Atorvastatin decreased plasma lysophosphatidic acid level in patients with ischemic stroke

Zhen-Guang Li1, Zhan-Cai Yu1, Yong-Peng Yu1, Dao-Zhen Wang1, Wei-Ping Ju1, Qi-Zhuang Wu2

1 Department of Neurology, Wendeng Center Hospital of Weihai, the Affiliated Hospital of Weifang Medical College and Teaching Hospital of Dalian Medical University, Shandong Province 264400, PR China; 2 Lab. of Neurochemistry, the First Hospital of Beijing University, Beijing 100034, China

Corresponding to: Yong-Peng Yu
Tel.: +86 06318474031
Fax: +86 06318476036
E-mail: yypeng6688@126.com
Received: 2011-06-20
Accepted: 2011-06-30

Abstract
Lysophosphatidic acid (LPA) is released from activated platelets. Statins are the commonly used anti-atherosclerotic drug. The purpose of this study is to observe whether atorvastatin could decrease the plasma LPA levels in ischemic stroke patients. A total of 386 subjects, including the 247 ischemic stroke cases and 139 healthy controls, were enrolled in this study. The 247 ischemic stroke cases were divided into Group A (n=109) and Group B (n=138) who had and had not received atorvastatin treatment before a stroke respectively. The plasma LPA levels of all the subjects were measured using chromatography. There was significant difference in the LPA levels between cases and controls (3.22 ±1.51μmol/L vs. 1.83±1.07μmol/L, p<0.01). The plasma LPA level in Group A was lower than that of Group B (2.66±1.23 umol/L vs. 3.83±1.14 umol/L, p<0.01). Atorvastatin (20mg/d) significantly reduced LPA levels in ischemic stroke
patients (n=138) compared with that before atorvastatin administration (1.96±0.87μmol/L vs. 3.83±1.14μmol/L, p<0.01). However, the LPA levels re-elevated after atorvastatin withdrawal for one month. Atorvastatin could decrease the plasma LPA levels in patients with ischemic stroke, which providing a better understanding of how statins protect against ischemic stroke. It is plausible to speculate that statins might have an effect of anti platelet activation.

**Keywords:** lysophosphatidic acid; stroke; ischemic; atorvastatin; platelet activation

**Introduction**

Lysophosphatidic acid (LPA) is a family of molecules with the general form of 1-O-acyl-2-hydroxy-sn-glyceryl-3-phosphate\[1\]. LPA is produced by activated cells, notably platelets\[2,3\], fibroblasts and leukocytes. In most circumstance platelets are the main source of plasma LPA. Mauco et al\[4\] showed that LPA is produced by human platelets after the cells were treated with exogenous phospholipase C from Clostridium welchii. LPA is also produced by platelets when they are activated by thrombin\[5,6\]. Sano et al reported that LPA is produced during the course of blood coagulation in humans\[7\]. LPA also acts as a strong agonist for platelet aggregation and intracellular Ca\(^{2+}\) mobilization\[8\]. Since platelet activation is a crucial mechanism in arterial thrombogenesis and in the pathophysiology of ischemic stroke\[9\], and LPA is produced by activated platelet, LPA may be produced by platelets in the courses of ischemic heart attack and ischemic cerebrovascular diseases. It is known that many of the key components of the cardiovascular system harbor LPA receptors, and LPA plays important roles in morphogenesis of the cardiovascular system. LPA may contribute to angiogenesis and atherosclerosis\[10\].

The role of statins in the treatment and prevention of cardiovascular diseases, such as coronary artery disease, acute coronary syndromes, diabetes or stroke is well established. Recent trials demonstrated that statins lower the risk of stroke up to 42% in patients with coronary heart disease\[11\]. However,
both evidence from mechanism and clinical studies also supports the idea that reductions in cardiovascular risk are dependent on mechanisms beyond cholesterol reduction alone, such as the reduction of endothelial dysfunction, inhibition of inflammatory responses\(^{12}\), stabilization of atherosclerotic plaques, and modulation of procoagulant activity and platelet function\(^{13,14}\). A well-characterized pleiotropic effect of statins is the upregulation of endothelial nitric oxide synthase (eNOS)\(^{15}\). Haramaki et al\(^{16}\) showed that treatment with the HMG-CoA reductase inhibitor fluvastatin alters platelet aggregability in a cholesterol-lowering independent manner, but the mechanisms by which this pleiotropic effect is exerted are not known. There was a hypothesis that inhibition of phospholipase by satins might reduce LPA level in vivo\(^{17}\). We further hypothesized that satins inhibits LPA production yielding prohibiting platelet activation, which would be a new target for the treatment of ischemic stroke. In this study, we therefore investigated the effect of atorvastatin on the level of plasma LPA in patients with ischemic stroke.

