Caspase-Dependent Cell Death Mediates the Early Phase of Aortic Hypertrophy Regression in Losartan-Treated Spontaneously Hypertensive Rats

Eve-Lyne Marchand, Shant Der Sarkissian, Pavel Hamet, Denis deBlois

Abstract—Blockade of angiotensin type 1 (AT₁) receptors induces smooth muscle cell (SMC) death and regression of aortic hypertrophy in spontaneously hypertensive rats (SHR). We postulated that SMC death and vascular remodeling in this model may be attenuated by z-Val-Ala-Asp(OMe)-CH₂F (z-VAD-fmk), a tripeptide inhibitor of caspase enzymes mediating apoptosis. To determine the time course of SMC death and aortic remodeling, SHR were treated with losartan (30 mg/kg per day) for up to 9.5 days. Transient SMC apoptosis occurred in the aortic media with a peak around day 5 of treatment, with increases in the Bax to Bcl-2 protein ratio (3-fold), in active caspase-3 (5.6-fold), in TUNEL-positive nuclei (19-fold), preceding by 24 hours the peak activation of caspase-9 (3.8-fold), and significant reductions in SMC number (46%) and aortic cross-sectional area (8.5%) at 5.5 days. The decrease in total aortic DNA reached significance at 6.5 days (29%). Blood pressure reduction with losartan was progressive and reached significance at day 7 of treatment. Next, we examined the causal link between vascular apoptosis and remodeling. SHR received placebo or losartan (30 mg/kg per day) for 6 days. During the last 24 hours, a subgroup of losartan-treated rats received 3 IV injections of z-VAD-fmk (cumulative dose: 4.4 mg · kg⁻¹). All other rats received the vehicle, DMSO. The 24-hour cotreatment with z-VAD-fmk effectively prevented losartan-induced caspase-3 activation and internucleosomal DNA fragmentation, as well as SMC depletion and the reductions in aortic mass and DNA content. Together, these data suggest that caspase-dependent SMC death mediates the early phase of vascular remodeling in response to AT₁ receptor blockade in this model of hypertension. (Circ Res. 2003;92:777-784.)

Key Words: caspase ■ apoptosis ■ aorta ■ hypertrophy ■ hypertension

In recent years, programmed cell death has emerged as an important mechanism of cardiovascular remodeling. Apoptosis is a form of programmed cell death associated with the activation of caspases, notably caspase-3, which acts as a downstream key executioner of the death program and internucleosomal fragmentation of DNA. The prototypical irreversible inhibitor of caspases z-Val-Ala-Asp(OMe)-CH₂F (z-VAD-fmk) is a cell-permeable tripeptide with broad specificity for all known caspases. Z-VAD-fmk potently inhibits apoptosis in vitro as well as in vivo in cardiomyocytes, neurons, and hepatocytes.

The spontaneously hypertensive rat (SHR) is a genetic model of primary hypertension showing dysregulations of cell proliferation and death contributing to increased vascular mass and DNA content. We first reported that smooth muscle cell (SMC) death is enhanced transiently during the regression of aortic hypertrophy in SHR treated with antihypertensive agents, an effect that is not secondary to blood pressure reduction. Within 1 week of treatment with an antagonist of type 1 (AT₁) receptors for angiotensin II, SHR aortic DNA fragmentation is maximally increased.

The present study explored the causal relationship between SMC apoptosis and vascular remodeling induced by AT₁ antagonist treatment. We first determined the relative time course of blood pressure reduction, SMC apoptosis, and aortic mass regression in SHR treated with losartan. Second, z-VAD-fmk was administered in vivo in an attempt to block losartan-induced cell death and vascular mass regression. The results suggest that caspase-dependent SMC death plays an obligatory role in onset aortic remodeling induced by AT₁ receptor blockade in hypertension.

Materials and Methods

Male SHR (250 to 275 g) purchased from Charles-River (St-Constant, Quebec) were housed for at least 48 hours before initiation of drug treatment. Food and water were administered ad libitum. All animal manipulations were conducted according to institutional guidelines.
Effects of Losartan Treatment for 6 Days and z-VAD-fmk Cotreatment During the Last 24 Hours on Total SMC Nuclei and Morphometric Quantification in the Aortic Media of SHR

