Vagal Nerve Stimulation Markedly Improves Long-Term Survival After Chronic Heart Failure in Rats

Meihua Li, MS; Can Zheng, PhD; Takayuki Sato, MD; Toru Kawada, MD; Masaru Sugimachi, MD; Kenji Sunagawa, MD

**Background**—Diminished cardiac vagal activity and higher heart rate predict a high mortality rate of chronic heart failure (CHF) after myocardial infarction. We investigated the effects of chronic electrical stimulation of the vagus nerve on cardiac remodeling and long-term survival in an animal model of CHF after large myocardial infarction.

**Methods and Results**—Two weeks after the ligation of the left coronary artery, surviving rats were randomized to vagal- and sham-stimulated groups. Using an implantable miniature radio-controlled electrical stimulator, we stimulated the right vagal nerve of CHF rats for 6 weeks. The intensity of electrical stimulation was adjusted for each rat, so that the heart rate was lowered by 20 to 30 beats per minute. The treated rats had significantly lower left ventricular end-diastolic pressure (17.1 ± 5.9 versus 23.5 ± 4.2 mm Hg, *P* < 0.05) and higher maximum dp/dt of left ventricular pressure (4152 ± 237 versus 2987 ± 192 mm Hg/s, *P* < 0.05) than the untreated rats. Improvement of cardiac pumping function was accompanied by a decrease in normalized biventricular weight (2.75 ± 0.25 versus 3.14 ± 0.22 g/kg, *P* < 0.01). Although the 140-day survival of the untreated group was only half, vagal stimulation markedly improved the survival rate (86% versus 50%, *P* = 0.008). Vagal stimulation therapy achieved a 73% reduction in a relative risk ratio of death.

**Conclusions**—Vagal nerve stimulation markedly improved the long-term survival of CHF rats through the prevention of pumping failure and cardiac remodeling. *(Circulation, 2004;109:120-124.)*

**Key Words:** electrical stimulation ▪ heart failure ▪ myocardial infarction ▪ remodeling ▪ vagus nerve

A cute myocardial infarction occurs when blood supply to part of the heart muscle is severely reduced or stopped. Survivors after large myocardial infarction have a high risk for chronic heart failure (CHF), with poor prognosis. CHF is a clinical syndrome that is initiated by cardiac dysfunction and followed by activation of compensatory mechanisms such as the sympathoadrenal and renin-angiotensin-aldosterone systems. Apparently, activation of compensatory mechanisms during the early phase of CHF helps the heart compensate for deteriorating pumping function. However, excessive sustained activation has deleterious effects on cardiac function. Once such an excessive activation, on the contrary, worsens cardiac function, it triggers further activation of those compensatory mechanisms, which, in turn, further deteriorates cardiac function. This positive feedback mechanism leads the heart to decompensatory cardiac remodeling and failure at the end stage. Therefore, the maladaptation process is a key of pathophysiology of CHF.

In the maladaptation process, the cardiac autonomic nervous system also plays an important role. Clinical evidence from the Autonomic Tone and Reflexes After Myocardial Infarction study (ATRAMI) and the Cardiac Insufficiency Bisoprolol Study II (CIBIS II) indicates that diminished cardiac vagal activity and increased heart rate predict a high mortality rate of CHF. Based on this body of knowledge, it would be logical to clarify whether augmentation of vagal activity prevents cardiac remodeling and death. On the occurrence of life-threatening arrhythmias in acute ischemia, the effect of vagal stimulation has been reported to prevent ventricular fibrillation in dogs. The antianginal effect of vagal stimulation has been also shown in patients with coronary artery disease. However, its effect on CHF remains unknown. Therefore, in the present study, we examined the effects of vagal stimulation on cardiac remodeling after large myocardial infarction and on the long-term prognosis of CHF in rats.

