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Selegiline attenuates cardiac oxidative stress and apoptosis in heart failure: association with improvement of cardiac function☆

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Abstract

We have shown recently that selegiline exerts a cardiac neuroprotective effect in chronic heart failure. Since selegiline has an antioxidant antiapoptotic effect, we proposed to determine whether selegiline attenuates cardiac oxidative stress and myocyte apoptosis in chronic heart failure by modulating Bcl-2 and Bax protein expression, and whether the effects are associated with the improvement of cardiac function. Rabbits with rapid cardiac pacing (360 beats/min) and sham operation without pacing were randomized to receive oral selegiline (1 mg/day) or placebo for 8 weeks. Echocardiography was used to measure left ventricular fractional shortening. After 8 weeks of treatment, animals were studied for arterial norepinephrine and left ventricular systolic function (fractional shortening and dP/dt), and were then sacrificed for measuring the stable oxidative product of myocardial mitochondrial DNA (mtDNA) 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), myocyte apoptosis by monoclonal antibody to single stranded DNA, and Bcl-2 and Bax protein expression by Western blot and immunohistochemistry. Rapid cardiac pacing increased plasma norepinephrine, cardiac oxidative stress and myocyte apoptosis, reduced Bcl-2 and the Bcl-2 to Bax ratio. These changes were associated with decreased left ventricular fractional shortening and dP/dt. Selegiline treatment in chronic heart failure animals reduced plasma norepinephrine, cardiac oxidative stress and myocyte apoptosis, prevented the changes of Bcl-2 and Bcl-2 to Bax ratio, and improved left ventricular fractional shortening and dP/dt. The findings suggest that the reduction by selegiline of myocyte apoptosis is related to the decrease of cardiac oxidative stress and the modulation of apoptotic and antiapoptotic proteins. The antioxidant antiapoptotic effects of selegiline are potentially beneficial in the improvement of cardiac function in chronic heart failure.

Keywords: Selegiline; Oxidative stress; Apoptosis; Cardiac function; Heart failure

1. Introduction

Selegiline is a monoamine oxidase type B inhibitor used primarily for the treatment of Parkinson's disease (Ebadi et al., 1996). It facilitates dopaminergic neurotransmission and slows progressive loss of neurons (Birkmayer et al., 1975). It has also been used to increase the survivability of neurons independent of its effect on monoamine oxidase type B activity following chemical or mechanical injuries (Salo and Tatton, 1992), and prevents age-associated diminution in splenic sympathetic noradrenergic innervation (ThyagaRajan et al., 2000). This neuroprotective effect of selegiline may be mediated in part by stimulation of biosynthesis of target-derived growth factors (Semkova et al., 1996), but may also be related to the antioxidant, antiapoptotic effect of selegiline (Kitani et al., 1998, 1999), which has been shown to occur in both cultured neurons (Le et al., 1997) and animal models (Simon et al., 2001). In a recent intact animal study, we showed that selegiline exerts a sympatholytic and cardiac neuroprotective effect in congestive heart failure (Shite et al., 2000), but it is not known if the effects of selegiline are mediated via reduction of oxidative stress and myocyte apoptosis known to occur in heart failure (Hare, 2001). In this study, we proposed to determine whether selegiline affected cardiac oxidative stress by measuring mitochondrial DNA (mtDNA) 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), a guanine adduct formed by attack of ·OH, using high-performance liquid chromatography (HPLC) (Duez et

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al., 2000; Shigenaga et al., 1994), myocyte apoptosis utilizing monoclonal antibody against single-stranded DNA antibody labeling (Watanabe et al., 1999), and protein expression of antiapoptotic Bcl-2 and apoptotic Bax genes (Hockenbery et al., 1993; Oltvai et al., 1993) by immunohistochemistry and Western blot analysis. We also correlated the changes in apoptosis index to left ventricular systolic function in the chronic heart failure animals.

2. Materials and methods

2.1. Animal model

The study was approved by the University of Rochester Committee on Animal Resources and conformed to the guiding principles on animal experimentation approved by the Council of the American Physiological Society and the National Institutes of Health Guide on the humane care and use of laboratory animals.

Adult healthy New Zealand White rabbits [3.33 ± 0.04 kg (mean ± S.E.M.), 4–6 months old] were chosen for experimental heart failure using a modified rapid cardiac pacing technique (Kawai et al., 2000). Briefly, subxyphoid thoracotomy and pericardiotomy were performed under general isoflurane gas anesthesia. Two shielded pacing leads were sutured onto the left ventricular apex and left pectoral muscle, respectively, and exteriorized to the interscapular region. One week later, rabbits were randomly assigned to receive pacing at a rate of 360 beats/min (chronic heart failure animals) with an implantable model 8086 Prevail VHRP programmable pacemaker (Medtronic, Minneapolis, MN). Sham animals underwent identical surgery for wire placement but received no cardiac pacing.

2.2. Experimental protocol

One week after thoracotomy, the animals were assigned randomly to receive either oral selegiline (1 mg/day) or placebo for 8 weeks. Animals were examined regularly for clinical development of heart failure. After 8 weeks of treatment, cardiac pacing was discontinued, and animals were anesthetized for measuring arterial blood norepinephrine using the Cat-A-Kit assay system (Amersham, Arlington Heights, IL), left ventricular dimension and fractional shortening by echocardiography, and resting hemodynamics by direct catheterization. The animals were then sacrificed with a lethal dose (>100 mg/kg) of intravenous pentobarbital sodium. The heart was removed, weighed, and rinsed in ice-cold oxygenated normal saline. The left ventricular weight includes both the septum and the left ventricular free wall. Transmural muscle blocks of the left ventricle were stored in liquid nitrogen for later analysis. The persons performing the assays and measurements were blinded to the animal identity and drug assignment.

