Inflammation as a Mediator of the Link between Mild to Moderate Renal Insufficiency and Endothelial Dysfunction in Essential Hypertension

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The relationship among inflammation (plasma high-sensitivity C-reactive protein [CRP]), endothelial function (hemodynamic response to acetylcholine [ACh] in the forearm), and renal function (serum creatinine and GFR [Modification of Diet in Renal Disease formula]) was investigated in 264 never-treated individuals with uncomplicated essential hypertension and serum creatinine within the normal range. Multiple regression models of renal function (creatinine) were constructed in sequence including Framingham risk factors as well the hemodynamic response to ACh and plasma CRP. The inclusion of endothelial function into a model based on Framingham risk factors added highly significant (P < 0.001) power to this model (15%). Of note, in an alternative model that included CRP (instead of endothelial function), the creatinine variance explained by this factor was two times higher (10%) than that associated with endothelial function in the first model. In the full model that included both endothelial function and CRP, CRP maintained a much stronger independent link with the outcome measure than endothelial function. In individuals with untreated, uncomplicated essential hypertension, multivariate modeling indicated that inflammation is a crucial mechanism mediating the endothelial–renal function link. The proatherogenic potential of inflammation associated with subtle impairment in renal function may contribute to the cardiovascular risk of essential hypertension.


Mild to moderate renal insufficiency now has emerged fully as a major public health problem. Population-based studies (1) and secondary analyses of intervention studies in hypertensive patients (2) and in selected patients at high cardiovascular risk (3) have shown coherently that classical risk factors such as hypertension, diabetes, hyperlipidemia, smoking, and overweight/obesity represent major correlates of renal dysfunction. Although its importance for prevention is still debated, convincing evidence was provided recently that low-grade inflammation, as measured by highly-sensitivity C-reactive protein (CRP), is an early marker of renal dysfunction and of cardiovascular events in the general population (4) and a risk factor for cardiovascular complications and progressive renal insufficiency in patients with chronic kidney disease (CKD) (5). We recently reported an association between mild to moderate renal impairment and the hemodynamic response to acetylcholine (ACh), an established test of endothelial function, in a large group of never-treated individuals with uncomplicated essential hypertension (6). A corollary finding in this study was that renal function also was associated with serum CRP, suggesting that inflammation may be a critical factor mediating endothelial dysfunction and renal insufficiency in individuals with essential hypertension. Although merely associative, these observations in a carefully selected population of hypertensive individuals are important in that they may be hypothesis generating for understanding the high risk associated with mild to moderate renal insufficiency.

In this study, we therefore performed a secondary analysis in the same database to test the influence of inflammation, as measured by serum CRP, on the association between altered hemodynamic response to ACh and mild to moderate renal insufficiency in individuals with essential hypertension. This analysis, based on multivariate modeling, suggests that inflammation is a critical factor in the chain of events that lead to renal dysfunction in hypertensive individuals.

Materials and Methods

The local ethics committee approved the study, and all participants gave written, informed consent for all procedures.

Patients

A total of 246 never-treated individuals who had uncomplicated essential hypertension (Table 1) and had simultaneous measurements
Modification of Diet in Renal Disease equation developed by Levey and implemented in an autoanalyzer. The GFR was estimated by the automated technique based on the measurement of Jaffe chromogen ACh was considered for statistical analysis.

The measurement of the vasodilatory response to ACh, we adopted the protocol by Panza et al. (7), and the detailed description of these tests was described elsewhere (6). For our study, the maximal response to ACh was considered for statistical analysis.

