Donepezil Markedly Improves Long-Term Survival in Rats With Chronic Heart Failure After Extensive Myocardial Infarction

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Background: Vagal activation by electrical stimulation has been shown to improve the long-term survival of rats with chronic heart failure (CHF) after extensive myocardial infarction (MI). Acetylcholinesterase inhibition increases synaptic acetylcholine, and can disproportionately increase vagal tone. To develop an alternative therapy for CHF using a clinically available drug, the present study investigated whether oral donepezil, an acetylcholinesterase inhibitor, could reproduce the beneficial effects of electrical vagal stimulation in rats.

Methods and Results: At 2 weeks after ligation of the proximal left coronary artery, resulting in extensive MI, surviving rats were randomly assigned to donepezil-treated and untreated groups. Donepezil treatment started 14 days after MI significantly decreased the heart rate (325±6 vs. 355±10 beats/min, P<0.05) and improved 140-day survival (29% to 54%, P=0.03) by preventing pump failure (cardiac index: +29%, P<0.001; left ventricular dp/dt max: +18%, P<0.01; left ventricular end-diastolic pressure: −26%, P<0.01) and cardiac remodeling (biventricular weight: 2.73±0.04 vs. 3.06±0.08 g/kg, P<0.001). In addition, donepezil treatment lowered the levels of plasma arginine vasopressin, brain natriuretic peptide, catecholamine, and tissue pro-inflammation markers.

Conclusions: Oral donepezil markedly improved the long-term survival of CHF rats by preventing pump failure and cardiac remodeling, indicating that donepezil may be a new alternative therapy for CHF. (Circ J 2013; 77: 2519–2525)

Key Words: Donepezil; Heart failure; Myocardial infarction; Survival; Vagal activation
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Methods

The care and use of the animals in this study were in strict accordance with the guiding principles of the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee in the National Cerebral and Cardiovascular Center.

Experimental Heart Failure

Extensive left ventricular MI was induced by ligation of the proximal coronary artery in 8-week-old male rats (Sprague-Dawley; n=190; body weight: 250–280g; SLC, Hamamatsu, Japan). The immediate occurrence rate of lethal arrhythmia was 100% in 10 min after MI. We performed artificial heartmassage and successfully saved 90% of the MI rats. After 24 h

Figure 1. Study design and timeline. (A) Study design of donepezil therapy for post-MI rats. (B) Study protocol and timeline. MI, myocardial infarction; EF, left ventricular ejection fraction; BP, blood pressure; UT, untreated group; DT, donepezil-treated group.
had elapsed, 101 rats survived (53%). One week later, we measured the left ventricular ejection fraction (EF) by echocardiography (SSA-380A; Toshiba, Japan), and rats with EF <40% (n=100) were enrolled in this study. We confirmed the infarct size by postmortem examination as described previously.\textsuperscript{17}

**Study of Long-Term Hemodynamics**

One week after inducing extensive MI, to evaluate the long-term effects of donepezil on hemodynamics (blood pressure and heart rate) under non-stressful conditions, 31 rats were implanted with a blood pressure transmitter (TA11PA-C40, Data Sciences International). A Teflon tube of transmitter was placed in the abdominal aorta to record blood pressure and heart rate in real-time.

**Experimental Protocols**

As shown in Figure 1, 14 days after MI, survivors were randomly assigned to donepezil-treated (DT, n=50; 15 for remodeling study, 35 for prognosis study) and untreated (UT, n=45; 14 for remodeling study, 31 for prognosis study) groups. In the DT group, rats received an average dose of 5 mg/kg/day of donepezil in drinking water (50 mg/L). The dose of donepezil was chosen in order to decrease the heart rate by 20–30 beats/min without significant influence on normal growth, according to the findings in a preliminary study.\textsuperscript{22}

**Remodeling Study**

**Neurohumoral and Biochemical Study** Blood was collected from the cervical vein at 3 and 6 weeks in both groups (UT, n=10/14; DT, n=12/15) for neurohumoral and biochemical assays. The rats were temporarily anesthetized with a mixture of 1.2% halothane in oxygen-enriched air for 10 min. Blood samples were centrifuged at 14,000 rpm for 20 min, and the supernatant fluid was taken and stored at –80°C until assay. Transforming growth factor (TGF)-β1, interleukin (IL)-1β, and tumour-necrosis factor (TNF)-α were measured by ELISA assay (Rat TGF-β1, IL-1β and TNF-α immunoassay kit; CT).