2.3. Echocardiographic and hemodynamic measurements

Two-dimensional and M-mode echocardiograms were obtained using a 5-MHz transducer on a Toshiba Model SSH-60A sonographic system (Toshiba America Medical System, Tustin, CA). Maximal left ventricular end-diastolic dimension (EDD) and end-systolic dimension (ESD) were used to calculate left ventricular fractional shortening (FS) by the following equation: FS=[(EDD − ESD) × 100]/EDD.

For the hemodynamic studies, animals were anesthetized with katamine (35 mg/kg) and midazolam (0.8 mg/kg). A 20-guage fluid-filled catheter (Insyte; Deseret Medical, Becton, Dickinson and Company, Sandy, UT) was inserted into the left carotid artery for measuring aortic pressure, and a 2 French micromanometer-tipped catheter (Millar Instruments, Houston, TX) was introduced into the left ventricle via the right carotid artery for measuring the left ventricular pressure. Electrocardiograms, aortic pressure and the first derivative of left ventricular pressure (dP/dt) were recorded on a Brush Model 480 recorder (Gould, Instrument Systems Division, Cleveland, OH). Resting hemodynamic measurements were obtained in triplicate over a 20-min steady-state period at least 1 h after the catheterization. The averages were used for statistical analysis.

2.4. Myocardial mitochondrial DNA 8-oxo-dG content

Left ventricular muscle tissue was prepared for isolation of mitochondria (Vercesi et al., 1978). The mtDNA was extracted using QIAamp blood kit (Qiagen, Valencia, CA) per manufacturer's instructions and digested into deoxynucleosides by nuclease P1 and alkaline phosphatase. The deoxynucleoside mixture was filtered through a 0.22-μm Nylon filter and injected into a YMC Basic™ S 3μ 4.6 × 150 mm column (Water, Milford, MA) in a BAS 480 HPLC system (Bioanalytical Systems, West Lafayette, IN), with a mobile phase of 5% methanol in 100 mM lithium acetate buffer (pH 5.2) for measuring 8-oxo-dG and 2′-deoxyguanosine (dG). A Model 5200A Coulochem II electrochemical detector equipped with a Model 5011 analytical cell and Model 5021 guard cell (ESA, Chelmsford, MA) was used (De la Asuncion et al., 1996). Purified 8-oxo-dG (ESA) and dG (Sigma-Aldrich) were used for calibration.

2.5. Apoptosis detected by monoclonal antibody to single stranded DNA

Frozen cardiac muscle sections were fixed in 85% methanol in phosphate-buffered saline. The sections were incubated with anti-single-stranded mouse DNA monoclonal antibody (Mab, Chemicon International, Temecula, CA). The sections were then incubated with biotin-conjugated anti-mouse immunoglobulin M (Vector Laboratory, Burlingame, CA) and avidin and biotinylated horseradish peroxidase macromolecular complex (Vector Laboratory), and

Table 1
Body weight, resting hemodynamics, plasma norepinephrine and cardiac function

	Sham-operated		Chronic heart failure	
	Placebo	Selegiline	Placebo	Selegiline
N	10	8	9	8
Body weight (kg)	3.38 ± 0.11	3.34 ± 0.05	3.28 ± 0.07	3.32 ± 0.06
Heart rate (beats/min)	258 ± 10	280 ± 18	245 ± 11	251 ± 8
Mean aortic pressure (mm Hg)	94 ± 4	105 ± 5	85 ± 4	97 ± 6
Plasma norepinephrine (pg/ml)	87 ± 30	63 ± 24	411 ± 46[a]	231 ± 49[a,b]
LV end-diastolic pressure (mm Hg)	7.7 ± 0.6	6.6 ± 0.9	23.5 ± 2.0[a]	15.8 ± 2.9[a,b]
LV d*P*/d*t* (mm Hg/s)	3915 ± 209	4107 ± 205	1968 ± 178[a]	3134 ± 266[a,b]
LV end-diastolic dimension (mm)	15.2 ± 0.2	15.4 ± 0.3	18.5 ± 0.3[a]	18.0 ± 0.5[a]
LV fractional shortening (%)	34.2 ± 0.9	32.6 ± 0.7	19.7 ± 1.5[a]	22.4 ± 1.2[a,b]

Values are means ± S.E.M.; LV, left ventricular.
[a] $P<0.05$ compared to sham.
[b] $P<0.05$ compared to chronic heart failure placebo.

stained with 3-amino-9-ethylcarbazole (Vector Laboratory) and hematoxylin (Vector Laboratory). Mab was omitted for negative control. For positive control, the sections were incubated with proteinase K (20 μg/ml) to induce apoptotic changes. The samples were examined under light microscopy. Four sections randomly picked from each of four pieces were analyzed per animal. Myocyte nuclei were determined by random counting of 10 fields per section. The number of Mab-positive nuclei was calculated per 10,000 cardiomyocytes.

