Increased Levels of Atherosclerosis Markers in Salt-Sensitive Hypertension

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Background: Salt sensitivity in essential hypertension is associated with both endothelial dysfunction and increased cardiovascular risk. We evaluated several serum markers of atherosclerosis and endothelial function in a group of essential hypertensive patients classified on the basis of their salt sensitivity.

Methods: Forty-three patients were classified as having salt-sensitive (20 subjects) or salt-resistant (23 subjects) hypertension on the basis of their 24-h blood pressure (BP) response from low salt (50 mmol/d) to high salt (250 mmol/d) intake. Endothelium-dependent and independent responses were measured in the forearm previously to salt manipulation. High-sensitivity C-reactive protein (CRP), soluble intercellular adhesion molecule type 1 (sICAM-1), soluble vascular cell adhesion molecule type 1 (sVCAM-1), e-selectin, p-selectin, interleukin-6 (IL-6), monocyte chemotactic protein type 1 (MCP-1), matrix metalloproteinases types 1, 2, and 9 (MMP-1, MMP-2, and MMP-9), and the tissue inhibitor of metalloproteinases type 1 (TIMP-1) were measured in serum on the last day of both low salt and high salt intakes.

Results: Compared to salt-resistant patients, salt-sensitive hypertensives showed age-adjusted increased levels of p-selectin ($P = .006$), e-selectin ($P = .042$), and MCP-1 ($P = .036$), although differences in e-selectin were not maintained after adjustment for BP values. Moreover, salt-sensitive subjects exhibited decreased serum levels of MMP-9 ($P = .007$) and increased levels of TIMP-1 ($P = .045$). No differences in serum CRP, sICAM-1, sVCAM-1, or IL-6 were observed between salt-sensitive and salt-resistant patients. Finally, maximal acetylcholine-induced vasodilation (319% ± 153% vs 414% ± 178% increase in forearm blood flow; $P = .022$ age-adjusted) was significantly impaired in salt-sensitive hypertensives.

Conclusions: Serum markers of inflammation, especially selectins and chemokines, as well as markers of vascular remodeling, and endothelium-dependent vasodilation are altered in salt-sensitive hypertension. These alterations could help to explain the greater target organ damage and cardiovascular risk observed in salt-sensitive subjects.

Key Words: Cell adhesion molecules, inflammation, extracellular matrix, endothelium, dietary sodium, sodium-dependent hypertension, atherosclerosis.

Various epidemiologic and interventional studies have demonstrated a clear relationship between salt intake and hypertension. However, blood pressure (BP) response to increased dietary salt is heterogeneous among individuals, a phenomenon known as salt sensitivity. Although salt sensitivity is well established in experimental and human hypertension, the pathophysiologic mechanisms leading to such individual susceptibility remain unresolved. It has been suggested that abnormalities in renal sodium regulation, in the renin-angiotensin system, in the sympathetic nervous system, and in the transmembrane sodium transport, are all involved in the pathogenesis of salt sensitivity.

Two independent groups have suggested that the presence of salt sensitivity is associated with an increased incidence of death or cardiovascular complications, representing an accelerated atherosclerotic process in the vascular wall. Moreover, salt-sensitive patients tend to be nondippers, and more frequently exhibit left ventricular hypertrophy and microalbuminuria. In addition, salt-sensitive hypertension is accompanied by more pronounced endothelial dysfunction.

This endothelial dysfunction may be the pathophys-
ologic link between salt sensitivity and accelerated atherosclerosis. It is well known that the intact endothelium not only contributes to vasodilation, but also has antiagregant, anti-inflammatory, and antiproliferative properties and that endothelial dysfunction is associated with an increased risk of atherosclerotic events. Several acute phase reactants, soluble adhesion molecules, chemokines, and metalloproteinases are altered in patients at risk of cardiovascular events, such as hypertensives, diabetics, or those with left ventricular hypertrophy. Moreover, in patients with coronary heart disease some of these serum markers have prognostic implications.

One previous study measured soluble intercellular and vascular cell adhesion molecules and e-selectin in patients classified on the basis of salt sensitivity, finding an elevation of the latter. We hypothesized that not only inflammation, but also metalloproteinases, could be altered in salt-sensitive patients compared to salt-resistant ones. Thus, the aim of the study was to compare levels of soluble adhesion molecules, selectins, chemokines, and metalloproteinases in essential hypertensive patients classified on the basis of salt sensitivity.

