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α₁-Microglobulin/albumin ratio may improve interpretation of albuminuria in statin-treated patients

Abstract

Background: Statins can cause tubular proteinuria by inhibiting tubular reabsorption of urinary proteins. To distinguish between microalbuminuria originating from glomerular leakage of albumin and tubular microalbuminuria due to statin therapy, the α₁-microglobulin/albumin ratio is evaluated in patients taking statins and compared to untreated patients.

Methods: Ten apparently healthy subjects were given 40 mg of simvastatin and tested for urinary α₁-microglobulin, albumin, creatinine and cystatin C, up to 24 h after administration. Additionally, urine samples of 76 statin-treated and 456 untreated patients presenting with micro-albuminuria (albuminuria range between 20 and 200 mg/L) were tested for α₁-microglobulin and albumin. α₁-Microglobulin/albumin ratios were compared. Total cholesterol was measured in 50 patients on statin therapy.

Results: In the 10 apparently healthy subjects, a significant temporary increase of α₁-microglobulin, albumin and α₁-microglobulin/albumin ratio was observed after statin intake. In the group of 532 patients showing microalbuminuria, those treated with statins showed a significantly higher mean urinary α₁-microglobulin/albumin ratio then untreated patients. Urinary albumin concentrations were significantly higher in patients taking simvastatin than in patients on rosuvastatin treatment and they were also higher in patients on statin therapy with a total serum cholesterol concentration below 3.88 mmol/L than in patients with a total serum cholesterol concentration above 5.17 mmol/L.

Conclusions: Tubular proteinuria, caused by the use of statins, can be distinguished from glomerular proteinuria by a higher urinary α₁-microglobulin/albumin ratio.

Keywords: albumin; α₁-microglobulin; proteinuria; statins.

Introduction

Statins lower cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase [1], which converts HMG-CoA to mevalonate. Derivates of mevalonate, such as geranylgeranyl pyrophosphate (GGPP) play a major role in post-translational modification of a lot of proteins, especially GTP-binding proteins [2]. These proteins are involved in the process of receptor-mediated endocytosis (RME), which occurs in the proximal tubular cells for albumin uptake [3–6]. This process involves megalin and cubilin receptors, which require the presence of prenylated GTP-binding proteins [7].

By inhibiting HMG-CoA reductase and reducing prenylation of GTP-binding proteins, statins inhibit protein uptake by the human proximal tubule. The reduced uptake of plasma proteins by the tubules may result in proteinuria in some patients treated with statins [2, 8]. Patients on statin therapy often suffer from conditions that can cause proteinuria, such as diabetes and arterial hypertension. In these patients, interpretation of microalbuminuria is hampered by the fact that microalbuminuria may either originate from glomerular leakage of albumin or from a statin-induced reduction of albumin uptake by the renal tubules. Proteinuria associated with statins may be a physiologic and benign response, related to altered protein reabsorption rather than an indication of diminished glomerular membrane integrity or frank toxicity. As microalbuminuria monitoring is recommended in the
follow-up of patients with diabetes and arterial hypertension, correct interpretation is of practical importance [9–11].

Correcting albuminuria for a disturbed tubular reabsorption can theoretically be achieved by comparing albuminuria to the concentration of a tubular marker protein which is taken up by the same receptor protein. Urinary α1-microglobulin is a very stable protein and is considered as a robust marker for tubular reabsorption of proteins [12].

In the present study, we evaluated the α1-microglobulin/albumin ratio to differentiate between proteinuria caused by risk factors and proteinuria caused by use of statins. Albumin and α1-microglobulin are both ligands of the cubilin/megalin receptors and increased urinary concentrations of these proteins will be found in statin-induced tubular proteinuria [12, 13].

Materials and methods

A group of 10 apparently healthy subjects (6 males, 4 females; 31±13 years) who were not treated with statins, angiotensin converting enzyme inhibitors or angiotensin receptor blockers were administered 40 mg of simvastatin. Due to ethical concerns, we have only administered very modest amounts of weak statins (simvastatin is tered 40 mg of simvastatin. Due to ethical concerns, we have only enzyme inhibitors or angiotensin receptor blockers were adminis-

years) who were not treated with statins, angiotensin converting specimens were collected prior to the intake of the drug, then hourly by use of statins. Albumin and proteinuria caused by risk factors and proteinuria caused microglobulin/albumin ratio to differentiate between

The increase of α1-microglobulin/creatinine, albumin/creatinine and α1-microglobulin/albumin ratio was significant (p=0.004, p=0.048 and p=0.002). Twenty-four hours after the administration of 40 mg of simvastatin, the levels of α1-microglobulin and albumin returned to levels not significantly different from those prior to administration. All urinary cystatin C levels were below 1.04 mg/L at baseline, during the 6 h after administration and also 24 h after administration of simvastatin.

