Insulin-Like Growth Factor-1 Attenuates the Detrimental Impact of Nonocclusive Coronary Artery Constriction on the Heart

Baosheng Li, Manabu Setoguchi, Xiaowei Wang, Anna Maria Andreoli, Annarosa Leri, Ashwani Malhotra, Jan Kajstura, Piero Anversa

Abstract—Coronary artery narrowing (CAN) induces tissue injury, which may involve myocyte necrosis and apoptosis. Insulin-like growth factor (IGF)–1 may counteract cell death, modifying the detrimental effects of myocardial ischemia. On this basis, CAN was produced in female FVB/\text{Igf}^+/– mice and nontransgenic littermates, and the animals were euthanized 7 days later. CAN consisted of an 82% reduction in the vessel luminal cross-sectional area in both groups of mice. Severe left ventricular dysfunction was present in CAN nontransgenic and transgenic mice, but heart and left ventricular weights increased more in littermates than in FVB/\text{Igf}^+/– mice. Similarly, the changes in chamber volume and diastolic wall stress were greater in nontransgenic mice. Subacute tissue injury, represented by foci of replacement fibrosis, was 2.6-fold higher in CAN littermates than in FVB/\text{Igf}^+/– mice. Ongoing myocyte necrosis was 5-fold greater in nontransgenic mice, whereas apoptosis was low and did not differ in the 2 groups of mice. In CAN nontransgenic mice, myocyte necrosis was 12-fold more frequent than apoptosis but, in CAN transgenic mice, these 2 types of cell death were comparable. \text{\alpha}-Myosin and \text{\beta}-myosin isoform mRNAs were affected by CAN, but \text{\alpha}-myosin mRNA was reduced more in nontransgenic mice. In conclusion, myocyte necrosis and replacement fibrosis are the prevailing forms of myocardial damage induced by CAN. Constitutive overexpression of IGF-1 attenuates myocyte necrosis and tissue injury, having no effect on cell apoptosis. These factors limit ventricular dilation, myocardial loading, cardiac hypertrophy, and alterations in \text{\alpha}- and \text{\beta}-myosin isoform expression. (\textit{Circ Res.} 1999;84:1007-1019.)

Key Words: myocardial ischemia ■ myocyte death ■ ventricular remodeling ■ myosin isoform ■ insulin-like growth factor-1 transgenic mice

Ischemic heart disease sustained by nonocclusive coronary artery constriction is characterized by replacement fibrosis across the ventricular wall, cavity dilatation, and mural thinning.1 These modifications are associated with cardiac dysfunction and elevation in diastolic stress. Myocyte loss is modest, but it may continue with time.1 Dropout of myocytes, in combination with defects in coronary perfusion, may counteract reactive growth, increase chamber volume, and promote further elevation in wall stress and oxygen consumption. Cell death, whether apoptotic or necrotic, may have a differential impact on the evolution of the ischemic myopathy. Myocyte necrosis leads to an inflammatory reaction, vessel proliferation, macrophage and fibroblast activation, and scar formation.2 The increase in fibrous tissue alters muscle mechanical performance; force development is depressed, and the compliance properties of the ventricle are impaired.3 More complex is the understanding of the consequences of myocyte apoptosis. After apoptosis, the reparative process does not involve healing, and apoptotic bodies are removed by neighboring cells4 with no changes in the morphology of the myocardium.5 However, apoptosis is implicated in acute restructuring of the wall, downward shift in the length-tension curve, and decreased active tension generation of the myocardium.6