Serum creatinine was measured in the routine laboratory by an automated technique based on the measurement of Jaffé chromogen and implemented in an autoanalyzer. The GFR was estimated by the Modification of Diet in Renal Disease equation developed by Levey et al. (8). CRP was measured by a high-sensitivity turbidimetric immunoassay (Behring, Marburg, Germany).

of high-sensitivity CRP and forearm blood flow studies were selected from a population of approximately 3500 individuals who were referred to the hypertension clinic of the University Hospital of Catanazaro University between September 1994 and January 2003. To be selected, patients had to have newly diagnosed essential hypertension, and gender. Weaker associations existed with systolic pressure and heart rate, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and serum glucose. Multiple regression models of serum creatinine and GFR (including all variables of the “basic” model and either the GFR or creatinine) were confronted by comparing the corresponding R² and associated P values. Data are expressed as standardized regression coefficient (β), partial correlation coefficient, and P value. All calculations were made using a standard statistical package (SPSS for Windows Version 9.0.1, Chicago, IL).

Results

In Table 1, we categorized hypertensive individuals on the basis of the GFR into four quartiles. As expected, the GFR was strongly associated (in an inverse manner) with age and male gender. Weaker associations existed with systolic pressure and fasting plasma glucose (P = 0.12 and 0.08 for trend, respectively). These associations were substantially confirmed by linear correlation analyses, which again identified age (r = −0.27) and gender (r = 0.46) as fundamental correlates of the GFR. Furthermore, the link between renal function and these risk factors (age, gender, smoking, body mass index, systolic and pulse pressure, heart rate, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and serum glucose). Multiple regression models of serum creatinine and GFR (including all variables of the “basic” model and either the GFR or creatinine) were confronted by comparing the corresponding R² and associated P values. Data are expressed as standardized regression coefficient (β), partial correlation coefficient, and P value. All calculations were made using a standard statistical package (SPSS for Windows Version 9.0.1, Chicago, IL).

Table 1. Main demographic, somatometric, clinical, and biochemical data of patients divided into GFR quartiles

<table>
<thead>
<tr>
<th>GFR Quartile</th>
<th>1 (&lt;71.1 ml/min per 1.73 m²)</th>
<th>2 (71.2 to 84.4 ml/min per 1.73 m²)</th>
<th>3 (84.5 to 97.0 ml/min per 1.73 m²)</th>
<th>4 (&gt;97.0 ml/min per 1.73 m²)</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>52.8 ± 10.6</td>
<td>47.7 ± 10.8</td>
<td>46.1 ± 8.9</td>
<td>44.7 ± 9.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender (n [%])</td>
<td>20 (30)</td>
<td>19 (30)</td>
<td>50 (71)</td>
<td>52 (81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers (n [%])</td>
<td>17 (26)</td>
<td>11 (17)</td>
<td>18 (26)</td>
<td>15 (27)</td>
<td>0.95</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 ± 3.8</td>
<td>26.9 ± 3.5</td>
<td>27.9 ± 3.5</td>
<td>26.8 ± 3.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>151.4 ± 14.3</td>
<td>152.9 ± 15.3</td>
<td>151.0 ± 17.3</td>
<td>147.6 ± 14.9</td>
<td>0.12</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>93.0 ± 10.1</td>
<td>93.7 ± 12.3</td>
<td>93.7 ± 12.4</td>
<td>90.9 ± 10.8</td>
<td>0.32</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>58.4 ± 11.1</td>
<td>59.2 ± 10.7</td>
<td>57.3 ± 12.3</td>
<td>56.7 ± 10.3</td>
<td>0.26</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>71.4 ± 8.8</td>
<td>73.2 ± 8.7</td>
<td>73.4 ± 9.4</td>
<td>71.3 ± 10.5</td>
<td>0.98</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.15 ± 0.17</td>
<td>0.91 ± 0.12</td>
<td>0.88 ± 0.10</td>
<td>0.78 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>207.6 ± 31.9</td>
<td>210.8 ± 32.2</td>
<td>206.5 ± 33.1</td>
<td>202.7 ± 32.9</td>
<td>0.29</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>131.4 ± 33.8</td>
<td>136.9 ± 31.7</td>
<td>127.8 ± 33.2</td>
<td>126.7 ± 31.5</td>
<td>0.20</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>51.5 ± 12.1</td>
<td>51.1 ± 13.7</td>
<td>53.9 ± 11.2</td>
<td>51.2 ± 11.5</td>
<td>0.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>119.8 ± 39.1</td>
<td>114.0 ± 39.6</td>
<td>123.2 ± 37.7</td>
<td>116.6 ± 40.6</td>
<td>0.98</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>95.6 ± 12.5</td>
<td>92.2 ± 9.5</td>
<td>95.2 ± 11.9</td>
<td>98.3 ± 11.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Maximal vasodilatory response to ACh (%)</td>
<td>254 (150 to 336)</td>
<td>242 (152 to 381)</td>
<td>300 (222 to 439)</td>
<td>400 (247 to 477)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.6 (3.3 to 6.4)</td>
<td>4.2 (2.4 to 5.7)</td>
<td>3.3 (2.3 to 4.9)</td>
<td>2.4 (1.5 to 4.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD, median (interquartile range) or as percentage of frequency, as appropriate. ACh, acetylcholine; CRP, C-reactive protein.
factors held true also when plasma creatinine was used as the outcome measure of renal function (creatinine versus age: \( r = 0.14, P = 0.02 \); creatinine versus gender: \( r = 0.12, P = 0.06 \)). Remarkably, both on categorical (Table 1) and linear correlation analyses (Figure 1), plasma CRP and the vasodilatory response to ACh correlated with the GFR, CRP being progressively higher as the GFR declined and the response to ACh being consistently higher in individuals with relatively higher GFR values. These correlations again were confirmed fully when plasma creatinine was used as an indicator of renal function (\( r = 0.32 \) and \( r = -0.28, P < 0.001 \)).