From the group of patients who were treated with statins (76 patients), 28 patients showed microalbuminuria (albuminuria between 20 and 200 mg/L) and 10 patients had albuminuria >200 mg/L. The median albuminuria was 20.2 mg/L (IQR=9.9–105.0 mg/L) in the statin group vs. 35.3 mg/L (IQR=9.1–103.0 mg/L) in the non-statin group (p=0.59). Median urinary concentration of α1-microglobulin was 9.3 mg/L (IQR=3.6–21.9 mg/L) in the statin group vs. 4.0 mg/L (IQR=1.3–9.7 mg/L) in the non-statin group (p<0.0001). Median ratio of urinary α1-microglobulin/albumin in the statin group was 0.4 (IQR=0.1–0.8) vs. 0.1 (IQR=0.0–0.4) in the non-statin group. The ratios differed significantly between the treated and the untreated group (p<0.0001) (Table 1).

Linear regression for the urinary albumin and α1-microglobulin concentrations was performed for the statin group and the non-statin group. The regression equation for the non-statin group was y = 20.2 mg/L + 0.6×(urinary α1-microglobulin, mg/L), for the statin group y = 74.3 mg/L + 1.2×(urinary α1-microglobulin, mg/L). As the slopes differed between the two groups, a mathematical correction could be proposed in order to compensate for

Results

In the group of apparently healthy subjects who had been administered a single dose of 40 mg of simvastatin, a transient increase of both α1-microglobulin and albumin was observed (Figure 1). At baseline, all 10 subjects had urinary albumin levels below 20 mg/L and urinary α1-microglobulin concentrations below 8 mg/L. Maximal concentrations were reached after 3±1 h (albumin/creatinine) and after 4±2 h (α1-microglobulin/creatinine and α1-microglobulin/albumin). Maximal values for urinary α1-microglobulin and albumin were respectively 16.7±9.8 mg/g creatinine and 14.5±5.0 mg/g creatinine.

The study was approved by the Ethics Committee review board of the University Hospital of Ghent (EC 2012–374). The study complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. A written informed consent was obtained from all participants. Statistical analysis was performed with the statistical program Medcalc (Medcalc, Mariakerke, Belgium). p-Values lower than 0.05 were considered statistically significant. Data are reported as mean±SD or median with interquartile range (IQR). Wilcoxon test for paired samples, Mann-Whitney U-test and Kruskal-Wallis tests were used as appropriate.
the effect of tubular reabsorption of albumin. As every mg increase of urinary α₁-microglobulin corresponded to a microalbuminuria of 74.3 mg + 0.6×(urinary α₁-microglobulin) in the non-statin group vs. a microalbuminuria value of 76.5 mg + 1.2×(urinary α₁-microglobulin) in the statin group, the following mathematical correction could be made in patients on statin therapy: microalbuminuria (corrected, mg/L) = microalbuminuria (measured, mg/L) − 0.6×(urinary α₁-microglobulin, mg/L).

Urinary α₁-microglobulin, albumin and α₁-microglobulin/albumin ratios were compared between the different types of statins: α₁-microglobulin levels did not differ significantly between the patients taking atorvastatin, pravastatin, rosuvastatin and simvastatin (p=0.9). Albuminuria was significantly different among the various types of statins prescribed (p=0.04): urinary albumin was significantly higher in patients taking simvastatin (n=43) than in patients taking rosuvastatin (n=12) (Table 2). No significant difference was found for the urinary α₁-microglobulin/albumin ratio between the different type of statins (p=0.3).

From 50 patients who were on statin therapy, the concentration of total serum cholesterol was measured. Patients were divided in three groups: patients with total serum cholesterol below 3.88 mmol/L, total serum cholesterol between 3.88 mmol/L and 5.17 mmol/L and total serum cholesterol exceeding 5.17 mmol/L. The urinary albumin concentration was significantly higher in patients with serum cholesterol values below 3.88 mmol/L than in patients with a serum cholesterol concentration exceeding 5.17 mmol/L (p=0.02). Urinary albumin levels were not significantly different between the patients with a serum cholesterol level between 3.88 and 5.17 mmol/L and the two other groups. No significant difference was found for urinary α₁-microglobulin levels and α₁-microglobulin/albumin ratio between the three groups.