2.6. Immunohistochemistry for Bcl-2 and Bax expression

Frozen heart tissue sections, 6 μm thickness, were thawed, air-dried and then fixed in pre-cooled acetone at − 20 °C. The sections were incubated with 0.3% H_2O_2 in methanol to quench endogenous peroxidase. The sections were incubated with 10% goat serum, and subsequently incubated with anti-mouse Bcl-2 antibody or anti-Bax antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and biotin-conjugated anti-mouse immunoglobin G (Vector Laboratory) and avidin and biotinylated horseradish peroxidase macromolecular complex (Vector Laboratory), and then stained with 3-amino-9-ethylcarbazole and hematoxylin (Vector Laboratory). For negative control, normal mouse immunoglobulin G was used instead of the primary antibodies. The samples were examined under light microscopy.

2.7. Western blot for Bcl-2 and Bax protein expression

Frozen myocardial tissue was homogenized in a lysis buffer and centrifuged. Protein concentration of the supernatant was determined using a bicinchoninic acid protein assay reagent (Pierce, Rockford, IL). Protein was separated by electrophoresis and transferred to polyvinylidene fluoride membrane. Equal loading of myocardial protein was confirmed by Coomassie blue staining. Anti-mouse Bcl-2 monoclonal antibody and anti-mouse Bax monoclonal antibody (Santa Cruz Biotechnology) were used to detect the protein levels of Bcl-2 and Bax. The Phototope-HRP Western Blot Detection Kit (New England Biolab, Beverly, MA) was used to visualize the bands. Autoradiograms were scanned by a GS-700 Imaging Densitometer and the bands were quantified using Quantity One Program (Bio-Rad Laboratories, Hercules, CA).

2.8. Statistical analysis

Results are presented as means ± S.E.M. Student's *t*-test was used to determine the statistical significance of a

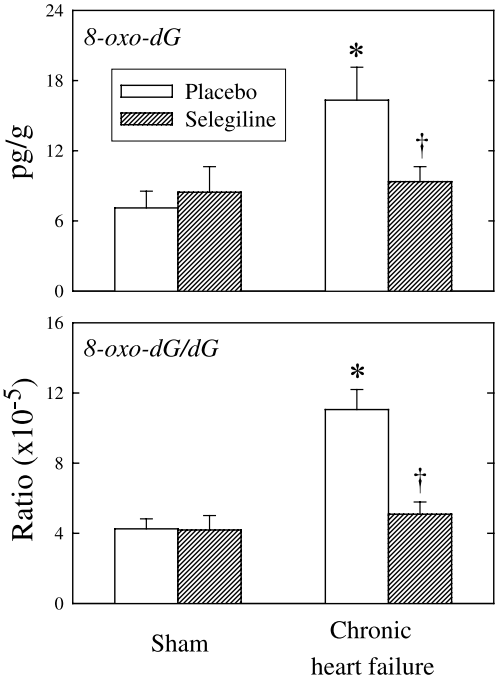

Fig. 1. Changes in mitochondrial DNA 8-oxo-7,8-dihydro-2′deoxyguanosine (8-oxo-dG) and 8-oxo-dG to 2′deoxyguanosine (dG) ratio in sham and chronic heart failure animals with and without selegiline treatment. $N=8$–10. Bars indicate S.E.M. *$P<0.05$ compared with sham placebo. †$P<0.05$ compared with chronic heart failure placebo. Two-way analysis of variance showed a significant difference of 8-oxo-dG between the sham and chronic heart failure animals ($F=6.52$, $P<0.015$), and a significant interaction between the group and treatment ($F=5.40$, $P<0.026$). Two-way analysis of variance also revealed significant differences in the 8-oxo-dG to dG ratio between the sham and chronic heart failure animals ($F=23.28$, $P<0.001$) and between the placebo and selegiline treatment ($F=16.39$, $P<0.001$). There was a significant interaction between the chronic heart failure group and selegiline treatment in the 8-oxo-dG to dG ratio ($F=15.72$, $P<0.001$).

difference between two means. Two-way analysis of variance was used to determine the statistical significance of differences between the sham and chronic heart failure groups, differences between the two treatment groups (placebo and selegiline), and the group and treatment interactions. Pearson product-moment correlation analysis was used to determine the relationship between cardiomyocyte apoptosis and left ventricular systolic function (dP/dt and fractional shortening). Values of $P < 0.05$ were considered significant.

3. Results

3.1. Plasma norepinephrine, cardiac function, and resting hemodynamics

Table 1 shows that body weight, heart rate and mean aortic pressure did not differ significantly among the sham-operated and chronic heart failure animals. As expected, rapid cardiac pacing produced clinical heart failure, which was associated with increased plasma norepinephrine, left ventricular end-diastolic pressure and end-diastolic dimension, and decreased left ventricular dP/dt and fractional shortening. There were no significant changes in body weight, heart rate and mean aortic pressure between the sham and chronic heart failure animals. Selegiline treatment produced no significant changes in any of these parameters in sham-operated animals. It also did not affect heart rate, mean aortic pressure and left ventricular end-diastolic dimension in chronic heart failure animals. However, selegiline attenuated the increases in plasma norepinephrine and left ventricular end-diastolic pressure and decreases of left ventricular dP/dt and fractional shortening in chronic heart failure animals.

3.2. Myocardial mitochondrial DNA 8-oxo-dG content

Fig. 1 shows the changes in myocardial mtDNA 8-oxo-dG. Chronic heart failure increased mtDNA 8-oxo-dG by two- to threefold. Since there was no change in mtDNA dG, the ratio of mtDNA 8-oxo-dG to dG was also increased in chronic heart failure animals. Selegiline treatment did not affect mtDNA 8-oxo-dG/dG ratio in sham animals, but abolished the increase of mtDNA 8-oxo-dG/dG in chronic heart failure animals.