Coronary artery narrowing (CAN) in mice leads to decompensated eccentric hypertrophy, tissue damage, and abnormalities in loading that mimic human ischemic cardiomyopathy.7 Moreover, overexpression of insulin-like growth factor (IGF)–1 in myocytes abolishes the activation of myocyte necrosis and apoptosis in the viable myocardium after infarction, attenuating reactive hypertrophy, ventricular dilation, and wall stress.8 Apoptosis is the prevailing type of cell death after infarction, whereas the form of myocyte injury associated with CAN remains to be defined. This is relevant because defects in coronary blood flow are present with CAN, contrasting the lack of alterations in flow distribution in the noninfarcted myocardium. In the current study, we attempted to establish (1) whether myocyte necrosis and apoptosis occur in a mouse model of global ischemia in a manner comparable with that of the postinfarction heart; (2) whether IGF-1 can protect the underperfused ventricle by these forms of cell death; and (3) whether loading abnormal-
Figure 1. Light micrographs of paraffin-embedded segments of nonconstricted and constricted left coronary arteries near their origin. A and B, Two examples of coronary arteries in sham-operated nontransgenic (A; luminal diameter = 153 μm) and transgenic (B; luminal diameter = 165 μm) mice. C and D, Two examples of CAN in nontransgenic (C; luminal diameter = 76 μm) and transgenic (D; luminal diameter = 63 μm) mice. A 52% and a 61% reduction in luminal diameter is depicted in these 2 micrographs (C and D). Internal diameters of the nonconstricted portions of the vessels were 157 and 161 μm, respectively. An inflammatory reaction is noted around the vessel wall. Fragments of the ligature are also visible (arrows). Empty space corresponds to the location of the suture that detached in part during sectioning. Magnification, A, B, C, and D, ×190.
was placed under the control of a rat α-myosin heavy chain promoter. The overexpression of human IGF-1B in the mouse heart did not express in the heart the human IGF-1B gene.9 Myocytes from transgenic mice at 75 days secreted 4.3-fold more IGF-1 than nontransgenic littermates and transgenic mice. Results are presented as mean ± SD. *Significant difference, P < 0.05. SO indicates sham-operated mice; LVEDP, left ventricular end-diastolic pressure; and LVSP, left ventricular systolic pressure. n = 18 in each group.

Figure 2. Effects of CAN on ventricular hemodynamics in nontransgenic littermates and transgenic mice. Results are presented as mean ± SD. *Significant difference, P < 0.05. SO indicates sham-operated mice; LVEDP, left ventricular end-diastolic pressure; and LVSP, left ventricular systolic pressure. n = 18 in each group.

Materials and Methods

Animals

Experiments were performed in female FVB Igf1–/– mice and nontransgenic littermates at 75 days of age.9 These transgenic mice were obtained with the use of a cDNA for the human IGF-1B, which was placed under the control of a rat α-myosin heavy chain promoter. The overexpression of human IGF-1B in the mouse heart increased postnataally, reaching its peak at 75 days; a 12-fold higher value of IGF-1B than at 1 day was found at this interval. Littermates did not express in the heart the human IGF-1B gene.9 Myocytes from transgenic mice at 75 days secreted 4.3-fold more IGF-1 than myocytes from littermates of the same age.9 For functional and anatomic studies, CAN was surgically induced in 213 animals, and 96 survived the operation, indicating a 55% mortality; 60 mice were excluded, and 18 littermates and FVB Igf1+/– mice each were analyzed. An identical number of sham-operated mice was examined in each group. Cell death was assessed in separate groups of mice, because frozen sections of myocardium were required. For the evaluation of myocyte necrosis, 10 CAN littermates and 10 CAN FVB Igf1+/– mice were injected with 10 μg of myosin monoclonal antibody (clone CCM-52) 24 hours before death.6 Control animals, consisting of 7 littermates and 6 FVB Igf1+/– mice, were injected also with myosin antibody. Apoptosis was measured in these 33 mice. DNA laddering was examined in myocardial samples obtained from mice used for the measurements of myocyte death; 5 CAN and 5 sham-operated mice each in nontransgenic mice and transgenic mice were studied. DNA laddering was confirmed in isolated myocytes; 4 animals in each group were included. The expression of α- and β-myosin was determined in isolated myocytes; 8 CAN and 8 sham-operated animals in each group were investigated. These 44 CAN mice required successful surgery in 92 of 168 animals.

CAN

Under ether anesthesia, thoracotomy was performed, the atrial appendage was elevated, and the left coronary artery was partially occluded. The chest was closed, the pneumothorax was reduced, and the mice were allowed to recover. To reduce pain, buprenorphine hydrochloride, 0.65 mg/kg body weight, was injected intramuscularly (Buprenex, Reckitt and Colman Pharmaceuticals). Sham-operated mice were treated similarly, but the ligature was not tied. Details of the procedure have been published.7 Experimental protocols were approved by New York Medical College.

