Deregulation of Soluble Adhesion Molecules in Resistant Hypertension and Its Role in Cardiovascular Remodeling

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Background: Resistant hypertension (RHTN) and target organ damage are linked to increased inflammatory biomarkers, which may regulate adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1); vascular cell adhesion molecule-1 (VCAM-1); and the platelet (P-selectin) and endothelial (E-selectin) selectins. We investigated a previously unknown relationship between soluble P-selectin (sP-selectin), E-selectin (sE-selectin), ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) with RHTN and target organ damage.

Methods: We included 110 subjects diagnosed for true RHTN and 112 mild-moderate hypertensive (HTN) patients. Blood pressure parameters, pulse wave velocity and left ventricular mass index (LVMI) were measured. Adhesion molecules were measured on ELISA. Both sP-selectin and sE-selectin were increased; in contrast, sICAM-1 was reduced in RHTN compared with HTN patients, while similar sVCAM-1 was noted in the groups. sP-selectin and sVCAM-1 were elevated in the presence of arterial stiffness (sP-selectin: 104±47 vs. 89±54 ng/ml, P<0.05; sVCAM-1: 1,189±411 vs. 1,060±412 ng/ml, P<0.05) and cardiac hypertrophy (sP-selectin: 105±51 vs. 88±43 ng/ml, P<0.05; sVCAM-1: 1,170±433 vs. 1,040±383 ng/ml, P<0.05) in all HTN patients. sP-selectin was associated with target organ damage after adjustment for age and BP. Apart from potential confounders, sE-selectin was a significant indicator of RHTN.

Conclusions: The adhesion molecule sP-selectin plays a role in cardiovascular damage, and sE-selectin in resistance to antihypertensive therapy. (Circ J 2016; 80: 1196–1201)

Key Words: Adhesion molecule; Arterial stiffness; Cardiac hypertrophy; Resistant hypertension
BP Measurement

BP measurements were performed according to the European Society of Hypertension guidelines. Office systolic BP (SBP) and diastolic BP (DBP) were evaluated using a validated digital sphygmomanometer (HEM-907XL, OMRON Healthcare, Bannockburn, IL, USA) with patients in the sitting position after a 10-min rest. Three consecutive measurements with a 3-min interval between them were assessed by a trained health professional. Twenty-four-hour ABPM was carried out using automatic oscillometric monitor (Spacelabs 90207, Spacelabs, Redmond, WA, USA). Patients were instructed to perform normal daily activities and to record activity in a personal diary during the 24-h recording.

Echocardiography

Left ventricular (LV) dimensions were measured using 2-D M-mode echocardiography according to the American Society of Echocardiography (ASE) recommendations. LV mass index (LVMI) was calculated, and patients grouped according to LVMI >95 g/m$^2$ (female patients) and >115 g/m$^2$ (male patients) for comparison of cardiac hypertrophy. Two blinded independent investigators evaluated the echocardiographic measurements using a cardio-vascular ultrasound machine.
(Siemens Acuson CV 70, Munich, Bavaria, Germany) with a multifrequency sector transducer (2–4 MHz). The intraobserver and interobserver coefficients of variation were <9.5% for LVMIs.

### Pulse Wave Velocity (PWV)

PWV is a non-invasive and reproducible method to estimate arterial stiffness. We used the Sphygmocor System (Artcor, Sidney, NSW, Australia) to assess pulse waves transcultaneously using the right common carotid and femoral arteries with patients in the supine position. PWV was calculated as the distance between the supra-sternal notch and the femoral recording site minus the distance from the supra-sternal notch to the carotid recording site, divided by the transit time (distance in meters/time in seconds). We used the mean of 2 PWV and defined PWV ≥10 m/s as high arterial rigidity for comparison of vascular damage. We performed 3 consecutive readings and took the median, if the difference between the 2 measurements was >0.5 m/s.

### Biochemistry

Blood samples were collected at 08.00 hours after overnight fasting with the patients in the seated position. Aldosterone and renin were measured on radioimmunoassay (Immunotech SAS, Marseille, France) and sP-selectin, sE-selectin, sICAM-1 and sVCAM-1 were measured using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA), according to the manufacturer’s instructions.

