The Lipid independent Effect of Statins on Albuminuria in Type-2 Diabetes Mellitus

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Abstract
Inflammation is associated with diabetic nephropathy. Statins have shown to have an anti-inflammatory effect and to ameliorate renal disease. The aim of this work was to evaluate if the effect of atorvastatin on albuminuria is due to a lipid dependent or independent mechanism. Twenty normotensive type-2 diabetic patients received atorvastatin 10 mg c/24 hrs for 3 months. In all of them 24 hrs urinary albumin excretion, lipid profile and the levels of soluble adhesion molecules (SAMs) were measured at the beginning and end of the study. In all patients 24 hrs urinary albumin excretion was reduced (566.08 ± 85.8 mg to 292.8 ± 60.1mg (p=0.002), and SAM levels too. ICAM-1 decreased from 326.4 ± 53 to 289.6 ± 32 ng/ml (p=0.05), VCAM went down from 964.6 ± 93.3 to 610.8 ± 83.3ng/ml (p=0.022), and e-selectin was reduced from 68.9 ± 16.4 to 29.8 ± 6.5ng/ml (p=0.01). Reduction in urinary albumin excretion correlated with the fall in VCAM-1 (R=0.65, p <0.001). Our results suggest that the effect of atorvastatin on urinary albumin excretion is mainly due to an anti-inflammatory action.

Keywords - Adhesion molecules, Albuminuria, Atorvastatin, Diabetic Nephropathy, Low density lipoproteins.

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I. INTRODUCTION

Type-2 diabetic patients usually present endothelial dysfunction, which stimulates vascular inflammation; this pathway has been implicated in diabetic microvascular and macrovascular complications. Normal arterial endothelium does not adhere leukocytes, but in the presence of endothelial dysfunction, adhesion molecules are expressed on the arterial endothelium, and soluble forms of adhesion molecules (SAMs) appear in the circulation, perhaps as a result of a proteolytic process that releases SAMs from the endothelial cells surface. SAMs facilitate the attachment and emigration of leukocytes into the arterial wall, once there, leukocytes infiltrate normal tissues and can produce proteolytic enzymes that lead to tissue and organ damage. Indeed, Nguyen and colleagues found leukocyte infiltration in the diabetic kidney, and secondary tissue damage due to leukocyte proteases. Dyslipidemia also contributes to renal damage in diabetic patients, the proposed mechanisms are described in table 1, all of those pathways lead to alteration in the glomerular selective barrier to proteins and finally to albuminuria, which is considered not only a marker of endothelial dysfunction in type-2 diabetic patients, but also a predictor of nephropathy and coronary heart disease. Interestingly, Cases and colleagues reported that lipid lowering therapies ameliorate renal damage. Statins, beyond their cholesterol lowering effects, have pleiotropic (or lipid-independent) properties, as reduction of oxidative stress, normalization of endothelial function, stimulation of fibrinolysis and anti-inflammatory actions. Several studies demonstrated that statins are able to slow the progression of kidney disease. However, renoprotection by statins may be secondary to their lipid-lowering effects or by their pleiotropic actions.

Table 1. Mechanisms of renal damage induced by dyslipidemia.

<table>
<thead>
<tr>
<th>DEPLETION OF NEPHRIN</th>
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<tr>
<td>PODOCYTE APOPTOSIS</td>
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<tr>
<td>INCREASE OF OXIDATIVE STRESS</td>
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<tr>
<td>INACTIVATION OF NITRIC OXIDE</td>
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<tr>
<td>PROMOTION OF INTRARENAL ATHEROSCLEROSIS</td>
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<tr>
<td>EXPRESSION OF NUCLEAR FACTOR-KAPPA B AND INTERLEUKINE 6</td>
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<tr>
<td>REABSORPTION OF FATTY ACIDS, PHOSPHOLIPIDS AND CHOLESTEROL STIMULATES TUBULO-INTERSTITIAL INFLAMMATION</td>
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<tr>
<td>UPREGULATION OF INTRACELULAR SIGNALING IN THE MESANGIUM, INVOLVED IN FIBROGENIC RESPONSES</td>
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<tr>
<td>GLOMERULOSCLEROSIS INDUCED BY ACCUMULATION OF LIPOPROTEINS IN THE GLOMERULAR MESANGIUM</td>
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<td>GLOMERULAR INFLAMMATION INDUCED BY OXIDATED LIPOPROTEINS, NUCLEAR FACTOR-KAPPA B AND INTERLEUKINE 6 WITH RECRUITMENT OF MACROPHAGES AND T LIMPHOCYTES.</td>
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The use of statins in type 2 diabetic patients is effective and safe; in fact, the American Diabetes Association (ADA) recommends that statins should be added, regardless of lipid values, in those diabetic patients with overt cardiovascular disease, or in those without cardiovascular disease, but older than 40 years and with one more cardiovascular risk factor. The Collaborative Atorvastatin Diabetes Study (CARDS) found that atorvastatin 10 mg daily is safe and efficacious in reducing the risk of cardiovascular events in type 2 diabetic patients without high low-density lipoprotein (LDL) levels. The aim of this study is to evaluate if the renoprotective actions of atorvastatin are due to their lipid lowering effects or to its anti-inflammatory actions.

