Serum Lipoprotein(a) Concentration and Apo(a) Isoform under The Condition of Renal Dysfunction

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A serum lipoprotein(a) (Lp(a)) is an independent risk factor for cardiac events. It is well known that the patients with chronic renal failure (CRF) have a high concentration of serum Lp(a). The purpose of this study was to indicate the relationship between serum Lp(a) concentration and apoprotein(a) (apo(a)) isoforms under the condition of renal dysfunction. One-hundred thirty patients having hypertension, hyperlipidemia, diabetes mellitus and/or CRF were selected in this study. All patients were divided into two groups according to the level of serum creatinine. Serum Lp(a) concentration in the CRF patients (Cr > 2.0 mg/dl) was significantly higher than that in the controls (Cr < 1.2 mg/dl). Many CRF patients had high molecular weight (HMW)-apo(a). This study showed that the increase in HMW-apo(a) was closely accompanied by the increase in serum creatinine levels, and the serum Lp(a) concentration with HMW-apo(a) was higher according to their creatinine levels. J. Atheroscler Thromb, 2003; 10: 283–289.

Key words: Lp(a), Chronic renal failure, High molecular weight (HMW)-apo(a), ApoB

Introduction

High concentration of serum lipoprotein(a) (Lp(a)) is a risk factor for cardiovascular disease (1–3). In recent years, several reports have indicated that serum Lp(a) concentration is markedly high in patients with end-stage renal disease (4, 5). Lp(a) concentration is an independent factor contributing to the risk for atherosclerotic disease in both hemodialysis and continuous ambulatory peritoneal dialysis patients, which is the major cause of morbidity and mortality in these groups (6).

Lp(a) is a low-density lipoprotein (LDL)-like particle in which apoprotein(a) (apo(a)) is linked to apoB-100 by a disulfide bridge (7). Apo(a) has six different isoforms designated F, B, S1, S2, S3 and S4 according to different electrophoretic mobilities, which vary in size from approximately 300 to greater than 800 kd (4). However little is known about Lp(a) metabolism, and the reason why serum Lp(a) concentration is high in patients with chronic renal failure (CRF). The aim of this study is to indicate the relationship between serum Lp(a) concentration and apoprotein(a) (apo(a)) isoforms in CRF patients.

Subjects

One-hundred eighty-five patients who were treated at our hospital more than twice, or were admitted for treatment in the period of time between April 2000 and March 2001, for hypertension, hyperlipidemia, diabetes mellitus and/or chronic renal failure, were selected for this study. Serum concentrations of Lp(a), lipid, apoproteins, total protein, albumin, BUN and creatinine were measured at fasting in all subjects. Subjects having family history of primary hyperlipidemia, excessive alcohol consump-
tion, liver disease, malignant disease, inflammatory diseases with high C-reactive protein levels over 2.0 mg/dl, and chronic renal failure (CRF) patients who were treated by hemodialysis or continuous ambulatory peritoneal dialysis or renal transplantation were excluded from this study.

Then, the subjects were divided into three groups according to their serum creatinine levels. The CRF group had 64 patients whose serum creatinine levels were higher than 2.0 mg/dl, and the control group had 66 subjects whose serum creatinine levels were below 1.2 mg/dl. The other group had 55 patients with mild renal insufficiency (serum creatinine levels over 1.2 mg/dl and under 2.0 mg/dl), and the group was excluded in this study. Population characteristics are listed in Table 1.

