Effect of Aspirin Treatment on Serum Concentrations of Lipoprotein(a) in Patients with Atherosclerotic Diseases

Masashi Akaike,1* Hiroyuki Azuma,1 Ayako Kagawa,1 Kazuya Matsumoto,1 Ikuro Hayashi,2 Katsuya Tamura,3 Takeshi Nishiuchi,4 Takahiko Iuchi,1 Nobuyuki Takamori,1 Ken-ichi Aihara,1 Tomonori Yoshida,1 Yasuhiko Kanagawa,1 and Toshio Matsumoto1

Background: Increased serum lipoprotein(a) [Lp(a)] is an independent risk factor for atherosclerosis. We previously reported that aspirin reduced Lp(a) production by cultured hepatocytes via the reduction of apolipoprotein(a) [apo(a)] gene transcription.

Methods: We evaluated both the effect of aspirin treatment (81 mg/day) on serum Lp(a) concentrations and the correlation between the degree of reduction in serum Lp(a) and the type of apo(a) isoform in 70 patients with coronary artery disease or cerebral infarction.

Results: Aspirin lowered serum Lp(a) concentrations to ~80% of the baseline values in patients with high Lp(a) concentrations (>300 mg/L). The percentage of decrease in serum Lp(a) was larger in patients with high Lp(a) than in patients with low Lp(a) (<300 mg/L), irrespective of apo(a) isoform size. The decreases in serum Lp(a) in high Lp(a) patients with both the high-molecular-weight and the low-molecular-weight isoforms were positively correlated with the baseline Lp(a) concentrations.

Conclusions: Because the secretory efficiencies of apo(a) in the same isoform are likely to be similar, the difference in serum Lp(a) concentrations in patients having the same apo(a) isoform depends on the transcriptional activity of the apo(a) gene. These findings suggest that aspirin decreases serum Lp(a) concentrations via a decrease in apo(a) gene transcription more effectively in patients with high transcriptional activity of this gene.

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Lipoprotein(a) [Lp(a)] is a LDL-like particle in which apolipoprotein (apo) B-100 is disulfide linked to a single large glycoprotein, apo(a) (1). apo(a) contains multiple repeats of a cysteine-rich protein motif resembling the kringle-4 structure of plasminogen. There is a large variation in the molecular weight of apo(a), from ~300 000 to 800 000, which is determined by the number of kringle-4 repeats (2, 3). Serum Lp(a) concentrations are highly heritable, and Boerwinkle et al. (4) reported that ~90% of the determinant factors are genetically dependent on sequences of the apo(a) gene in chromosome 6q26-27. In particular, the number of kringle-4 repeats accounts for 78% of the genetic variance in Lp(a) concentrations (5). It has also been reported that there is a strong inverse correlation between the size of the apo(a) isoform (i.e., the number of kringle-4 repeats) and serum Lp(a) concentrations (5–7). Because apo(a) is highly homologous to plasminogen (2), it competes with plasminogen for binding to plasminogen receptors distributed on peripheral blood cells and vascular endothelial cells, thus inhibiting plasmin generation (8) and transforming growth factor-β activation (9). These properties of Lp(a) lead to retardation of fibrinolysis and acceleration of cell growth of vascular smooth muscle cells (10, 11). In addition, Lp(a) has been shown to be deposited in atherosclerotic lesions.

1 Department of Medicine & Bioregulatory Sciences, University of Tokushima Graduate School of Medicine, Tokushima 770-8503, Japan.
2 Department of Cardiology, Tokushima Prefectural Hospital, Tokushima 770-859, Japan.
3 Department of Cardiology, Health Insurance Naruto Hospital, Naruto 772-8503, Japan.
4 Kawashima Cardiovascular Clinic, Tokushima 770-0011, Japan.
*Address correspondence to this author at: Department of Medicine & Bioregulatory Sciences, University of Tokushima Graduate School of Medicine, 3-18-15 Kuramoton-cho, Tokushima 770-8503, Japan. Fax 81-88-603-7121; e-mail akaike@clin.med.tokushima-u.ac.jp.

