanisms of the cognitive dysfunction, including memory impairment, in this disease. Donepezil, which is a piperidine-derivative cholinesterase inhibitor (ChEI), has been prescribed to improve cognitive function in patients with AD (Dooley & Lamb 2000). The sys-
temic administration of donepezil increases the extra-
cellular acetylcholine level of the hippocampus (Kosasa
et al. 1999a) and the cortex (Kosasa et al. 1999b). The
systemic administration of ChEIs like donepezil that
penetrate into the central nervous system from the sys-
temic blood flow is clinically used to improve the symp-
toms associated with AD.

We have reported that the central injection of neo-
stigmine induced the elevation of plasma norepineph-
rine, presumably via spill-over from the sympathetic
terminals that reflected the sympathetic nerve-
vous activity (Umegaki et al. 2000; Umegaki et al. 2009;
Khookhor & Umegaki 2013; Zhu et al. 2001a). Several
other studies have also indicated that central activation
of the cholinergic system induces sympathetic nerve-
ous activation (Risch et al. 1986) and blood pressure
elevation (Savci et al. 1998; Milutinović et al. 2006). The
intravenous injection of the short-acting ChEI activates
the hypothalano-pituitary-adrenal system through
central cholinergic neurons in young humans (Rubin et
al. 2006) and patients with Alzheimer’s disease (Peskin
et al. 1996). The central pathway, predominantly the
central cholinergic system, probably leads to an increase
in the activity of the sympathetic-adrenal system (Savci
et al. 1998). Donepezil, which elevates extracellular ACh
in the brain, appears to activate the sympathetic-adrenal
system through the central nervous system mechanism
(Sato et al. 2010).

On the other hand, all regions of the mammalian
heart are innervated by parasympathetic (vagal) nerves,
and the supraventricular tissues are more densely
innervated than the ventricles. Vagal activation causes
stimulation of cardiac muscarinic acetylcholine recep-
tors that modulate pacemaker activity, and directly (in
the atria) or indirectly (in the ventricles) the force of
contraction (Dhein et al. 2001). Therefore, ChEIs may
activate parasympathetic (vagal) nerves by the periph-
eral mechanism. The systemic administration of anti-
AD ChEIs, which transition highly but not perfectly
to the CNS, may activate the parasympathetic nervous
system. Indeed, ChEIs-associated bradycardia has been
reported (Hernandez et al. 2009).

Because most patients with AD are old and often
have cardiovascular comorbidity, the effects on the
cardiovascular system of centrally acting ChEI are very
important.

Heart rate variability (HRV) is a useful non-invasive
measure of autonomic activity. HRV is the temporal
variation between sequences of consecutive heart beats.
Power spectral analysis produces power bands that con-
sist of high and low frequencies. The high frequency
(HF) band is influenced by cardiac parasympathetic
tone, the low frequency (LF) band is influenced by both
sympathetic and parasympathetic activity, and the ratio
of LF and HF (LF/HF) is used for the assessment of the
sympathetic nervous system (Lombardi et al. 1996).
Our previous study demonstrated that HRV could
detect the changes in the autonomic nervous system
induced by the central infection of ChEI (Khookhor &
Umegaki 2013).

The goal of this study was to investigate the effects of
the systemic administration of donepezil on the auto-
nomic nervous system through HRV.

MATERIALS AND METHODS

Animals

Male Wistar/ST rats (Keary Co., Nagoya, Japan),
9 weeks of age (270–300 g), were housed at a room
temperature of 23±1°C and relative humidity of
55±10%, under a 12-h light/dark cycle (lighting from
6:00 to 18:00) with standard diet pellets (Oriental Yeast
Co., Ltd) and water available ad libitum. This study was
approved by the Animal Care and Use Committee of
Nagoya University.

Procedures

Thirty-four rats were divided into 2 groups (16 in the
treatment group and 18 in the control group). Done-
pezil hydrochloride was provided from Eisai Co Ltd.
(Tokyo, Japan). The treatment group was further
divided into 3 subgroups, and donepezil 3 mg/kg/ml
saline was intraperitoneally (i.p.)-administered as fol-
 lows (Kosaka et al. 1999): (1) group - single dose (n=6),
(2) group - daily for 7 days (n=5), and (3) group - daily
for 14 days (n=5). In the control group (n=18) was only
saline i.p.-administered. All reagents were of bio-
chemical grade.