### Methods

Serum concentrations of total cholesterol (TC) and triglyceride (TG) were measured by enzymatic colorimetric assay using an auto-analyzer (Hitachi 7250, Japan), whereas high-density lipoprotein (HDL)-cholesterol concentrations were determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate magnesium. Serum LDL-cholesterol concentration was calculated using the Friedewald formula (8). Serum apoprotein A-I, A-II, B and E levels were measured using immunonephelometry with a Toshiba TBA 30FR. Serum Lp(a) concentration was measured by latex agglutination method, and apo(a) phenotyping was performed using the analyzer kit of Lp(a) produced by Shima Institute. Plasma samples were diluted with a reducing buffer. The mixture was heated at 56°C for 10 minutes. Electrophoresis was performed on an SDS-agarose in tris-glycine for 90 minutes at a constant 20 mA for a gel at room temperature. After the protein transfer, the protein transfer, the membrane was placed in blocking solution containing casein to stop blotting. The filter was then immersed for 1 hour in a polyclonal anti-apo (a) antibody derived from a sheep. After extensive washing, the filter was incubated for 1 hour with a second antibody, antisheep immunoglobulin G, conjugated with peroxidase (POD) derived from rabbits. Then, enzyme reaction of the POD was used for revelation. Apo(a) isoforms were determined by comparing to the standard, consisting of F, B, S1, S3 isoforms. We determined all subjects who had at least one of the F, B, S1 or S2 isoforms belong to the low molecular weight (LMW) group, whereas the patients who had S3 and/or S4 isoforms belong to the high molecular weight (HMW) group. And some patients who didn’t have a clear band of isoforms were determined as the Null type, and this group was excluded in this study.

Statistical analyses were performed with Statistical Package for the Stat view for Macintosh version 5.0. Univariate comparisons of continuous variables between the control subjects and the CRF patients were done by unpaired t-test. Dichotomized variables were compared using simple regression, respectively. For multiple group comparisons, analysis of variance (ANOVA) was used in this study. Values were expressed as mean ±SE, except for sex which was expressed in the number. Significance levels were set at 0.05 in all cases.

### Results

Lipid parameters in the CRF patients and the controls are listed in Table 2. The CRF patients had a significantly higher concentration of serum Lp(a) than the control patients, while they had lower concentrations of serum apo

### Table 1. Characteristics of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 66)</th>
<th>CRF (n = 64)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.5 ± 1.5</td>
<td>68.3 ± 1.6</td>
<td>0.0026</td>
</tr>
<tr>
<td>Sex (M / F)</td>
<td>39 / 27</td>
<td>41 / 23</td>
<td>0.5602</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>16.2 ± 0.6</td>
<td>62.9 ± 3.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.73 ± 0.02</td>
<td>5.77 ± 0.48</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

mean ± S.E.

BUN: blood urea nitrogen, Cr: creatinine, M: male, F: female

### Table 2. Lipid parameters in the CRF patients and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 66)</th>
<th>CRF (n = 64)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>27.9 ± 3.3</td>
<td>47.1 ± 5.4</td>
<td>0.0028</td>
</tr>
<tr>
<td>apoA-I (mg/dl)</td>
<td>134.0 ± 2.2</td>
<td>114.5 ± 3.4</td>
<td>&lt; 0.0003</td>
</tr>
<tr>
<td>apoA-II (mg/dl)</td>
<td>23.2 ± 0.8</td>
<td>15.4 ± 0.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>apoB (mg/dl)</td>
<td>92.2 ± 2.6</td>
<td>93.7 ± 3.4</td>
<td>0.7203</td>
</tr>
<tr>
<td>apoE (mg/dl)</td>
<td>4.14 ± 0.12</td>
<td>3.94 ± 0.20</td>
<td>0.3876</td>
</tr>
<tr>
<td>T-Cho (mg/dl)</td>
<td>197.7 ± 4.8</td>
<td>193.3 ± 7.3</td>
<td>0.6076</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>135.9 ± 10.0</td>
<td>145.4 ± 10.4</td>
<td>0.5108</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>53.7 ± 1.6</td>
<td>46.1 ± 2.0</td>
<td>0.0023</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>117.3 ± 4.4</td>
<td>117.0 ± 6.1</td>
<td>0.9661</td>
</tr>
</tbody>
</table>

mean ± S.E.

protein A-I, A-II and HDL-cholesterol as compared with the controls. In the control group, the serum Lp(a) concentration with LMW-apo(a) was significantly higher than that with HMW-apo(a) (51.6 ± 9.0 mg/dl versus 22.5 ± 4.3 mg/dl, p = 0.0038). But this rule did not fit the CRF patients (Fig. 1). In the CRF group, the serum Lp(a) concentration with LMW-apo(a) was 42.0 ± 6.4 mg/dl and that with HMW-apo(a) was 34.5 ± 4.6 mg/dl. There were no significant differences between the serum Lp(a) concentration of their apo(a) isoforms in the CRF patients.

