Effects of ACE inhibition on cardiomyocyte apoptosis in dogs with heart failure

ANASTASSIA GOUSSEV, VICTOR G. SHAROV, HISASHI SHIMOYAMA, MITSUHIRO TANIMURA, MICHAEL LESCH, SIDNEY GOLDSINSTEIN, AND HANI N. SABBAH
Division of Cardiovascular Medicine, Department of Medicine, Henry Ford Heart and Vascular Institute, Detroit, Michigan 48202

Goussev, Anastassia, Victor G. Sharov, Hisashi Shimoyama, Mitsuhiro Tanimura, Michael Lesch, Sidney Goldstein, and Hani N. Sabbah. Effects of ACE inhibition on cardiomyocyte apoptosis in dogs with heart failure. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H626–H631, 1998.—Cardiomyocyte apoptosis or programmed cell death has been shown to occur in end-stage explanted failed human hearts and in dogs with chronic heart failure (HF). We tested the hypothesis that early long-term monotherapy with an angiotensin-converting enzyme (ACE) inhibitor attenuates cardiomyocyte apoptosis in dogs with moderate HF. Left ventricular (LV) dysfunction (ejection fraction 30–40%) was produced in dogs by multiple sequential intracoronary microembolizations. Dogs were randomized to 3 mo of therapy with enalapril (Ena, 10 mg twice daily, n = 7) or to no therapy at all (control, n = 7). After 3 mo of therapy, dogs were euthanized and the hearts removed. Presence of nuclear DNA fragmentation (nDNAf), a marker of apoptosis, was assessed in frozen LV sections using the immunohistochemical deoxynucleotidolysis (nDNAf) method. Sections were also stained with ventriculoverase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) method. Sections were also stained with ventricular anti-myosin antibody to identify cells of cardiocyte origin. From each dog, 80 fields (×40) were selected at random, 40 from LV regions bordering old infarcts and 40 from LV regions remote from any infarcts, for quantifying the number of cardiomyocyte nDNAf events per 1,000 cardiomyocytes. The average number of cardiomyocyte nDNAf events per 1,000 cardiomyocytes was significantly lower in Ena-treated dogs compared with controls (0.81 ± 0.13 vs. 2.65 ± 0.81, P < 0.029). This difference was due to a significantly lower incidence of cardiomyocyte nDNAf events in LV regions bordering scarred tissue (infarcts) in Ena-treated dogs compared with controls. We conclude that early long-term Ena therapy attenuates cardiomyocyte apoptosis in dogs with moderate HF. Attenuation of cardiomyocyte apoptosis may be one mechanism by which ACE inhibitors preserve global LV function in HF.

ventricular function; angiotensin-converting enzyme inhibitors; programmed cell death

LONG-TERM THERAPY with angiotensin-converting enzyme (ACE) inhibitors has been shown to reduce mortality and morbidity in patients with heart failure (HF) (15, 21, 35) and attenuate left ventricular (LV) remodeling (21) and chamber sphericity (18). In dogs with moderate HF, produced by multiple sequential intracoronary microembolizations, we also showed that early long-term therapy with enalapril prevents progressive LV systolic dysfunction and chamber dilation (26). Whereas these studies provided strong evidence that ACE inhibition can attenuate the progressive deterioration of global LV function, a complete understanding of the mechanism(s) that mediates this beneficial effect of ACE inhibition in HF remains elusive. A possible working hypothesis is that the progressive deterioration of LV function in HF is mediated, in part, by ongoing loss of cardiomyocytes and that therapy with ACE inhibitors positively impacts this process. Studies in our laboratory in dogs with chronic HF (32) and in end-stage explanted failed human hearts (19, 20, 32) have documented the occurrence of cardiomyocyte apoptosis or programmed cell death, a feature that may account, in part, for the progression of global LV dysfunction (19, 20, 32). Whereas necrosis occurs in response to lethal injury, apoptosis is an active, energy-requiring process that appears to be under genetic control (9, 36). Apoptosis differs from necrosis in that cell death occurs in the absence of cell membrane rupture and inflammation and is characterized by nuclear DNA fragmentation (nDNAf) (13, 14). In most normal adult tissue consisting of nonterminally differentiated cells, apoptosis plays a crucial role in maintaining cell population homeostasis and is balanced by mitosis (4, 10, 13). Recent studies have suggested that hypertrophic stimuli, including angiotensin II, can activate several protein serine/threonine kinases which, in turn, can phosphorylate nuclear transcriptional factors such as c-myc, c-jun, and c-fos (28), which are involved in cellular transformation, mitogenesis, and programmed cell death (1). In the present study, we tested the hypothesis that early long-term treatment with ACE inhibition in dogs with moderate HF is associated with reduced cardiomyocyte apoptosis as evidenced by a reduced incidence of cardiomyocyte nDNAf.

