Time Course of Vascular Structural Changes During and After Short-Term Antihypertensive Treatment

Taben M. Hale, Martin J. Shoichet, Terri L. Bushfield, Michael A. Adams

Abstract—The present study characterized the persistent changes (ie, off-treatment) resulting from short-term antihypertensive treatments on mean arterial pressure (MAP) and structurally based vascular resistance. Rats were treated for 14 days with enalapril (30 mg · kg⁻¹ · d⁻¹) with regular (ENAL, 0.4%) or low salt (ELS, 0.04%) diets, or a triple therapy (Triple: hydralazine 45 mg · kg⁻¹ · d⁻¹, hydrochlorothiazide 100 mg/L, and nifedipine 200 mg/d). MAP was continuously recorded via radiotelemetry. Structurally based hindlimb vascular resistance properties (resistance at maximum dilation [Max Dil]; resistance at maximum constriction [Max Con]) were assessed after 14-day enalapril treatment and 2 to 3 weeks after all drugs were withdrawn. Aortic urokinase plasminogen activator (uPA) activity was measured by zymography after 14 days of ELS. All treatments induced a significant, persistent decrease in the off-treatment MAP (ENAL ↓ 12±4.6%, ELS ↓ 16±2.6%, Triple ↓ 5±4.17%). During treatment (14 days) the enalapril group had significant changes in the index of medial bulk (Max Con ↓ 15±2.6%), but only minimal changes in lumen properties (Max Dil ↓ 3±6.5%, NS). After stopping therapy, vascular properties at Max Dil were significantly decreased only in the 2 enalapril groups (ENAL ↓ 15±7.9%, P<0.05; ELS ↓ 9±6.0%, P<0.05; Triple ↓ 2±9.8%, NS), whereas Max Con was significantly decreased in all groups (ENAL ↓ 12±8.0%, ELS ↓ 16±6.1%, Triple ↓ 7±5.4%). At 14 days of ELS treatment, there was increased aortic uPA activity (1.6-fold). The findings reveal that various short-term antihypertensive treatments can produce persistent long-term changes in MAP and vascular structure. Further, the magnitude of the depressor response may be as important in inducing persistent changes as is the removal of angiotensin II. (Hypertension. 2003;42:171-176.)

Key Words: rats, inbred SHR ■ vascular resistance ■ arterial pressure ■ antihypertensive agents ■ hypertension, experimental ■ sodium, dietary

Experimentally, long-term antihypertensive treatment, specifically with inhibitors of the renin angiotensin system (RAS) has been shown to induce both a regression of vascular structure (↓ medial thickness and/or ↑ lumen diameter) and a reduction in mean arterial pressure (MAP) that persist even after withdrawal of treatment.¹⁻⁴ These effects have been shown in several models of experimental hypertension, including the Prague hypertensive rat⁵ and the genetically hypertensive rat,⁶⁻⁷ although this is best documented in the spontaneously hypertensive rat (SHR).¹⁻³,⁸⁻⁹ The concept that the persistent reduction in MAP and the regression of vascular structure are linked mechanistically has also been widely discussed, although without resolution.¹⁻⁴,¹⁰⁻¹⁴ Further, the vascular structural changes that persist off-treatment have been proposed to result from extended inhibition of the trophic actions of angiotensin II,¹⁻³,⁸⁻¹⁶ prolonged reduction in arterial pressure,¹⁶⁻¹⁸ or from the combined effects. Regardless of the mechanism of action, the result is a reduced thickness of the medial layer and an increase in the lumen size of the vessels⁹,¹⁶,¹⁹ that lasts long after the cessation of therapy.