Median total serum cholesterol concentrations of six patients taking rosuvastatin (1×10 mg/day, 3×20 mg/day

Table 1 Comparison of α₁-microglobulin, albumin and the α₁-microglobulin/albumin ratio in urine for the statin vs. the non-statin group.

<table>
<thead>
<tr>
<th></th>
<th>Statin group (n=76)</th>
<th>Non-statin group (n=456)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₁-Microglobulin, mg/L</td>
<td>9.3 (3.6–21.9)</td>
<td>4.0 (1.3–9.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin, mg/L</td>
<td>20.2 (9.9–105.0)</td>
<td>35.3 (9.1–103.0)</td>
<td>0.59</td>
</tr>
<tr>
<td>Ratio α₁-microglobulin/albumin</td>
<td>0.4 (0.1–0.8)</td>
<td>0.1 (0–0.4)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

α₁-Microglobulin concentrations and the α₁-microglobulin/albumin ratio were significantly higher in the statin vs. the non-statin group (p<0.0001). Data were reported as median and interquartile range.
Table 2 Urinary α₁-microglobulin, albumin, α₁-microglobulin/albumin ratio and total serum cholesterol levels in the different types of statins.

<table>
<thead>
<tr>
<th></th>
<th>Simvastatin (n=43)</th>
<th>Atorvastatin (n=18)</th>
<th>Rosuvastatin (n=12)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary albumin, mg/L</td>
<td>26.7 (11.5–135.7)</td>
<td>26.0 (9.1–135.0)</td>
<td>14.4 (9.1–20.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Urinary α₁-microglobulin, mg/L</td>
<td>11.2 (4.2–21.5)</td>
<td>7.6 (4.9–20.6)</td>
<td>4.7 (2.0–30.5)</td>
<td>0.9</td>
</tr>
<tr>
<td>α₁-Microglobulin/albumin ratio (urine)</td>
<td>0.4 (0–0.8)</td>
<td>0.2 (0.1–0.5)</td>
<td>0.5 (0.2–1.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>Total serum cholesterol, mmol/L</td>
<td>4.14 (2.59–6.23)</td>
<td>4.16 (3.44–4.32)</td>
<td>5.56 (4.76–5.66)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Urinary albumin was significantly higher in patients taking simvastatin than in patients taking rosuvastatin (p=0.04). Data were reported as median with interquartile range. Pravastatin was not included in the data because the group is too small (n=3).

and 2×40 mg/day) and 26 patients on simvastatin therapy (19×20 mg/day and 7×40 mg/day) were compared because the urinary albumin concentration was significantly different in both groups. Median total serum cholesterol level was 5.56 mmol/L (IQR=4.76–5.66 mmol/L) in the patients on rosuvastatin therapy vs. 4.14 mmol/L (IQR=2.59–6.23 mmol/L) in patients on simvastatin therapy.

**Discussion**

The aim of the study was to distinguish microalbuminuria originating from glomerular leakage of albumin and tubular microalbuminuria due to statin therapy. All 10 healthy subjects who were administered a single dose of 40 mg simvastatin, showed a significant increase in urinary α₁-microglobulin, albumin and α₁-microglobulin/albumin ratio. This increase was transient as 24 h after administration, all urinary protein levels decreased to levels as before administration. Urinary cystatin C levels at baseline were all below 1.04 mg/L. After administration, up to 24 h after administration, levels never exceeded this value.

In patients, treated with statins, microalbuminuria can be observed in a large number of patients. These data are in agreement with earlier findings [2, 8, 15]. In a study of van der Tol et al. [16], statins were independently associated with an increased prevalence of microalbuminuria, even after correction for ‘bias by indication’. The researchers created a propensity score for statin use to correct for this ‘bias by indication’. Propensity score analysis is a well-established method to adjust for confounding factors by indication in observational trials [17, 18]. The propensity model included the following variables that were deemed to be possibly related to statin use: age, gender, body mass index (BMI), waist circumference, systolic blood pressure, previous cardiovascular event, C-reactive protein (CRP), fasting glucose, diabetes, serum uric acid, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, use of angiotensin converting enzyme inhibitor/angiotensin receptor blocker (ACE-I/ARB) and smoking. Except for uric acid, the authors did not take renal function parameters into account, such as cystatin C.