**Materials and methods**

**Study subjects**

This study was approved by the Regional Research Ethics Committee of the local region and all examinations were performed after obtaining informed consent from patients and control individuals. A total of 386 subjects including the 247 ischemic stroke cases and 139 healthy controls were enrolled in this study. The 247 ischemic stroke cases were divided into Group A (n=109) and Group B (n=138) who had and had not received atorvastatin, aspirin and anticoagulation treatment before a stroke respectively. Patients in Group B took atorvastatin 20mg daily for one month, and then stopped taking atorvastatin for one month. The plasma LPA was assayed at three time points viz., before, after and stopping taking atorvastatin. A normal neurological examination with no focal deficit at the time of entry was required. 139 healthy controls aged from 44 to 75 that had no history of cerebral or cardiac vascular diseases and no symptoms mentioned
above within one month were matched to patients by age and gender.

Ischemic stroke (including TIA) was diagnosed according to the currently generally accepted criteria[18]. All the patients recruited for this study had one or more than one ischemic stroke symptom, including transient numbness or weakness of the face, arm or leg, especially on one side of the body; transient confusion, trouble speaking or understanding; transient trouble seeing in one or both eyes; transient trouble walking, dizziness, loss of balance or coordination; transient severe headache with no known cause. Exclusion criteria: Patients with ischemic stroke, who had any of the following illnesses, conditions or requirement: (1) Rheumatic or congenital valvular heart disease. (2) After heart valve replacement. (3) Congestive heart failure and severe heart diseases. The diagnosis of congestive heart failure and severe heart diseases was confirmed by a medical evaluation, medical history and physical examination as well as various tests including echocardiogram and a chest X-ray to detect abnormal function of the left ventricle and/or heart valves and the size and shape of the heart. (4) Coagulation abnormalities. Coagulation abnormalities were defined as an INR spontaneously >1.6 and/or an APTT >60s and/or a platelet count <150×10^9 /L and/or a fibrinogen less than 1.0g/L. (5) Intracranial or systemic infection. (6) Obvious organs dysfunction (such as liver, kidney, lung). (7) Receiving antithrombotic or warfarin therapy. (8) Hypertension with carotid atherosclerosis or hemal stricture revealed by carotid color ultrasonic examination. (9) Female in the menstruation period. Other causes which may cause similar symptoms, such as cold, were excluded. Information about clinical characteristics in each group such as arterial hypertension (AH), diabetes mellitus (DM), dyslipidemia and smoking habit were recorded. Cranial MRI was performed in all participants at entry to the study. All subjects were examined by the same investigator who was blinded to clinical characteristics.

Sample Collection and LPA Analysis
The plasma LPA levels of all the subjects were measured using chromatography as previously reported\[19\]. Briefly, Patients were told to stay away from fatty food and alcohol several days before blood samples were obtained in the morning. Four milliliters of venous blood were drawn from each participant into commercially available anticoagulant tubes (Two-fish Comp.). Whole blood was centrifuged at 3500g for 10 minutes after lipid extraction. The supernatant was transferred to a microcentrifuge tube and centrifuged at 8000g for 10 minutes. Lipids were extracted and the resulting organic extracts were pooled and dried in vacuo. Each sample was resuspended in 0.3ml of chloroform/methanol/ water/28% NH4OH (250:100:15:0.3, v/v) and was immediately filtered by an Econosphere 3µm, 50mm×4.6mm silica column (Alltech Associates, Deerfield, IL). Compounds were eluted with a mobile phase, and the source was maintained at 250°C with a drying gas flow of 10L/h.

Comparisons of the baseline characteristics in each group were performed by multivariate logistic regression. For comparisons in LPA levels among the groups, analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test (equal variances assumed) or Dunnett's T3 test (equal variances not assumed) and student's t test were performed. All statistical analyses was performed with SPSS software package for Windows version11.5. Data was expressed as mean±SEM and statistical significance was set at p<0.05.

**Results and Discussion**

There were 247 patients and 139 controls similar in baseline characteristics (Table 1).

There was significant difference in the LPA levels between cases (n=247) with ischemic stroke and healthy controls (n=139) (3.22±1.51µmol/L vs. 1.83±1.07µmol/L, p<0.01). The ischemic stroke patients in Group A had a lower plasma level of LPA compared with that of Group B (2.66±1.23 umol/L vs. 3.32±1.51 umol/L p<0.01).
Administration of atorvastatin (20mg/d) for one month significantly reduced LPA levels in patients (n=138) (1.96±0.87μmol/L) compared with that before taking atorvastatin (3.83 ± 1.14μmol/L) (p<0.01). However, the LPA levels re-elevated after stopped taking atorvastatin for one month. After one month atorvastatin withdrawal, LPA level was higher than that after taking atorvastatin for one month (3.58±1.06μmol/L vs 1.96±0.87μmol/L, p<0.01).

Blood platelets of patients with ischemic stroke could be activated in most circumstances, which is followed by the release of LPA from these activated platelets and then plasma LPA levels should increase in patients with ischemic stroke as evidenced by the observation of the current study. LPA might be used as a marker for the evaluation on status of platelet activation.

