Reversal of interstitial fibroblast hyperplasia via apoptosis in hypertensive rat heart with valsartan or enalapril

Shant Der Sarkissian a, Eve-Lyne Marchand a, David Duguay a, Pavel Hamet b, Denis deBlois a, *

 a Department of Pharmacology, University of Montreal, University of Montreal Hospital (CHUM) Research Center, 3840 St-Urbain Street, Room 7-132B, Montreal, Quebec H2W 1T8, Canada
 b Department of Medicine, University of Montreal, University of Montreal Hospital (CHUM) Research Center, 3850 St-Urbain Street, Montreal, Quebec H2T 1T7, Canada

Received 26 June 2002; accepted 14 November 2002

Abstract

Objective: Renin–angiotensin system inhibitors transiently induce apoptosis at the onset of cardiac hypertrophy regression in spontaneously hypertensive rats (SHRs). The focus of this study is to evaluate the cell selectivity of this response. Methods: SHRs were treated with valsartan or enalapril (30 mg kg -1 day -1 ) or placebo for 1 to 4 weeks. Stereological and morphological data were obtained from immunohistological analyses. Apoptosis was quantified by DEVDase (caspase-3-like) activity assay and immunoblot analysis of apoptosis-regulatory proteins (Bax and Bcl-2). Identification of the apoptotic cell type was conducted using in situ TUNEL labeling, in conjunction with α-sarcomeric actin or lectin immunoreactivity as markers for cardiomyocytes and endothelial cells, respectively.

Results: Stereological analysis of the left ventricle revealed significant non-cardiomyocyte hyperplasia in placebo-treated SHRs (239 ± 29 × 10 3 nuclei) as compared to untreated age-matched normotensive Wistar–Kyoto (WKY) rats (107 ± 12 × 10 3 ). In contrast, the number of cardiomyocyte nuclei was comparable between untreated SHRs (48 ± 4 × 10 3 ) and WKY rats. After 4 weeks of valsartan or enalapril treatment, SHRs showed significant reductions in systolic blood pressure (28%), left ventricular hypertrophy (9%) and cardiomyocyte cross-sectional area (17%). Moreover, these treatments abolished non-cardiomyocyte hyperplasia in SHR left ventricle without affecting cardiomyocyte number, capillary density or number of capillary per cardiomyocyte nucleus. As a mechanism of cell deletion consistent with apoptosis induction, ventricles showed increased caspase-3 activation (4.5-fold) as well as Bax to Bcl-2 protein ratio (3.2-fold) within 2 weeks of valsartan or enalapril treatment. Immunohistological analysis revealed a significant increase in TUNEL-positive, lectin-negative non-cardiomyocytes, suggesting a rise in apoptotic interstitial fibroblasts in the left ventricle within 2 weeks of treatment with valsartan or enalapril (63%), with a return to baseline (0.033 ± 0.003%) at 4 weeks. Treatments did not affect right ventricular mass, apoptosis or cellularity. Conclusion: Cardiac apoptosis induction during regression of left ventricular hypertrophy reverses interstitial fibroblast hyperplasia in SHRs treated with inhibitors of the renin–angiotensin system.

© 2003 European Society of Cardiology. Published by Elsevier Science B.V. All rights reserved.

Keywords: ACE inhibitors; Antihypertensive/diuretic agents; Apoptosis; Hypertension; Hypertrophy

1. Introduction

In recent years, apoptosis, a gene-regulated process of cell self-destruction associated with caspase activation and internucleosomal DNA fragmentation, has emerged as an important mechanism contributing to cardiac remodeling [1,2]. Several studies have associated enhanced levels of apoptosis with the pathogenesis of cardiac disease, e.g., in the loss of cardiomyocytes and neurons leading to heart failure and cardiac conduction system disorders [3–5]. However, the role of apoptosis in cardiac remodeling is context-dependent and may participate in normal cardiac
remodeling as illustrated by the differential regulation of cardiomyocyte apoptosis between the right and left ventricles during the post-natal period [6].