Although the majority of investigations have indicated that treatment of hypertensive rats with single agents that do not directly inhibit the RAS do not induce a persistent lowering of blood pressure⁵,²⁰,²¹ others have shown some persistent effects, at least in young, growing SHR.²²,²³ Heller et al.² using adult Prague hypertensive rats, showed that persistent pressure lowering occurred with both ACE inhibitors and AT₁ blockers but not with other agents. In contrast, we recently demonstrated in adult SHR that a 6-week antihypertensive treatment using a combination of agents (diuretic, calcium channel blocker, and vasodilator) was able to induce a persistent reduction in MAP.⁹ In analyzing data from publications before 1997, Lundie et al² found that the longer the treatment, the greater the effect on MAP and vascular structure, and suggested that a minimum of 4 weeks was required to induce persistent changes in the circulation. More recently, however, experimental evidence has revealed that only 2 weeks of treatment using an angiotensin converting enzyme (ACE) inhibitor was required to induce regression of both hindlimb and penile structurally based vascular resistance²,³⁴ and persistent reduction in MAP.³
Based on these recent findings, the main objective of the present study was to characterize the persistent impact of different short-term antihypertensive treatments on MAP and structurally based vascular resistance properties after stopping therapy. Initially, adult SHR were assessed at the end of a 2-week treatment with the ACE inhibitor, enalapril, to provide a comparison for the off-treatment results. The persistent impact of 2-week treatments on MAP and structurally based vascular resistance properties was then assessed during the off-treatment period in separate groups of animals treated with enalapril, enalapril plus low sodium diet, or the previously described triple therapy (hydrochlorothiazide, nifedipine, and hydralazine). These treatments were chosen to examine the relative importance of blood pressure lowering and removal of the trophic factor angiotensin II in inducing persistent changes in the cardiovascular system. Thus, ACE inhibitor treatment was done in 2 groups, with one of these groups also receiving a low sodium diet to enhance the depressor response\(^\text{25}\) while maintaining the same level of angiotensin II reduction. In the third group, the triple therapy regimen was used to lower blood pressure by a means that does not directly target the removal of angiotensin II. Finally, as a further assessment of the treatment-induced vascular changes, the matrix degrading activity of urokinase plasminogen activator (uPA) was assessed in the aortas of SHR treated for 2 weeks with enalapril/low salt.

**Methods**

**Animals and Treatment**

Male SHR (Charles River, Montréal, Quebec), were housed for at least 7 days before initiation of treatment. Control animals received water and standard rat chow (0.4% NaCl) ad libitum. Treatment involved either enalapril (ENAL 30 mg·kg\(^{-1}\)·d\(^{-1}\)), triple therapy (hydralazine 45 mg·kg\(^{-1}\)·d\(^{-1}\), hydrochlorothiazide 100 mg/mL, and nifedipine 200 mg/d), or enalapril plus low sodium (0.04%) diet (ELS). After 6 days of ELS treatment, rats received 4-hour daily enalapril, enalapril plus low sodium diet, or enalapril/low sodium plus nifedipine. All drugs were from Sigma Chemical Co and were mixed in drinking water, except nifedipine, which was provided in gelatin capsules. Treatment was started on the 7th day of life. All SHR were fed commercial chow and water and standard rat chow (0.4% NaCl) ad libitum. Treatment was administered in 15-week-old rats for 14 days. After stopping treatment, all rats were given tap water and standard rodent chow (0.4% NaCl). All procedures were in accordance with the Canadian Council on Animal Care.

**Mean Arterial Pressure**

Using previously established protocols, MAP was continuously monitored using radiotelemetry (Data Sciences International) throughout the treatment period and for at least 2 weeks after treatment stopped.\(^\text{7}\) Pressure transducers (TA11PA-C40; Data Sciences International) were implanted in the abdominal aorta of SHR (ENAL, n=7; ELS, n=5; triple, n=5) as described previously.\(^\text{3}\) Data from age-matched control SHR (n=12) were used for comparison.

**Hindlimb Vascular Resistance**

Assessments were made both during treatment (ENAL, n=7; control, n=7) and 2 to 3 weeks after stopping treatment (ENAL, n=7; ELS, n=5; triple, n=7; control, n=27) using a previously described hindlimb perfusion technique.\(^\text{24}\) Briefly, the right hindquarter was isolated and perfused with Tyrode’s plus dextran buffer, maximum vasodilation was induced (sodium nitroprusside 20 μg/mL), and a flow rate–perfusion pressure relationship (0.5 to 4.0 mL/min per 100 g BW) and cumulative \(α\)-adrenoceptor concentration-response (methoxamine, 0.5 to 64 μg/mL) were determined. Finally, a cocktail of vasoconstrictors (vasopressin, 21 μg/mL; angiotensin II, 200 ng/mL; and methoxamine, 64 μg/mL; all from Sigma) produced maximum constriction (ie, “yield” response). This “yield” response correlates with the bulk of medial vascular smooth muscle in the resistance vasculature.\(^\text{27}\)