II. METHODS
A total of 20 normotensive patients with type-2 diabetes mellitus with duration of more than 12 months, and without previous treatment with thiazolidinediones, statins or inhibitors of the renin angiotensin system were included. All patients received atorvastatin 10 mg daily; the duration of the study was 3 months. The diagnosis of type-2 diabetes was performed according to the American Diabetes Association criteria. To avoid influence of physical activity level on albuminuria, patients were recommended not to perform exercise in the 3 days previous to urine recollection.

In all the subjects, vascular-cellular adhesion molecule-1, (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin circulating levels were measured in duplicate by commercial ELISA kits (R&D Systems, Minneapolis, MN) at the beginning and the end of the study, all venous samples were collected in the morning, after 12 h overnight fast, intra-assay variation was 3.1 for VCAM-1, 4.1 for ICAM-1, and 3.8 for E-selectin, whereas interassay variation was 7 for VCAM-1, 7.3 for E-selectin and 7.4 for ICAM-1.

Fasting glycemias, HbA1c, and lipid profile also were measured. And twenty-four hour urinary albumin excretion (nephelometry) was also performed, all of them at the beginning and the end of the study. All the determinations were carried out by personal blinded to the study.

Patients with any of the following diagnoses were excluded from the study: Decompensated diabetes mellitus (fasting blood glucose >250 mg/dl); heart, hepatic, or renal failure (Serum Creatinine > 1.5 mg/dl); evidence of valvular heart disease; heart block or cardiac arrhythmia; hypertension; acute coronary syndrome or cerebrovascular disease six months before the study’s initiation; autoimmune disease, pregnancy; urinary tract infection, fever or a history of alcohol abuse and/or psychotropic drugs.

The study was conducted with the approval of the Research and Medical Ethics Committee of our hospital, in accordance with the Helsinki declaration. Participants gave informed, written consent before their inclusion in the study protocol.

Statistical analysis
Data are presented as the mean ± standard deviation, statistical analysis was performed with ANOVA, a P < 0.05 was considered significant. The relationship between the differences (δ) in circulating SAMs levels, lipid profile and 24 h albumin excretion before and after the study, was assessed by the Pearson correlation coefficient.

III. RESULTS
Basal characteristics of patients are shown in Table 2. In all patients, LDL levels were reduced (154.35 ± 18.8 to 104.75 ± 12.5 mg/dl p<0.01), also, SAMs levels were down, ICAM-1 decreased from 326.4 ± 53 to 289.6 ± 32 ng/ml (p=0.05), VCAM went down from 964.6 ± 93.3 to 610.8 ± 83.3 ng/ml (p=0.022), and e-selectin was reduced from 80 ± 48 to 29.8 ± 6.5 ng/ml (p=0.01).

24 hrs urinary albumin excretion was reduced from 566.08 ± 85.8 mg to 292.8 ± 60.1 mg (p=0.002).

Table 2

<table>
<thead>
<tr>
<th>Basal characteristics of the patients</th>
<th>54 ± 2</th>
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<tbody>
<tr>
<td>Age (Years)</td>
<td>8/12</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>137.1 ± 27</td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>6</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>154.35 ± 18</td>
</tr>
<tr>
<td>Low Density Lipoproteins (mg/dl)</td>
<td>32.4 ± 5.1</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td>122/72</td>
</tr>
<tr>
<td>History of DM2 (years)</td>
<td>8.8</td>
</tr>
</tbody>
</table>

When we correlated the reduction of SAMs with the decrease in albuminuria, we did not find any correlation between the changes in ICAM-1, nor with e-selectin and urinary albumin excretion. However, we found a significantly correlation between the reduction in albuminuria and the decrease of VCAM-1 (r=0.65, p<0.001, Figure 1). When the reduction on albuminuria was correlated with the fall in LDL, we did not find a significantly correlation (R=0.25, p> 0.1, Figure 2).