The spontaneously hypertensive rat (SHR) is a model of primary hypertension in which an imbalance between cell growth and apoptosis favors increased cardiac mass and DNA content in neonates and adults [7–9]. Adult SHRs show cardiac fibroblast hyperplasia [10], a common feature of pathological cardiac remodeling leading to increased extracellular matrix deposition and cardiac fibrosis [11,12]. With aging, the hypertensive heart also shows increased cardiomyocyte apoptosis [4,13,14], a feature that is attenuated following angiotensin converting enzyme inhibition or angiotensin II type 1 (AT<sub>1</sub>) receptor blockade [4,10,14,15]. Liu et al. [10] reported an age-dependent increase in cardiac apoptosis in young SHRs, mainly in cardiomyocytes, concomitant with the development of fibroblast hyperplasia. Prophylactic treatment of hypertension with the angiotensin converting enzyme inhibitor ramipril reduces cardiac apoptosis in the long term and prevents the development of fibroblast hyperplasia [10]. Likewise, initiating hydralazine treatment during the pre-hypertensive phase can prevent the development of cardiac hyperplasia in SHRs (deBlois et al., unpublished data). In contrast, hydralazine does not reverse cardiac hyperplasia in adult SHRs [16]. A key question is whether established fibroblast hyperplasia can be reversed in the hypertensive adult. We previously reported that a transient increase in apoptosis occurs at the onset of cardiac hypertrophy regression in SHRs [16]. In this model, intercellular fragmentation of ventricular DNA peaks at 1 week of treatment with an AT<sub>1</sub> receptor antagonist, or at 2 weeks of treatment with an equipotent antihypertensive dose of an angiotensin converting enzyme inhibitor. Current evidence suggests that angiotensin II regulation of apoptosis in the heart is cell type-specific. In vitro, angiotensin II increases cardiomyocyte apoptosis, an effect dependent on AT<sub>1</sub> receptor activation [17,18]. In contrast, cultured cardiac fibroblasts undergo AT<sub>1</sub> receptor-dependent proliferation in response to angiotensin II [19].

We therefore postulated that the early phase of cardiac hypertrophy regression induced by inhibitors of the renin–angiotensin system in adult SHR involved the selective deletion of fibroblasts via apoptosis. The present study provides evidence for a beneficial role of selective apoptosis up-regulation in cardiac remodeling induced by anti-hypertensive treatments, namely the reversal of fibroblast hyperplasia.

2. Methods

2.1. Animal procedures

Forty-two male SHRs and six male Wistar–Kyoto (WKY) rats (10 weeks old; weighing ~250 g) were purchased from Charles-River (St.-Constant, Canada) and housed for at least 1 week before treatment. Food and water were administered ad libitum. SHRs (n=6/group) were randomly assigned to treatment with the selective AT<sub>1</sub> antagonist valsartan (30 mg kg<sup>-1</sup> day<sup>-1</sup> p.o.; gift of Novartis, Toronto, Canada) or the angiotensin converting enzyme inhibitor enalapril (30 mg kg<sup>-1</sup> day<sup>-1</sup> p.o.; Sigma Chemicals, St. Louis, MO, USA) in the drinking water for 1, 2 or 4 weeks. Control SHRs and WKY rats received vehicle. Systolic blood pressure was determined by the tail-cuff method at 12 and 26 days of treatment (n=6/group) and tissues were isolated after 1 and 2 weeks as we described previously [16]. Briefly, rats were anesthetized with a single i.m. injection of ketamine (80 mg kg<sup>-1</sup>; Ayerst, Guelph, Canada), xylazine (4 mg kg<sup>-1</sup>; Bayer, Etobicoke, Canada), and acepromazine (2 mg kg<sup>-1</sup>; Ayerst, Montreal, Canada), and killed by exsanguination via the jugular vein. Both ventricles were isolated together, weighed and an equatorial cross-section (3 mm) was fixed in paraformaldehyde (4%). In SHRs killed after 4 weeks of treatment and age-matched WKY (n=6/group) anesthesia was followed by an i.v. injection of 1.5 ml KCl (100 mmol l<sup>-1</sup>) via the vena cava to induce diastolic cardiac arrest. Ventricles were isolated together, weighed and fixed overnight in 4% paraformaldehyde. Equatorial cross-sections of ventricles were paraffin-embedded. All animal manipulations were conducted in accordance with the Care and Use of Laboratory Animals guidelines published by the National Institutes of Health (NIH).