**Cardiac Mass**

Hearts were excised and blotted dry and right ventricle and left ventricle plus septum were then separated and weighed. Analysis of the left ventricle–to–body weight ratio was used as an index of change in cardiac structure.\(^\text{28}\)

**Urokinase Plasminogen Activator Activity**

Urokinase plasminogen activator (uPA, 54 kDa) activity was determined in aortas from control (n=4) and treated (14-day ELS, n=4) SHR using a previously described casein zymography assay.\(^\text{29}\) Protein (10 μg) samples were loaded onto SDS/polyacrylamide gels containing 2 mg/mL α-casein (Sigma) and 0.025 U/mL plasminogen (American Diagnostica). Densitometric analysis was performed using Un-Scan-It software. Each lane was digitized, and a background value was subtracted from the value obtained for each band. Samples from untreated rats were included in each zymogram to ensure appropriate normalization.

**Data Analysis**

Values are expressed as mean±SD. Data were analyzed for between-group comparisons using a one-way ANOVA with the Neuman Keuls post hoc test or an unpaired Student \(t\) test where appropriate. A paired Student \(t\) test was used for within-group analysis of MAP. A value of \(P<0.05\) was considered significant.

**Results**

**Mean Arterial Pressure Assessment**

All treatments induced a significant decrease in MAP during treatment compared with pretreatment levels (ENAL \(\downarrow 27±3.4\%\), ELS \(\downarrow 47±3.6\%\), triple \(\downarrow 18±3.5\%\); \(P<0.05\) versus pretreatment and between treatments) (Figure 1). Although all treatments resulted in a significant depressor response, the magnitude of this effect was significantly

![Figure 1. Treatment with enalapril, enalapril/low salt, and triple therapy induced a significant reduction in MAP that persisted for at least 2 weeks following cessation of therapy. Dotted line during enalapril and triple treatment represents an interruption in data recording. Data are presented as 24-hour mean±SD.](http://hyper.ahajournals.org/doi/10.1161/01.HYP.0000368174.60353.E2)
The effects of enalapril during treatment were used to compare with off-treatment changes. After 14 days of enalapril treatment there was a significant decrease in structurally based vascular resistance properties. Although there was a minimal impact on the level at which maximum dilation is reached (Control 30±1.6 mm Hg, ENAL 29±2.0 mm Hg, NS), there was a significant reduction in the perfusion pressure at maximum constriction (Control 364±14.9 mm Hg, ENAL 310±9.4 mm Hg, \(P<0.05\)) (Figure 2). These data suggest that the early pattern of changes in vascular structure initially involve a reduction in the overall medial bulk with a delay in the changes in overall lumen size.

**Structurally Based Vascular Resistance Off-Treatment**

**Enalapril**

Two to three weeks after stopping enalapril treatment, there was a persistent reduction in resistance, both at maximum dilation (Control 34±3.5 mm Hg, ENAL 29±2.7 mm Hg, \(P<0.05\)) and maximum constriction (Control 369±48.3 mm Hg, ENAL 323±29.7 mm Hg, \(P<0.05\)) (Figure 2). There was no change in the EC\(_{50}\), the slope of the concentration-response, or the constrictor response to the \(\alpha\)-adrenoceptor agonist, methoxamine, when assessed either during the on- or off-treatment period (Table).

**Enalapril/Low Salt**

Two to three weeks after stopping treatment, there was a significant change in the index of lumen diameter (maximum dilation: Control 30±1.7 mm Hg, ELS 28±1.4 mm Hg, \(P<0.05\)) (Figure 2) and medial bulk (maximum constriction: Control 338±27.7 mm Hg, ELS 280±19.1 mm Hg, \(P<0.05\)) (Figure 2). This more aggressive treatment resulted in a greater impact on structurally based vascular resistance assessed at maximum constriction than did enalapril treatment alone (ELS 280±20.1 versus ENAL 323±29.6 mm Hg, \(P<0.05\)). There was no change in assessments of reactivity and sensitivity to \(\alpha\)-adrenoceptor stimulation (Table).