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5 Nonstandard abbreviations: Lp(a), lipoprotein(a); apo, apolipoprotein; HMW, high molecular weight; and LMW, low molecular weight.
of the aorta and to enhance foam cell formation (12). Epidemiologic studies have revealed that high serum Lp(a) is an independent risk factor for atherosclerotic diseases, including coronary artery disease and stroke (13–15). Therefore, it is clinically important to reduce high serum Lp(a) concentrations by intervention. However, because the serum Lp(a) concentration is strongly influenced by genetic factors such as those described above and is not influenced by age, foods, exercise, environmental conditions (4, 5), or most lipid-lowering drug therapies other than niacin (16), drugs that can effectively and safely lower high serum Lp(a) concentrations are needed.

We previously reported that aspirin decreases Lp(a) production from cultured human hepatocytes through reduction of the transcriptional activity of the apo(a) gene with suppression of apo(a) mRNA expression (17, 18). In this study, we investigated whether aspirin can decrease serum Lp(a) in patients with atherosclerotic diseases and analyzed the effect of aspirin on serum Lp(a) in terms of the differences in apo(a) isoforms.

Materials and Methods

Participants and Aspirin Treatment

We studied 37 Japanese patients (20 males and 17 females) with high serum Lp(a) concentrations (>300 mg/L) and 33 Japanese patients (20 males and 13 females) with low serum Lp(a) concentrations (<300 mg/L), who consecutively visited to our institutions for the treatment of coronary artery disease or cerebral infarction. All patients were given 81 mg of aspirin daily. The clinical characteristics of these two groups are summarized in Table 1. No significant intergroup differences in the characteristics listed in Table 1, except for the serum Lp(a) concentration, were observed. To determine whether aspirin has a real effect on serum Lp(a), we also performed a placebo-controlled study in patients without established atherosclerosis. We examined 22 Japanese individuals (12 males and 10 females; mean ± SE age, 62.3 ± 1.8 years) with high serum Lp(a) and 34 Japanese individuals (20 males and 14 females; age, 61.0 ± 1.9 years) with low serum Lp(a), all of whom consecutively visited to our institutions. Patients with unstable angina, acute myocardial infarction, acute cerebral vascular accident, malignancy, infectious disease, liver cirrhosis, or renal failure (serum creatinine >20 mg/L) were excluded from the present study. Medications for cardiovascular disease, hyperlipidemia, or diabetes mellitus were not changed during this study. Fasting venous blood samples were obtained before the start of aspirin treatment and at 1, 3, and 6 months after the start of treatment. For individuals participating in a placebo-controlled study, fasting venous blood samples were obtained at the start of the observation period and at 1 and 3 months during the period. Informed consent was obtained from all participants.

Procedure

Serum Lp(a) was measured by a turbidimetric immunoassay using a commercially available reagent set (Chugai Co.). The intra- and interassay CVs were <10%, and the lower limit of detection in the assay was 10 mg/L. Total cholesterol, triglycerides, HDL-cholesterol, and blood sugar were measured enzymatically. apo(a) isoforms were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by immunoblotting using a commercial reagent set (Sanwa Chemical Co.) (19). Briefly, serum was treated with sodium dodecyl sulfate and 2-mercaptoethanol and then subjected to electrophoresis in 5% polyacrylamide gels. After transfer to a nitrocellulose membrane, apo(a) bands were detected by a double-antibody procedure and visualized by histochemical staining for peroxidase. According to their electrophoretic mobilities relative to molecular mass markers for apo(a), the apo(a) isoform were categorized into F (faster than apoB-100); B (similar to apoB-100); or S1, S2, S3, and S4 (progressively slower than apoB-100), as previously described by Utermann et al. (3). In the present analysis, apo(a) isoforms F, B, S1, S2, S3, and S4 correspond to the number of kringle-4 repeats (11–15, 15–19, 19–23, 23–27, 27–31, and 31–35, respectively) (20). The other allele, “null”, was defined as a lack of detectable bands because Lp(a) concentrations were below the detection limit of the assay (40 mg/L). On the basis of a report by Mölgaard et al. (21), these apo(a) isoforms were grouped into two types: a high-molecular-weight (HMW) isoform group (S3/null, S4/null, S3/S3, S3/S4, or S4/S4) and a low-molecular-weight (LMW) isoform group (in either case for F, B, S1, or S2 as a haplotype).