3.3. Myocyte apoptosis

Fig. 2A shows the positive control of immunohistochemical staining of single-stranded DNA of rabbit ventricular muscle tissue section after proteinase K treatment. Representative pictures of the Mab staining of ventricular sections from a sham, a chronic heart failure and a chronic heart failure plus selegiline animal are shown in Fig. 2B–D. Two-way analysis of variance indicates that significant differences exist in the number of apoptotic myocytes between the sham and chronic heart failure groups ($F = 29.18$, $P < 0.0001$), between the placebo and selegiline treatments ($F = 11.49$, $P = 0.002$), and in the interactions between the animal group and drug assignment ($F = 11.84$, $P = 0.002$). Fig. 3 shows that the number of Mab-positive nuclei was

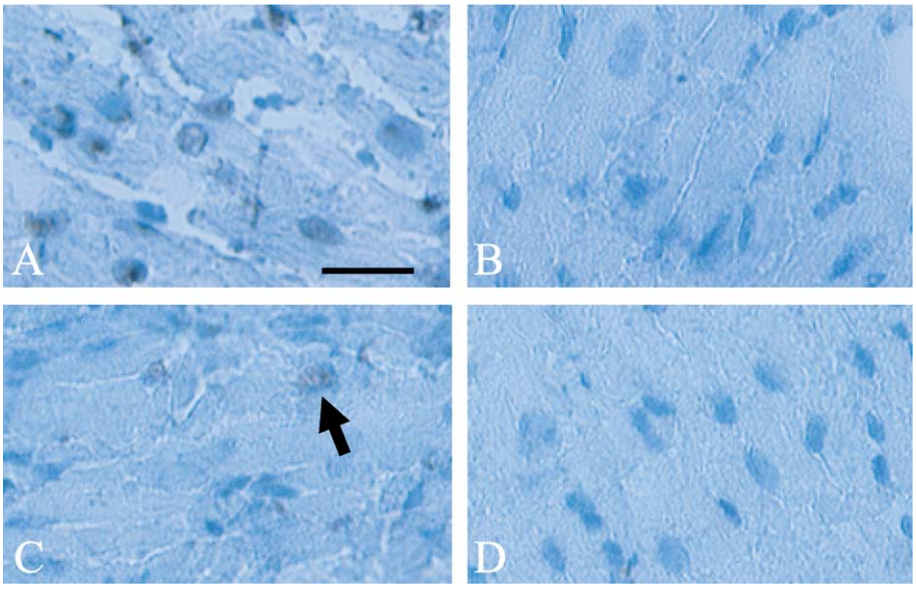

Fig. 2. Photomicrographs of rabbit left ventricular tissue sections showing monoclonal antibody to single-stranded DNA (Mab) staining. The nuclei stained with Mab were shown brownish. Other nuclei stained with hematoxylin were shown blue. Panel A illustrates positive control with brownish Mab-positive staining after proteinase K treatment. Panels B, C and D show the tissue sections obtained from a sham, a chronic heart failure animal and a chronic heart failure + selegiline animal. A brownish nucleus (arrow) showed Mab-positive staining in the chronic heart failure animal. The bar in Panel A indicates 20 μm and accounts for all photomicrographs.

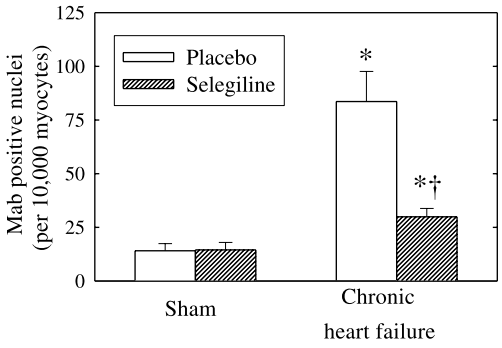

Fig. 3. The number of Mab-positive nuclei in sham and chronic heart failure animals with and without selegiline treatment. $N = 8-10$. Bars indicate S.E.M. $^{*}P < 0.05$ compared with sham placebo. $^{\dagger}P < 0.05$ compared with chronic heart failure placebo.

increased in chronic heart failure compared to the sham animals. Selegiline treatment had no effect in sham rabbits, but reduced the number of apoptotic myocytes in chronic heart failure animals.

3.4. Bcl-2 and Bax protein expression

Fig. 4 shows representative Western blots of Bcl-2 protein expression and Bax protein expression in sham and chronic heart failure animals with and without selegiline treatment. Densitometric analysis demonstrated a reduction of Bcl-2 protein in chronic heart failure (0.77 ± 0.02 arbitrary optical units, $t = 5.67$, $P < 0.001$) compared to the sham-operated animals (1.0 ± 0.03). Selegiline treatment abolished the reduction of Bcl-2 in the chronic heart failure animals. Two-way analysis of variance revealed statistically signifi-