We tried to confirm the relationship between the percentage of HMW-apo(a) and their renal function. The CRF patients were divided into two groups according to their serum creatinine levels, and the percentages of HMW- or LMW-apo(a) were indicated in each group (Fig. 2). One group had moderate-stage renal failure (Group 1: 2.0 ≤ Cr < 5.0 mg/dl, n = 32), and the other group had end-stage renal failure (Group 2: Cr ≥ 5.0 mg/dl, n = 32). In the control group, the frequency of LMW-apo(a) was 44.7% and that of HMW-apo(a) was 55.3%. Increase in the percentage of HMW-apo(a) was closely accompanied by the increase in serum creatinine levels. The percentage of HMW-apo(a) in Group 1 and 2 was 77.8% and 89.5%. We were not able to find a significant difference in the percentage of HMW-apo(a) between Group 1 and the controls (p = 0.0961), but we found a difference between Group 2 and the controls (p = 0.0061). Fig. 3 shows the serum Lp(a) concentration with LMW-apo(a) or HMW-apo(a) in each group. There was no constant tendency of serum Lp(a) concentration with LMW-apo(a) among the three groups, but the Lp(a) concentration with HMW-apo(a) was higher according to their creatinine levels. Serum Lp(a) concentration with HMW-apo(a) in Group 2 was higher than that in the controls (p = 0.0378).

There was significant positive correlation between the serum concentrations of apoB and TC in all subjects (Fig. 4A). It is natural that there was not significant positive correlation between apoB and TG in the control group (p = 0.07). However, a positive correlation between the concentrations of apoB and TG was observed in the CRF patients (n = 62, r = 0.583, p < 0.0001 in Fig. 4B).

**Discussion**

It is well known that there is a negative correlation between the molecular weight of apo(a) and serum Lp(a) concentrations (9–12). Patients with renal failure have a high concentration of serum Lp(a) (4, 5). Actually, serum Lp(a) concentration in the CRF patients were significantly higher than that in the controls (Table 2). The percentage of HMW-apo(a) was also increased in the CRF patients (Fig. 2) and there was not a significant difference of Lp(a) concentration between LMW-apo(a) and HMW-apo(a) in the CRF patients (Fig. 1). We can find a significant difference between serum Lp(a) concentration and creatinine levels if we focus on HMW-apo(a). The serum Lp(a) concentration with HMW-apo(a) in Group 2 was higher than that in the controls (p = 0.0378, Fig. 3). It seems that the high concentration of serum Lp(a) with HMW-apo(a) is

![Fig. 1. Serum concentrations of Lp(a) in the controls and the CRF patients](image)
Fig. 2. Percentages of LMW-apo(a) and HMW-apo(a) in the controls and the two groups of CRF patients. Cr: creatinine, Control: serum creatinine was under 1.2 mg/dl, Group 1: serum creatinine was over 2.0 mg/dl and under 5.0 mg/dl, Group 2: serum creatinine was over 5.0 mg/dl. The percentage of HMW-apo(a) was closely accompanied by the increase in serum creatinine levels.

Fig. 3. The distribution of Lp(a) concentration according to their creatinine levels in the patients with LMW-apo(a) and with HMW-apo(a). Cr: creatinine, Control: serum creatinine was under 1.2 mg/dl, Group 1: serum creatinine was over 2.0 mg/dl and under 5.0 mg/dl, Group 2: serum creatinine was over 5.0 mg/dl. There were no significant differences in Lp(a) concentrations among the three groups in the patients with LMW-apo(a). Serum Lp(a) concentration with HMW-apo(a) in Group 2 was higher than in the controls ($p = 0.0378$).
related to their renal function in the CRF patients. We wished to know the reason why the serum Lp(a) concentration with HMW-apo(a) is high in the CRF patients (13). In this study, a significant difference between serum Lp(a) concentration and the molecular weight of apo(a) isoforms was not observed in the CRF patients, whereas it was present in the controls (Fig. 1). Although the CRF patients had a high concentration of serum Lp(a), the result was not due to LMW-apo(a) (14, 15). The CRF patients were further classified into two groups according to their serum creatinine levels in order to show the relationship between apo(a) isoforms (HMW or LMW) and their renal function. The percentage of LMW-apo(a) was gradually decreased and that of HMW-apo(a) was increased, accompanying the increase in serum creatinine levels (Fig. 2). Several reports showed that the CRF patients had a high concentration of Lp(a) with HMW-apo(a) (13, 15). Little was known about the reason why many CRF patients had HMW-apo(a) and a high concentration of serum Lp(a).