α₁-Microglobulin is a marker of tubular proteinuria which occurs in patients with a normal glomerular filtration rate, but with a diminished capacity of the proximal tubules to reabsorb and catabolize proteins [12]. The quantitative measurement in urine of total protein, albumin, α₁-microglobulin, IgG and α₁-macroglubulin is useful to differentiate the origin of proteinuria and hematuria [19–23]. Apart from α₁-microglobulin, many other urinary proteins are catabolized by the same megalin/cubulin pathway: vitamin D-binding protein [24, 25], β₂-microglobulin [26], transferrin [27] and retinol-binding protein [28], which are all markers of tubular proteinuria. The excretion of urinary α₁-microglobulin was significantly higher in statin-treated patients compared to statin untreated patients. Kostapanos et al. showed that rosuvastatin induced a small but significant increase in the excretion of α₁-microglobulin (by 16%, p<0.05), indicating that statin-related proteinuria involves low-molecular-weight proteins and is of proximal tubular origin [29]. The present study demonstrated that the urinary α₁-microglobulin/albumin ratio differs significantly between statin-treated and untreated individuals. One explanation may be that albumin and α₁-microglobulin are taken up by the same megalin/cubulin receptor, localized in the renal tubules, which requires the presence of small GTP-binding proteins. However, as mentioned earlier, statins inhibit the biosynthesis of several intermediates of the mevalonate pathway. Statin-induced microalbuminuria should also be differentiated from a possible renal bleeding, which is characterized by an α₁-microglobulin/albumin ratio <0.7 in combination with a positive urine dip test result (test strip for hemoglobin positive + + +). The additional measurement of the
IgG/albumin and α₁-microglobulin/albumin ratios might be helpful in this condition [22, 23]. However as the clinical presentation of a patient with a renal hemorrhage is totally different from the presentation of a patient with microalbuminuria from tubular overflow (statin)-induced microalbuminuria, it should be easy for clinicians to differentiate both conditions.

Although the urinary albuminuria values induced by statins are only slightly above the reference range, our mathematical formula is important as it makes it possible to distinguish this mild proteinuria from microalbuminuria caused by glomerular damage. The reported changes in urinary α₁-microglobulin concentration (and in urinary α₁-microglobulin/albumin ratio) are statistically significant as the values increase by a factor of 2.32. For a sensitive assay with a CV of a few percent, this significant temporary increase illustrates an impaired protein reabsorption capacity by the tubules. In the present study, starting α₁-microglobulin levels of the volunteers taking statins were within the reference values (as expected for a good control group). However, a 232% increase remains impressive in people with a normal tubular function. Due to ethical concerns, only very modest amounts of weak statins (simvastatin is only a weak statin) were administered in a single dose regimen.

There was a difference between the urinary albumin levels in patients taking either rosuvastatin or simvastatin. Patients on simvastatin therapy had significantly more urinary albumin than patients on rosuvastatin therapy. When we investigated the cholesterol levels in serum in 50 patients on statin therapy, lower serum cholesterol levels (below 3.88 mmol/L) were significantly associated with higher urinary albumin concentrations than in patients with serum cholesterol values exceeding 5.17 mmol/L. This could be explained by a more profound effect of statins in these patients and as a consequence a higher prevalence of microalbuminuria. So the urinary albumin levels are not dependent on the drug but on the cholesterol levels.

The data clearly highlight the difficulties in interpreting microalbuminuria values in patients treated with statins, since two distinct mechanisms might be involved: glomerular damage and an impaired tubular reabsorption. As the statin-induced microalbuminuria is mostly of tubular origin, it is uncertain and rather unlikely whether it has the same prognostic impact for renal and cardiovascular disease as endothelial dysfunction-induced glomerular microalbuminuria [16]. The suggested correction factor is based on the difference between the slopes of urinary albumin and α₁-microglobulin concentrations between the treated and untreated patients. When microalbuminuria is measured in a patient on statin therapy, this value could be corrected with the following mathematical correction: microalbuminuria (corrected) (mg/L) = microalbuminuria (measured) (mg/L) − 0.6 × (urinary α₁-microglobulin, mg/L). This formula is particularly useful for patients with microalbuminuria to estimate the effect of statin use on the proteinuria level. Also in conditions of mixed (glomerular and tubular) proteinuria, our findings should be taken into account. However in case of macroalbuminuria, the clinician should look for an underlying renal pathology. It was not the aim of our study to differentiate statin-induced proteinuria from other conditions associated with tubular proteinuria. However, we definitely propose to include the urinary α₁-microglobulin/albumin ratio in a standard laboratory form, as this ratio could be helpful in distinguishing glomerular proteinuria from tubular overflow (statin)-induced albuminuria.

In conclusion, albuminuria caused by increased glomerular leakage can better be distinguished from the effect of concomitant tubular proteinuria by using a mathematical correction when microalbuminuria is measured in a patient on statin therapy.

Conflict of interest statement

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

Received November 22, 2012; accepted December 13, 2012; previously published online January 11, 2013

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