### Statistical Analysis

Continuous variables are expressed as mean±SD or median (IQR), according to Kolmogorov-Smirnov test. Clinical and biochemistry data for RHTN and HTN were compared using Student’s t-test or Mann-Whitney test. Categorical variables were presented as frequencies and percentages and were compared using chi-squared or Fisher’s exact test. Arterial and biochemistry data for RHTN and HTN were compared using chi-squared or Fisher’s exact test. Arterial and biochemistry data for RHTN and HTN were compared using chi-squared or Fisher’s exact test.

### Results

Table 1 lists the main clinical characteristics of both the HTN and RHTN subjects. RHTN subjects were found to have impaired glycemic and lipid profiles as well as higher aldosterone when compared with HTN subjects (Table 1). Antihypertensive use was also different between the groups. RHTN individuals used more antiplatelet drugs and a greater number of almost all the antihypertensive medication classes than their HTN counterparts. In contrast, the HTN group used more statins, and glucose-lowering drugs were similar between the groups (Table 2).

In addition, both sP-selectin and sE-selectin were increased, while sICAM-1 was reduced in RHTN compared with HTN patients (Figure). Similar sVCAM-1 was seen between the groups.

sP-selectin and sVCAM-1 were elevated in all hypertensive patients with either arterial stiffness (PWV ≥10 m/s) or cardiac hypertrophy (Table 3), but sP-selectin was still associated with target organ damage after adjustment for age and BP. On logistic regression analysis, sP-selectin, sE-selectin and sICAM were independently associated with resistance to antihypertensive therapy. In another model, adjusted for both cardiac and vascular damage, only sE-selectin remained as a significant indicator (Table 4).

### Discussion

Accumulating evidence suggests that RHTN is associated with inflammation and oxidative stress. Because these processes are accompanied by infiltration of immune cells into target tissues including the perivascular sites and the kidney, adhesion molecules are likely to be deregulated in RHTN. Inflammatory cells release mediators including cytokines and metalloproteinases that promote cardiovascular remodeling. We found that sP-selectin and sE-selectin are increased, while sICAM-1 is reduced and sVCAM-1 is similar in RHTN compared with BP-controlled peers. Moreover, apart from potential confounders, sP-selectin was associated with target organ damage and sE-selectin with resistance to antihypertensive therapy.

Adhesion molecules play major roles in the preservation of tissue integrity, mediation of cellular interactions and supply of extracellular matrix contact. Studies have indicated higher levels of adhesion molecules in hypertension. As an example, increased circulating levels have been observed in obese subjects and in patients with target organ damage with coexisting hypertension. It is therefore not surprising that we observe even greater levels of these in RHTN, given that there is a high prevalence of obesity and cardiovascular remodeling in this condition. The mechanisms leading to increases in these are unclear, and might not be similar for the specific markers. In contrast, in vivo and in vitro studies have shown that levels of soluble CAM are elevated in states of increased cell membrane expression in response to endothelial structure and function.

sE-selectin is produced exclusively by endothelial cells, suggesting that it would provide a circulating surrogate indicator of endothelial activation and damage. sE-selectin plays a major role in leukocyte attachment to the endothelium. In doing this, the entry of leukocytes into the subendothelial space is increased, promoting oxidative stress and atherosclerosis. A previous study has shown that sE-selectin is associ-

### Table 2. Medication Use

<table>
<thead>
<tr>
<th>AntiHTN drugs</th>
<th>HTN (n=112)</th>
<th>RHTN (n=110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. classes</td>
<td>2±1</td>
<td>4±1*</td>
</tr>
<tr>
<td>Diuretics</td>
<td>77 (69)</td>
<td>96 (87)*</td>
</tr>
<tr>
<td>MRA</td>
<td>02 (02)</td>
<td>40 (36)*</td>
</tr>
<tr>
<td>ACEI</td>
<td>18 (16)</td>
<td>41 (37)*</td>
</tr>
<tr>
<td>ARB</td>
<td>82 (73)</td>
<td>58 (53)*</td>
</tr>
<tr>
<td>CCB</td>
<td>48 (43)</td>
<td>88 (80)*</td>
</tr>
<tr>
<td>β-blockers</td>
<td>15 (13)</td>
<td>76 (70)*</td>
</tr>
<tr>
<td>Others</td>
<td>01 (01)</td>
<td>31 (28)*</td>
</tr>
<tr>
<td>Statins</td>
<td>87 (78)</td>
<td>57 (52)*</td>
</tr>
<tr>
<td>Glucose-lowering drugs</td>
<td>42 (38)</td>
<td>53 (48)</td>
</tr>
<tr>
<td>Antiplatelet drugs</td>
<td>19 (17)</td>
<td>62 (56)*</td>
</tr>
</tbody>
</table>