**Prognosis Study**

We observed a 140-day survival rate. Animal cages were inspected daily, and any dead rats were weighed and underwent a gross postmortem examination. The heart was removed for subsequent determination of the infarct size.

**Determination of Infarct Size**

The right and left ventricles, including the interventricular septum, were dissected, separated, and weighed. The heart was fixed in 4% phosphate-buffered paraformaldehyde solution. The left ventricle was cut from the apex to the base into 4 transverse slices. Sections of 4-μm thickness were cut and stained by Masson trichrome method. Histological images were digitized through a frame grabber and analyzed. Infarct size was calculated from the 4 slices by dividing the sum of the endocardial lengths of the infarcted regions by the sum of the total endocardial circumferences.\textsuperscript{17}

**Statistical Analysis**

All values are expressed as mean±SEM. For the hemody-
U-test was used to detect any difference between the 2 groups. Survival data are presented as Kaplan-Meier curves; the effect of treatment on 140-day survival was analyzed by a Fisher exact test. P<0.05 was considered statistically significant.

Results

Remodeling Study

Of the 14 animals in the UT group, 10 survived; 1 rat died from implanted device-related complications and 3 rats died from CHF. Of the 15 animals in the DT group, 13 survived; 1 rat died from implanted device-related complications and 1 died from CHF.

Neurohumoral, Biochemical, and Cytokine Studies

Figure 2 shows the neurohumoral measurements at the third and the sixth weeks of the cardiac remodeling study. Compared with the UT rats, DT rats had lower plasma levels of AVP (Figure 2A, 1,746±510 vs. 2,160±232 pg/ml, P<0.05) on the third and (1,667±468 vs. 1,421±99 pg/ml, P<0.05) sixth weeks. Furthermore, the DT rats had significantly lower plasma levels of BNP (Figure 2B, 413±21 vs. 602±29 pg/ml, P<0.001) on the third and (389±23 vs. 498±45 pg/ml, P<0.05) the sixth week.

Table 1. Plasma and Serum Variables at the End of 6 Weeks of Donepezil Treatment (DT) or Control (Untreated, UT) After Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>UT group (n=10/14)</th>
<th>DT group (n=12/15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>1,746±510</td>
<td>497±126*</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>1,667±468</td>
<td>495±81*</td>
</tr>
<tr>
<td>Serum osmolality (mEq/L)</td>
<td>306±6</td>
<td>308±14</td>
</tr>
<tr>
<td>Serum Na⁺ (mEq/L)</td>
<td>137±1</td>
<td>143±1*</td>
</tr>
<tr>
<td>Serum K⁺ (mEq/L)</td>
<td>6.8±0.3</td>
<td>6.2±0.3</td>
</tr>
</tbody>
</table>

It was not possible to obtain blood samples from 1 animal in the DT group. Values are mean±SEM, *P<0.05 from UT group by Mann-Whitney U-test.

Table 2. Inflammatory Factors at the End of 6 Weeks of Donepezil Treatment (DT) or Control (Untreated, UT) After Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>UT group (n=10/14)</th>
<th>DT group (n=13/15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart (pg/g tissue)</td>
<td>TGF-β1</td>
<td>258±18</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>146±18</td>
</tr>
<tr>
<td></td>
<td>IL-1β</td>
<td>340±31</td>
</tr>
<tr>
<td>Liver (pg/g tissue)</td>
<td>TGF-β1</td>
<td>106±18</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>(42±2)×10³</td>
</tr>
<tr>
<td></td>
<td>IL-1β</td>
<td>1,471±90</td>
</tr>
<tr>
<td>Lung (pg/g tissue)</td>
<td>TGF-β1</td>
<td>403±56</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>191±15</td>
</tr>
<tr>
<td></td>
<td>IL-1β</td>
<td>3,164±296</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.05 from UT group by Mann-Whitney U-test.