<table>
<thead>
<tr>
<th>Measurements on cross sections</th>
<th>Control (n=15)</th>
<th>Losartan (n=12)</th>
<th>Losartan+z-VAD-fmk (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial cross-sectional area, ( \mu m^2 \times 10^{-4} )</td>
<td>45±1</td>
<td>40±3*</td>
<td>46±1†</td>
</tr>
<tr>
<td>Measurements on dissectors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total volume of dissectors, ( \mu m^3 \times 10^{-6} )</td>
<td>112±9</td>
<td>143±7*</td>
<td>96±12†</td>
</tr>
<tr>
<td>No. of nuclei in top sections</td>
<td>67±4</td>
<td>88±4*</td>
<td>61±3†</td>
</tr>
<tr>
<td>No. of nuclei still present in bottom sections</td>
<td>39±2</td>
<td>66±5*</td>
<td>39±5†</td>
</tr>
<tr>
<td>No. of nuclei absent from bottom sections</td>
<td>28±3</td>
<td>22±3*</td>
<td>22±4</td>
</tr>
<tr>
<td>Average nuclear width, ( \mu m )</td>
<td>6±1</td>
<td>3.2±0.3*</td>
<td>7±1†</td>
</tr>
<tr>
<td>Cell numerical density, ( \mu m^{-3} \times 10^{-3} )</td>
<td>0.25±0.01</td>
<td>0.15±0.01*</td>
<td>0.23±0.01†</td>
</tr>
<tr>
<td>Combined measurements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cells/\mu m of aorta</td>
<td>114±19</td>
<td>61±3*</td>
<td>104±14†</td>
</tr>
<tr>
<td>Percent reduction in cell number</td>
<td>...</td>
<td>47*</td>
<td>9†</td>
</tr>
</tbody>
</table>

*Significantly different (P<0.05) vs control; †significantly different (P<0.05) vs losartan.

Time Course Study

In order to assess the time-course of losartan effects, SHR received losartan (30 mg/kg per day; gift of Merck-Frosst, Montreal, Canada) dissolved in the drinking water for 0, 4.5, 5.5, 6.5, 7.5, or 9.5 days (n=6 per group). In the subset of rats killed at day 9.5 of losartan treatment, systolic blood pressure was evaluated by tail-cuff plethysmography, as we described previously, on each day between days 0 and 7 of treatment.

In order to evaluate vascular DNA synthesis in vivo at 1.5 hour before death, all rats received a single intravenous bolus of \([1^H]\)-thymidine (0.5 mCi/kg; New England Nuclear) after induction of anesthesia with a single IM injection of a mixture of ketamine (80 mg/kg; MTC Pharmaceuticals), xylazine (4 mg/kg; Bayer), and acepromazine (2 mg/kg; Ayerst). Death was induced by exsanguination with retrograde perfusion of isonic saline (200 mL) through the abdominal aorta and draining via the jugular vein. The thoracic aorta was isolated, cleaned of adherent tissue, and a vascular segment between the third and fourth intercostal arteries was fixed in 4% paraformaldehyde for histological studies. The distal segment of the aortic media (between the fourth intercostal arteries and the diaphragm) was denuded of endothelium, snapped frozen and pulverized in liquid nitrogen using a mortar and pestle and stored at \(-80^\circ C\).

Histological Studies

Detailed histological methods are described in the online data supplement.

Determination of Smooth Muscle Cell Number

SMC number per unit length was determined as we previously described using the 3-dimensional disector method, a quantification procedure independent of nucleus orientation, shape, or size. Detailed formulae are described in the online data supplement. Calculations were performed in parallel to evaluate SMC number per unit length for each animal. Final values as well as intermediate values at selected steps of the calculations (averaged for each experimental group) are presented (Table).