Methods

Patient Selection

The study population included 43 nontreated, newly diagnosed essential hypertensive patients consecutively recruited from the Hypertension Unit, Hospital Clinic, Barcelona, Spain. There were 29 men and 14 women with a mean age of 40 years (range, 26 to 58 years). Subjects with hypercholesterolemia (total cholesterol >6 mmol/L [230 mg/dL]), diabetes mellitus, impaired renal function (serum creatinine >132 µmol/L [1.5 mg/dL]), or previous history of coronary or cerebrovascular disease were excluded from the study, as were patients who drank more than 40 g of ethanol per day, those under chronic treatment with nonsteroidal anti-inflammatory drugs, and women taking oral contraceptives or estrogen replacement therapy.

Assessment of Salt Sensitivity

All patients gave informed consent. The protocol was approved by the Ethics Committee of the Hospital Clinic and by the Spanish Health Authority (Protocol F.I.S. 00/0435). Essential hypertensive patients were placed on a baseline low salt diet containing 50 mmol of Na+ during 14 days. This diet was supplemented in a random, single-blind fashion by placebo tablets during 7 days (low salt period) and by NaCl tablets, 200 mmol/d (high salt period) during another 7 days. Thus, total NaCl intake during the high salt period was 250 mmol/d.

On the last day of both low salt and high salt periods, 24-h ambulatory BP monitoring (ABPM) was performed with an automated, noninvasive oscillometric device (SpaceLabs 90217; SpaceLabs Inc., Redmon, WA). Blood pressure was registered automatically at 15-min intervals for 24 h. Salt-sensitive hypertension was defined as a significant increase (P < .05; >4 mm Hg) of 24-h mean BP from low to high salt intake.

Measurement of Endothelial Function

These procedures have been previously described in detail. Briefly, before dietary manipulations forearm endothelium-dependent and independent vasodilation were determined by measuring forearm blood flow by strain-gauge venous plethysmography (EC5R-Hokanson, Bellevue, WA) at baseline (0.9% saline infusion), after increasing doses of acetylcholine (from 0.15 to 15 µg/100 mL forearm tissue/min), and after increasing doses of sodium nitroprusside (from 1 to 4 µg/100 mL of forearm tissue/min). The acetylcholine dose–response curve was repeated after the addition of 100µg/100 mL of forearm tissue/min of the nitric oxide (NO) synthase inhibitor L-NMMA (N-nmonomethyl-L-arginine).

Endothelium-dependent vasodilation (EDV), endothelium-independent vasodilation (EIV), and the effect of L-NMMA on acetylcholine response were presented as a percentage increase in forearm blood flow above the baseline.

Measurement of Atherosclerosis Markers

Fasting venous blood samples were drawn on the last day of both low salt and high salt periods between 7 AM and 9 AM. Sera were obtained by centrifugation and stored at −80°C until analysis. High-sensitivity C-reactive protein (hs-CRP) was determined by immunonephelometry (Dade-Behring S.A., Barcelona, Spain). Adhesion molecules, selectins, chemokines, and metalloproteinases were determined in duplicate wells using commercially available ELISA assays from R & D Systems, Minneapolis, MN (soluble vascular cell adhesion molecule type 1 [sVCAM-1], soluble intercellular adhesion molecule type 1 [sICAM-1], e-selectin, p-selectin, interleukin-6 [IL-6], and monocyte chemotactic protein type 1 [MCP-1]) or reagents from Amersham Biosciences (matrix metalloproteinases types 1, 2, and 9 [MMP-1, MMP-2, MMP-9] and tissue inhibitor of metalloproteinases type 1 [TIMP-1]).

Statistical Analysis

Values are expressed as mean ± SD or median (interquartile range). At least, 20 patients in each group (salt sensitive and salt resistant) were needed to detect a 25% difference in serum atherosclerosis markers assuming a common standard deviation of 25% of the mean with a 80% statistical power and 0.05 α error.

Differences between biochemical markers obtained at low and high salt intakes in salt-sensitive and salt-resistant patients were calculated by analysis of variance with the repeated measures design (for CRP and MMP-1 after log
transformation). The effect of both salt sensitivity and dietary salt manipulation was tested for all variables. Moreover, differences between salt-sensitive and salt-resistant subjects were adjusted by age and 24-h mean BP obtained at both low salt and high salt intakes (covariates). Differences in endothelium-dependent and independent responses between salt-sensitive and salt-resistant subjects were analyzed by means of one-way ANOVA adjusted by age. The relationship between serum markers and changes in 24-h BP was assessed by a stepwise linear regression analysis.