Dynamic and remodeling study, the UT and DT groups were compared using Student’s t-test. Changes in heart rates and blood pressures before and during treatment in each group were examined by one-way repeated measures ANOVA with a post-hoc Dunnett’s test. For biochemical data, a Mann-Whitney U-test was used to detect any difference between the 2 groups. Survival data are presented as Kaplan-Meier curves; the effect of treatment on 140-day survival was analyzed by a Fisher exact test. P<0.05 was considered statistically significant.

Remodeling Study

Of the 14 animals in the UT group, 10 survived; 1 rat died from implanted device-related complications and 3 rats died from CHF. Of the 15 animals in the DT group, 13 survived; 1 rat died from implanted device-related complications and 1 died from CHF.

Neurohumoral, Biochemical, and Cytokine Studies

Figure 2 shows the neurohumoral measurements at the third and the sixth weeks of the cardiac remodeling study. Compared with the UT rats, DT rats had lower plasma levels of AVP (Figure 2A, 1,549±175 vs. 2,160±232 pg/ml, P<0.05) on the third and (1,043±90 vs. 1,421±99 pg/ml, P<0.05) sixth weeks. Furthermore, the DT rats had significantly lower plasma levels of BNP (Figure 2B, 413±21 vs. 602±29 pg/ml, P<0.001) on the third and (389±23 vs. 498±45 pg/ml, P<0.05) the sixth week.

Table 1 shows the effects of chronic acetylcholinesterase inhibition with donepezil on biochemical data for CHF rats in the sixth week. Plasma norepinephrine and epinephrine levels were lower in the DT rats compared with UT rats, and serum Na⁺ levels were maintained in the DT rats. Furthermore, the DT rats had lower levels of inflammatory TGF-β1, TNF-α, and IL-1β than the UT rats in the heart, liver, and lung (Table 2).

Hemodynamic and Remodeling Study

Although CHF rats had high heart rates prior to treatment, donepezil significantly lowered the heart rate. The difference in heart rate between DT and UT groups reached approximately 30 beats/min at the end of treatment (Figure 3A, P<0.01). However, donepezil did not affect mean blood pressure during the 6-week treatment period (Figure 3B).

The hemodynamic and remodeling parameters measured at the eighth week after extensive MI are shown in Table 3. Although there were no significant differences in body weight or infarct size between the UT and DT groups, the DT rats had...
Donepezil and HF after MI

a significantly higher cardiac index (CI), lower left ventricular end-diastolic pressure (LVEDP), lower right atrial pressure (RAP), and higher maximum dp/dt of left ventricular pressure (LV+dp/dtmax) than the UT rats. Prevention of cardiac dysfunction in the DT rats was accompanied by a significant decrease in normalized biventricular weight (HW).

Prognosis Study
Rats with extensive MI were enrolled in the prognosis study, with 35 and 31 rats in the DT and UT groups, respectively. Donepezil was administered accordingly throughout the 140-day study period. As shown in Figure 4, donepezil markedly suppressed the mortality rate of CHF rats. The CHF rat survival was 54% in the DT group compared with 29% in the UT group (P=0.03). Donepezil therapy achieved a 35% reduction in the relative risk of death ([71−46]/71 = 35%). Excluding the surviving rats, dead DT rats had a significantly smaller normalized biventricular weight at the time of death (3.07±0.09 g/kg vs. 3.45±0.06 g/kg, P<0.001). There was no significant difference in the infarct size between DT and UT rats (53±1% vs. 53±1%, NS).