Data given as mean±SD or n (%). *P<0.05. ACEI, angiotensin-converting enzyme inhibitors; AntiHTN, antihypertensive; ARB, angiotensin receptor blockers; CCB, calcium channel blockers; MRA, mineralocorticoid receptor antagonists. Other abbreviations as in Table 1.
Adhesion Molecules in RHTN

Table 3. Adhesion Molecule Level in Target Organ Damage (n=222)

<table>
<thead>
<tr>
<th></th>
<th>PWV</th>
<th></th>
<th>LVMI</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10 m/s</td>
<td>≥10 m/s</td>
<td>&lt;95/115 g/m²</td>
<td>≥95/115 g/m²</td>
</tr>
<tr>
<td>sP-selectin</td>
<td>89±45</td>
<td>104±47*</td>
<td>88±43</td>
<td>105±51*</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>43±17</td>
<td>47±16</td>
<td>44±16</td>
<td>45±17</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>302±115</td>
<td>299±117</td>
<td>301±116</td>
<td>300±112</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>1,060±412</td>
<td>1,189±411</td>
<td>1,040±383</td>
<td>1,170±433</td>
</tr>
</tbody>
</table>

*P<0.05 for †PWV ≥10 vs. PWV <10 m/s and ‡LVMI ≥95/115 vs. LVMI <95/115 g/m². sE-selectin, soluble endothelial selectin; sICAM-1, soluble intracellular adhesion molecule-1; sP-selectin, soluble platelet selectin; sVCAM-1, soluble vascular cell adhesion molecule-1. Other abbreviations as in Table 1.

Table 4. Indicators of RHTN

<table>
<thead>
<tr>
<th></th>
<th>Median cut-offs</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sP-selectin &gt;89.14 (ng/ml)</td>
<td>2.58 (1.22–5.44)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>sE-selectin &gt;44.28 (ng/ml)</td>
<td>2.08 (1.03–4.20)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>sICAM-1 &lt;291.5 (ng/ml)</td>
<td>2.17 (1.05–4.49)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>sVCAM-1 &gt;1,068 (ng/ml)</td>
<td>1.20 (0.56–2.56)</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sP-selectin &gt;89.14 (ng/ml)</td>
<td>1.32 (0.56–3.01)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>sE-selectin &gt;44.28 (ng/ml)</td>
<td>2.81 (1.23–6.44)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>sICAM-1 &lt;291.5 (ng/ml)</td>
<td>2.04 (0.88–4.72)</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>sVCAM-1 &gt;1,068 (ng/ml)</td>
<td>1.32 (0.54–3.18)</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

†Adjusted for age, gender, race and BMI; ‡adjusted for age, gender, race, BMI, PWV and LVMI. Abbreviations as in Tables 1,3.
ated with higher SBP and DBP as well as body mass index (BMI), fasting glucose and C-reactive protein in otherwise healthy subjects, suggesting that E-selectin might be a useful biomarker for metabolic disorders related to RHTN. This supports the present finding that sE-selectin is associated with resistance to antihypertensive therapy and thus, increased sE-selectin is associated with high cardiovascular risk due to the lack of BP control. HTN might not be severely affected by elevated BP, or other risk factors may have to accompany HTN in order to increase E-selectin expression.

sP-selectin – a plasma marker of platelet activation that facilitates the adhesion of platelets to leukocytes and endothelium – is associated with hypertension and adverse cardiovascular prognosis. P-selectin was independently associated with arterial stiffness and intima-media thickness. The present study identified the role of sP-selectin in cardiac and vascular damage in all hypertensive subjects (RHTN and HTN) after adjustment for age and BP, which are known to contribute to the development and progression of cardiovascular remodeling. In this context, inhibition of neointimal formation after arterial injury in an experimental design of P-selectin deficiency, and the secretion of thromboxane A2 by activated platelets, may explain the mechanism of involvement of sP-selectin in functional alteration of target organ damage. Moreover, synthesis of P-selectin is increased by some inflammatory mediators. Upregulation of this in endothelial cells has been attributed to tumor necrosis factor (TNF)-α, being mediated by nuclear factor-κB transcription factor. Indeed, we previously demonstrated that RHTN patients have increased TNF-α, as well as target organ damage compared with normotensive subjects, which could explain the present findings and also infer that increased platelet activation is occurring in the RHTN group. The relative contribution of inflammation and endothelial activation in the structural vascular and cardiac changes, however, beyond the BP-mediated effects, in hypertension remains unclear.