2.2. Hypertrophy and hyperplasia measurements

We used the NIH Image 1.61 program (http://rsb.info.nih.gov/nih-image/) to measure the cross-sectional area of the ventricles, ventricular chambers and cardiomyocytes at 4 weeks. Three sets of randomly chosen pictures (from the left and right ventricular sub-epicardium, sub-endocardium and mid-myocardium) were captured from hematoxylin stained histological sections and digitally enhanced. The total number of cardiomyocytes within each ventricle was assessed stereologically as per Anversa et al. [20]. A similar procedure was used to calculate total non-cardiomyocyte number with the exception that the area covered by the (unstained) cell body of non-cardiomyocytes was estimated using the average nuclear length measurements (10.5±0.3 μm in controls; no change with treatment), extended half a time in the x and y axes around each nucleus.

2.3. DEVDase activity

Ventricles were pulverized in liquid nitrogen and an aliquot (5 mg) was lysed to measure DEVDase activity using the fluorogenic substrate DEVD-AMC (40 μM) in the presence or absence of the Ac-DEVD-CHO (1 μM), a caspase-3 inhibitor as previously described [21]. Caspase-
3-like activity was defined as the Ac-DEVD-CHO-sensitive activity.

2.4. Expression of apoptosis-regulatory proteins

The protein levels of active caspase-3 17–20 kDa fragments, Bcl-2 and Bax in cardiac tissue were examined by Western Blot analysis. Total heart was pulverized in liquid nitrogen and a 50 mg aliquot was lysed in extraction buffer [10 mmol l⁻¹ Tris–HCl, pH 7.5, 1% Triton X-100, 4 mmol l⁻¹ β-Glycerophosphate, 4 mmol l⁻¹ sodium fluoride, 1 mmol l⁻¹ EDTA, 1 mmol l⁻¹ EGTA, 200 μmol l⁻¹ sodium orthophosphate, 51 μmol l⁻¹ benzamidine, 0.5 mmol l⁻¹ phenylmethylsulfonylfluoride, 21 μmol l⁻¹ leupeptin, 5 mmol l⁻¹ dithiothreitol (DTT) and 1 μmol l⁻¹ microsystin (Sigma–Aldrich, Oakville, Canada)]. Western Blot was carried out with equal amounts of proteins (25 μg) loaded onto a 15% sodium dodecyl sulfate (SDS)–polyacrylamide gel and transferred to Hybond-C extra membrane (Amersham Bioscience, London, UK). Membranes were hybridized with anti-caspase-3 (1:1000; BD Pharmingen, Mississauga, Canada), anti-Bax or anti-Bcl-2 (1:1000; Santa-Cruz Biotech, Santa Cruz, CA, USA) antibodies followed by incubation with goat anti-rabbit (caspase-3) or goat anti-mouse (Bax, Bcl-2) horseradish peroxidase-conjugated secondary antibodies (1:2000; Santa-Cruz Biotech) according to manufacturer’s protocol. Membranes were then incubated with ECL Plus (Life Science Products, Boston, MA, USA), exposed to film and then developed. The image was digitized and band intensity was quantified using NIH Image 1.61 program. For caspase-3, the 32 kDa (procaspase-3) and 17–20 kDa (indicative of enzyme activation) fragments were measured using NIH Image 1.61 program. The amount of tdt enzyme (20 units; Roche, Indianapolis, IN, USA), biotin-16-dUTP (0.5 nmol l⁻¹; Roche) and FITC-labeled extravidin (0.06 μg ml⁻¹; Sigma Chemicals) were used as a marker of apoptosis. Modifications from the published protocol [17] included pre-incubation at room temperature for 15 min in a 0.1% saponin-EGTA solution, amount of tdt enzyme (20 units; Roche, Indianapolis, IN, USA), biotin-16-dUTP (0.5 nmol l⁻¹; Roche) and FITC-labeled extravidin (0.06 μg ml⁻¹; Sigma Chemicals). Negative controls had water instead of tdt. As a second step, immunoreactivity for α-sarcomeric actin was used as a marker for cardiomyocytes. A primary mouse IgG antibody directed against α-sarcomeric actin (1:40; SC5 clone, Sigma Chemicals) and a TRITC-labeled goat IgG secondary antibody directed against mouse IgG (1:100; Sigma Chemicals) were used. As a third step, 5 nmol l⁻¹ propidium iodide (ICN Biomedicals, Aurora, OH, USA) was used for total cardiac nuclei staining. In a duplicate set of sections, endothelial cells were labeled using 5 μg ml⁻¹ TRITC-labeled lectin (Bandeiraea simplicifolia; Sigma Chemicals) and TUNEL positive nuclei were labeled using 0.1 μg ml⁻¹ AMCA-labeled streptavidin (Vector Laboratories). Appropriate filters were used to quantify labeled cells by fluorescence microscopy separately in right and left ventricles. All TUNEL-positive nuclei (cardiomyocyte, non-cardiomyocyte: endothelial and fibroblast) in each section were counted. The epicardium was analyzed separately. Total number of cell specific nuclei per cross-section was estimated by counting profiles in 3 randomly chosen fields from both ventricles (250×; 0.02 mm²/field) and multiplying the average numerical density by the ventricular cross-sectional area. An average of 500 endothelial cell profiles were counted for each rat and results were expressed as number of capillaries/mm². In the epicardium, all nuclei were counted. A separate set if sections were stained with Sirius red to evaluate the percentage of ventricular area occupied by collagen [22].