Heart rate variability

HRV was measured at day 1, day 7, and day 14 after
the administration of donepezil for 60 minutes. The
AD Instruments HRV module signal was recorded
using commercial software, with sampling at 50 Hz.
A time spine of 40 minutes of RR interval data were
digitized and stored on a computer for subsequent off-
line analysis (Lab Chart® 7, AD Instruments, Pty Ltd.,
Australia).

The collected data were analyzed using custom-
ized Lab Chart software to obtain spectral components
of HRV. Spectral components for HRV analysis were
expressed as absolute units and normalized units and
calculated as power in the LF (0.04–0.15 Hz), and the
HF (0.15–0.40Hz) range was calculated for HRV.

Statistical analysis

The ratios of the HRV parameters at each time point
after the injection of donepezil to those at the baseline
(just before the injection, 0 minutes) were analyzed
using a two-way repeated measures ANOVA followed
by a post-hoc Fisher’s PLSD test. For all inferential
analyses, the probability error was set at 0.05. Results
were reported as means ± standard deviation unless
otherwise noted. Statistical analysis of variance was
carried out to test for differences in frequency domain
between the treatment groups and control group.
RESULTS

The HRV parameters at day 1, day 7, and day 14 are shown in Tables 1, 2, 3, respectively. The frequency domain parameters of heart rate variability (HRV) were significantly increased as determined by LF/HF on the first day, but the activation was attenuated at day 7 and day 14. The ratios of the HRV parameters after donepezil injection to those at baseline are shown in Figure 1 (LF/HF) and Figure 2 (HF) (LF/HF ratios for day 1 vs. day 14 and day 1 vs. day 7, $p=0.0088$ and $p=0.0247$, respectively). HF absolute power increased after the injection of donepezil in the groups treated with 3 mg daily for 1 week and for 2 weeks compared to the single dose group (HF ms$^2$ for day 1 vs. day 14 and day 14 vs. day 7, $p=0.0097$ and $p=0.0049$, respectively).

<table>
<thead>
<tr>
<th>Tab. 1. HRV variabilities after the i. p. injection of donepezil at the dose of 3mg/kg or saline at day 1.</th>
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<tr>
<td><strong>LF/HF(ratio)</strong></td>
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</tr>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>0min 10min 20min 30min 40min</td>
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<tr>
<td>2.188±0.31 2.652±0.52 3.576±0.46 2.703±0.58 2.622±0.99</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>1.842±1.23 3.965±2.76 6.666±2.53 4.048±0.54 2.899±0.49</td>
</tr>
<tr>
<td><strong>Low frequency HRV (ms$^2$)</strong></td>
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<tr>
<td>Saline</td>
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<tr>
<td>0.040±0.02 0.056±0.02 0.077±0.06 0.070±0.06 0.110±0.13</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>0.033±0.02 0.099±0.10 0.207±0.12 0.110±0.07 0.096±0.63</td>
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<tr>
<td><strong>High frequency HRV (ms$^2$)</strong></td>
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<td></td>
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<tr>
<td>Saline</td>
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<tr>
<td>0.017±0.01 0.018±0.00 0.022±0.01 0.030±0.02 0.040±0.03</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>0.020±0.01 0.024±0.01 0.029±0.02 0.023±0.01 0.027±0.02</td>
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<th>Tab. 2. HRV variabilities after the i. p. injection of donepezil at the dose of 3 mg/kg or saline at day 7.</th>
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<tr>
<td><strong>LF/HF(ratio)</strong></td>
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<td></td>
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<tr>
<td>Saline</td>
</tr>
<tr>
<td>1.418±0.34 1.456±0.48 2.044±0.41 1.559±0.09 1.710±0.42</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>1.952±0.74 2.941±1.22 5.657±2.79 2.347±0.87 2.134±0.81</td>
</tr>
<tr>
<td><strong>Low frequency HRV (ms$^2$)</strong></td>
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<tr>
<td>Saline</td>
</tr>
<tr>
<td>0.051±0.02 0.088±0.06 0.158±0.16 0.136±0.01 0.104±0.04</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>0.146±0.19 0.082±0.04 0.137±0.07 0.088±0.02 0.070±0.02</td>
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<tr>
<td><strong>High frequency HRV (ms$^2$)</strong></td>
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<tr>
<td>Saline</td>
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<tr>
<td>0.033±0.01 0.060±0.03 0.046±0.03 0.088±0.00 0.058±0.03</td>
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<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>0.033±0.01 0.033±0.01 0.033±0.02 0.050±0.03 0.033±0.01</td>
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<tr>
<th>Tab. 3. HRV variabilities after the i. p. injection of donepezil at the dose of 3 mg/kg or saline at day 14.</th>
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<td><strong>LF/HF(ratio)</strong></td>
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<tr>
<td>Saline</td>
</tr>
<tr>
<td>2.649±0.84 2.482±0.90 3.026±1.36 2.860±1.10 2.289±0.53</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>2.049±1.39 2.400±1.70 4.112±2.94 2.263±1.32 1.575±1.04</td>
</tr>
<tr>
<td><strong>Low frequency HRV (ms$^2$)</strong></td>
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<tr>
<td>Saline</td>
</tr>
<tr>
<td>0.054±0.02 0.085±0.03 0.083±0.02 0.076±0.05 0.073±0.03</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>0.089±0.11 0.100±0.09 0.156±0.12 0.114±0.08 0.095±0.08</td>
</tr>
<tr>
<td><strong>High frequency HRV (ms$^2$)</strong></td>
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<tr>
<td>Saline</td>
</tr>
<tr>
<td>0.046±0.05 0.024±0.01 0.030±0.01 0.025±0.01 0.032±0.01</td>
</tr>
<tr>
<td>Donepezil 3 mg/kg</td>
</tr>
<tr>
<td>0.032±0.03 0.046±0.03 0.057±0.04 0.064±0.04 0.069±0.04</td>
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DISCUSSION