METHODS

Animal model. The canine model of chronic heart failure used in this study was previously described in detail (27). In this model, chronic LV dysfunction is produced by multiple sequential intracoronary embolizations with polystyrene latex microspheres (77–102 µm diameter). A unique feature of this model is the continued progressive deterioration of LV function long after cessation of coronary embolizations (26, 27). In the present study, 14 dogs weighing between 18 and 31 kg underwent multiple intracoronary microembolizations ~1–3 wk apart. Embolizations were performed during cardiac catheterization under general anesthesia and sterile conditions. Anesthesia consisted of a combination of intravenous oxymorphone (0.22 mg/kg), diazepam (0.17 mg/kg), and pentobarbital sodium (150–250 mg to effect). This anesthesia regimen was previously shown to have no effect on global LV function (26). Dogs underwent an average of five coronary microembolization procedures each. Embolizations were discontinued when LV ejection fraction, determined angiographically, was between 30 and 40%. The cohort of dogs used in the
present study represents a subset of a previously published larger study (26).

Study protocol. Three weeks after the last embolization, the dogs underwent a left and right heart catheterization to establish hemodynamic status. One day later, dogs were randomized to 3 mo of oral monotherapy with enalapril (10 mg twice daily, n = 7) or no therapy at all (controls, n = 7). Hemodynamic and angiographic measurements were made during cardiac catheterization 1 day before therapy was initiated and were repeated during a cardiac catheterization performed at the end of 3 mo of therapy. At the end of the 3-mo therapy or follow-up, the heart was harvested and tissue prepared for histological examination. The study was approved by the Henry Ford Hospital Care of Experimental Animals Committee and conformed to the "Position of the American Heart Association on Research Animal Use" and to the guiding principles of the American Physiological Society.

Hemodynamic and ventriculographic measurements. Aortic and LV pressures were measured with catheter-tip micromanometers (Millar Instruments). Cardiac output was measured in duplicate using the thermodilution method. Systemic vascular resistance was calculated as previously described (27). Left ventriculograms were obtained after hemodynamic measurements were completed in the dog placed on its right side and were recorded on 35-mm cine at 30 frames/s during the injection of 20 ml of contrast material (Hypaque meglumine 60%, Winthrop Pharmaceutical). Correction for image magnification was made with a radiopaque calibrated grid placed at the level of the left ventricle. LV end-systolic and end-diastolic volumes were calculated from ventricular silhouettes using the area-length method (7). Ejection fraction was calculated as previously described (27).

In situ labeling of nDNAf. At the end of the final hemodynamic and angiographic assessment with the dogs under general anesthesia, we opened each dog's chest via a left thoracotomy, the pericardium was opened, and the heart was rapidly removed and placed in ice-cold cardioplegia solution. From each heart, transverse slices (3–4 mm thick) were obtained from the LV midventricular level and cut into several blocks, each labeled for anatomic location. Transmural blocks were mounted on cork with Tissue Teck embedding medium, embedded in isopentane cooled to −160°C in liquid nitrogen, and stored at −70°C until ready for use.