2.6. Statistical analysis

Results were analyzed using analysis of variance and unpaired Student’s t-test with Bonferroni correction for multiple comparisons where appropriate. TUNEL assay was analyzed with the nonparametric Mann–Whitney test. Values are presented as mean±S.E.M. P<0.05 was considered statistically significant.

3. Results

Systolic blood pressure (mmHg) was significantly decreased by therapy after 12 days (controls: 206±9; valsartan: 166±10; enalapril: 155±9) and 26 days (controls: 212±10; valsartan: 152±8; enalapril: 148±9). Body weight was not significantly affected after 4 weeks of treatment with valsartan (285±12 g) or enalapril (277±11 g), compared with controls (295±14 g), whereas left ventricular weight to body weight ratio was significantly reduced with valsartan (>9%) and enalapril (>17%).

Morphological data obtained on equatorial sections of diastole-arrested hearts are shown in Table 1. As compared to WKY rats, untreated SHRs showed increased left ventricular cross-sectional area and cardiomyocyte cross-sectional area. SHRs tended to have a (non-significant) larger chamber cross-sectional area in right ventricle as compared to WKY rats. Unexpectedly, the only difference in relative collagen content was in the right ventricle of WKY rats where there was a slight increase over SHRs. After 4 weeks, valsartan tended to reduce and enalapril completely normalized left ventricular cross-sectional area without affecting ventricular chamber size or right ventricular parameters. Valsartan and enalapril significantly
Table 1
Ventricular morphology of SHRs treated with valsartan or enalapril for 4 weeks or age-matched untreated WKY rats

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR control</th>
<th>SHR valsartan</th>
<th>SHR enalapril</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left ventricle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue cross-sectional area (mm²)</td>
<td>47±3*</td>
<td>60±1</td>
<td>54±1*</td>
<td>47±1*</td>
</tr>
<tr>
<td>Chamber cross-sectional area (mm²)</td>
<td>15±2</td>
<td>21±1</td>
<td>20±1</td>
<td>21±1</td>
</tr>
<tr>
<td>Relative collagen content (%)</td>
<td>15.5±0.4</td>
<td>15.2±0.5</td>
<td>16.1±0.2</td>
<td>14.4±0.4</td>
</tr>
<tr>
<td>Cardiomyocyte cross-sectional area (μm²)</td>
<td>182±13*</td>
<td>240±11</td>
<td>177±4*</td>
<td>169±6*</td>
</tr>
<tr>
<td><strong>Right ventricle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue cross-sectional area (mm²)</td>
<td>9.3±1.3</td>
<td>7.5±0.5</td>
<td>7.1±0.2</td>
<td>7.8±0.7</td>
</tr>
<tr>
<td>Chamber cross-sectional area (mm²)</td>
<td>5.7±0.6</td>
<td>5.4±0.4</td>
<td>6.4±0.5</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>Relative collagen content (%)</td>
<td>15.9±0.3*</td>
<td>14.4±0.2</td>
<td>14.1±0.3</td>
<td>14.0±0.5</td>
</tr>
</tbody>
</table>

Equatorial sections of diastole-arrested, paraformaldehyde-fixed hearts were studied.