**Methods**

**Experimental Heart Failure**

The care and use of the animals were in strict accordance with the guiding principles of the Physiological Society of Japan. Left ventricular myocardial infarction was induced by coronary artery ligation in 8-week-old male Sprague-Dawley rats (SLC, Hamamatsu, Japan). The mortality rate in animals with myocardial infarction was ~60% within the first 24 hours. One week later, we checked the infarct size by echocardiography (SSA-380A, Toshiba), as described previously. The rats with infarcted area >40% of the left ventricular...
Vagal Nerve Stimulation

To stimulate the vagal nerve and to monitor blood pressure and heart rate in freely moving rats, we developed a remote system controlled by a computer (Figure 1). The computer commands an implantable and radio-controlled pulse generator (ISE1010C, Unimec) to stimulate the vagal nerve while sensing blood pressure and heart rate through an implantable transmitter (TA11PA-C40, Data Sciences International). The miniature pulse generator and transmitter were subcutaneously implanted in the abdomen in 7 days after myocardial infarction. A pair of Teflon-coated stainless steel wires for electrical stimulation was looped around the right vagal nerve in the neck; a Teflon tube for blood pressure recording was placed in the abdominal aorta.

Experimental Protocols

At 14 days after myocardial infarction, the survivors were randomized into groups treated with sham and active stimulation. In the actively treated group, we stimulated the vagal nerve with electrical rectangular pulses of 0.2-ms duration at 20 Hz for 10 seconds every minute for 6 weeks. The electrical current of pulses was adjusted for each rat, so that the heart rate was lowered by 20 to 30 beats per minute. This resulted in the ranges of 0.1 to 0.13 mA. Mean blood pressure and heart rate were recorded every minute for 6 weeks. In a preliminary study, we confirmed that the chronic vagal stimulation at this intensity did not alter feeding behavior and did not evoke any signs of pain reaction such as an increase in plasma epinephrine level.

Hemodynamic and Remodeling Study

To evaluate the effect of vagal stimulation on cardiac remodeling, at the end of the 6-week stimulation period we measured hemodynamics and heart weights of sham-operated and sham-stimulated rats, untreated CHF rats, and treated CHF rats. Anesthesia was maintained through the use of 1.2% halothane during surgical procedures and 0.6% halothane during data recording. Left ventricular and arterial pressures were measured with a 2F catheter-tipped micromanometer (SPC-320, Millar Instruments). Pressure signals were digitized at a rate of 1 kHz for 5 minutes. After hemodynamic measurement, the heart was excised for subsequent determination of infarct size.

Figure 1. Neural interface approach to stimulate the vagal nerve. While monitoring heart rate through an implantable transmitter, a remote control system adjusted the intensity of electrical pulses of an implantable miniature radio-controlled electrical stimulator.

Prognosis and Neurohormone Study

To examine the effect of 6-week vagal stimulation on prognosis, we observed a 20-week survival rate in treated and untreated CHF rats. Because of the life of the battery of the implantable pulse generator, the treatment period was limited to 6 weeks. Each cage was inspected daily for the rat that had died. The heart was removed from the dead animal for subsequent determination of infarct size.

At the end of the observation period, blood for neurohormone assays was sampled. The surviving rat was placed in a glass jar, where it inspired a mixture of 1.2% halothane in oxygen-enriched air for 5 to 10 minutes. To avoid the modification of neurohumoral states by invasive manipulation, immediately after the induction of anesthesia, we quickly collected 3 mL of a blood sample from the left ventricular cavity through a transsthoracic approach without measuring hemodynamics. After blood sampling, the heart was excised for subsequent determination of infarct size.

Plasma concentrations of norepinephrine were measured by high-performance liquid chromatography with electrochemical detection. Plasma levels of brain natriuretic peptide were determined by radioimmunoassay.

Determination of Infarct Size

As described previously,9 the right ventricle and the left ventricle including the interventricular septum were dissected, separated, and weighted. The heart was fixed in 10% buffered formalin. The left ventricle was cut from apex to base into 4 transverse slices. Sections 4 μm thick 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 infarcted regions by the sum of the total endocardial circumferences.

Statistical Analysis

For data of the hemodynamic and remodeling study, differences among 3 groups were tested by ANOVA, with a Scheffé multiple comparison test. Differences in heart rates before and during treatment in each group were examined by a 1-way ANOVA with repeated measures, followed by a post hoc Dunnett test.

For a neurohormonal data, differences between two groups were examined by a Mann-Whitney U test. Survival data are presented as Kaplan-Meier curves; the effect of treatment on 140-day survival was analyzed by a Fisher exact test. Differences were considered significant at a value of P<0.05.
Results

Hemodynamic and Remodeling Study
Although CHF rats (untreated, n = 13; treated, n = 11) had a higher heart rate than sham-operated rats (n = 9) before the treatment, vagal stimulation significantly slowed the heart rate of CHF rats (Figure 2). The difference in heart rate between untreated and treated CHF rats reached ~40 beats per minute at the end of treatment (P < 0.05). CHF rats had significantly lower blood pressure, but the vagal stimulation did not affect blood pressure during the 6-week treatment period (Table).