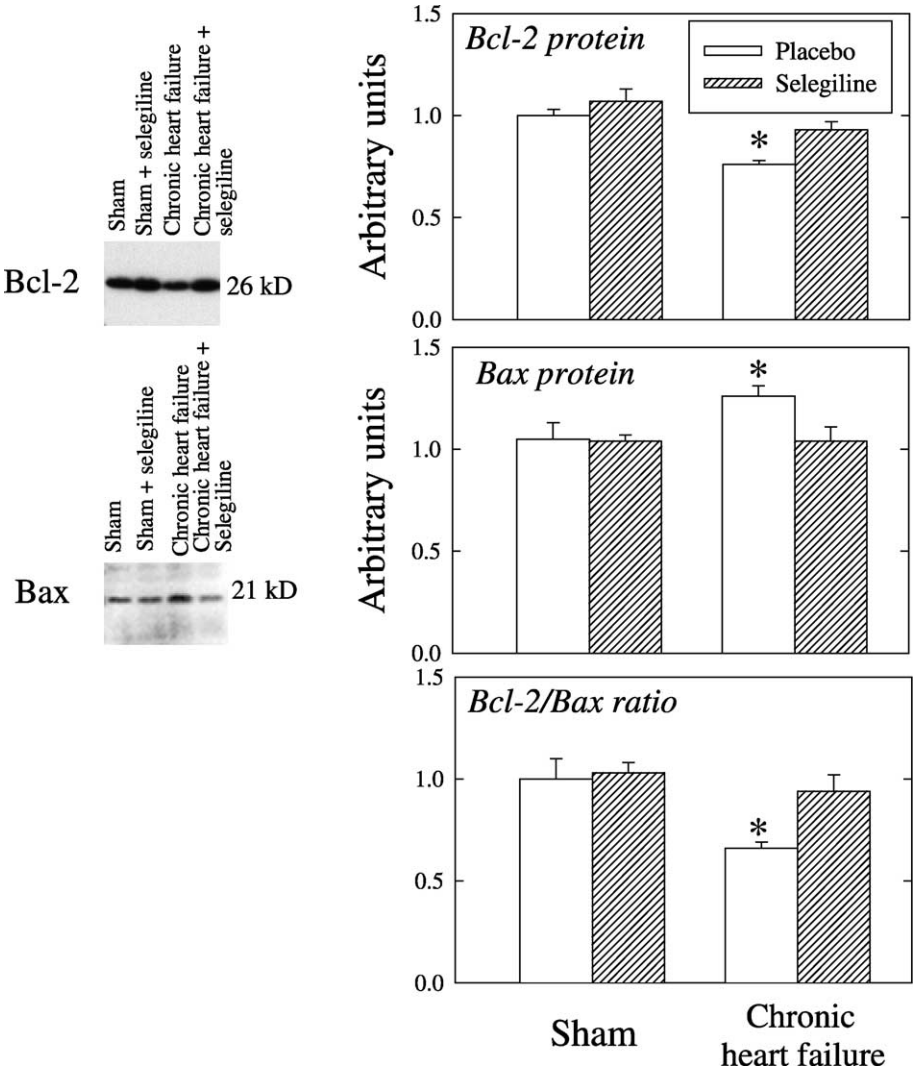

Fig. 4. Changes in Bcl-2 protein, Bax protein and Bcl-2/Bax ratio in sham and chronic heart failure animals with and without selegiline treatment. Results were normalized by arbitrarily setting the densitometry of a control sample to 1. $N = 8-10$. Bars indicate S.E.M. $^{*}P < 0.05$ compared with sham placebo. $^{\dagger}P < 0.05$ compared with chronic heart failure placebo.

cant differences between the sham and chronic heart failure animals ($F = 20.61$, $P < 0.0001$) and between the placebo and selegiline treatments ($F = 8.31$, $P = 0.007$), but the interaction between the animal group and drug treatment was not statistically significant ($F = 1.35$, $P = 0.255$). Bax protein increased in the chronic heart failure animals (1.26 ± 0.05, $t = 2.21$, $P < 0.05$) compared to the sham-operated animals (1.05 ± 0.08). The increase in Bax in chronic heart failure was abolished by selegiline (1.04 ± 0.07). The changes in Bcl-2 and Bax were confirmed by immunohistochemistry (Fig. 5).

The changes in Bcl-2 protein were magnified by incorporating the changes of Bax in the same animals. Fig. 4 shows that the Bcl-2 to Bax ratio was reduced by 34% in the chronic heart failure animals. Selegiline treatment abolished the decrease of Bcl-2 to Bax ratio in chronic heart failure animals. Two-way analysis of variance revealed statistically significant differences between the sham and chronic heart