* Significantly different (P<0.05) from SHR control group (n=6/group).

Decreased cross-sectional area of left ventricular cardiomyocytes down to levels observed in WKY rats. The relative collagen content in either ventricle of SHRs was maintained after valsartan or enalapril for 4 weeks. Ultimately, both valsartan and enalapril significantly decreased the total number of non-cardiomyocytes in the left ventricle by 37% (P=0.002). Levels reached that of age-matched normotensive WKY rats (107±12×10⁶) without affecting the total number of cardiomyocyte nuclei at 4 weeks (Fig. 1A). Thus, the ratio of cardiomyocyte to non-cardiomyocyte nuclei, which was low in controls (0.17±0.01), was normalized with valsartan (0.24±0.01; P<0.01) and enalapril (0.25±0.02; P<0.01) and increased to values similar to those of the WKY rats (0.22±0.02; P<0.05 versus control SHRs). In contrast, the right ventricle showed no significant change in cellularity with therapy (Fig. 1B). At 4 weeks, capillary density (capillary/mm²) and capillaries per myocyte in the left ventricular myocardium remained unchanged by treatments (versus respective controls: 1405±39 and 1.01±0.05, Fig. 1C, D). The absolute number of epicardial cells per cross-section at 4 weeks was reduced, though the change was not significant (valsartan: 559±85; enalapril: 676±58; controls: 756±91; NS).

![Diagram A](https://example.com/A.png)  ![Diagram B](https://example.com/B.png)  ![Diagram C](https://example.com/C.png)  ![Diagram D](https://example.com/D.png)

Fig. 1. Changes in the total number of cardiomyocyte nuclei (□) and non-cardiomyocyte nuclei (■) in the (A) left ventricle and (B) right ventricle of SHR treated for 4 weeks with valsartan or enalapril or age-matched untreated WKY rats. Changes in the total number of (C) capillaries per cross-section (capillary density), (D) capillary per myocyte, in the left ventricle of SHR treated for 4 weeks with valsartan or enalapril. * Significantly different (P<0.003) from SHR control group.
Our previous time course studies showed that DNA fragmentation indicative of apoptosis is maximally increased in the SHR heart after 1 week of AT$_1$ antagonist treatment and 2 weeks of angiotensin converting enzyme inhibitor treatment during regression of hypertrophy [16]. Thus, these time points for valsartan (1 week) and enalapril (2 weeks) were selected for apoptosis study and in situ determination of cell type undergoing apoptosis in the ventricles.

Consistent with these previous observations, ventricular DEVDase (caspase-3-like) activity was increased by more than 4.5-fold after 1 week of valsartan or 2 weeks of enalapril treatment (Fig. 2A). These results were corroborated with immunoblot analyses showing transient 3.6- and 4.2-fold increases in caspase-3 cleavage fragments (bands at 17–20 kDa) with valsartan and enalapril, respectively, without alteration in pro-caspase-3 (32 kDa) expression levels (not shown). In addition, measurement of cardiac pro- (Bax) and anti-apoptotic (Bcl-2) protein levels showed significant 3.4- and 3.2-fold increases in the Bax to Bcl-2 ratio with valsartan and enalapril, respectively (Fig. 2B).