In the current study we demonstrated that systemic administration of donepezil, a ChEI, induced the sympathetic nervous activation measured by HRV, and chronic administration for 2 weeks reduced the sympathetic nervous activation but induced parasympathetic nervous activation.

McLaren et al. reported that the oral administration of donepezil induced the sympathetic nervous activation measured by HRV in dementia patients (McLaren et al. 2003). The current results partly agreed with this finding. The activation of the sympathetic nervous system may occur through the central mechanism. On the other hand, the administration of donepezil may result in peripheral cholinergic side-effects that include vagal activation (Nordberg & Svensson 1998); these side effects may result from peripheral direct cholinergic activation peripheral direct cholinergic activation.

The current study suggested that donepezil administration induced both the sympathetic nervous activation due to central cholinergic stimulation and the parasympathetic activation mediated by a peripheral mechanism, which showed different time-course profiles.

As we have previously reported, the central cholinergic stimulation elicited the activation of the HPA axis and the sympathetic nervous system (Umegaki et al. 2000; Khookhor & Umegaki 2013). In our previous study we reported that the intragastric administration of 3 mg/kg of donepezil significantly increased the plasma adrenocorticotropic hormone levels and c-Fos expression in the paraventricular nucleus (HPA axis activation) (Zhu et al. 2001a;b) and decreased the food intake on the first day (Umegaki et al. 2009). The increase in adrenocorticotropic hormone and the loss of appetite after intragastric administration of the drug were attenuated after daily administration for 2 weeks (Umegaki et al. 2009). The current observation of the attenuation of the sympathetic activity after the administration of donepezil is similar to the profile of the HPA axis activation. Pharmacologically, donepezil has a half-life of approximately 70 h, lending itself to once-daily administration (Wilkinson 1999); therefore, the cholinergic system in the CNS was chronically activated for the treatment period. This system may be desensitized by chronic cholinergic stimulation.

The time course of the parasympathetic nervous activation was different in two ways from that of the sympathetic nervous system; first, the peak of the activity was later, at 40 min after the administration, and second, it was reinforced from the first day to day 14. The mechanism of parasympathetic activation after the administration of donepezil remains to be elucidated. The reasons for the reinforcement by means of chronic administration should also be investigated.

ChEIs reportedly induce atrial fibrillation (Constable et al. 1990; Bordier et al. 2005). Atrial fibrillation may be induced by the alteration of the activity of the autonomic nervous system (Park et al. 2012). Both the sympathetic and parasympathetic nervous systems can induce arrhythmia including atrial fibrillation. Age is the strongest risk factor for AD, and elderly patients often exhibit a comorbidity of heart disease; when prescribing ChEIs, medical care providers should keep in mind these effects on the autonomic nervous system.

In conclusion, we have demonstrated that acute administration of donepezil, a ChEI, induced sympathetic

![Fig. 1. Changes in time course of LF/HF ratio between day 1 vs. day 14 and day 1 vs. day 7 significantly different p=0.0088 and p=0.0247, respectively.](image1)

![Fig. 2. Changes in time course of HF absolute power HF day 1 vs. day 14 and day 14 vs. day 7 significantly different p=0.0097 and p=0.0049, respectively.](image2)
nervous activation, and repeated administration of donepezil for 14 days attenuated the sympathetic activation and induced parasympathetic activation.

ACKNOWLEDGEMENT

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REFERENCES