Expression of Apoptosis-Regulatory Proteins

The protein levels of the latent caspase-3 fragment (32 kDa), active caspase-3 fragments (17 to 20 kDa), active caspase-9 fragment (38 kDa), as well as Bcl-2 and Bax in the aortic media were examined by immunoblot analysis. The distal segment of the aortic media was pulverized in liquid nitrogen using a mortar and pestle, and an aliquot (25 mg) of the pulverized tissue was lysed in extraction buffer [10 mmol/L \( \text{Tris-HCl} \) pH 7.5, 1% Triton x-100, 4 mmol/L \( \beta \)-glycerophosphate, 4 mmol/L \( \text{NaF} \), 1 mmol/L \( \text{EDTA} \), 1 mmol/L \( \text{EGTA} \), 200 mmol/L \( \text{L}^- \)phenylmethylsulfonylfluoride (PMSF), 21 mmol/L \( \text{leupeptin} \), 5 mmol/L \( \text{DTT} \) and 1 mmol/L \( \text{microystin} \) (Sigma-Aldrich Canada Ltd)]. Protein concentrations were determined with the Bio-Rad Assay (Bio-Rad Laboratories). Equal amounts of proteins (25 μg) separated on 15% SDS-polyacrylamide gel were transferred to Hybond-C extra membrane (Amersham, Bioscience). Membrane were blocked in 5% nonfat milk and incubated with anti-caspase-3 (1:1000 BD Pharmingen), anti-active caspase-9 (1:1000; New England Inc), anti-Bax (1:1000 Santa-Cruz Biotech), or anti-Bcl-2 (1:1000 Sata-Cruz Biotech) followed by incubation with goat anti-mouse or goat anti-rabbit horseradish peroxidase-conjugated antibody (1:2000 Santa-Cruz Biotech) and then enhancement medium ECL Plus (Life Science Products) according to manufacturer’s protocol. Intensity of each band was quantified using NIH Image program 1.61.

Caspase Inhibitor Study

In order to assess the role of caspases in losartan-induced vascular remodeling, male SHR (250 to 275 g) were randomized to treatment with placebo (n=11) or losartan (30 mg/kg per day; n=23) in the drinking water for 6 days. At 24 hours before death, a subset of losartan-treated animals (n=12) was randomly selected for treatment with the caspase inhibitor z-VAD-fmk (cumulative dose: 4.4 mg/kg; Enzyme System Products) given fractionally in three intravenous injections at 24, 16, and 8 hours before death. All other rats received the DMSO vehicle (100 μL per intravenous bolus) according to the same administration schedule (final group size was n=14 in placebo group, n=12 in losartan group, and n=11 in losartan+z-VAD-fmk) group. Animals were subjected to brief anesthesia with inhalation enflurane, and the right femoral vein was exposed for the intravenous injections.

Tissue isolation procedures were exactly as described above, including \([1^H]\)-thymidine administration at 1.5 hours before death by exsanguination in anesthetized animals, and sampling of vascular sections for histological studies. In this study, 25 mg of the frozen aortic media was used as a source of material to examine both synthesis (\([1^H]\)-thymidine incorporation) and internucleosomal fragmentation of vascular DNA.

The internucleosomal DNA fragmentation index was quantified as we previously described. Briefly, after terminal deoxynucleotidyl transferase-mediated dUTP-[\( ^32 \text{P} \)] nick end labeling of extracted DNA, increasing amounts of DNA (0.05 to 0.4 μg) were fractionated...
by 1.5% agarose gel electrophoresis. Radioactivity associated with small DNA fragments (from 150 to 1500 bps) was evaluated with a PhosphoImager (Molecular Dynamics). An aliquot of the pulverized aortic media (10 mg) from the distal segment of the vessel (between the fourth intercostal arteries and diaphragm) was lysed and DEV-Dase (caspase-3-like) activity was measured using the fluorogenic substrate Ac-DEVD-AMC (1 μmol · L⁻¹) (BioMol Research Labs Inc) in the presence or absence of the caspase-3 inhibitor DEVD-CHO (1 μmol · L⁻¹), as we previously described for cultured SMCs. Fluorescence was calibrated with AMC (10 to 100 nmol · L⁻¹). Caspase-3-like activity was defined as the DEVD-CHO-sensitive activity.

Statistical Analysis
Values are presented as mean±SEM. Results were analyzed using analysis of variance followed by unpaired Student’s t test with Bonferroni correction for multiple comparisons. A value of P<0.05 was considered statistically significant.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results

Time Course Study
Pretreatment values of body weight were 238±3 g (n=36). All rats gained weight during the experiment period but final values of body weight were similar between all groups (not shown). Average pretreatment values of systolic blood pressure were 183±9 mm Hg (n=6) in the subset of rats randomly selected for hemodynamic monitoring by tail cuff plethysmography. Systolic blood pressure showed a gradual decrease that reached statistical significance after 7 days of losartan treatment (Figure 1).