Fig. 3A shows a representative photomicrograph of an area within the myocardium in which all (propidium iodide-positive) nuclei appear in bright red, well delineated spots, whereas nuclei within the α-sarcomeric actin-positive area (diffuse red area) were identified as cardiomyocyte nuclei. In Fig. 3B, the same field examined under a green filter to detect FITC-labeling shows a TUNEL-positive non-cardiomyocyte nucleus. For each animal, a subset of sections was stained for identification of endothelial cells using lectin as a marker (bright red spots in Fig. 3C). In Fig. 3D, the same field examined under a blue filter to detect AMCA-labeling shows a TUNEL-positive, lectin-negative nucleus, considered as an apoptotic fibroblast. Treatment with valsartan for 1 week or enalapril for 2 weeks each caused a significant twofold increase in TUNEL-positive fibroblasts (Fig. 4A) selectively in the interstitium of the left ventricle. In contrast, TUNEL-positive endothelial nuclei remained unchanged versus controls (Fig. 4B). TUNEL-positive fibroblasts returned to basal values (0.026±0.005%) after 4 weeks of valsartan (0.033±0.003%) or enalapril (0.034±0.004%) treatment. TUNEL-positive cardiomyocyte nuclei were increased with enalapril at 2 weeks (by 75% in the left ventricle only) (Fig. 4C). Valsartan-treated hearts at 1 week showed a similar though non-significant tendency. After 4 weeks, TUNEL-positive cardiomyocyte nuclei were not significantly different between rats treated with placebo (0.008±0.002%), valsartan (0.006±0.001%) or enalapril (0.006±0.001%). As shown in Fig. 3E, epicardial cells showed by far the greatest frequency of TUNEL-positive nuclei. TUNEL-positive epicardial cells were increased with valsartan at 1 week (45±2 vs. 38±2% in age-matched controls; P<0.05) and enalapril at 2 weeks (46±1 vs. 32±2% in age-matched controls; P<0.05), with
Fig. 3. Photomicrographs showing (A) areas immunoreactive for α-sarcomeric actin (cardiomyocyte cell body in diffuse red) and areas positive for the total nuclear stain propidium iodide (bright red, sharply delineated spots). The arrow points a non-cardiomyocyte nucleus (located outside the α-sarcomeric actin stained area) (×600). (B) The same field visualized under an FITC filter showing the same nucleus as a TUNEL-positive (bright green, sharply delineated) nucleus (×600). (C) Endothelial cells (bright red, sharply delineated spots) after staining with TRITC-labeled lectin (×600). (D) In the same field, visualized under a DAPI filter, the arrow points a TUNEL-positive (bright blue, sharply delineated), lectin-negative non-cardiomyocyte nuclei, indicating an interstitial fibroblast (×600). (E) TUNEL-positive nuclei prevalent in the epicardium (×400).
was restricted to interstitial fibroblasts in the left ventricle of SHRs treated with valsartan or enalapril. As a result, fibroblast hyperplasia in the adult SHR was normalized within 4 weeks of treatment, down to levels seen in WKY rats. Apoptosis increase was transient even though anti-hypertensive treatments were maintained, consistent with our previous observations [16]. Both drugs had a tendency to transiently increase TUNEL staining also in cardiomyocytes (significant with enalapril). The cumulative effect was marginal, however, since the total number of cardiomyocyte nuclei was not significantly decreased after 4 weeks of treatment in the left ventricle. Administration of quinapril decreases the percentage of tetraploid cardiomyocytes in SHRs [23], an effect that reflects either nuclear division or polyploid cell deletion. Cardiomyocyte polyploid nuclei may be removed by apoptosis during treatment with renin–angiotensin system inhibitors. Alternatively, TUNEL staining may label nuclei undergoing DNA repair [24]. After the 4-week treatments, the number of cardiomyocytes in the right and left ventricles of SHRs remained unchanged and higher than in WKY rats, possibly reflecting compensated cardiac hypertrophy in SHR at that age. In humans, right septal endomyocardial biopsies from hypertensive patients show greater caspase-3 immunoreactivity, Bax to Bcl-2 protein ratio and TUNEL staining in both cardiomyocytes and interstitial fibroblasts as compared to tissues from normotensive volunteers [25]. These high levels of apoptosis in cardiomyocytes and interstitial fibroblasts are attenuated significantly, though not normalized, following administration of losartan for 12 months. The discrepancy with the present results may reflect species specificities, different stages of the disease or time-dependent regulation of cardiac apoptosis during treatment.

The beneficial effects of angiotensin pathway inhibitors include the amelioration of oxygen diffusion in the left ventricular myocardium. Previous reports show that cardiac capillaries are not decreased in SHRs receiving renin–angiotensin system inhibitors [26,27]. Consistent with this, the treatments in this study did not increase TUNEL-positive endothelial cells or decrease capillary number or density at 4 weeks. In the non-cardiomyocyte population of the heart, fibroblasts, representing the main cell type, play a key role in cardiac connective tissue regulation [28].