Ventricular Hemodynamics

Mice were anesthetized with chloral hydrate (400 mg/kg body weight, IP), and the right carotid artery was cannulated with a microtip pressure transducer catheter (model SPR-671, Millar Instruments). The catheter was advanced into the left ventricle (LV) for the evaluation of LV pressures and dP/dt in the closed-chest preparation.

Fixation for Anatomic Measurements

The abdominal aorta was cannulated with a PE-50 catheter, and the heart was arrested in diastole through the aortic injection of 0.15 mL of cadmium chloride (100 mmol/L). The myocardium was perfused retrogradely through the aorta. The LV chamber was filled with fixative and kept at a pressure equal to end-diastolic pressure throughout the fixation procedure.7,8 After fixation, the heart was excised and cardiac weights were recorded.

Coronary Artery Diameter

The proximal 0.5- to 1.0-mm segment of the left coronary artery was isolated and cut transversely to expose the level of the ligature. The diameter of the lumen adjacent to the narrowed site and at the constricted portion were measured. Constriction was evaluated by comparing the vessel diameter above the stenosis with the diameter at the level of stenosis.7

Ventricular Dimensions

The major intracavitary axis of LV was measured. Each LV was then cut transversely to obtain a 1.5-mm section, halfway between the base and the apex, in which the average thickness of the free wall and septum and chamber luminal diameter were measured. Longitudinal and transverse diameters were used to calculate chamber volume.7,8 Measurements of wall thickness, chamber radius, and end-diastolic pressure were used to compute diastolic stress.

Myocardial Damage

Three slices of each LV, from the basal, middle, and apical portions, were embedded in paraffin and stained with hematoxylin and eosin. Sixty fields were examined at ×400 with a 42-point reticle, defining
a tissue area of 39 205 μm². The fraction of points lying over sites of replacement fibrosis and the number of these foci in the myocardium were measured.7

Myosin Antibody Labeling and Terminal Deoxynucleotidyld Transferase (TdT) Assay

Frozen tissue sections were exposed to TRITC-labeled anti-mouse IgG and fixed in 1.5% paraformaldehyde. The TdT assay was performed by incubating sections with 5 U of TdT, 2.5 mmol/L Tris-HCl, 0.25% BSA, and 0.5 mmol/L biotinylated 2′-deoxyuridine-5′-triphosphate (biont-16-dUTP). After exposure to 5 μg/mL of FITC-Extravidin (Avidin, Sigma), myocytes were stained with α-sarcomeric actin and nuclei with propidium iodide.8,10 For the TdT assay, nuclei were labeled by TdT in each LV, and the fraction of myocyte profiles stained by myosin antibody was assessed in a similar manner.

In Situ Ligation Assay

Polymerase chain reaction (PCR) was performed as previously described.11 After heating to 80°C, Taq polymerase, 2.5 U, was added. Gel electrophoresis documented a single PCR product that was subsequently used in our laboratory.8,9 Contamination from nonmyocardial tissue was repeatedly used in our laboratory.8,9 DNA Gel Electrophoresis

Tissue homogenates and isolated myocytes were fixed in 70% ethanol. Fixed material was incubated in 40 μL of phosphate-citrate buffer (pH 7.8). Supernatant was digested with RNase and proteinase K, and samples were subjected to electrophoresis.8,10

Northern Blot

Myocytes were frozen in liquid nitrogen and were homogenized in Tri reagent (Molecular Research Center). Homogenates were precipitated by isopropanol. Pellets were washed with ethanol and dissolved in diethyl pyrocarbonate–treated water. RNA amounts were determined by A₂₆₀/A₂₃₀ (nm) ratio and by hybridization of RNA blots with GAPDH cDNA probe. Oligonucleotide probes for α- and β-myosin heavy chain mRNAs (5′-CGAACGTTCACCTACCT-3′ and 5′-GGTGCGTCTCCTGCGGGCGTTTCTGACCCCTG-3′ complementary to pBluescript-bSDI plasmid.11

Myocytes were dissociated by collagenase following a procedure repeatedly used in our laboratory.8,9 Contamination from nonmyocytes ranged from 1% to 3%.8,9 Myocyte Isolation