**Multivariate Analyses**

To test the influence of inflammation (as measured by CRP) in the statistical association between endothelial and renal function (serum creatinine), we constructed hierarchical multivariate models based on Framingham risk factors as well as the hemodynamic response to ACh. In multivariate modeling, we elected to use creatinine rather than the GFR as an outcome measure to avoid the analytical distortion that is derived by the use of GFR (i.e., a variable calculated on the basis of age and gender, which also are independent covariates in the same models). A basic model that included these risk factors (age, gender, BP, LDL cholesterol and triglycerides, body mass index, and smoking) explained 8% of the creatinine variance. As shown in Figure 2, the addition of the forearm blood flow response to ACh added significant information to the model in that the explained creatinine variance rose to 13% \( (P < 0.001) \). CRP seemed to be a stronger independent correlate of creatinine than the response to ACh because a model that included this variable (instead of the response to ACh) explained an even higher proportion of serum creatinine variance (18%; \( P < 0.001 \)). Of note, in a model that included both covariates (the response to ACh and CRP), the independent association between CRP with creatinine lost modest strength (partial \( r = 0.27, P < 0.001 \)), whereas the endothelial function–creatinine link, although still significant, lost substantial strength (partial \( r = -0.15, P = 0.02 \)) so that CRP remained a much stronger correlate of serum creatinine than endothelial function \( (P < 0.001) \). Notwithstanding the possible analytical distortion that is derived by the use of the GFR as an outcome measure, the results of GFR modeling did not materially differ from the previously described creatinine modeling (data not shown).

**Discussion**

This study shows that endothelial dysfunction and inflammation are associated with renal insufficiency in cardiovascular events–free individuals with essential hypertension. Furthermore, modeling of renal function by multiple regression analysis suggests that these two risk factors are in the same causal pathway conducive to renal damage in primary hypertension.

In the past decade, convincing evidence has been accrued that even minor degrees of renal insufficiency as estimated on the basis of plasma creatinine predict adverse cardiovascular outcomes in uncomplicated essential hypertension (9,10) and in

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**Figure 1.** Relationship between maximal vasodilatory response to acetyl choline (ACh) with the GFR (left) and with plasma C reactive protein (CRP; right).