**Table 1. Clinical characteristics of the participants.**

<table>
<thead>
<tr>
<th></th>
<th>High Lp(a) (n = 37)</th>
<th>Low Lp(a) (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F, n</td>
<td>20/17</td>
<td>20/13</td>
</tr>
<tr>
<td>Age, years</td>
<td>67.4 ± 1.9</td>
<td>61.2 ± 2.1</td>
</tr>
<tr>
<td>Serum Lp(a), mg/L</td>
<td>627 ± 54</td>
<td>120 ± 13</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.5 ± 0.5</td>
<td>24.7 ± 0.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>135 ± 2.8</td>
<td>112 ± 8.0</td>
</tr>
<tr>
<td>Total cholesterol, mg/L</td>
<td>2090 ± 82</td>
<td>2020 ± 59</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/L</td>
<td>560 ± 25</td>
<td>490 ± 24</td>
</tr>
<tr>
<td>Triglycerides, mg/L</td>
<td>1160 ± 89</td>
<td>1370 ± 100</td>
</tr>
<tr>
<td>Fasting blood sugar, mg/L</td>
<td>1310 ± 92</td>
<td>1120 ± 80</td>
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</table>

*Values for characteristics are the mean ± SE.*

**Statistical Analyses**

All data are expressed as the mean ± SE. Means were compared by the Wilcoxon test or the Mann–Whitney test, as appropriate. Comparisons between different groups were made using ANOVA and multiple comparison tests. Correlations between the decreases in serum Lp(a) at 6 months after the start of aspirin treatment and the baseline serum Lp(a) concentrations were determined by linear regression analysis. P <0.05 was considered statistically significant.
Results
Serum Lp(a) concentrations before the start of aspirin treatment in patients with high Lp(a) and low Lp(a) were 627 ± 54 and 120 ± 13 mg/L, respectively (Table 1). As shown in Fig. 1A, aspirin treatment significantly decreased serum Lp(a) in patients with high Lp(a), to 88.3% ± 2.7% of the baseline values after 1 month (P < 0.01). Serum Lp(a) concentrations in these patients remained low at 3 and 6 months after the start of treatment (85.0% ± 3.4% and 81.7% ± 2.6% of the baseline values, respectively; P < 0.01). In contrast, no significant changes in serum Lp(a) concentrations in patients with low Lp(a) were observed during 6 months of aspirin treatment (95.2% ± 4.0%, 101.9 ± 5.7%, and 96.5% ± 3.0% after 1, 3, and 6 months, respectively; Fig. 1B). Other variables, including serum total cholesterol, HDL-cholesterol, and fasting blood sugar, did not change in patients with either high or low Lp(a) during the 6-month treatment period (data not shown). In a placebo-controlled study, serum Lp(a) concentrations at the start of the observation period in individuals with high Lp(a) and low Lp(a) were 623 ± 18 and 137 ± 19 mg/L, respectively. No significant changes in serum Lp(a) concentrations in individuals with high or low Lp(a) were observed during 3 months of the observation period (103.0% ± 7.7% and 103.0% ± 8.7% in the high Lp(a) group and 100.6% ± 9.4% and 101.1% ± 9.8% in low Lp(a) group at 1 and 3 months during the period, respectively).