**Results**

**General Characteristics of Essential Hypertensive Patients**

Table 1 shows the clinical characteristics of the 43 essential hypertensive patients included in the study. No differences were observed in terms of gender distribution, body mass index (BMI), and baseline office systolic BP between salt-sensitive and salt-resistant essential hypertensive patients. However, salt-sensitive patients were slightly older and had higher diastolic BP values.

**Diagnosis of Salt-Sensitive Hypertension**

Salt-sensitive hypertension was diagnosed in 20 patients in whom 24-h mean BP significantly increased ($P < .05$) when switched from low to high salt intake. The mean increase in 24 h mean BP was $10.7 \pm 4.5$ mm Hg (from $101 \pm 11$ mm Hg at the end of the low salt period to $112 \pm 11$ mm Hg at the end of the high salt period) (Table 2). The remaining 23 patients were diagnosed as having salt-resistant hypertension. The change in 24 h mean BP was $-0.9 \pm 4.1$ mm Hg (from $101 \pm 10$ mm Hg to $100 \pm 11$ mm Hg). Table 2 also shows systolic and diastolic BP and heart rate during low and high salt intakes in salt-sensitive and salt-resistant essential hypertensive patients.

To check adherence of patients to dietary changes, urinary sodium excretion was measured at the end of both periods (Table 2).

**Differences in Endothelium-Dependent and Independent Response Between Salt-Sensitive and Salt-Resistant Hypertensive Subjects**

As mentioned previously, EDV and EIV were estimated as the percentage increase in forearm blood flow in response to acetylcholine or sodium nitroprusside. As shown in Fig. 1, salt-sensitive patients exhibited an impaired maximal acetylcholine-induced vasodilation ($319\% \pm 153\% v 414\% \pm 178\%; P = .022$ adjusted by age), compared to salt-resistant hypertensives. Moreover, the effect of L-NMMA infusion on this endothelium-dependent response was blunted in the former group ($72\% v 170\%; P = .002$). No differences were observed in endothelium-independent response ($417\% \pm 139\% v 394\% \pm 131\%; P = .550$) between both groups of patients.

**Differences in Serum Inflammatory Markers Between Salt-Sensitive and Salt-Resistant Subjects During Low and High Salt Intakes**

Table 3 shows values of inflammatory markers in salt-sensitive and salt-resistant patients measured at low and high salt intakes. No effect of dietary salt changes was observed in any of the measured parameters, but the comparison of salt-sensitive and salt-resistant patients revealed differences in the inflammatory markers measured. Thus, age-adjusted p-selectin ($P = .006$) and MCP-1 ($P = .036$) were significantly higher in salt-sensitive hypertensive patients and this significance was maintained after adjustment for BP differences. Values of e-selectin were also higher in salt-sensitive subjects ($P = .042$), although differences lost their statistical significance after adjustment for BP differences.

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**Table 1.** Baseline clinical characteristics of salt-sensitive and salt-resistant essential hypertensive patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salt sensitive</th>
<th>Salt resistant</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>12 / 8</td>
<td>17 / 6</td>
<td>.515</td>
</tr>
<tr>
<td>Age (y)</td>
<td>42.8 $\pm$ 7.6</td>
<td>36.9 $\pm$ 7.9</td>
<td>.016</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.8 $\pm$ 17.5</td>
<td>80.9 $\pm$ 15.8</td>
<td>.829</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>28.7 $\pm$ 7.9</td>
<td>27.5 $\pm$ 5.2</td>
<td>.522</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>15</td>
<td>26</td>
<td>.234</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>162.6 $\pm$ 16.2</td>
<td>157.8 $\pm$ 10.6</td>
<td>.247</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>96.9 $\pm$ 7.9</td>
<td>92.4 $\pm$ 6.3</td>
<td>.039</td>
</tr>
<tr>
<td>Urinary albumin excretion (mg/24 h)</td>
<td>7.03 [3.7–15.6]</td>
<td>4.6 [2.5–17]</td>
<td>.382</td>
</tr>
</tbody>
</table>

BMI = body mass index; BP = blood pressure. Values as mean $\pm$ SD, or median [interquartile range].
Differences in Metalloproteinases Between Salt-Sensitive and Salt-Resistant Subjects During Low and High Salt Intakes

Table 4 shows the values of the three metalloproteinases measured (MMP-1, MMP-2, and MMP-9) and their tissue inhibitor (TIMP-1) in salt-sensitive and salt-resistant subjects during low and high salt intakes. No effect of dietary salt changes was observed in any of these parameters, but age-adjusted values of MMP-9 were significantly lower ($P = .007$) and TIMP-1 significantly higher ($P = .045$) in salt-sensitive, compared to salt-resistant patients. These differences were maintained after adjustment for mean BP.