As shown in Figure 2, treatment with losartan induced abrupt changes in aortic cellularity and mass. Whereas the aorta showed no change in SMC number or CSA at 4.5 days of treatment, the vessel showed a marked reduction in SMC number (46%) and CSA (8.5%) after an additional 24 hours of treatment (at 5.5 days), as measured between the third and fourth intercostal arteries. Medial CSA was 0.472±0.006 mm² at day 0 and 0.464±0.004 mm² at day 4.5 (NS versus day 0), whereas it was 0.432±0.007 mm² at day 5.5 (P=0.0007 versus day 0; n=6 per group). No further significant modification of vascular cellularity or mass was observed throughout the rest of the 9.5-day treatment. Total DNA extracted from the distal segment of the thoracic aorta showed a decrease that reached significance at day 6.5 (1.09±0.05 g/mm) as compared with day 0 (1.464±0.06 g/mm) with no further reduction up to 9.5 days of treatment (Figure 2C). Overall, the decrease in aortic DNA correlated significantly with the depletion of SMCs (P=0.001; R=0.76).
The time course of aortic SMC depletion suggested transient SMC apoptosis induction, as we previously reported. 8,9 Consistent with this, the number of TUNEL-positive nuclei in the aortic media was markedly elevated (19-fold) at 4.5 days (Figures 2D and 3D). At that time, there was not a detectable change in vascular mass or SMC number. The increase in TUNEL-positive SMC nuclei at 4.5 days preceded the reduction of aortic cellularity and hypertrophy by at least 24 hours, and it decreased gradually over the rest of the treatment period (Figures 2D and 3F). Western blot analysis and quantification of caspase-3 cleavage fragments (17 to 20 kDa) in aortic protein extracts suggested a transient increase (5.6-fold at day 4.5) in active caspase-3 as compared with control group (Figure 4A). There was no alteration in procaspase-3 (32 kDa) expression levels (Figure 4A). Likewise, quantification of caspase-9 cleavage fragments (38 kDa) suggested a transient increase (3.8-fold at day 5.5) in active caspase-9 as compared with control group (Figure 4B). The similarities between the kinetics of activation for caspase-3 and -9 and the induction of SMC death in response to losartan suggested a possible role for caspases in this model. Further evidence for the transient induction of SMC apoptosis was provided by the increased levels of proapoptotic protein Bax (>2-fold) at days 4.5 and 5.5 (significant at 5.5 days only; \( P<0.007 \); Figure 4C) but not thereafter, and by the decreased levels of antiapoptotic protein Bcl-2 (50%) at 4.5 days but not thereafter (Figure 4D). As a result, the Bax to Bcl-2 ratio was significantly increased (>4-fold) at day 4.5.

In summary, antihypertensive treatment with losartan in SHR elicited an abrupt profile of vascular mass regression that was associated temporally with caspase activation and SMC deletion.

Caspase Inhibitor Study
To validate the implication of caspase-dependent cell death in the early phase of vascular hypertrophy regression in this model, the caspase inhibitor z-VAD-fmk was administered to SHR during the last 24 hours of a 6-day losartan-treatment. This schedule of z-VAD-fmk administration was selected based on data obtained using tissue extracts from the distal segment of the thoracic aorta in the time course study, which suggested recruitment of several apoptosis pathways (activa-
tion of caspase-3 and -9, increased Bax levels, and a decrease in DNA content) around day 5.5 of losartan treatment (Figures 2D and 4A through 4C). Subsets of age-matched SHR (n=3 per experimental group) were used for hemodynamic monitoring by telemetry during the 24-hour period covering the three intravenous injections of z-VAD-fmk or DMSO vehicle. As shown in Figure 5, systolic blood pressure, diastolic blood pressure, and heart rate showed no significant alteration during this period.

As compared with vehicle, losartan increased internucleosomal DNA fragmentation 2-fold ($P=0.026$), an effect that was attenuated by z-VAD-fmk (Figure 6). The results obtained using a fluorogenic caspase substrate provided additional evidence of increased caspase-3 activity (>3-fold) in aortic protein extracts after losartan treatment and confirmed the effectiveness of z-VAD-fmk as a caspase inhibitor in our in vivo model (Figure 7A). Consistent with the time course data described above, treatment with losartan for 6 days caused a significant reduction in aortic SMC number (47%), medial CSA (11%), and media DNA content (20%) (Figure 7). All these effects of losartan were suppressed by z-VAD-fmk (Figure 7). As a further confirmation that z-VAD-fmk suppressed vascular mass regression, the aorta to body weight ratio showed a significant 16% reduction with losartan (4.1±0.2 mg/mm per g; $P=0.007$) as compared with control values (4.9±0.2 mg/mm per g; $P=0.0001$). The incorporation of [3H]-thymidine into aortic DNA was not significantly affected with losartan (93±9 CPM/10 μg; NS) or by z-VAD-fmk (85±9 CPM/10 μg; NS), as compared with control values (106±19 CPM/10 μg).