Discussion
The major findings reported here are that donepezil treatment started 14 days after extensive MI significantly reduced the heart rate and prevented progressive cardiac remodeling and dysfunction; suppressed plasma BNP and AVP levels and tissue pro-inflammation markers; and as a result, effectively improved the long-term survival in CHF rats.

Bradycardic Effects and Prevention of Cardiac Remodeling
Acetylcholinesterase is found primarily in the blood and neurosynapses. Donepezil may increase the concentration of ACh in the synaptic clefts through reversible inhibition of cholinesterase activity. Increased ACh in various tissues may induce beneficial effects for CHF from several aspects as will be discussed.

The first mechanism may be associated with the enhancement of vagal efferent effects. Although there is no direct proof for increased vagal discharge in the present study, our previous study demonstrated that donepezil treatment increased high-frequency components of heart rate variability, indicating increased vagal efferent effects. Heart rate is the simplest indicator of vagal efferent function. This study used telemetry to record blood pressure and heart rate in conscious freely-moving rats. The results of the present study demonstrated that donepezil treatment significantly decreased weekly averaged heart rate without decreasing blood pressure in CHF rats. In contrast, a study in mice indicated that donepezil does not decrease heart rate measured by a tail cuff method, but the restraint of mice during heart rate measurements might have masked the bradycardic effect of donepezil in that study.

The bradycardic effect of donepezil may appear to be similar to that of β-blockers. By inducing bradycardia, β-blockers have become very important in treating patients with CHF. However, because β adrenergic receptors exist on both sinus node and cardiac myocytes, β-blockers also decrease heart rate and suppress myocardial contractility. Hence, these negative

![Figure 4. Effects of donepezil on survival rates of CHF rats. UT (blue line, n=31) and DT (red line, n=35). Treatment started 14 days after MI (14-day post-MI). Donepezil significantly improved 140-day survival rate (DT, 54% vs. UT, 29%, P=0.03). UT, untreated group; DT, donepezil-treated group.](image)

<table>
<thead>
<tr>
<th>Prognosis Study</th>
<th>Table 3. Hemodynamic and Remodeling Parameters at the End of 6 Weeks of Donepezil Treatment (DT) or Control (Untreated, UT) After Myocardial Infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UT group (n=10)</td>
</tr>
<tr>
<td>BW (g)</td>
<td>445±12</td>
</tr>
<tr>
<td>HW (g/kg)</td>
<td>3.06±0.08</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>51±1</td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>78±4</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>27±2</td>
</tr>
<tr>
<td>LV+dp/dtmax (mmHg/sec)</td>
<td>3,200±196</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>6.8±1.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM. <sup>*</sup>P<0.05 from UT group by unpaired t-test. BW, body weight; HW, biventricular weight normalized by body weight; CI, cardiac index; LV+dp/dtmax, maximum dp/dt of left ventricular pressure; RAP, right atrial pressure.
inotropic effects make β-blockers less suitable for patients with decompensated conditions or preexisting myocardial dysfunction, because the maintenance of cardiac output in such patients depends in part upon sympathetic drive.

In contrast, because the vagal nerve poorly innervates the ventricles, donepezil increases synaptic ACh and reduces the heart rate with less negative effects on cardiac function. The result from the DT group showing decreased heart rate without significant effects on arterial pressure may partly support this interpretation. Furthermore, because all preganglionic fibers are cholinergic, donepezil can theoretically increase synaptic transduction in both sympathetic and parasympathetic nerves.26,27 Our previous study showed that VNS prevents cardiac remodeling in MI rats in the presence of background β-receptor blockade, which suggested that VNS may work on the failing heart beyond bradycardic effects.28 Therefore, donepezil may be an alternative to β-blockers for severe CHF patients.

Several studies have shown that efferent vagal stimulation blunts the activation of nuclear factor-κB in the liver via nicotinic receptors, thus attenuating the systemic inflammatory response to endotoxins or acute hemorrhagic shock through reduced hepatic production of TNF-α.29,30 Pro-inflammatory markers, including TNF-α, are also involved in cardiac remodeling and the progression of CHF.31 Low levels of tissue inflammatory factors in the DT group (Table 2) indicated that an anti-inflammatory pathway might also be involved in the beneficial effects associated with donepezil in CHF rats.