When compared with sham-operated rats, untreated CHF rats had low blood pressure (Figure 3a), high left ventricular end-diastolic pressure (LVEDP) (Figure 3b), a depressed maximum dp/dt of left ventricular pressure (LV+dp/dt max) (Figure 3c), and an increased heart weight (Figure 3d). On the other hand, CHF rats treated with vagal nerve stimulation had significantly lower LVEDP (17.1 ± 5.9 versus 23.5 ± 4.2 mm Hg, P < 0.05) and higher LV+dp/dt max (4152 ± 237 versus 2987 ± 192 mm Hg/s, P < 0.05) than untreated CHF rats. Improvement of pumping function in treated CHF rats was accompanied by a significant decrease in normalized biventricular weight (2.75 ± 0.25 versus 3.14 ± 0.22 g/kg, P < 0.01). There was no significant difference in infarct size between treated and untreated CHF rats (53 ± 7% versus 53 ± 6%).

Prognosis and Neurohormone Study
Although 60 rats with CHF after large myocardial infarction were enrolled in the prognosis study, 8 of the 30 rats assigned to the treated group were excluded from the results because of the breaking down of electrode wires during vagal stimulation for 6 weeks. Vagal nerve stimulation markedly suppressed the mortality rate of CHF rats (Figure 4); there were only 3 deaths among the 22 treated rats versus 15 deaths among the 30 untreated rats (14% versus 50%, P < 0.008). Vagal stimulation therapy achieved a 73% reduction in a relative risk ratio of death.

Shown in Figure 5, improvement of survival in treated CHF rats was accompanied by a significant decrease in normalized biventricular weight (2.63 ± 0.38 versus 3.17 ± 0.42 g/kg, P < 0.01). When compared with untreated CHF rats, treated CHF rats had lower levels of plasma norepinephrine (426 ± 102 versus 1182 ± 260 pg/mL, P < 0.01) and brain natriuretic peptide (251 ± 31 versus 363 ± 82 pg/mL, P < 0.01). There was no significant difference in infarct size between treated and untreated CHF rats (54 ± 8% versus 53 ± 7%).

Discussion
The prognosis of patients with CHF is still poor, even though various therapeutic approaches with a β-adrenergic receptor

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SO-SS indicates sham-operated rats treated with sham stimulation (SS); CHF-SS, CHF rats treated with sham stimulation; CHF-VS, CHF rats treated with vagal stimulation. Values are mean ± SD of the 24-hour average of mean blood pressure.

*P < 0.01 from SO-SS group.

**Figure 3.** Effects of vagal nerve stimulation on a, mean blood pressure; b, LVEDP; c, maximum dp/dt of left ventricular pressure (LV+dp/dt max); d, biventricular weight normalized by body weight in sham-operated (SO-SS, hatched bar, n = 9) rats treated with sham stimulation, CHF rats treated with sham (CHF-SS, open bar, n = 13), and vagal stimulation (CHF-VS, closed bar, n = 11). Assessment was made at the end of 6-week treatment. Data are expressed as mean ± SD. *P < 0.05; †P < 0.01.

**Figure 4.** Effects of vagal nerve stimulation on survival curves of CHF rats treated with sham (broken line, n = 30) and vagal stimulation (solid line, n = 22). Treatment started 14 days after coronary artery ligation. Vagal stimulation significantly (P = 0.008) improved survival rate.
when the basal level of cAMP is elevated by other hand, the negative inotropic effect can be only achieved inotropic and chronotropic effects; in the ventricle, on the stimulation of muscarinic M 2 receptors causes direct negative M2–subtype, which couples through a pertussis toxin other species, muscarinic receptors are predominantly of the effects on adrenergic signaling cascade through G-protein interactions. In human hearts as well as those of several other species, muscarinic receptors are predominantly of the M 2–subtype, which couples through a pertussis toxin–sensitive G i protein to inhibit adenyl cyclase. In the atrium, stimulation of muscarinic M 2 receptors causes direct negative inotropic and chronotropic effects; in the ventricle, on the other hand, the negative inotropic effect can be only achieved when the basal level of cAMP is elevated by β-adrenoceptor agonists. These mechanisms are known as accentuated antagonism.