There is convincing experimental evidence, which indicates that statins have various potentially neuroprotective properties, including amelioration of glutamate-mediated excitotoxicity, attenuated production of reactive oxygen species, upregulation of endothelial nitric oxide synthase with favorable effects on the microcirculation, diminished inflammatory reaction by modulation of the cytokine response and promotion of angiogenesis, which could improve the availability of collateral vessels[20,21,22]. Flow in the microcirculation could also be enhanced by statins due to antiplatelet and profibrinolytic effects[23]. Furthermore, statins could positively impact brain regeneration by stimulating neurogenesis and angiogenesis[20].

The present study indicates that atorvastatin could reverse the elevated LPA levels in the patients with stroke. How does the LPA release and why atorvastatin can decrease the LPA level? It was reported that most of the platelet-derived LPA was produced in a two-step process: lysophospholipids were released from activated platelets by the actions of two phospholipases, group IIA secretory phospholipase A₂ (sPLA₂-IIA) and phosphatidylserine-specific phospholipase A₁ (PSPLA₁), which were abundantly expressed in
the cells. Then these lysophospholipids were converted to LPA by the action of plasma lysophospholipase D (lysoPLD)\(^{[24]}\). The mechanisms by which statins inhibit thrombosis have been extensively investigated and several pathways appear to be involved\(^{[22]}\). In particular, statins have been proposed to reduce platelet activation and to exert favorable effects on fibrinolysis. It is generally acknowledged that atorvastatin exerts its major antithrombotic, at least in part, by inhibiting thrombin generation\(^{[25,26]}\). It inhibits phospholipases (e.g., sPLA\(_2\)-IIA) and thrombin generation, which inhibits LPA production in the process of platelet activation.

It is unexpected that the lowered LPA levels with concomitant 20mg atorvastatin administration re-elevated by stopping atorvastatin administration for one-month in patients with ischemic stroke. This result suggests that the origin of platelet activation cannot be diminished by atorvastatin administration, at least not by short term administration. We presumably concluded that this might be the possible reason for the limitation of atorvastatin’s effectiveness. Gertz et al found that acute withdrawal of statin treatment may impair a vascular function independent of lipid lowering, which may be of high relevance for the clinical use of these drugs\(^{[27]}\). There is increasing evidence that acute statin withdrawal can increase the prevalence cerebrovascular and coronary ischemic events in patients with vascular risk\(^{[28,29]}\). The molecular mechanism by which withdrawal of statin treatment re-elevated LPA level may be partly associated with a positive feedback regulation of the signal pathway for production of LPA\(^{[30]}\).

One of the limitations in our study was that it is not a randomized controlled trial, but an observational study. Indeed data from a randomized controlled trial would be more reliable. Secondly, certain diseases including inflammatory processes, hypercholesterolemia or diabetes mellitus might involve in the alteration of LPA levels. Thirdly, the effect of atorvastatin on the LPA levels of patients with ischemic stroke was
assessed only one month after atorvastatin administration or withdrawal in the present study. The long-term effect of atorvastatin administration on the LPA levels should be evaluated, which is more clinically relevant. Based on the data from the previous and the present studies\(^\text{1,2}\), we can conclude that LPA may play an important role in platelet activation. Atorvastatin could decrease the plasma LPA levels in patients with ischemic stroke, which providing a better understanding that the beneficial effects of statins on ischemic stroke might be mediated, at least in part, by inhibiting platelet activation. We presumably hypothesized that there might be a pathway underlying the anti platelet activation effect of statins, which has not been recognized yet. Atorvastatin might be involved in the positive-feedback loop in production of LPA due to activated platelet. Furthermore, our results provide a new perspective to understand the beneficial effects of satins on ischemic stroke. It is plausible to speculate that satins might have an effect of anti platelet activation, but our results need further study.

Acknowledgments
This study was supported by grants from the Chinese Science and Technology Ministry (G2000056905). We are very obliged to Tang Chao-shu as an Expert providing the technical assistance help.

References
4. Mauco G, Chap H, Simon MF and Douste-Blazy L. Phosphatidic and lysophosphatidic acid production in phospholipase C and
thrombin-treated platelets.


14. Ludman A, Venugopal V, Yellon DM, Hausenloy DJ. Statins and cardioprotection—more than just


Table 1. Baseline characteristics in each group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=247)</th>
<th>Controls (n=139)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean±SD)</td>
<td>66.9 ± 7.0</td>
<td>67.5 ± 6.8</td>
<td>ns</td>
</tr>
<tr>
<td>Male</td>
<td>130 (52.6%)</td>
<td>64 (54.0%)</td>
<td>ns</td>
</tr>
<tr>
<td>Hypertension</td>
<td>116 (47.0%)</td>
<td>65 (46.8%)</td>
<td>ns</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>50 (20.2%)</td>
<td>26 (18.7%)</td>
<td>ns</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>62 (25.1%)</td>
<td>31 (22.3%)</td>
<td>ns</td>
</tr>
<tr>
<td>Smoking</td>
<td>45 (18.2%)</td>
<td>23 (16.5%)</td>
<td>ns</td>
</tr>
</tbody>
</table>