In summary, a caspase inhibitor prevented the early regression of vascular hypertrophy and hyperplasia induced by losartan without affecting hemodynamic parameters.

**Discussion**

The present study tested the hypothesis that caspase-dependent cell death plays an obligatory role in the early
phase of vascular remodeling induced by losartan in SHR. The early time courses of vascular apoptosis induction and regression of medial hypertrophy were examined in the aorta of SHR treated with the AT1 receptor blocker. We found that losartan induced a synchronous wave of SMC apoptosis, as evidenced by a transient increase in Bax to Bcl-2 ratio, caspase-3 and -9 activation profiles, accumulation of TUNEL-positive nuclei, as well as a decrease in SMC number and vascular DNA content between days 4.5 and 6.5 of treatment. Histological analysis showed a close temporal association between SMC number decrease and vascular mass reduction, suggesting that transient SMC apoptosis induction may contribute to the early phase of vascular hypertrophy regression in this model. To test this hypothesis, we gave the pan-caspase inhibitor z-VAD-fmk between days 5 and 6 of losartan treatment, ie, within the period of recruitment of several apoptosis pathways (caspase-3 and -9 and Bax) and decrease in vascular DNA and SMC number. We found that treatment with z-VAD-fmk within this critical period prevented the effects of losartan on aortic remodeling, including increased caspase-3–like enzymatic activity, internucleosomal DNA fragmentation, SMC depletion, vascular DNA reduction, and aortic mass reduction. Taken together, these results suggest that caspase-dependent cell death plays an obligatory role in the early phase of vascular mass regression induced by losartan in this model of hypertension.

SMCs have been shown to act as amateur phagocytes in the presence of apoptotic bodies in vitro. In rabbit carotid arteries undergoing remodeling in response to reduction in flow, Cho et al reported that removal of apoptotic SMCs occurred with a half-life of 1 to 2 hours in vivo. However, the estimated frequency of apoptotic SMCs in the rabbit arteries (0.13%) was lower than in the present study (approximately 30%). We noted that the magnitude of SMC depletion, assessed histologically, was slightly larger than the significant reduction of total vascular DNA, possibly reflecting incomplete removal of remnant apoptotic bodies in the arterial wall. Consistent with this, we reported that after 4 weeks of losartan treatment in SHR, the decrease in aortic DNA content is comparable (or slightly greater) that the decrease in SMC number. It may be relevant that SMC nuclear profiles and total DNA content were quantified in different aortic segments (proximal and distal to the heart, respectively). In the early phase of vascular regression, the incidence of SMC apoptosis may be lower or slower to develop at the more distal levels of the thoracic aorta. The formation of polyploid SMC by nuclear fusion may also reduce the number of SMC nuclear profiles. Although it cannot be formally excluded, we consider this possibility unlikely because of the low incidence (%1%) of multinucleated SMC in 3-month-old SHR aorta. Moreover, the enhanced accumulation of polyploid SMC in the untreated SHR aorta is reversed rather than increased by AT1 receptor antagonists.

In other acute in vivo models of apoptosis induction such as in rat ischemic cardiomyocytes, Fas-stimulated mouse hepatocytes, and ischemic mouse neurons, z-VAD-fmk effectively inhibits apoptosis with preservation of functionality in the later two models. The plasma z-VAD-fmk concentration likely reached in the present study (25 μmol/L range) is in the lower range of the concentrations used in vitro to inhibit caspases. Moreover, inhibition of ischemic cardiomyocyte apoptosis in vivo is dose-dependent in the range of the z-VAD-fmk dose used in the present study.