There is no direct evidence that donepezil treatment activated the afferent vagal pathway. However, we found that DT rats had decreased plasma AVP (Figure 2A), consistent with the findings from our other VNS study.32 Vagal afferent activation evokes the cardiopulmonary reflex and modulates neuronal activity in several hypothalamic nuclei involved in cardiovascular regulation. It is well known that cholecroceptors in the cardiopulmonary region of vagal afferents regulate the release of plasma AVP from the hypotalamus, which controls renal function and blood volume in CHF.33 Further studies are required to determine whether such neurohumoral response is a direct effect of donepezil or secondary to improved cardiac function.

As a centrally-acting reversible acetylcholinesterase inhibitor, donepezil’s main therapeutic use is to treat Alzheimer’s disease by increasing cortical ACh.34,35 Therefore, another mechanism of donepezil action may be related to restored equilibrium of autonomic tone in favor of parasympathetic activity through its central effects.

Recently, new reports have focused on the cardiac protective mechanisms of VNS and ACh at the cellular and molecular levels.36,37 and also from the aspect of cardiac metabolism.38 Those studies have helped expand our knowledge of the mechanism of cholinergic effects in CHF.

**Literature**

A recent study indicated that pyridostigmine, an acetylcholinesterase inhibitor, improves tachycardia in postural tachycardia syndrome.41 Others report that pyridostigmine improves heart rate recovery after maximal exercise in patients with CHF and protects against exercise-induced myocardial ischemia.1,6

Acetylcholinesterase inhibition with donepezil may also improve ventricular efficiency by decreasing the heart rate,41 as high heart rates are associated with decreased ventricular efficiency.42 Therefore, by preventing tachycardia after MI, donepezil increases the efficiency of the failing heart and thus protects against cardiac remodeling and dysfunction (Table 3).

In a leading study, Pfeffer et al43 examined the effects of long-term therapy with captopril, an angiotensin-converting enzyme inhibitor, in CHF rats after MI. In a follow-up of 1 year, the investigators reported that captopril therapy markedly improved survival from 20% to 50% in rats with a moderate infarct size (20% to 39.9%); however, there was no significant improvement in those with a large infarct (≥40%). Their protocol was similar to their study except pharmacologic therapy was initiated 14 days after MI. In comparison, we found that donepezil therapy markedly suppressed the 140-day mortality rate from 71% to 46% (P=0.03) in CHF rats with extensive MI (average infarct size, 53%). Although the beneficial effects of direct electrical VNS were not perfectly reproduced, donepezil may be a clinically promising, novel therapy for severe CHF patients.

**Study Limitations**

Beneficial effects of donepezil on cardiac function, remodeling, and survival of CHF rats were shown in the present study. However, the mechanism is not definitive, and it is unknown whether other acetylcholinesterase inhibitors would exert similar cardioprotective effects. Further research is necessary to determine the mechanism of action of acetylcholinesterase inhibition in treating CHF.

We noticed that the untreated group survival was lower in this study (29%) than that previously reported (50%) in our VNS study.37 We could not completely eliminate technical modifications in the animal preparation, and in particular, there were different interventions. In the present study, the MI rats in the prognosis study group did not undergo a second surgery for device implantation. In contrast, MI rats in the previous VNS study had to survive a second surgery for implantation of the stimulation device and blood pressure telemetry and hence rats that were too weak to survive were not enrolled in the prognosis analysis.

**Conclusions**

Acetylcholinesterase inhibition with donepezil started 14 days after extensive MI was highly effective in preventing cardiac remodeling and, as a result, improved the long-term survival of CHF rats. We suggest that pharmacological vagal modulation by donepezil may be a new alternative therapy for CHF after MI.

**Acknowledgments**

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**Conflict of Interest**

None.

**References**

3. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ.