Correlation Between Atherosclerosis Markers and Changes in BP Induced by High Salt Intake

The correlation between atherosclerosis markers and salt sensitivity was also assessed by a stepwise multiple linear regression using the 24-h mean BP increase with salt intake as the dependent variable, and atherosclerosis markers (the mean of both measures) as independent variables. We found that after age adjustment, p-selectin ($\beta = .035; P = .028$), sVCAM-1 ($\beta = .037; P < .001$), and MMP9 ($\beta = -.017; P = .029$) were associated with a BP increase induced by dietary salt (adjusted $R^2 = 0.602$).

Discussion

This study shows that salt sensitivity in essential hypertension is associated with increased circulating levels of atherosclerosis markers. These biomarkers include inflammatory mediators, such as selectins and MCP-1, and a lack of equilibrium between metalloproteinases and their tissue inhibitor TIMP-1, favoring the latter. These findings were demonstrated by comparing salt-sensitive and salt-resistant hypertensives before and after BP adjustment. These
Table 3. Serum inflammatory markers during low and high salt intake in salt-sensitive and salt-resistant hypertensive patients

<table>
<thead>
<tr>
<th></th>
<th>Salt Sensitive</th>
<th>Salt Resistant</th>
<th>P (effect of salt sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Salt</td>
<td>High Salt</td>
<td>Adjusted by Age</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.56 ± 0.39</td>
<td>0.55 ± 0.46</td>
<td>0.36 ± 0.27</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td>205 ± 83</td>
<td>204 ± 95</td>
<td>209 ± 50</td>
</tr>
<tr>
<td>sVCAM-1 (ng/mL)</td>
<td>359 ± 92</td>
<td>353 ± 64</td>
<td>327 ± 73</td>
</tr>
<tr>
<td>p-Selectin (ng/mL)</td>
<td>200 ± 62</td>
<td>193 ± 57</td>
<td>140 ± 51</td>
</tr>
<tr>
<td>e-Selectin (ng/mL)</td>
<td>44.1 ± 13.0</td>
<td>44.3 ± 12.6</td>
<td>35.8 ± 12.5</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.48 ± 0.73</td>
<td>2.63 ± 1.03</td>
<td>2.17 ± 0.68</td>
</tr>
<tr>
<td>MCP-1 (pg/mL)</td>
<td>314 ± 56</td>
<td>340 ± 50</td>
<td>266 ± 80</td>
</tr>
</tbody>
</table>

Table 4. Metalloproteinases and their tissue inhibitor measured during low and high salt intake in salt-sensitive and salt-resistant hypertensive patients

<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Low Salt</td>
<td>High Salt</td>
<td>Adjusted by Age</td>
</tr>
<tr>
<td>MMP-1 (ng/mL)</td>
<td>12.5 ± 7.5</td>
<td>17.5 ± 7.2</td>
<td>13.4 ± 11.5</td>
</tr>
<tr>
<td>MMP-2 (ng/mL)</td>
<td>1057 ± 154</td>
<td>1253 ± 154</td>
<td>1005 ± 217</td>
</tr>
<tr>
<td>MMP-9 (ng/mL)</td>
<td>185 ± 87</td>
<td>215 ± 92</td>
<td>339 ± 142</td>
</tr>
<tr>
<td>TIMP-1 (ng/mL)</td>
<td>668 ± 152</td>
<td>688 ± 178</td>
<td>549 ± 197</td>
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</tbody>
</table>
results strengthen the idea that endothelial dysfunction plays a role in the pathogenesis of salt-sensitive hypertension, as indicated by previous results from our group showing impaired endothelial-dependent vasodilation in the forearm circulation of salt-sensitive hypertensive patients, which have been confirmed in the present study. Increased levels of atherosclerosis markers and impaired endothelium-dependent vasodilation may help to explain the greater frequency of target organ damage and the poorer prognosis in hypertensive subjects classified as having salt-sensitive hypertension.7,8