4. Discussion

The present results indicate significant cell deletion by apoptosis during onset of cardiac hypertrophy regression...
reported that AT1 receptor blockade for 14 weeks stimulates collagenase activity and reduces fibrosis in SHR left ventricles. As an additional mechanism, the present data suggest that a reduction of fibroblast hyperplasia may contribute to decreased myocardial extracellular matrix mass.

The induction of fibroblast apoptosis and the reduction in ventricular cross-sectional area occurred only in the left ventricle, suggesting a possible causal relationship between these two processes. The absence of right ventricular apoptosis also suggests a role for blood pressure reduction. It is important to note, however, that effective antihypertensive treatment with hydralazine does not induce cardiac apoptosis or mass regression, suggesting pressure-independent influences [16]. We previously reported that transient apoptosis induction during regression of aortic hypertrophy is dependent on pro-apoptotic AT2 receptors for angiotensin II in SHRs treated with the AT1 antagonist valsartan but not enalapril [32]. We recently observed that transient cardiac apoptosis induced by either valsartan or enalapril was not affected by AT2 receptor blockade (Der Sarkissian et al., unpublished data), suggesting a role for downregulation of AT1 receptor signaling in the induction of the time window of cardiac apoptosis in this model. The stimulation of AT1 receptors activates pro-growth and anti-apoptotic signaling pathways in mesenchymal cells such as fibroblasts and smooth muscle cells, including Akt activation, reactive oxygen species generation, and growth factor expression [33,34]. One candidate is insulin-like growth factor 1, which is induced by angiotensin II [35] and which inhibits apoptosis in cardiac fibroblasts via a mechanism up-stream of caspase-3 but independent of Bax or Bcl-2 expression [36]. Cardiac Bax to Bcl-2 ratio was increased during apoptosis in the present study, however, suggesting an alternative pathway. Tissue inhibitor of metalloproteinase-4 (TIMP-4), which shows dysregulated expression during pathological cardiac remodeling [37], was recently shown to induce apoptosis in transformed, although not in normal, cardiac fibroblasts [38]. Pathways involved in regulating fibroblast apoptosis during cardiac remodeling remain to be better defined.

The present results confirm our previous observations that cardiac mass regression is associated with induction of apoptosis in the epicardial region [16]. The epicardium, which is part of the cardiac pleura, is populated by mesothelial cells and is a major expression site for matrix metalloproteinases [39] and several factors modulating cardiomyocyte and non-cardiomyocyte behavior, including angiotensin II [40]. The contribution of mesothelial cells to ventricular hypertrophy regression is not clear but may reflect proportional remodeling of the pleura to accommodate the change in ventricular mass. Interestingly, epicardial apoptosis was similar between the right and left ventricles, thus ruling out a direct causal relationship between these events. Post-operative fibrosis of the cardiac pleura is associated with increased local expression of angiotensin-converting enzyme [41] and AT1 receptors [42]. Considering the present findings, it is intriguing to speculate that the suppression of post-operative pericardial fibrosis by AT1 receptor blockade [43] involves enhanced mesothelial cell death by apoptosis. Also intriguing, are the high basilar levels of TUNEL-positive mesothelial cells (30% range), which may suggest a high turnover rate.

Several studies showed that inhibition of the renin-angiotensin system decreases cardiomyocyte apoptosis in hypertension [4,10,14,15]. Considering these findings with the present data, we propose that the beneficial cardiac effects of angiotensin II pathway inhibitors may involve cell type-specific regulation of apoptosis, with an early decrease in fibroblast survival and a long-term increase in cardiomyocyte survival. If confirmed in humans, the ability to reduce fibroblast hyperplasia via apoptosis may have important implications in the treatment of advanced cardiac fibrotic diseases.

Acknowledgements

This work was supported by a grant from the Canadian Institute of Health Research (CIHR; MOP-42452). D.D. is a scholar of the Fonds de la Recherche en Santé du Québec. S.D.S. and E.L.M. both hold an award from the Heart and Stroke Foundation of Canada/CIHR. We would like to acknowledge the help of Dr. Maria Antonia Fortuno and Dr. Martin G. Sirois in developing the immunohistochemistry protocols and of Marie-France Ross in conducting the quantitative image analysis.

References