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Results

Perfusion Fixation and CAN

Figure 1 illustrates degrees of CAN ranging from 52% to 61%. To reduce the variability of CAN, only mice with reduction in luminal diameter ≥49% and ≤70% were included in all cases. On this basis, 60 mice were excluded from a total of 96 for anatomic studies, and 48 from a total of 92...
for measurements of cell death and biochemical parameters. Anatomic determinations were obtained in 36 mice, 18 nontransgenic and 18 transgenic. Sham-operated groups also included 18 animals each. In comparison with the nonconstricted portion of the vessel, the procedure resulted in a 59±6% (proximal, 151±18 μm; constricted, 62±12 μm; \( P<0.001 \)) and 58±6% (proximal, 157±17 μm; constricted, 66±13 μm; \( P<0.001 \)) reduction in luminal diameter of the coronary artery in littermates and FVB.1g1/– mice, respectively. Corresponding decreases in luminal area were 83±5% (proximal, 18 169±4233 μm²; constricted, 3067±1234 μm²; \( P<0.001 \)) and 82±5% (proximal, 19 541±4295 μm²; constricted, 3548±1349 μm²; \( P<0.001 \)).

### Physiological Measurements
LV end-diastolic pressure increased 145% (\( P<0.001 \)) in CAN nontransgenic mice, whereas LV peak systolic pressure decreased 12% (\( P<0.005 \)), and +dP/dt and –dP/dt, 34% (\( P<0.001 \)) and 29% (\( P<0.001 \)), respectively (Figure 2). Corresponding changes in CAN transgenic mice were 137% (\( P<0.001 \)), 12% (\( P<0.01 \)), 31% (\( P<0.001 \)), and 30% (\( P<0.001 \)). No difference in these indices of LV function was found between littermates and FVB.1g1/– mice before and after CAN. In summary, CAN resulted in severe LV dysfunction that affected nontransgenic and transgenic mice.

### Body Weight and Heart Weight
CAN for 7 days was characterized by a 7% (\( P<0.01 \)) and 5% (\( P<0.05 \)) decrease in body weight in nontransgenic mice (control, 22.8±0.86 g; CAN, 21.3±2.0 g) and transgenic mice (control, 24.4±1.7 g; CAN, 23.1±1.5 g, respectively). Heart weight increased 29% (\( P<0.001 \)) and 19% (\( P<0.005 \)), resulting in a 38% (\( P<0.001 \)) and 25% (\( P<0.001 \)) increase in heart weight–to–body weight ratio in CAN littermates and CAN FVB.1g1/– mice (Figure 3). In CAN littermates, LV increased 34% (\( P<0.001 \)) and right ventricle 14% (\( P<0.05 \)).
Figure 6. Confocal microscopy of myosin antibody labeling of necrotic myocytes (A, green fluorescence) and $\alpha$-sarcomeric actin antibody staining of the myocyte cytoplasm (B, red fluorescence) in a CAN nontransgenic mouse. These 2 confocal images are combined in panel C: red fluorescence corresponds to myocytes and yellow fluorescence reflects necrotic cells. D through F, Similarly, localization of necrotic myocytes in a CAN transgenic mouse. Panels G through I illustrate nuclear fragmentation in a CAN nontransgenic mouse by confocal microscopy (G, red fluorescence of propidium iodide), TdT labeling of nuclei (H, green fluorescence) and $\alpha$-sarcomeric actin staining of the myocyte cytoplasm (I, red fluorescence). Yellow fluorescence in panel I reflects the combination of propidium iodide and TdT labeling shown in panels G and H. Arrows indicate the position of the apoptotic nucleus. J through L, Similarly, apoptosis of a myocyte nucleus in a CAN transgenic mouse. Loss of DNA and features preceding nuclear fragmentation are apparent. Arrows indicate position of the apoptotic nucleus. M through O, In a CAN nontransgenic mouse by confocal microscopy nuclei (M, red fluorescence of propidium iodide), Pfu labeling of a nucleus (N, green fluorescence), and $\alpha$-sarcomeric actin staining of the myocyte cytoplasm (O, red fluorescence). Yellow fluorescence in panel O reflects the combination of propidium iodide and Pfu labeling shown in panels M and N. Arrows indicate position of the necrotic nucleus. P through R, Similarly, necrosis of a myocyte nucleus in a CAN transgenic mouse. S through U, In a CAN nontransgenic mouse by confocal microscopy nuclei (S, red fluorescence of propidium iodide), Taq labeling of a nucleus (T, green fluorescence), and $\alpha$-sarcomeric actin staining of the myocyte cytoplasm (U, red fluorescence). Yellow fluorescence in panel U reflects the combination of propidium iodide and Taq labeling shown in panels S and T. Arrows indicate position of the apoptotic nucleus. V through X, Similarly, apoptosis of a myocyte nucleus in a CAN transgenic mouse. Magnification: panels A through F, $\times1500$; panels G through I and panels M through X, $\times2000$; and panels J through L, $\times2500$. 
whereas, in FVB_IGF1−/− mice, these increases were 21% (P<0.001) and 8% (NS), respectively. These responses resulted in a 44% (P<0.001) and 22% (P<0.005) increase in LV and right ventricle weight-to-body weight ratios in littermates and in a 29% (P<0.001) and 10% (P<0.05) increase in these parameters in FVB_IGF1−/− mice. In comparison with CAN transgenic mice, 53% (P<0.05), 62% (P<0.04) 52%, (P<0.03), and 52% (P<0.03) higher heart weight, LV weight, and ratios of heart weight and LV weight to body weight were found in CAN nontransgenic mice. In summary, CAN resulted in a greater magnitude of cardiac hypertrophy in nontransgenic than in transgenic mice.