Cryostat sections were triple stained as shown in Fig. 1A–C. First, sections were stained using the ApopTag in situ apoptosis fluoroscein detection kit (Oncor, Gaithersburg, MD) to identify cells showing nDNAf (32). In this process, residues of digoxigenin nucleotide are catalytically added to DNA by terminal deoxynucleotidyl transferase. The deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling is referred to as the TUNEL method (Fig. 1A). Second, to identify cardiomyocytes or cells of cardiomyocyte origin, sections were then stained overnight at 4°C with a monoclonal anti-myosin antibody (Chemicon, Temecula, CA) (Fig. 1C). For visualization, a rhodamine-conjugated secondary anti-mouse antibody was used. Finally, to identify nuclei, sections were stained using the specific nuclear stain Hoechst 33342 (Molecular Probes, Eugene, OR) (Fig. 1B). With the use of the above sequence of staining, nDNAf events were visualized microscopically as yellow-green under fluorescein light (Nikon DM 510 filter), myosin was seen as red under rhodamine light (Nikon DM 580 filter), and cell nuclei were seen as blue (Nikon DM 455 filter). From each section, 80 light microscopic fields (magnification ×40) were used to count the number of cells positively labeled for nDNAf. Forty fields were selected at random from myocardial regions bordering scar tissue (old infarcts) and 40 fields from myocardial regions remote from scars. The selection of regions bordering scars was made due to the high incidence of cardiomyocyte apoptosis previously identified in these regions (1). The number of cardiomyocytes positively labeled for nDNAf was calculated per 1,000 cardiomyocytes. LV sections from six normal dogs were prepared and examined in an identical fashion. In these normal dogs, 40 microscopic fields were selected at random for quantitation of the number of nDNAf events. In frozen tissue sections stained with anti-myosin antibody, a scar or infarct was defined as a myocardial region devoid of myocytes and occupying a surface area equivalent to that of at least 1,000 myocytes, as visualized at a magnification of ×10. Confirmation that these regions indeed represented scar tissue was made by immunostaining sections with antibodies for collagens I and III. The region bordering a scar or infarct was defined using anti-myosin-stained sections as a peri-infarct region consisting of 10 rows of myocytes adjacent to the scar counted radially, starting at the scar to viable tissue interface. A remote myocardial region was defined as any area that was at least 100 myocytes away from any infarct in all directions. These definitions, while empirical, are consistent with previous work from our laboratory (31).

Data analysis. Comparisons of the hemodynamic and angiographic measures of LV function were made between values obtained before therapy and those obtained at the end of therapy within each of the study groups. For these comparisons, a Student’s paired t-test was used and a probability of 0.05 or less was considered significant. A t-statistic for two means was used to examine the two study groups with respect to the number of nDNAf events observed at the end of 3 mo of therapy. For this test, a probability of 0.05 or less was considered significant. All data are reported as means ± SE.

RESULTS

Hemodynamic and angiographic findings. There were no significant differences in any of the hemodynamic and angiographic measures at the time of randomization (before therapy was initiated) between control dogs and enalapril-treated dogs. In untreated control dogs, as expected based on previous observations with this animal model, LV ejection fraction decreased significantly during the 3 mo of follow-up from a pretreatment value of 36 ± 1 to 26 ± 1% (P < 0.001) and was associated with a significant increase in LV end-diastolic volume (61 ± 6 vs. 78 ± 8 ml, P < 0.001), end-systolic volume (39 ± 4 vs. 57 ± 6 ml, P < 0.001), and systemic vascular resistance (2,620 ± 6 vs. 3,000 ± 147 dyn·s·cm⁻⁵, P < 0.05). In contrast, enalapril therapy prevented the progressive decline in LV ejection fraction (35 ± 1 vs. 38 ± 3%) and attenuated the progressive rise in LV end-diastolic volume (61 ± 6 vs. 65 ± 5 ml), LV end-systolic volume (40 ± 4 vs. 40 ± 3 ml), and systemic vascular resistance (2,386 ± 5 vs. 2,247 ± 205 dyn·s·cm⁻⁵).

Effect of treatment with enalapril on nDNAf. There was no evidence of nDNAf in cardiomyocytes of normal dogs. In contrast, in dogs with HF, regardless of treatment, nDNAf was identified in cardiomyocytes remotely located from any scars as well as in constituent cardiomyocytes of regions bordering scars. In untreated dogs, consistent with earlier observations (32), the number of nDNAf events of cardiomyocyte origin was significantly higher in LV regions bordering scars.
compared with LV regions remote from any scars (5.32 ± 0.77 vs. 0.39 ± 0.12 nDNAf events/1,000 cardiomyocytes, P < 0.001). A typical high-powered micrograph depicting nDNAf in a cardiomyocyte is shown in Fig. 1. In dogs treated long term with enalapril, the overall number of nDNAf events of cardiomyocyte origin was significantly lower than that in untreated dogs (0.81 ± 0.13 vs. 2.65 ± 0.81 nDNAf events/1,000 cardiomyocytes, P < 0.001).
events/1,000 cardiomyocytes, P < 0.03) (Table 1). There was a significantly lower incidence of nDNAf events of cardiomyocyte origin in regions bordering old infarcts of dogs treated with enalapril compared with control dogs (Table 1). There was no statistically significant difference in the number of cardiomyocyte nDNAf events in LV regions remote from infarcts between the two study groups (Table 1).