**Triple Therapy**

There was no impact on structurally based vascular resistance at maximum dilation (Control 30±2.2 mm Hg, Triple 30±2.7 mm Hg, NS), but resistance at maximum constriction was significantly lower than controls (Control 374±17.3 mm Hg, Triple 355±14.2 mm Hg, \(P<0.05\)). There was no change in assessments of reactivity and sensitivity to \(\alpha\)-adrenoceptor stimulation (Table).

**Timecourse of Vascular Remodeling**

Comparing the vascular changes revealed that there were differences in the impact on the lumen and medial bulk.

### Hindlimb Vascular Resistance Properties

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight, %</th>
<th>Slope: FFlow-Pressure, %</th>
<th>Slope: C-R Curve, %</th>
<th>Log EC(_{50}), %</th>
<th>Max MXA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-day ENAL, on-tx</td>
<td>7.2±8.22</td>
<td>-8.6±15.29</td>
<td>17.2±25.59</td>
<td>-3.4±23.73</td>
<td>-10±4.15*</td>
</tr>
<tr>
<td>14-day ENAL, off-tx</td>
<td>-0.28±1.79</td>
<td>-2.9±9.34</td>
<td>-32.3±22.38</td>
<td>5.4±15.42</td>
<td>-14.2±9.02</td>
</tr>
<tr>
<td>14-day ELS, off-tx</td>
<td>0.9±8.31</td>
<td>-9.1±13.33</td>
<td>-19.8±26.86</td>
<td>5.0±10.79</td>
<td>-10.3±4.27*</td>
</tr>
<tr>
<td>14-day Triple, off-tx</td>
<td>1.4±3.95</td>
<td>-4.4±20.14</td>
<td>-2.3±27.93</td>
<td>7.8±30.31</td>
<td>0.3±9.05</td>
</tr>
</tbody>
</table>

Impact of treatment on reactivity to \(\alpha\)-adrenoceptor stimulation to methoxamine (MXA). Data are presented as mean percentage change from time control±SD. C-R indicates concentration response to MXA; on-tx, on-treatment; off-tx, off-treatment.

\*\(P<0.05\).
Table 1. Summary of percentage change of left ventricular mass during enalapril treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>ENAL</td>
<td>-0.12 ± 0.07</td>
</tr>
<tr>
<td>ELS</td>
<td>-0.10 ± 0.05</td>
</tr>
<tr>
<td>Triple</td>
<td>0.10 ± 0.05</td>
</tr>
</tbody>
</table>

Discussion

A major finding of the present study was that, regardless of the mechanism of blood pressure reduction, after only 2 weeks of effective antihypertensive treatment, there was both a persistent lowering of the off-treatment level of MAP and a regression of structurally based vascular resistance. In addition, increasing the magnitude of the depressor response during treatment resulted in a greater off-treatment effect on blood pressure and structurally based vascular resistance properties, although the relationship may not be directly proportional. Further, the findings revealed that the process of vascular remodeling appears to continue even in the absence of drug treatment, based on the finding that the index of resistance properties of the vascular lumen changed significantly between the on- and off-treatment periods. The suggestion of ongoing vascular structural changes is supported by the finding of increased uPA activity at the end (14 days) of the treatment period.

Previously, it was suggested that 4 weeks of antihypertensive therapy in adult SHR was the minimum time required to induce even minimal persistent alterations in either MAP or vascular structure. In the present study, using radiotelemetry to provide an accurate and sensitive measurement of MAP, a 5% to 16% reduction in the off-treatment level of MAP was revealed after only 2 weeks of antihypertensive therapy. In addition, using an approach that characterizes structurally based changes in the hindlimb vasculature, a significant decrease in vascular resistance was detected. Although it has been well established previously that RAS inhibitors induce regression of vascular structure, the present data suggest that the magnitude of the reduction in MAP may be a critical component in inducing these changes. Specifically, the present results show that a combination therapy, not directly targeting the RAS, resulted in a reduction in both structurally based vascular resistance and left ventricular mass, albeit to a lesser extent than the RAS inhibitors.