**Ventricular Dimensions**

CAN produced a 20% (P<0.05) and 16% (P<0.05) increase in LV longitudinal intracavitary axis in littermates and FVB_IGF1−/− mice (Figure 4). Chamber diameter increased 18% (P<0.05) and 11% (P<0.005), and cavity volume expanded 68% (P<0.001) and 37% (P<0.001) in nontransgenic mice and transgenic mice. Thus, chamber volume increased 84% (P<0.02) more in littermates than in FVB_IGF1−/− mice. LV mass-to-chamber volume ratio decreased 23% (P<0.005) with CAN only in nontransgenic mice. Additionally, LV thickness decreased 10% (P<0.05) and 9% (P<0.05), and wall thickness-to-chamber radius ratio decreased 28% (P<0.001) and 20% (P<0.005) in littermates and FVB_IGF1−/− mice, respectively. These anatomic properties and the measurements of LV end-diastolic pressures allowed the computation of diastolic wall stress. CAN resulted in a 263% (P<0.001) and 189% (P<0.001) increase in diastolic stress in nontransgenic mice and transgenic mice, indicating a 1.4-fold (P<0.01) higher level of
wall stress in littermates than in FVB.Igf+/– mice. In summary, CAN produced greater ventricular dilation and higher diastolic stress in nontransgenic mice than in transgenic mice.

**Ventricular Damage**
CAN was characterized by sites of replacement fibrosis in various phases of healing across the LV wall (Figure 5A through 5D). The number of lesion profiles per mm² of myocardium was 1.6-fold \( (P<0.001) \) greater in CAN nontransgenic mice than in transgenic mice (Figure 5E). Similarly, the volume percentage of scarring was 2.6-fold \( (P<0.001) \) more extensive in CAN littermates (Figure 5F). Fibrosis represented the damage accumulated from the time of CAN to euthanization. However, this analysis did not include ongoing myocyte death. In summary, CAN resulted in areas of subacute myocardial injury in the LV wall that were more severe in littermates than in FVB.Igf+/– mice.

**Ongoing Myocyte Death**
Hearts were not fixed by perfusion, because myocyte necrosis was identified by myosin labeling in frozen sections of myocardium. Adjacent sections were processed for the detection of apoptosis by TdT. CAN involved a decrease in luminal diameter of 56% in nontransgenic mice (proximal segment, 146±20 μm; constricted segment, 64±14 μm; \( P<0.001 \)) and 60% in transgenic mice (proximal segment, 151±19 μm; constricted segment, 60±15 μm; \( P<0.001 \)). Low levels of myocyte necrosis were observed in both control animals. After CAN, single cells and groups of
myocytes were labeled by myosin in littermates (Figure 6A through 6C) and FVB.Igf+/– mice (Figure 6D through 6F). In the absence of CAN, myocyte apoptosis was identified in nontransgenic mice (Figure 6G through 6I) and transgenic mice (Figure 6J through 6L). CAN had little effect on apoptosis in both groups of mice.