It is difficult to identify whether the above phenomenon is the result of renal dysfunction or not. There are two factors involved in this mechanism. First, the biochemical or structural characteristics of the Lp(a) in the CRF patients are different from that in the controls. Lp(a) is described to be a dominantly-inherited LDL-like particle in human plasma. It differs in structure from LDL by the highly polymorphic glycoprotein apo(a), which is linked to the apoB moiety of LDL by a single disulfide bridge (7, 16). Lp(a) is synthesized primarily in the liver (18), but the metabolism in humans is not well understood. It was generally accepted that CRF patients have a characteristic dyslipidemia consisting of hypertriglyceridemia and/or low concentration of HDL-cholesterol (19–21). Lipid abnormalities such as high levels of serum cholesterol, LDL and apoB of renal insufficiency contribute to the progression of renal failure in human chronic renal disease (22).

In this study, there was a positive correlation between the concentration of apoB and TG in the CRF patients (Fig. 4B). The lipid composition of LDL-like particles in Lp(a) seemed to be different between the controls and the CRF patients.

Second, this paper showed that the increase in the percentage of HMW-apo(a) was closely accompanied by the increase in serum creatinine level. And the serum Lp(a) concentration with HMW-apo(a) in Group 2 was higher than that in the controls ($p = 0.0378$). The serum Lp(a) clearance system in the CRF patients is different from

**Fig. 4.** The relationship between serum concentrations of apoB and T-cho, TG apoB: apoprotein B, T-cho: Total Cholesterol, TG: Triglyceride. There was a significant positive correlation between the serum concentration of apoB and T-cho in all subjects. Although there was not so strong a correlation between apoB and TG in the control group ($n = 64, r = 0.334, p = 0.070$), a positive correlation was observed in the CRF patients ($n = 62, r = 0.583, p < 0.0001$).
the controls. A striking feature of Lp(a) metabolism is that fragments of apo(a) but not of apoB are excreted into urine. The excretion rate of apo(a) fragments is very high in spite of their large size and is much higher than any other serum proteins which have the same molecular size as apo(a) (23). Therefore, it is argued that active transport mechanisms are concerned in an apo(a)-excreting mechanism. Several studies showed that serum Lp(a) binds to the LDL receptor about the catabolism of Lp(a) (24, 25), but other studies found no or negligible clearance of Lp(a) via this receptor (26). Concerning the molecular weight of apo(a), the molecular mass in the patients’ serum is larger than that in their urine (23). The excretion rate of Lp(a) also correlates with creatinine clearance (27, 28). The role of the kidney in Lp(a) catabolism is very important in controlling serum Lp(a) levels. A high concentration of serum Lp(a) may be observed when glomerular function is poor. When intact Lp(a) is cleared from patients’ circulation, molecular sizes of serum apo(a) are not changed for at least 24 hours (29). It is hard to accept that serum apo(a) isoforms are modified or structural by-changed in the biological circulation system of humans. Strong intracellular immunostaining for apo(a) is observed in the cytoplasm of proximal tubular cells. ApoB is colocalized with glomerular apo(a), but renal capillaries and tubules remain negative (29). It is possible that the high concentration of serum Lp(a) with HMW-apo(a) results from reducing affinity to its clearance system. Renal dysfunction is accelerated by the accumulated serum Lp(a) with HMW-apo(a). High concentration of serum Lp(a) with HMW-apo(a) in the patients with end-stage renal failure is the result of renal dysfunction. From a clinical standpoint, the inhibition of the increase in serum Lp(a) with HMW-apo(a) can be an intensive therapy for CRF patients. If it is possible, cardiovascular events in CRF patients will be prevented.

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