Previous studies suggested that transient induction of SMC apoptosis was a common feature of aortic hypertrophy regression in SHR treated with different classes of antihypertensive drugs, including AT1 antagonists, angiotensin converting enzyme inhibitors, and dihydropyridine calcium channel blockers. These drugs also induce a transient increase in cardiac apoptosis during regression of cardiac hypertrophy, a response that results in selective reversal of fibroblast hyperplasia in the SHR heart. Evidence suggest that apoptosis is also increased by losartan treatment in small mesenteric arteries of SHR, although with a delayed kinetic of induction. Nifedipine treatment increases SMC apoptosis...
and reduces SMC number in the carotid artery neointima of SHR and of Wistar-Kyoto rats but not in the underlying media. The possible role of blood pressure reduction in SMC apoptosis induction during aortic mass regression is worth considering. Arterial unloading and reduction in blood flow can induce SMC apoptosis. In vitro studies have established, however, that angiotensin II can modulate SMC apoptosis negatively (via AT_1 receptors) or positively (via AT_2 or AT_1 receptors) in the absence of hemodynamic influences. In SHR treated with the AT_1 antagonist valsartan, coadministration of the AT_2 antagonist PD123319 prevents both SMC apoptosis and vascular mass regression, although it does not affect blood pressure reduction. Moreover, treatment with the angiotensin converting enzyme inhibitor enalapril for one week markedly reduces blood pressure without affecting SMC apoptosis at that time in the SHR aorta. Likewise, marked reduction of high blood pressure with hydralazine is not associated with vascular apoptosis or remodeling within 4 weeks in SHR. In the present study, the decline in high blood pressure over the first week of losartan treatment contrasted with the sudden onset and subsequent stabilization of SMC deletion and regression of vascular mass. Collectively, these data suggest that the regulation of SMC apoptosis and early vascular mass regression in this model show a strong pressure-independent component, although a role for hemodynamic factors cannot be formally excluded. It is however important to note that z-VAD-fmk suppressed apoptosis without affecting key hemodynamic parameters in SHR.

The time-dependent changes in apoptosis-related proteins suggest that caspase-3 activation and cleavage downregulation acted upstream of Bax and caspase-9. After a death signal, Bax can associate with the mitochondria to induce the release of cytochrome c and formation of the apoptosome complex in association with caspase-9 activation. The signaling pathways responsible for cell death in the present model remain incompletely defined, but evidence suggests a role for AT_1 receptors for angiotensin II. In recent years, it has become apparent that angiotensin II modulates apoptosis in a cell type- and receptor subtype-dependent manner. Acting via AT_1 receptors, angiotensin II can suppress apoptosis in SMCs but stimulate apoptosis in endothelial cells and cardiomyocytes. AT_2 receptors can however induce apoptosis in SMCs, endothelial cells, cardiomyocytes, and cardiac fibroblasts. AT_2 receptor-dependent apoptosis is associated with Bcl-2 downregulation and executioner caspase-3 activation. Other key intracellular pathways implicated in mediating AT_2-dependent apoptosis include de novo ceramide generation and protein phosphatase activation. In vivo, the role of AT_2 receptors is more controversial. With its rapid kinetics, the present in vivo model provides a framework for studying the molecular regulation of AT_2 receptor- and caspase-dependent SMC death during onset vascular remodeling with AT_1 receptor antagonists in hypertension. To our knowledge, this model currently exhibits one of the largest synchronized induction of cell death by apoptosis in vivo. Massive cell death by apoptosis in vivo has been described in pathological conditions such as postangioplasty vascular remodeling and Fas-induced liver degeneration in mice. Considering the current focus on inhibitors of apoptosis as potential therapeutic agents, it is noteworthy that apoptosis may be viewed as therapeutically beneficial in the present model.

Some limitations of the present study should be addressed. First, the relative role of caspase subtypes remains to be determined because z-VAD-fmk is not specific for caspase subtypes. Second, long-term effects of caspase inhibition on SMC survival are unknown. Although apoptosis inhibition with z-VAD-fmk maintains functionality in ischemic hepatocytes, neurons, and cardiomyocytes in vivo, other in vitro studies have shown that apoptosis inhibition may eventually lead to noncaspase-dependent cell death. Third, nonspecific effects of z-VAD-fmk, for instance on lysosomal cysteine proteases of the cathepsin family, cannot be ruled out. The role of cathepsins in apoptosis is unclear, with studies in nonvascular cells showing stimulation, suppression, or no effect. Cathepsin-induced apoptosis may involve a limited caspase-processing activity. Overall, available evidence suggests that caspases act downstream of cathepsin-mediated apoptosis. Whatever the relationship between caspases and cathepsins, the present conclusion that SMC apoptosis inhibition prevents early vascular mass regression with an AT_1 antagonist is supported by our previous study in SHR cotreated with an AT_1 receptor antagonist.

In summary, the early phase of aortic mass regression in losartan-treated SHR occurred as an acute and rapid event synchronized with a transient increase in Bax to Bcl-2 ratio, caspase-3 activation, and SMC apoptosis. Caspase inhibition with z-VAD-fmk prevented losartan-induced apoptosis and regression of aortic hypertrophy. Together these results demonstrate that caspase-dependent SMC death mediates the early phase of vascular remodeling in response to AT_1 receptor blockade in this rat model of essential hypertension.

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References


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