Myocyte necrosis and apoptosis were detected by 2 additional methods, using probes generated by Pfu and Taq polymerase, respectively. Pfu labeled blunt-ended products of DNA damage (Figure 6M through 6R) that developed during necrosis. Conversely, Taq identified double–DNA strand cleavage with single-base 3’ overhangs (Figure 6S through 6X) that occurred with apoptosis. In no instance was myosin or Pfu labeling of myocytes associated with TdT staining. Figure 7A illustrates that myocyte necrosis, recognized by myosin antibody, was similar in sham-operated littermates and FVB.Igf+/– mice. CAN resulted in a 46-fold (P<0.001) and 9.4-fold (P<0.001) increase in myocyte necrosis in nontransgenic mice and transgenic mice, respectively. In comparison with CAN transgenic mice, the 5.2-fold higher magnitude of necrotic death in CAN nontransgenic mice was significant (P<0.001). Myocyte apoptosis, measured by TdT, was comparable in the 2 groups of mice at baseline (Figure 7B). CAN modestly increased myocyte apoptosis, 1.8-fold (P<0.05) in littermates and 3.0-fold (P<0.001) in FVB.Igf+/– mice. Apoptosis in CAN nontransgenic mice and CAN transgenic mice was not different (P=0.78). When necrosis was established by Pfu, CAN resulted in a 44-fold (P<0.001) and a 9.7-fold (P<0.001) increase of this form of cell death in littermates and
FVB. Igf−/− mice, respectively (Figure 7C). In a manner similar to TdT, apoptosis, measured by Taq, increased moderately with CAN in both groups of animals. Measurements of necrosis with myosin antibody and Pfu, and of apoptosis with TdT and Taq, were not statistically different. In CAN nontransgenic mice, necrosis was an average 12-fold (P<0.001) greater than apoptosis with both techniques. In CAN transgenic mice, the 2.3-fold higher value in necrosis than in apoptosis was not significant (P=0.43). In summary, myocyte necrosis was the predominant type of cell death with CAN, and this form of injury was more severe in FVB. Igf−/− mice.

DNA Damage
Agarose gel electrophoresis of DNA from LV samples (Figure 8A) and dissociated myocytes (Figure 8B) documented that CAN was associated with a diffuse DNA pattern in tissue preparations. This aspect reflected random fragmentation of the DNA that was consistent with cell necrosis.13 In myocytes, mononucleosomes and oligonucleosomes were detected, indicating that laddering and apoptosis were present in the electrophoretic profile of low molecular weight DNA.13 In summary, CAN was accompanied by myocyte necrosis and modest levels of apoptosis.

Myosin Isoenzymes
α- and β-myosin heavy chain mRNAs were detectable in LV myocytes from sham-operated littermates and FVB. Igf−/− mice (Figure 9). Similarly, these myosin isoforms were present in myocytes of both groups of animals after CAN (n=8 in each group of mice). However, expression of myosin isoforms differed between littermates and FVB. Igf−/− mice. In controls, β-myosin mRNA was 75% (P<0.04) higher and α-myosin mRNA was 7% (P<0.04) lower in nontransgenic mice compared to transgenic mice. CAN decreased 10% (P<0.002) α-myosin mRNAs (77±5%) and increased 64% (P<0.002) β-myosin mRNAs (32±5%) in littermates. In FVB. Igf−/− mice, CAN transgenic mice. However, α-myosin mRNA level was 9% (P<0.02) higher in CAN transgenic mice than in nontransgenic mice. In summary, expression of myosin isoforms was affected by CAN, but α-myosin mRNA was greater in transgenic mice at baseline and after CAN.
myocyte apoptosis. Distinct forms of cell death appear to be implicated in the restructuring of the wall with coronary artery disease in the absence or presence of a myocardial infarction.