No patient suffered adverse events.

Figure 1. Correlation between the reduction in albuminuria (6-albuminuria), and the reduction in VCAM-1 (6- VCAM-1).
IV. DISCUSSION

In this study we found that therapy with atorvastatin 10 mg daily given for 12 weeks reduces albuminuria, LDL levels and SAMs levels in normotensive, type 2 diabetic patients with diabetic nephropathy; it is important to say that in our work, circulating levels of SAMs were measured in duplicate, so intra-individual variation was taken into account when statistical analysis was performed. Type 2 diabetes may lead to renal damage by several mechanisms beyond hyperglycemia, as increase in oxidative stress, inflammation and the effect of some growth factors as transforming growth factor β-1 (TGF-β-1). The increase in oxidative stress leads to a decreased eNOS activity and inactivation of nitric oxide, these facts favors endothelial dysfunction, inflammation and vascular and end organ disease.

Inflammation has a role in the development of diabetic nephropathy; both VCAM-1 and ICAM-1 are responsible for leukocyte infiltration in the diabetic kidney, causing secondary tissue damage from leukocyte proteases, then the renoprotective effects of statins may be secondary to their anti-inflammatory properties, as we found in this paper, where the fall in urinary albumin excretion correlated with the decrease in VCAM-1 levels, but not with the reduction on LDL concentrations. Interestingly, we have found previously that the levels of VCAM-1 but not those from ICAM-1, nor e-selectin, correlated with albuminuria in diabetic patients.

Statins have shown to produce a protective renal effect, but the mechanisms for this renoprotective action is unclear, the lipid lowering actions of atorvastatin may lead to ameliorate glomerular injury by avoiding the pathways described in the table. However, their effects on renal damage may also be mediated by their pleiotropic properties, among which a reduction in the expression of nuclear factor κB (NFκB) has been reported. NFκB increases pro-inflammatory gene expression, including the expression of leukocyte adhesion molecules, which are expressed on the arterial endothelium, and also increases systemic concentrations of soluble forms of SAMs. Then it is expected that the administration of atorvastatin reduces the levels of al SAMs, as we found in this trial.

Douglas and colleagues reported a beneficial effect of statins on albuminuria. However, the mechanisms for that reduction are unclear. The improvement of endothelial function and the reduction in serum oxidized LDL associated with the use of statins have been proposed as an explanation. In the present study, we found that the renoprotective effects of statins appear to depend more from a direct mechanism than from their lipid-lowering properties, since the reduction of urinary albumin excretion correlated more with the fall in VCAM-1 concentrations than with the decrease in LDL levels.

β2 adrenergic receptors (β2AR) play a role in the pathophysiology of hypertension and diabetes mellitus, several studies shown that reduced β2AR expression contributes to impaired glucose tolerance and hypertension with aging. In the kidney, β2AR are involved in the modulation of renal hemodynamics, participates in the development of salt-sensitive hypertension and modulates renin release, the reduction of β2AR in that organ may also be implicated in the presence of glomerulosclerosis. In rats, statins induced a significant increase in βAR density; potentially, the statin effects on microalbuminuria may me mediated, at least in part, by its effects on β2AR. However this aspect requires further investigation. Our results may have therapeutic implications: the addition of a statin to the management of patients with albuminuria unresponsive to treatment with an inhibitor of the renin angiotensin system seems to be a good option not only for its cardiovascular implications, but also for renoprotection, especially since the publication of both, the Ongoing Telmisartan Alone or in combination with Ramipril Global Endpoint Trial (ONTARGET), and the VA Nephron studies, where combination therapy of an ACEI with an ARB was associated with an increased risk of adverse events among patients, and now the combination of an ACE inhibitor with an ARB is not recommended. Our study has some limitations, as the small sample size, but the clinical implication is the recognition of a lipid-independent renoprotective effect of atorvastatin. However, if this finding translates into reduction of end stage renal disease requires further investigation.

V. CONCLUSIONS

Our results shown that atorvastatin decreases both, circulating SAMs levels and 24 hrs urinary albumin excretion in normotensive type-2 diabetic patients, and that the reduction in albuminuria correlates with VCAM-1 reduction. Statins ameliorate diabetic renal damage mainly by mechanisms not related to their lipid lowering properties, as their anti-inflammatory action, and, beyond their cardiovascular benefits, may be another option in the treatment of albuminuria that not respond in adequate form to monotherapy with an inhibitor of the renin-angiotensin system.

References