DISCUSSION

The results of the present study indicate that early long-term therapy with the ACE inhibitor enalapril attenuates cardiomyocyte apoptosis in the LV myocardium of dogs with HF. In dogs treated with enalapril, the reduction in the overall incidence of cardiomyocyte apoptosis was due to a substantial reduction in the number of cardiocyte apoptotic events of LV regions bordering scars or old infarcts. The overall incidence of cardiomyocyte apoptosis in the present study was nearly 2.7 cardiomyocytes per 1,000 cardiomyocytes. This is consistent with findings by Olivetti et al. (20) in failed human hearts due to ischemic or idiopathic dilated cardiomyopathy in which the incidence of cardiomyocyte apoptosis was reported to be nearly 2.4 cardiomyocytes per 1,000 cardiomyocytes.

The apparent ability of enalapril to reduce apoptotic markers in LV myocardial regions bordering infarcts was associated with the preservation of global LV function and attenuation of LV chamber remodeling compared with untreated dogs; a feature consistent with results of major clinical trials (15, 18, 21, 35). Even though the results of the present study indicate that cardiocyte apoptosis is associated with progression of LV dysfunction and remodeling and the attenuation of these processes by enalapril, these findings in and of themselves do not imply a cause and effect relationship. Further studies aimed at elucidating the importance of cardiomyocyte apoptosis in the overall process of progressive deterioration of LV function are needed to arrive at such a conclusion. The observations made in the present study are, nonetheless, consistent with recent findings by Li et al. (17), who demonstrated a reduction in the extent of cardiomyocyte apoptosis in cardiocytes of spontaneous hypertensive rats with HF treated with the ACE inhibitor captopril.

A wide gap of knowledge has existed for many years with respect to the underlying factors responsible for the progressive deterioration of LV function in patients with HF. In the absence of any established mechanism or mechanisms that can account for this progressive deterioration, the following working hypothesis can be put forth; namely, that progressive LV dysfunction results, in part, from ongoing intrinsic contractile dysfunction of residual viable cardiomyocytes and/or from ongoing degeneration and loss of viable cardiomyocytes. In the present study, comparisons of the extent of cardiomyocyte apoptosis were made in both study groups at the same time point, namely, 3 mo after randomization and initiation of therapy. This protocol did not allow for determination of the rate at which apoptosis occurs during the progressive phase of the disease and, therefore, no implications can be derived with regard to the role of ACE inhibition in modulating cardiomyocyte apoptosis in the course of progressive LV dysfunction. In a recent study in dogs with HF, however, we showed that the rate of cardiomyocyte apoptosis is essentially constant during the progressive phase of LV dysfunction (24). The results of the present study cannot be used to distinguish whether cardiomyocyte apoptosis, observed in this canine model of HF, results from HF and LV dysfunction per se or from repeated coronary microembolizations and persistent myocardial ischemia.

It has become abundantly clear in recent years that interference with the renin-angiotensin system in the form of ACE inhibition in HF can positively impact LV chamber remodeling and function. The mechanism or mechanisms responsible for this beneficial action of ACE inhibitors in HF, however, are not fully understood. Studies in animal models support the concept that treatment with ACE inhibitors can modulate cardiomyocyte hypertrophy and the accumulation of collagen in the cardiac interstitium termed reactive interstitial fibrosis (5, 37). In HF, interstitial fibrosis was shown to be associated with reduced capillary density and increased oxygen diffusion distance (25), factors that can lead to hypoxia of the collagen-encircled cardiomyocyte (22, 33). In the present study, the highest incidence of cardiocyte apoptosis occurred in regions bordering scars or old infarcts that are characterized by severe interstitial fibrosis (19, 33) and are susceptible to ischemia/hypoxia (33). Severe structural degeneration and the presence of ultrastructural features consistent with apoptosis have also been described in HF among constituent myocytes of LV regions bordering old infarcts (31, 32). ACE inhibitors can also reduce myocardial oxygen demands, secondary to afterload and preload reduction, which, in turn, can increase the ischemic threshold for apoptosis to occur.

Studies in our laboratories in dogs with HF have shown that long-term treatment with ACE inhibitors is associated with reduced volume fraction of replacement fibrosis (30), reduced volume fraction of interstitial fibrosis (11), attenuation of cardiomyocyte hypertrophy (11), and enhanced capillary density (23) compared with those studies in untreated dogs. These beneficial effects, when considered in aggregate, can presumably limit hypoxia/ischemia of the collagen-encircled cardiomyocyte and its adverse effects on cellular structure.