IGF-1 overexpression markedly attenuated the extent of myocardial fibrosis and the magnitude of ongoing cell necrosis after CAN; volume fraction of reparative fibrosis was reduced 62% and ongoing necrotic myocyte death 80%. IGF-1 interferes with the activation of myocyte necrosis during ischemia reperfusion injury and myocardial infarction. However, the mechanism of this protective effect on cell viability has not been defined. The current results also leave this issue unresolved. The growth factor may increase the vascular component of the myocardium, minimizing the influence of global ischemia. On the other hand, this possibility is not supported by differences in infarction size with coronary artery occlusion. IGF-1 enhances Bcl-2 expression that increases the stability of cellular membranes. Intracellular Ca²⁺ homeostasis and compartmentalization of this cation are preserved by the influence of IGF-1 on antagonists of Bcl-2 such as Bad and Bax. These factors may inhibit Ca²⁺ overload–mediated cell necrosis. Bax may form pH- and voltage-dependent ion-conducting channels, altering membrane permeability. Bcl-2 antagonists of Bcl-2 such as Bad and Bax may promote antithrombotic and antiinflammatory reactions, decreasing necrotic myocyte cell death.

Myocyte apoptosis was low in FVB IGF+/− mice and nontransgenic animals at baseline, and CAN increased this parameter modestly. Overexpression of IGF-1 did not reduce this form of cell death in the myocardium. Apoptosis represented a minimal component of the total myocyte loss in the heart of littermates, and the amount of apoptosis and necrosis was limited in FVB. IGF-1 releases nitric oxide, and this may promote antithrombotic and antiinflammatory reactions, decreasing necrotic myocyte cell death.

**Discussion**

The results of this study indicate that severe reductions in luminal diameter of a major epicardial coronary artery led to chronic loss of myocytes that altered cardiac anatomy and impaired ventricular function. Cell death occurred by apoptosis and necrosis, but necrosis was the predominant form of myocyte injury. Cavitory dilation, reactive hypertrophy, elevated diastolic stress, increased β-myosin mRNA, and decreased α-myosin mRNA developed with CAN. Overexpression of IGF-1 in myocytes attenuated the magnitude of accumulated damage and the extent of ongoing myocyte death. This protective effect of IGF-1 on cell survival limited the changes in chamber volume, myocardial hypertrophy, ventricular loading, and α- and β-myosin mRNAs. Thus, cell death appears to be a critical component of the onset and early evolution of ischemic cardiomyopathy, and interference with this process by IGF-1 may positively influence the short- and long-term outcome of ventricular remodeling.

**CAN, Myocyte Cell Death, and IGF-1**

Current results and previous observations after infarction indicate that differences exist between the impact of restrictions in coronary blood flow and the consequences of a segmental loss of tissue with coronary occlusion. In the latter case, myocyte apoptosis occurs in the surviving myocardium and markedly exceeds cell necrosis. Myocyte apoptosis is responsible for side-to-side slippage of myocytes, mural thinning, and cavitory dilation acutely after infarction. Additionally, myocar dial scarring is not present in the viable tissue of the postinfarction heart shortly after coronary artery ligation. Conversely, myocyte necrosis and tissue fibrosis characterize the cardiac myopathy mediated by coronary artery stenosis. Foci of replacement fibrosis in the myocardium detected here reflected an immediate activation of myocyte necrosis, evolving with time in multiple areas of reparative scarring. Collagen accumulation is not the consequence of myocyte apoptosis. Distinct forms of cell death appear to be implicated in the restructuring of the wall with coronary artery disease in the absence or presence of a myocardial infarction.