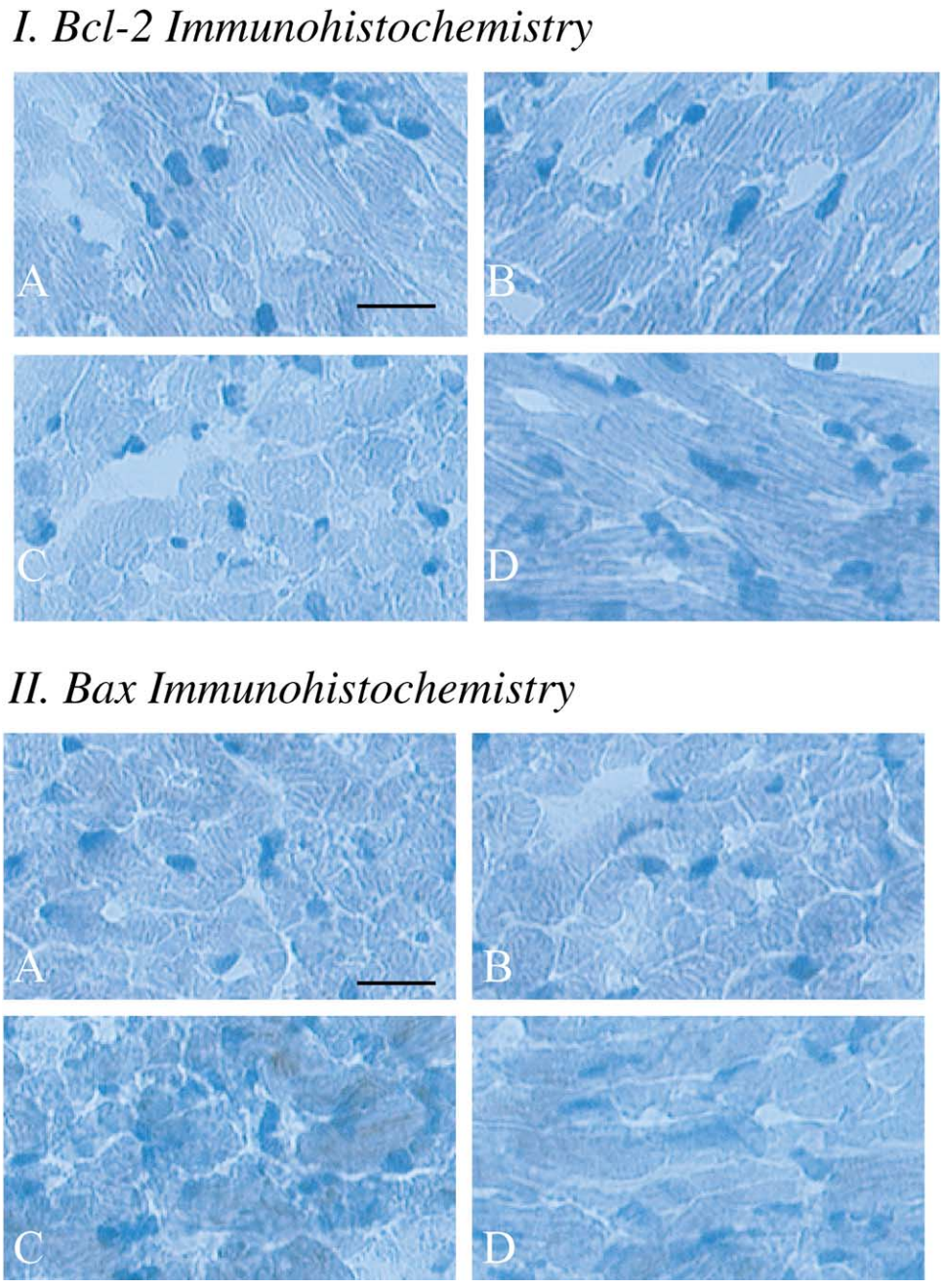

Fig. 5. Photomicrographs of rabbit left ventricular tissue sections showing Bcl-2 expression (Section I) and Bax expression (Section II). The nuclei stained with hematoxylin were shown blue. Panels A, B, C and D show the tissue sections obtained from a sham, a sham + selegiline animal, a chronic heart failure animal and a chronic heart failure + selegiline animal. The bar in Panel A indicates 20 μm and accounts for all photomicrographs.

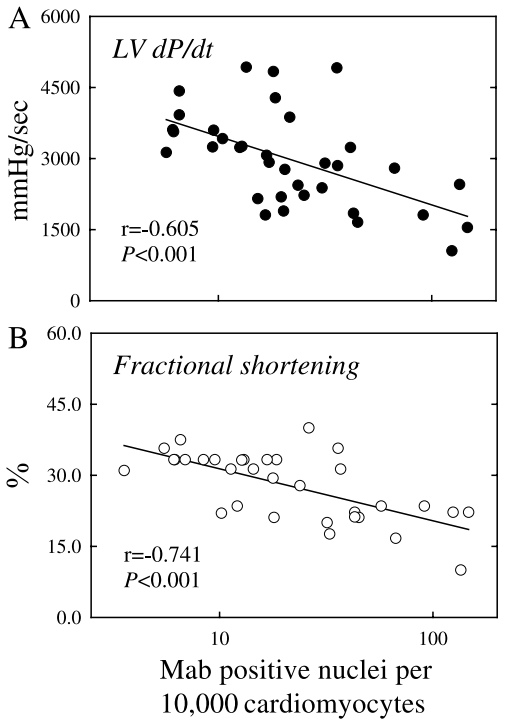

Fig. 6. Correlation between myocyte apoptosis and left ventricular systolic function using either left ventricular dP/dt (Panel A) or fractional shortening (Panel B). Each data point represents one animal. r indicates coefficient of correlation.

failure animals ($F=9.40$, $P<0.005$) and between the placebo and selegiline treatments ($F=4.54$, $P=0.042$). The group and treatment interaction was not statistically significant ($F=3.09$, $P=0.09$).

3.5. Correlation between cardiac apoptosis and function in chronic heart failure

Fig. 6 shows that cardiomyocyte apoptosis correlates significantly with left ventricular dP/dt and fractional shortening. The quantities r^2 indicate that 36–50% of the variances of left ventricular systolic function are associated with linear regressions on myocyte apoptosis.

4. Discussion

Rapid ventricular pacing produced clinical heart failure, cardiac dilation and decreases in left ventricular dP/dt and fractional shortening. These changes were associated with increases in plasma norepinephrine, oxidative stress, Bax to Bcl-2 ratio, and myocyte apoptosis. Selegiline treatment reduced plasma norepinephrine and produced antioxidant and antiapoptotic effects on the failing heart. These changes were associated with improved left ventricular function in chronic heart failure, as characterized by a decrease of left ventricular end-diastolic pressure, and increases of left ventricular dP/dt, and fractional shortening.