Increased salt intake is related to the development of hypertension and confers an increased risk of cardiovascular disease to individuals who are more salt sensitive.7,8 Salt-sensitive hypertension is also related to increased target organ damage, including left ventricular hypertrophy, renal impairment, and vascular endothelial dysfunction.12 This association between salt sensitivity and endothelial dysfunction was the basis of the hypothesis of this study, which was that abnormal endothelium in salt-sensitive hypertensive patients may be responsible for enhanced vascular inflammation leading to greater cardiovascular risk.

We found that salt-sensitive hypertensive patients have increased levels of several inflammatory markers, especially selectins and MCP-1. Moreover, p-selectin was associated with a BP increase with salt intake in the multivariate analysis. E-selectin is produced in endothelial cells, whereas p-selectin is found in both platelets and endothelial cells. They are markers of either endothelium or platelet activation and they both act in the first steps of the atherosclerotic process by favoring rolling of leukocytes to the endothelial surface.8 Increased serum p-selectin levels have been related to the development of future cardiovascular events in healthy women.30 Moreover, e-selectin levels are associated with poor prognosis in patients with coronary artery disease.22 The higher levels of both p-selectin and e-selectin found in salt-sensitive hypertensives in the present study are in agreement with a previous study from Ferri et al.,25 who also found increased serum e-selectin in salt-sensitive patients. In that study levels of p-selectin were not measured.

We also found increased serum MCP-1 in salt-sensitive patients. The MCP-1 is responsible for monocyte recruitment and trafficking, has been found in human and animal atherosclerotic lesions, and has recently been associated with a poor prognosis in patients with acute coronary syndromes.23 The MCP-1 production clearly increases in the presence of endothelial dysfunction and classic atherosclerotic risk factors, of which both situations are more common in salt-sensitive subjects.6,12

We found no difference in levels of soluble adhesion molecules (sICAM-1 and sVCAM-1) or IL-6 between patients with or without salt-sensitive hypertension, although in the multiple linear regression analysis sVCAM-1 was associated with the BP increase with dietary salt. Although they have been associated with hypertension and cardiovascular disease, they do not represent biomarkers of a more subtle cardiovascular risk related to the presence of salt sensitivity in hypertension. In a previous study of salt-sensitive hypertension, no differences in sICAM-1 and sVCAM-1 were observed between patients with salt-sensitive or salt-resistant hypertension.25

Finally, we found that, compared to salt-resistant, salt-sensitive hypertensive patients had lower levels of MMP-9 and higher levels of the tissue inhibitor of metalloproteinases TIMP-1. The MMP-9 was also inversely associated with BP increase with salt in the multivariate analysis. Matrix metalloproteinases are produced by macrophages and smooth muscle cells and play an important role in vascular remodeling. Two studies have found lower levels of various metalloproteinases (MMP-1, MMP-2, and MMP-9), and increased levels of the tissue inhibitor (TIMP-1) in essential hypertensive patients compared to normotensive subjects. Moreover, TIMP-1 has been related to left ventricular hypertrophy in essential hypertensives. The present study also confirms that, in salt-sensitive hypertensive subjects, the relationship between extracellular matrix degradation (as represented by low MMP-9 levels) and the inhibition of such degradation (as represented by high TIMP-1 levels) is skewed to the latter, probably representing more pronounced collagen deposition in the vascular wall.

The present study has some limitations due to the low number of patients included, the fact that salt-sensitive patients were somewhat older and more severe hypertensive compared to salt-resistant ones, and the evaluation of target organ damage did not include the measurement of left ventricular mass. However, all the differences between salt-sensitive and salt-resistant patients were maintained after adjustment by age and BP. Moreover, the relationship between salt sensitivity and left ventricular hypertrophy has been previously reported.10,11

In conclusion, salt-sensitive hypertension is related to several abnormalities in biomarkers of atherosclerosis and remodeling, including inflammation (high levels of selectins and chemokines) and extracellular matrix degradation (low MMP-9 and high TIMP-1). Salt-sensitive hypertension is also characterized by an impaired endothelial dysfunction. These alterations could help to explain the greater target organ damage and cardiovascular risk observed in salt-sensitive subjects.

References