Table 1. Number of nDNAf per 1,000 cardiomyocytes in untreated dogs and enalapril-treated dogs

<table>
<thead>
<tr>
<th>Number of nDNAf per 1,000 Cardiomyocytes</th>
<th>Enalapril</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Border zone</td>
<td>0.81 ± 0.09</td>
<td>0.77 ± 0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Remote zone</td>
<td>0.81 ± 0.26</td>
<td>0.12 ± 0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Overall</td>
<td>0.81 ± 0.13</td>
<td>0.81 ± 0.26</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE for left ventricular regions bordering old infarcts (Border zone), regions remote from any infarcts (Remote zone) and overall incidence of nuclear DNA fragmentation (nDNAf). NS, not significant.
and function. Some evidence exists that supports the concept that hypoxia and/or ischemia can trigger cardiomyocyte apoptosis (4, 12, 34). Exposure of cultured rat neonatal cardiocytes to hypoxia was shown to induce apoptosis as evidenced by positive in situ nick-end labeling (34). Exposure of cultured rat cardiomyocytes to hypoxia has also been shown to activate Raf-1 and mitogen-activated protein kinase (29). The latter can regulate the expression of protooncogenes such as c-myc, c-jun, and c-fos that also have been implicated in the induction of cell cycle progression and apoptosis (1, 3, 8, 28, 38). Others have shown that hypoxia/reoxygenation can lead to Jun kinase activation in cardiomyocytes, an event that has been linked to apoptosis through redox signaling (16). The above rationale for ACE inhibitors to attenuate cardiomyocyte apoptosis by limiting exposure of cardiomyocytes to a hypoxic stimulus is one possible indirect mechanism by which ACE inhibitors act to preserve cardiomyocytes in HF. Another possible direct action may be mediated by prevention of the formation of angiotensin II. Angiotensin II-induced activation of mitogen-activated protein kinase has been shown to occur in primary cultures of cardiac myocytes (28), a step that can also lead to apoptosis. Finally, it is by no means certain that the modulation of cardiomyocyte apoptosis in the infarct border zone seen in the present study is unique to ACE inhibition and interference with the renin-angiotensin system signaling. It is possible, but yet unestablished, that other drugs, such as β-blockers, which are known to improve LV function in heart failure, can also have a beneficial effect on cardiomyocyte apoptosis. It is also possible that favorable perfusion and subsequent histological remodeling of the peri-infarct region by ACE inhibition could be responsible for the reduced incidence of apoptosis rather than a direct effect of prevention of angiotensin II formation. The latter possibility, albeit speculative, could also explain the lack of difference in the incidence of cardiomyocyte apoptosis between treated and untreated dogs in myocardial regions remote from any infarcts.

The present study is not without limitations. Nuclear DNA breaks using the TUNEL method were used as the basis for identifying cardiomyocytes undergoing apoptosis. Several studies have suggested that internucleosomal DNA cleavage can occur in both apoptosis and necrosis (2, 6). The study is also limited by the lack of confirmation of the results using other markers of apoptosis in addition to nDNAf. In the present investigation, cardiomyocyte nDNAf was examined in dogs with HF studied 3 mo after the last microembolization when all infarcts were healed. In several studies in which this canine model was used, we were not able to identify cardiomyocyte necrosis in the late chronic stage of HF, namely, long after (3–4 mo) completion of intracoronary microembolizations (25, 27, 31–33). There is also another limitation to the use of the TUNEL method to quantify the absolute number of cardiomyocytes undergoing apoptosis. Not all cardiomyocytes have their nuclei in the plane of the histological section and, for this reason, cannot be judged as undergoing apoptosis or not. Nevertheless, this limitation tends to be minimized when comparisons are performed among study groups, as in the present work, using identical histological techniques.

In conclusion, the observations made in this study indicate that long-term treatment with the ACE inhibitor enalapril in dogs with moderate HF attenuates cardiomyocyte apoptosis as evidenced by reduced cardiomyocyte nDNAf events in viable myocardial regions that border scar tissue (old infarcts). The attenuation of cardiomyocyte apoptosis with ACE inhibition therapy was associated with prevention of progressive LV dysfunction and attenuation of LV chamber remodeling. Reduction of ongoing loss of functional cardiac units in HF through apoptosis may be one mechanism by which ACE inhibitors preserve LV function and attenuate the progression of LV chamber remodeling in the failing heart.

This study was supported in part by National Heart, Lung, and Blood Institute Grant HL-49090–04. Address for reprint requests: H. N. Sabbah, Cardiovascular Research, Henry Ford Hospital, 2799 West Grand Blvd., Detroit, MI 48202.

Received 25 September 1997; accepted in final form 29 April 1998.

REFERENCES