**Figure 2.** Explained creatinine variance in statistical models that included classical risk factor (basic model) and CRP or endothelial function or both factors.
the general population (11). The strength of this association is such that guidelines that are promoted by major scientific societies now recommend GFR estimates that are based on serum creatinine for refining risk stratification in essential hypertension and in CKD as well. Renal endothelial dysfunction as an expression of systemic endothelial dysfunction is suspected to be a contributory factor to kidney disease in primary hypertension (12). This hypothesis has experimental support in animal models in that in the rat, the nitric oxide inhibitor NG-nitro-L-arginine methyl ester induces an arteriopathy very similar to nephroangiosclerosis in humans (13).

Endothelial dysfunction that is measured by the hemodynamic response to ACh in the forearm is a very consistent finding in essential hypertension (14). This alteration is only in minor part accounted for by the severity of hypertension and other risk factors, such as overweight, smoking, and hypercholesterolemia (15). The systemic nature of this disturbance (15) is highlighted by the fact that it affects disparate vascular beds, including peripheral vessels, the coronary circulation (16), and the kidney (17). In line with a primary analysis reported elsewhere (6), this secondary analysis confirms that the forearm blood flow response to ACh is strongly associated with renal function in essential hypertensives.

Inflammation as measured by serum or plasma CRP concentration is associated with BP levels in seemingly healthy individuals (4). CRP levels are considered a risk marker for cardiovascular disease in the general population (18) as well as in patients with cardiac ischemia (3). The importance of this risk factor is highlighted by the fact that in intervention studies, changes in CRP concentration are paralleled by coherent changes in the rate of cardiovascular events (19). Notably, CRP was associated with the brachial artery flow-mediated dilation in a community-based study (20). Such an association suggests that a microinflammatory state, perhaps triggered by the synthesis of inflammatory cytokines in abdominal and visceral fat cells (21), may alter the hemodynamic control of the circulation by the endothelium. Renal insufficiency, a condition whereby endothelial dysfunction is pervasive (22), very frequently is accompanied by raised CRP. CRP was found recently to be associated with a subtle reduction in creatinine clearance in the Prevention of Renal and Vascular Endstage Disease (PREVEND) study (23). In keeping with these observations, we reported that CRP was related inversely both with the vasodilatory response to ACh and with renal function. These coherent findings are compatible with the hypothesis that endothelial dysfunction may be an intermediate mechanism mediating the effect of inflammation on renal function. To explore this hypothesis in our study, we modeled renal function by sequentially including endothelial function and CRP in multiple regression analyses. This technique can be used for investigating causal inferences in mechanistic hypotheses (24). It is intriguing that this analysis indicated that CRP is a stronger correlate of renal function and showed that endothelial function loses considerable explanatory power for the creatinine variance when tested in a model that includes both factors, CRP retaining a much stronger association with the outcome measure. Such statistical observation suggests that CRP and endothelial dysfunction are in the same causal pathway that leads to renal insufficiency in hypertensive individuals and that CRP may be a causative factor for endothelial dysfunction in the pathogenetic sequence that causes renal damage.

Our observations are cross-sectional and therefore need to be confirmed in prospective and in interventional studies. In this regard, it is worth noting that secondary analyses of clinical trials (25) demonstrated that interventions that are known to modify CRP, such as statins, also reduce the risk for progression of CKD in patients with hypercholesterolemia. Such a favorable effect may not depend entirely on amelioration of hypercholesterolemia because, independent of changes in serum cholesterol, reductions in CRP that are induced by statins predict a reduction in adverse cardiovascular outcomes (19).

Conclusion

CRP and endothelial dysfunction are markers of renal function loss in patients with essential hypertension. Inflammation signaled by high serum CRP may be the trigger of systemic endothelial dysfunction and renal insufficiency in these patients. Intervention studies aimed at modifying inflammation are needed to confirm whether this risk factor is a crucial element in the perversive cardiovascular–renal connection that is conducive to cardiovascular complications in hypertension.

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References