Vagal stimulation is also postulated to improve ventricular efficiency by slowing heart rate. Burkhoff et al 18 showed that the ventricular efficiency, that is, the ratio of ventricular stroke work to ventricular oxygen consumption, is adjusted to be maximal under physiological conditions and that the efficiency of the failing heart is more sensitive to changes in heart rate than that of the normal heart. Prevention of tachycardia after myocardial infarction by vagal stimulation would optimize the efficiency of the failing heart and thus protect the heart against remodeling.

Apparantly, vagal efferent stimulation is considered to act on the ventricle of CHF like a β-adrenergic blocker. However, in rats, β-blockade therapy rather failed to exert a beneficial effect on the cardiac remodeling or hemodynamics after myocardial infarction (for review, see Gaballa and Goldman19). Litwin et al 20 showed that chronic propranolol treatment did not improve cardiac remodeling and worsened pumping function in rats with postinfarction CHF. Wei et al 21 also demonstrated that metoprolol deteriorated ventricular remodeling in CHF rats. Therefore, in addition to antagonism against sympathetic effects, unique actions of vagal stimulation would be important in providing the favorable outcome for CHF rats. A facilitatory effect of vagal stimulation on nitric oxide release from the coronary endothelium could also have an antiremodeling action through improvement of viable myocardial conditions.22

In addition to the effects of electrical stimulation of vagal efferents on the heart, vagal afferent effects7–23 are also considered because afferent stimulation would evoke cardio-pulmonary reflex and modulate neuronal activity in several hypothalamic nuclei involved in cardiovascular regulation. As shown in Figure 5c, vagal stimulation lowered the plasma norepinephrine level. Therefore, vagal stimulation therapy would terminate the vicious circle of maladaptation in CHF through the suppression of chronic excessive activation of the sympathetic nervous system.24,25

A more recent study by Guarini et al 26 has shown that efferent vagal fiber stimulation blunts activation of nuclear factor-κB in the liver through nicotinic receptors and then reduces the hepatic production and the plasma level of tumor necrosis factor-α during acute hemorrhagic shock. It has been reported that these factors are also involved in cardiac remodeling and the poor prognosis of CHF.27 Therefore, the hepatic effect of vagal stimulation would prevent cardiac remodeling and improve survival of CHF.

It is also noted that short-term vagal stimulation for 6 weeks after myocardial infarction prevented long-term cardiac remodeling (Figure 5a) and improved the long-term survival. There may be a critical period during which short-term treatment against cardiac dysfunction and remodeling will ensure the long-term survival of CHF.

A pioneer work by Pfeffer et al 28 examined the effect of long-term therapy with captopril in CHF rats after myocardial infarction. As well as vagal stimulation in the present study, oral captopril administration started at 14 days after ligation of the left coronary artery. Pfeffer et al observed 1-year survival and found that the median survival was 146 and 181 days for untreated and treated CHF rats with large infarcts, respectively. Thus, the survival curve of untreated CHF rats with large infarcts in their study was quite similar to our result of untreated CHF rats. On the other hand, the effect of captopril on survival in CHF rats with large infarcts appeared to be much different from that of vagal stimulation. Approximately 40% of captopril-treated CHF rats with large infarcts died at 140 days; vagal stimulation reduced the mortality rate to <20%. Therefore, vagal stimulation therapy may be promising for severe CHF after large myocardial infarction.
Limitations
The beneficial effects of vagal stimulation on cardiac function, remodeling, and survival of CHF rats were shown in the present study. However, its safety and adverse effects remain to be unclear. The appropriate protocol of treatment is also still unsettled and should be investigated. To establish the therapeutic strategy shown in this study, large-scale, long-term trials of vagal nerve stimulation with an animal model of CHF are required.

Clinical Implications
Our previous studies indicated that a pharmacological intervention in the central nervous system of CHF rats prevented the progression of cardiac dysfunction and remodeling. The therapeutic modality used in the present study also brought a favorable prognosis of CHF by manipulation of autonomic tone through vagal efferent and/or afferent mechanisms. We therefore propose the neural interface approach to optimize cardiac autonomic tone for the treatment of CHF. Technologies to materialize this neural interface strategy using totally implantable miniaturized systems are readily available.

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