4.1. Oxidative stress and myocyte apoptosis in chronic heart failure

Evidence has accumulated that oxidative stress is increased in chronic heart failure (Mak and Newton, 2001). Hydroxyl free radical formation is increased in the failing mouse heart due to myocardial infarction (Kinugawa et al., 2000). Plasma lipid-derived free radicals and plasma thiobarbituric acid reactive substances are elevated in patients with ischemic and nonischemic chronic heart failure (Ellis et al., 2000). Increased levels of plasma malondialdehyde and lipid peroxidation products and lower glutathione peroxidase activity also have been reported in patients with chronic heart failure (Castro et al., 2002). More recently, a direct demonstration of increased lipid peroxides in the heart also has been reported in human cardiomyopathic hearts using endocardial biopsy samples (Nakamura et al., 2002). Our present study demonstrates that myocardial mtDNA 8-oxo-dG was increased in pacing-induced heart failure in rabbits. This is consistent with myocardial mtDNA damage associated with oxidative stress in animals with chronic heart failure after myocardial infarction (Ide et al., 2001). Although our study does not address the sources of oxidative stress, other studies have shown that oxygen free radicals may derive from norepinephrine autoxidation products, angiotensin II and activated cytokines (Ferdinandy et al., 2000).

Increased oxidative stress induces myocyte apoptosis and importantly contributes to the pathophysiology of heart failure (Feuerstein and Young, 2000). Reactive oxygen products such as nitrotyrosine have been demonstrated to increase myocyte apoptosis in pacing-induced heart failure in dogs (Cesselli et al., 2001). Norepinephrine-derived oxidative stress also has been shown to induce myocyte apoptosis (Qin et al., 2001). The importance of oxygen free radicals in myocyte apoptosis is also supported by the findings that antioxidants attenuate myocyte apoptosis in the failing heart following myocardial infarction (Oskarsson et al., 2000). Furthermore, the concept that oxidative stress induces apoptosis is supported by the fact that exposure of cultured adult rat ventricular myocytes to H_2O_2 and $FeSO_4$ as a superoxide-generating system exerts myocyte apoptosis through mitochondrial pathway (Aoki et al., 2002).

4.2. Effects of selegiline on oxidative stress and apoptosis

Selegiline inhibits apoptosis in cultured neuronal cells and experimental brain ischemia (Le et al., 1997; Simon et al., 2001). This antiapoptotic effect of selegiline is independent of its monoamine oxidase type B inhibition, as the effective concentration for the inhibition of apoptosis is much lower than that required for monoamine oxidase inhibition activity (Tatton et al., 1994; Tatton and Chalmers-Redman, 1996; Xu et al., 1999). Neuronal protective effect also can be produced in PC12 cells by a propargylamine, which is structurally related to selegiline but does not inhibit monoamine oxidase B (Tatton et al., 2002). Also,

pretreatment with *N*-(2-aminoethy)-*p*-chlorobenzamide, a selective monoamine oxidase type B inhibitor, does not protect the cells from apoptosis (Le et al., 1997). The antiapoptotic effect of selegiline probably is related to its antioxidant property (Khaldy et al., 2000). This notion is further supported by studies showing that selegiline suppresses hydroxyl radical formation induced by 1-methyl-4-phenylpyridinium ion (Wu et al., 1994) or during iron-catalyzed dopamine autoxidation (Khaldy et al., 2000). Selegiline also diminishes cell death associated with glutathione depletion (Mytilineou et al., 1998). Selegiline not only increases the expression of superoxide dismutase and catalase (Tatton et al., 1996), but also elevates superoxide dismutase activity (Carrillo et al., 1994, 2000).

Studies have shown that selegiline reduces cytoplasmic oxidative stress and neuronal apoptosis (Maruyama et al., 2000). It also has been shown to reduce cell apoptosis by preventing decrease of mitochondrial membrane potential induced by oxidative stress (Maruyama et al., 2000) or serum and nerve growth factor withdrawal (Wadia et al., 1998). Loss of mitochondrial membrane potential is thought to result from dissipation of the H^+ gradient after opening of the permeability transition pore in the inner mitochondrial membrane (Bialik et al., 1999). The change will then result in increased mitochondrial membrane permeability, and release of mitochondrial cytochrome *c* into the cytoplasm, causing activation of apoptotic caspase 3 and cell apoptosis (Suuronen et al., 2000). Tatton et al. (1996, 2002) speculate that selegiline acts on gene expression and protein synthesis to maintain mitochondrial function. These effects of selegiline do not involve monoamine oxidase inhibition (Tatton and Chalmers-Redman, 1996), but the exact molecular mechanism by which selegiline selectively alters transcription to decrease cytoplasmic oxidative radical levels and apoptosis has not been fully elucidated. The protective actions of selegiline on mitochondrial damage and cellular apoptosis are consistent with our present study showing that selegiline reduced myocardial mtDNA 8-oxo-dG and myocyte apoptosis in chronic heart failure. These results suggest that the reduction by selegiline of myocyte apoptosis probably is associated with the decrease of oxidative stress in chronic heart failure.