ICAM-1 and VCAM-1, identified as endothelial dysfunction biomarkers, play a key role in the early stages of the inflammatory response to facilitate leukocyte adhesion to vascular endothelial cells. Interestingly, soluble ICAM-1 and VCAM-1 did not change after the invasive procedure of renal denervation. It was suggested that the prolonged endothelial remodeling process in atherosclerotic lesions might cause the persistence of increased biomarker concentrations, even apart from sympathetic stimulus. We found that ICAM-1 is reduced in RHTN compared with HTN, probably by the chronic status of resistance to therapy, implying that in essential hypertension there is a very early activation of the endothelial adhesion molecules that favors atherosclerosis. In contrast, no change in sVCAM-1 was found between the present groups. In another study age, but not BP, was the primary determinant of sVCAM-1 level. Similarly, we found that age was correlated with sVCAM-1 in both the RHTN and HTN patients (r=0.30; P<0.001), which also may explain why the controlled subjects had similarly levels of sVCAM-1 as RHTN, given that the former group was older. Finally supporting this, the noted relationship between sVCAM-1 and target organ damage was no longer significant after age and BP adjustment.

Current antihypertensive approaches exert pleiotropic cardiovascular effects due to their anti-inflammatory properties. Angiotensin-converting enzyme inhibitors (ACEI) and mineralocorticoid receptor antagonists, but not angiotensin receptor blockers, have significantly decreased plasma circulating adhesion molecule levels, including that of ICAM-1. These findings, in part, may explain the higher ICAM-1, in contrast to the other soluble adhesion molecules, in controlled HTN compared with RHTN, due to the lower prevalence of ACEI and spironolactone use in controlled HTN. Indeed, this may support the change in the expression of soluble molecules between the groups. On multivariate linear regression analysis adjusted for age, race, BMI, gender and resistance to antihypertensive treatment, neither the different classes of antihypertensive drugs, nor statins, glucose-lowering and antiplatelet drugs were independently associated with adhesion molecules. The only 2 exceptions were: (1) diuretics were negatively associated with sE-selectin (β coefficient=-15.6; SE=5.4; P=0.01) and (2) β-blockers were positively associated with sICAM-1 (β coefficient=73.0; SE=31.2; P=0.02). These possible sources of interference, however, did not affect the results, given that RHTN subjects had increased sE-selectin and reduced sICAM-1, despite the greater prevalence of diuretic and β-blockers agents. It is worth noting that the lack of standard therapy in the present RHTN and HTN groups was due to the individualized care. In addition, the antihypertensive and non-antihypertensive drugs could not be withdrawn due to ethical concerns. Therefore, further large clinical studies are required to determine the potential favorable effects of hypertensive treatments to clarify whether these biomarker changes are related to BP reduction or to additional effects.

Some limitations should be mentioned. This was a cross-sectional study therefore causal relationship cannot be inferred. It is not evident whether the adhesion molecules promote the target organ damage and hypertension or whether the high BP causes cardiovascular remodeling which then initiates activation of cell adhesion cascade. The negative finding for sVCAM-1 may be attributed to low power to detect differences between the groups.

The present findings have clarified the effects of adhesion molecules, in particular sP-selectin on arterial stiffness and cardiac hypertrophy, and sE-selectin on resistance to antihypertensive therapy. These findings raise the question of whether early treatment and intensive monitoring of hypertension is crucial reducing the risk for cardiovascular complications and to achieve BP control, although a normotensive group is needed to confirm this hypothesis. Prospective studies are needed to establish the biological functions of soluble forms and their association with cardiovascular risk, and the regulation of the adhesion process itself.

Acknowledgments
This study was supported by the São Paulo Research Foundation (FAPESP), the National Council for Scientific and Technological Development (CNPq); and Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil.

Disclosures
All authors declare no conflict of interest.

References