Shown in Fig. 2 are the individual changes in serum Lp(a) concentrations before and 6 months after the start of aspirin treatment in each apo(a) isoform group. To examine whether the suppressive effect of aspirin on serum Lp(a) was associated with the size of the apo(a) isoform, we then compared the percentage of decrease in Lp(a) at 6 months after the start of aspirin treatment in the following four patient groups: high Lp(a) with LMW isoform [25 patients; baseline Lp(a), 726 ± 69 mg/L]; high Lp(a) with HMW isoform [12 patients; baseline Lp(a), 421 ± 48 mg/L]; low Lp(a) with LMW isoform [7 patients; baseline Lp(a), 180 ± 42 mg/L]; and low Lp(a) with HMW isoform [26 patients; baseline Lp(a), 104 ± 10 mg/L]. Among the HMW isoform groups, the percentage of decrease in serum Lp(a) at 6 months after the start of aspirin treatment was significantly greater (P < 0.01) in the high Lp(a) group than in the low Lp(a) group (24.6% ± 4.5% vs 3.2% ± 3.0%, respectively; Fig. 3). Similarly, among LMW isoform groups, patients with a high Lp(a) concentration exhibited a significantly greater reduction (P < 0.01) compared with those with a low Lp(a) concentration (15.2% ± 3.1% vs 4.6% ± 9.4%, respectively; Fig. 3). In addition, the percentage of decrease tended to be higher in the high Lp(a) group with the HMW isoform than in the high Lp(a) group with the LMW isoform, but the difference was not statistically significant.

Finally, in an effort to elucidate the relationship between baseline serum Lp(a) concentrations and the decrease in serum Lp(a) produced by aspirin treatment, we analyzed the correlation between the baseline Lp(a) concentrations and the values of decrease in Lp(a) after 6 months of aspirin treatment in the four patient groups. As shown in Fig. 4, we found significant correlations in the high Lp(a) group with the HMW isoform (Fig. 4A; r = 0.818; P < 0.01) and the high Lp(a) group with the LMW isoform (Fig. 4B; r = 0.511; P < 0.01) but no correlations in
low Lp(a) group with the HMW isoform (Fig. 4C) or the low Lp(a) group with the LMW isoform (Fig. 4D).

Discussion

Serum Lp(a) concentrations are known to be determined genetically mostly by the rate of production of apo(a) in the liver but not by the catabolism of Lp(a) (7). In the present study, we demonstrated that low-dose aspirin treatment significantly lowered high serum Lp(a) in patients with coronary artery disease or cerebral infarction, to ~80–85% of the baseline values. Because high serum Lp(a) is an independent risk factor for atherosclerotic disease, these findings suggest that aspirin has beneficial effects in addition to its well-known effects on platelet aggregation in patients with cardiovascular disease or stroke, especially complicated with high serum Lp(a).

The production rate of apo(a) is controlled mainly by the efficiency of both synthesis and secretion of apo(a) in the liver (22, 23). The synthesis of apo(a) depends on the expression of apo(a) mRNA in hepatocytes (24), which is regulated by the transcriptional activity of the apo(a) gene (25, 26). It has been reported that not only sequence polymorphisms of the 5′ flanking regulatory region in the apo(a) gene (27, 28), but also unknown apo(a) gene sequence variations are important genetic factors affecting the expression of apo(a) mRNA (5). It has also been shown that the secretion of apo(a) by hepatocytes is affected by the molecular weight of the apo(a) isoform protein, which is determined by the number of kringle-4 repeats (22). Because higher molecular weight apo(a) isoforms require a longer residence time in the endoplasmic reticulum for proper folding and glycosylation of the apo(a) precursor, opportunities for the degradation of such apo(a) isoform proteins are greater during the maturation process, yielding a low secretion rate (22). On the other hand, because small apo(a) isoform proteins pass through the maturation process in the cells more quickly, the secretion rate of such proteins is considered high (22). These findings are thought to account for the inverse correlation between the molecular weight of apo(a) isoforms and serum Lp(a) concentrations (6, 7).