The magnitude of the changes in both vascular and left ventricular structure paralleled the reduction in the long-term level of arterial pressure. Although the concept is somewhat controversial, some investigators have maintained that there is a mechanistic association between regression of vascular structure and persistent reduction of MAP. For example, conclusions in some studies that did not support this causal link were based on a lack of statistical significance in vascular structural changes and yet the nonsignificant changes in vascular dimensions were in the order of 8% to 21%. It is important to recognize, based on the Poiseuille relationship, that the average vessel lumen diameter would only have to increase by about 4% to induce a 16% decrease in vascular resistance. Given that these changes are small, it is not surprising that the mechanisms involved in the process of vascular remodeling have not been well established, although
vascular smooth muscle apoptosis, matrix degradation, cell migration, and cellular atrophy have all been implicated.\textsuperscript{30–34}

In the present study, in addition to assessment of structurally based vascular resistance in the hindlimb, the characterization of uPA activity in the aorta of treated and untreated SHR provided an additional measure of vascular changes. The plasminogen activator system is regarded as an important regulator of matrix degradation in remodeling blood vessels.\textsuperscript{35,36} Thus, although at present there is only evidence for the aorta, it may be that the increased activity of uPA is indicative of ongoing remodeling. It is widely acknowledged that RAS inhibition induces remodeling of aortic and resistance vessel structure.\textsuperscript{1–4} The present data suggest a possible role for enhanced activity of the plasminogen activator system and the associated changes in the matrix as important components of this response.

The present findings indicate that there has been a change in structurally based vascular resistance, primarily suggesting a reduction in medial bulk, after only 2 weeks of treatment. Previous evidence supporting an early remodeling process has shown in aorta that there is a “burst” of vascular smooth muscle cell apoptosis within the first 2 weeks of antihypertensive treatment.\textsuperscript{30,31} Regardless of the precise mechanism of remodeling, the present findings describe a time course of vascular structural changes that occur during both the on- and the off-treatment time periods. Specifically, changes in the index of lumen dimensions were not fully consolidated until 2 to 3 weeks after stopping treatment. In contrast, regression of medial bulk, as demonstrated by changes in the resistance properties at maximum constriction, was already fully reduced by the end of the treatment period. It may be that the mechanisms involved in regulating lumen size and medial thickness are different. For example, regression of medial thickness may result from the reduction in the levels of angiotensin II or the decrease in wall stress, whereas the widening of the lumen may have resulted from the increase in blood flow associated with the increase in arterial pressure following cessation of therapy.\textsuperscript{37–39}

An additional objective was to determine whether the depressor response or the removal of a trophic factor was the critical component in producing persistent changes in MAP and vascular structure. Two groups of SHR received the same degree of ACE inhibition and therefore a similar reduction in the trophic factor angiotensin II, with one group receiving a diet that was low in sodium. Given that MAP is dependent on the level of sodium intake in the absence of a functional RAS, this paradigm allowed for the manipulation of pressure without using a higher dose of drug. The third group was administered a combination therapy that did not directly involve the removal of a trophic factor. In the enalapril/low salt group there was a significant enhancement of the blood pressure lowering, both during and after withdrawal of treatment, relative to both enalapril and triple therapy groups, but the magnitude of the persistent lowering of MAP was not proportional to the depressor response. It may be that 2 weeks is not sufficient time to allow the full magnitude of changes or that the removal of angiotensin II is of greater importance than the depressor response in inducing persistent changes.

**Perspectives**

The present findings reveal that changes in vascular structure occur much earlier in treatment than previously hypothesized. Based on these results, investigations into the mechanisms of antihypertensive drug-induced vascular remodeling in SHR can focus on a time frame spanning days to weeks and not months as has been done previously. In addition, particularly when combined with those of Woolard et al,\textsuperscript{9} the results emphasize that treatments that effectively reduce blood pres-
sure, but do not directly target the RAS, can also induce persistent changes in MAP and vascular structure. In a recent editorial, Julius indicated that new concepts resulting from experimental treatments of hypertension should provide an impetus for further studies in humans to optimize the usage of currently available antihypertensive agents.

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