**CAN, Ventricular Remodeling, and IGF-1**

Coronary constriction leads to a dilated myopathy in which the expansion of cavitory volume, in combination with relative thinning of the wall, results in a decrease in wall thickness–to–chamber radius ratio and decompensated eccentric hypertrophy. Cavitory dilation exceeds the increase in muscle mass generating a profound alteration in ventricular anatomy, characterized by a reduction in myocardial mass–to–chamber volume ratio. These cardiac changes occur in experimental and human ischemic cardiomyopathy. Moreover, end-diastolic pressure is increased, producing a marked elevation in diastolic wall stress, which is not necessarily accompanied by a change in systolic loading. These anatomic, physiological, and loading abnormalities have been
found here after CAN in nontransgenic and transgenic mice. However, the magnitude of ventricular dilation, diastolic wall stress, and reactive hypertrophy was attenuated by the constitutive overexpression of IGF-1 in myocytes. Comparable results have been obtained after myocardial infarction. Given that the most apparent effect of the growth factor involved the limitation in myocyte death by necrosis with CAN and apoptosis with infarction, the possibility may be advanced that ongoing, scattered myocyte loss may be crucial in the onset and progression of the ischemic cardiomyopathy. Although IGF-1 exerted a protective influence on the ischemic mycardium after CAN, the impairment in ventricular function was comparable in littermates and FVB.Igf1+/− mice. Despite a reduction in tissue fibrosis and necrotic cell death with IGF-1, the alterations in cardiac hemodynamics did not differ in the 2 groups of animals. IGF-1 was unable to diminish the impact of ischemia on myocardial performance. A similar adaptation was observed after myocardial infarction. Importantly, IGF-1 may increase the coronary vascularity and microvasculature of the ischemic myocardium, enhancing its viability.

**CAN, Myosin Heavy Chain Expression, and IGF-1**
Changes in the relative proportion of myosin isoenzyme mRNAs and proteins occur in the rat heart after pathological loads. A reduction in the expression of the predominant V1 isoform and an increase in the slow migrating V2 isoform have been observed. A transcriptional upregulation of β-myosin heavy chain has also been found in mice with aortic banding. A similar phenomenon has recently been described in the failing human heart. In the current study, CAN resulted in an increase in β-myosin heavy chain mRNA and a decrease in α-myosin mRNA in myocytes of littermates and FVB.Igf1+/− mice, paralleling the observations summarized above. However, IGF-1 maintained α-myosin heavy chain mRNA levels higher in transgenic mice than in nontransgenic mice at baseline and after CAN. Although these findings are consistent with a more efficient mechanical performance of myocytes in transgenic mice, the basis for the differential expression of these 2 myosin isoenzymes is unknown. A possible explanation may involve the ability of IGF-1 to promote myocyte proliferation during postnatal life, and this mechanism of cell growth may be coupled with a higher proportion of younger myocytes and a greater quantity of V1 isoform in the cells.

**Myocyte Necrosis, Oncosis, and Apoptosis**
The identification of myocyte death in ischemic heart disease by nick end labeling has been challenged. Similarly, the discrimination between necrotic and apoptotic myocyte death by the use of myosin monoclonal antibody has been questioned. This is relevant because cell death is a critical event of cardiac pathology. Morphological alterations of myocytes may help in the recognition of the form of cell death when combined with specific markers of DNA damage. However, the use of electron microscopy in immersion-fixed myocardium is unacceptable in view of the numerous artifacts identified with this preparation more than 25 years ago. In our experience, immersion fixation of rabbit myocardium, under control conditions and after acute and chronic pressure overload, results in poor preservation of myocytes, particularly of the mitochondria and sarcoplasmic reticulum compartments. To avoid possible misinterpretation of morphological images, a PCR-generated Pfu polymerase probe was used here to detect blunt DNA ends (ie, necrosis).

This methodology will also identify DNA fragmentation associated with possible oncrosis or cell swelling. Moreover, a PCR-generated Taq polymerase probe was used to identify double-strand cleavage of the DNA with single-base 3' overhangs that occur exclusively with apoptosis mediated by activation of Ca2+-dependent DNase I. As recently emphasized, probes capable of detecting different aspects of DNA fragmentation represent the most valid approach for the distinction between apoptosis and oncrosis. Oncosis is a form of cell death characterized by cellular swelling and increased membrane permeability, which evolves into typical necrosis. Conversely, “necrosis” should reflect the end stage of any type of cell death. However, the terms oncrosis and necrosis have been used interchangeably. Unfortunately, at times, they have been interpreted as separate forms of cell death. To avoid confusion and misinterpretation, “oncosis” should not be introduced in the absence of a clear definition of its significance. In the current study, 2 methodologies have each been applied for the quantitative estimation of myocyte apoptosis (TdT and Taq) and necrosis (myosin antibody and Pfu). Almost identical values were obtained with both techniques in each form of cell death in sham-operated and CAN littermates and FVB.Igf1+/− mice. Necrosis was demonstrated as the dominant mechanism of cell death with CAN, in contrast with the infarcted heart, in which apoptosis is the prevailing factor of wall restructuring.

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