We classified our patients into the following four groups, based on the concentrations of the serum Lp(a) and apo(a) isoforms: high Lp(a) concentration with a HMW isoform; high Lp(a) concentration with a LMW isoform; low Lp(a) concentration with a HMW isoform; and low Lp(a) concentration with a LMW isoform. This classification is useful for comparing the transcriptional activities of the apo(a) gene in patients with high Lp(a) and those with low Lp(a) in the same molecular weight isoform group. Because the secretory efficiencies of apo(a) proteins in the same molecular weight isoform group are likely to be similar because of the abovementioned mechanism, a difference in Lp(a) concentrations in the same molecular weight isoform group would represent a dif-

![Fig. 3. Percentages of decrease in serum Lp(a) after the start of aspirin treatment in four groups.](image)

In both the LMW isoform and HMW isoform groups, the percentage of decrease in serum Lp(a) at 6 months after the start of aspirin treatment was significantly higher in patients with high Lp(a) than in patients with low Lp(a) (P < 0.01). In the high Lp(a) groups, the percentages of decrease in serum Lp(a) tended to be higher in patients with a HMW isoform than in patients with a LMW isoform, but the difference was not statistically significant. Data are mean values. **, P < 0.01 compared with the value in the group with low Lp(a) and the HMW isoform; ##, P < 0.01 compared with the value in the group with low Lp(a) and a LMW isoform.

![Fig. 4. Correlation between baseline serum Lp(a) and the decrease in serum Lp(a) at 6 months after the start of aspirin treatment.](image)

The decrease in serum Lp(a) after the start of aspirin treatment was significantly correlated with the baseline concentration of serum Lp(a) in both the group with high Lp(a) and a HMW isoform (A; \( r = 0.819; P < 0.01 \)) and the group with high Lp(a) level and a LMW isoform (B; \( r = 0.511; P < 0.01 \)), whereas there was no correlation in the group with low Lp(a) and a HMW isoform (C) and the group with low Lp(a) and a LMW isoform (D).
ference in expression rates of apo(a) mRNA, which depends on the transcriptional activity of the apo(a) gene. Therefore, we believe that the group with high Lp(a) and a HMW isoform has the highest transcriptional activity of the apo(a) gene among the four groups. In fact, aspirin lowered serum Lp(a) concentrations most effectively in the group with high Lp(a) and a HMW isoform, and also greatly reduced these concentrations in the group with high Lp(a) and a LMW isoform. In addition, among patients with high serum Lp(a), the decreased values for serum Lp(a) after the start of aspirin treatment correlated with the baseline concentrations of serum Lp(a) in both the HMW and LMW isoform groups, and the correlation was most significant in the group with high Lp(a) and a HMW isoform. Taken together, it is conceivable that aspirin lowers serum Lp(a) through reduction of apo(a) gene transcription in patients with high serum Lp(a), in whom the transcriptional activity of the apo(a) gene is considered to be high.

We previously reported that aspirin reduced apo(a) production from cultured human hepatocytes through the suppression of apo(a) mRNA expression. Aspirin (0.05–5.0 mmol/L) also reduced the transcriptional activity of the apo(a) gene in HepG2 hepatoma cells transfected with plasmids containing the apo(a) gene promoter. We also demonstrated that sodium salicylate, which does not inhibit cyclooxygenase-1, suppressed the transcriptional activity of apo(a) gene, whereas inhibition of cyclooxygenase-1 by indomethacin had no effect on the transcriptional activities of apo(a) gene (17). In addition, our previous findings, obtained by deletion analysis, suggest that an unknown transcriptional factor(s) binding to a negative regulatory element(s) (nucleotides −30 and +139) of the apo(a) gene promoter may be affected by aspirin (17). The present results as well as these previous findings suggest that aspirin may lower high serum Lp(a) concentrations by a mechanism whereby aspirin acts preferentially on the reduction of apo(a) gene transcription rather than suppression of secretory processing of mature apo(a) protein, independent of cyclooxygenase-1. However, the aspirin concentrations in the culture medium in our previous study were higher than the peak plasma concentrations of aspirin after the oral administration of low-dose aspirin in humans (29). Although it is possible that local concentrations of aspirin and salicylate in the liver are higher, the molecular mechanism by which aspirin can reduce serum Lp(a) concentrations in vivo remains unclear.

In conclusion, aspirin lowers serum Lp(a) concentrations potentially through the reduction of apo(a) gene transcription in patients with high serum Lp(a). Although it is possible that this novel action may be one of the mechanisms for the antiatherosclerotic effect of aspirin in patients with cardiovascular and cerebrovascular disease, further investigation is needed to verify this possibility.

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