We have shown that selegiline decreased plasma norepinephrine in chronic heart failure (Shite et al., 2000). Selegiline also has been shown to reduce cardiovascular sympathetic reflex responses in patients with Parkinson's disease (Turkka et al., 1997). This effect of selegiline on the sympathetic nervous system probably is secondary to an action on the central nervous system (Cohen et al., 1982), but the molecular mechanism by which selegiline exerts this inhibitory action of the central sympathetic nervous system is not clear. Since increased norepinephrine is one of the sources of free radicals in chronic heart failure, the sympatholytic effect of selegiline may play a role on the reduction of myocyte apoptosis in chronic heart failure. However, the relative contributions of the sympathetic nervous system inhibition and the independent antioxidant action of selegiline to the antiapoptotic effects of selegiline have not been fully investigated. Chemical or immunological sympathectomy has been used to reduce cardiac and circulating norepinephrine, but no studies have been performed to study the effect of these interventions on myocyte apoptosis in heart failure.

Earlier studies have shown that selegiline alters the configuration of antiapoptotic Bcl-2 and proapoptotic Bax genes (Simon et al., 2001). In our studies of chronic heart failure, selegiline attenuated the decrease of Bcl-2 protein and the increase of Bax protein, indicating that the reversal of Bcl-2 and Bax may be involved in the antiapoptotic mechanism of selegiline. These findings are consistent with a recent report that selegiline-related propargylamines prevents the apoptotic decrease in Bcl-2 and increase in mitochondrial Bax in serum- and nerve growth factor withdrawn differentiated PC12 cells (Tatton et al., 2002). Likewise, antioxidant vitamins have been demonstrated to inhibit norepinephrine-derived oxidative stress-mediated myocyte apoptosis by the modulation of Bcl-2 and Bax proteins (Qin et al., 2001). Thus, we speculate that the antiapoptotic effect of selegiline is mediated via reduction of norepinephrine and oxidative stress and reversal of the Bcl-2 to Bax ratio.

A direct effect of oxygen-free radicals on Bcl-2 and Bax proteins has been demonstrated in cultured myocytes (Suzuki et al., 2001). However, unlike the marked changes that could be produced by pharmacological doses of H_2O_2 in the cultured myocytes, the apoptotic and antiapoptotic proteins changed only modestly (15–45%) in intact animals as shown in our present study of chronic heart failure. Similarly in a prior study with subhypertensive doses of norepinephrine, myocardial Bcl-2 decreased only 27% in norepinephrine-treated ferrets (Qin et al., 2001). Simultaneously, Bax protein increased 42%. However, these modest changes of apoptotic and antiapoptiotic proteins in intact animals probably are physiologically significant because of the concordant changes of myocyte apoptosis and left ventricular systolic function with the apoptotic proteins in our study. Similar changes of Bcl-2 and Bax proteins also have been shown to contribute to myocyte apoptosis after myocardial ischemia and reperfusion (Zhao et al., 2000).

The dose of selegiline used in the present study was based on the results of our previous study (Shite et al., 2000). The dose was sufficient to produce the desired antioxidant and antiapoptotic effects. The findings are consistent with previous reports (Kitani et al., 1999; Le et al., 1997). Selegiline at a dose of 0.25 or 0.5 mg/kg 3 days per week in rats and mice has been shown to produce long-term optimal antioxidant effects and increased life expectancy of the animals (Carrillo et al., 1996; Kitani et al., 1996, 1998). The dose we used in the present study (0.3 mg/kg/day) was within this range. Monoamine oxidase activity was not measured in this study. However, at the low doses, selegiline is not expected to produce significant monoamine oxidase inhibition (Tatton et al., 1994, 1996; Xu et al., 1999).

However, at larger doses, selegiline may block monoamine oxidase and prevent tissue breakdown and elimina-

tion of endogenous norepinephrine. The increase in tissue norepinephrine release may not only counteract the salutary effects of selegiline on antioxidant enzyme activities (Carrillo et al., 2000), but also cause cytotoxicity after long-term exposure (Maruyama et al., 2000; Scheinin et al., 1998). Selegiline at high concentrations has been shown to induce apoptosis in cultured neuronal cells (Le et al., 1997). These findings may help explain why selegiline produced less neuroprotective effects at high doses (e.g, 5 mg/kg) (Shite et al., 2000).

4.3. Reduction of apoptosis and improvement of cardiac function in chronic heart failure

Myocyte apoptosis contributes to progressive deterioration of cardiac function (Hare, 2001; Sabbah, 2000). Cardiac-selective overexpression of proapoptotic gene caspase-3 produces depression of cardiac function in a transgenic mouse model (Condorelli et al., 2001) and blocking caspase-activated apoptosis improves cardiac function in chronic heart failure (Laugwitz et al., 2001). The magnitude of myocyte apoptosis is associated with the severity of left ventricular dilation and systolic dysfunction in acromegalic cardiomyopathy in humans (Frustaci et al., 1999). Furthermore, oxidative stress-mediated myocyte apoptosis is a major determinant of ventricular dysfunction and failure in pacing-induced cardiomyopathy in dogs (Cesselli et al., 2001). Our present study showing a close association of reduction by selegiline of oxidative stress-mediated myocyte apoptosis and the improvement of cardiac function in chronic heart failure is consistent with an important functional role of myocyte apoptosis in pacing-induced cardiomyopathy.

4.4. Conclusion

Our present results indicate that the beneficial effects of selegiline on cardiac function correlates with the reduction of myocyte apoptosis in chronic heart failure. The antiapoptotic action of selegiline probably is related to the reduction of oxidative stress and the modulation of Bcl-2 and Bax genes in the chronic heart failure animals. Additional studies are needed to investigate the exact mechanisms by which selegiline prevents myocyte apoptosis produced by oxidative stress.

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