High Levels of Soluble Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 in Acute Stroke: An Age- and Sex-Matched Cross-Sectional Study

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Aim: Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is known to be a key molecule in the pathogenesis of atherosclerosis. Although high levels of serum soluble LOX-1 (sLOX-1) were demonstrated in patients with acute coronary syndrome, there are no reports about acute stroke patients. The aim of the present study was to evaluate the levels of sLOX-1 in acute stroke patients according to different stroke subtypes.

Methods: We enrolled a total of 377 patients with a stroke (men/women: 251/126; age: 40–79 years), 250 with ischemic stroke and 127 with intracerebral hemorrhage (ICH). Patients were admitted to our hospital within 3 days after the onset of stroke. As controls, we randomly selected age- and sex-matched subjects without a past history of cardiovascular disease according to stroke subtype from the community-based cohort of the Suita study. Serum LOX-1 levels were compared between stroke patients and healthy controls according to stroke subtype.

Results: Median values of serum sLOX-1 in stroke patients were significantly higher than those in controls (526 vs. 486 ng/L in ischemic stroke and 720 vs. 513 ng/L in ICH, respectively). Among subtypes of ischemic stroke, median sLOX-1 levels in atherothrombotic brain infarction (641 ng/L) only were significantly higher than those in controls (496 ng/L). Ischemic stroke [odds ratio (OR), 3.80; 95% confidence interval (CI), 1.86–7.74] and ICH (OR, 5.97; 95% CI, 2.13–16.77) were independently associated with high levels of sLOX-1 by multivariate logistic regression analysis.

Conclusions: Higher levels of sLOX-1 were observed in patients with acute stroke than in controls. High levels of sLOX-1 can be useful as biomarker for acute stroke.

Key words: Stroke, Biomarker, Atherosclerosis

Introduction

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), the major receptor for oxidized low-density lipoprotein (oxLDL) in endothelial cells, is a key molecule in the pathogenesis of atherosclerosis. Basal expression of LOX-1 is very low and up-regulation of endothelial LOX-1 is induced via oxLDL in proatherogenic conditions. Several reports showed that high levels of soluble LOX-1 (sLOX-1) are generated through proteolytic cleavage of the extracellular domain of LOX-1 and that sLOX-1 can be used as a diagnostic biomarker of acute coronary syndrome. On the other hand, the clinical implications of serum sLOX-1 levels in acute stroke patients have not been clarified. We hypothesized that serum sLOX-1 levels would be also used as a biomarker of acute stroke as in the case of acute coronary syndrome.
Clinical Examinations

The following information was collected from medical records of the stroke patients: height; weight; results of routine blood examinations including lipid profiles, high sensitive-C reactive protein (hs-CRP), medication for hypertension, dyslipidemia, and diabetes; and stroke severity assessed by National Institutes of Health Stroke Scale (NIHSS) scores on admission and stroke subtypes. Subtypes of ischemic stroke, such as atherothrombotic brain infarction (ABI; \(n=43\)), lacunar (\(n=66\)), cardioembolic (\(n=59\)), and other types (\(n=92\)), were diagnosed as previously described\(^8\). For controls, similar information was extracted from the Suita Study.

Morning blood samples after overnight fasting (within 3 days after onset of stroke for cases) were collected to be kept at \(-80^\circ\)C until measurement of sLOX-1 levels. In all patients, serum lipids levels were immediately measured at the same in-hospital laboratory as previously described\(^8\). Serum hs-CRP levels were measured using an ultra-sensitive latex-enhanced immunoassay with an automatic analyzer (BN ProSpec System, Siemens, Munich, Germany). sLOX-1 levels were measured by ELISA using 2 monoclonal antibodies against LOX-1 as described previously\(^6\) but using mouse anti-human LOX-1 monoclonal antibody (MAB1798, R&D, Minneapolis, Minnesota, USA) instead of TS92.

Table 1. Comparison of soluble LOX-1 (sLOX-1) levels between stroke cases and age-matched controls by stroke subtypes

|                        | Ischemic stroke | Control | \(P\) | Ischemic stroke | Control | \(P\) |
|------------------------|-----------------|---------|-------|-----------------|---------|-------|
| Number                 | 250             | 250     |       | 127             | 127     |       |
| Age, years             | 67.3 (8.6)      | 66.5 (9.0) |       | 66.5 (8.6)      | 66.0 (8.7) |       |
| Men (%)                | 70              | 70      |       | 61              | 61      |       |
| BMI (kg/m\(^2\))      | 23.3 (3.3)      | 22.9 (3.0) | 0.22  | 23.1 (4.1)      | 22.8 (2.8) | 0.57  |
| sLOX-1 (ng/L)          | 526 (330, 883)  | 486 (321, 703) | 0.009 | 720 (459, 1125) | 513 (307, 770) | <0.001 |
| sLOX-1 \(\geq\) 1177 ng/L (%) | 18 | 6 | <0.001 | 24 | 7 | <0.001 |
| TC (mg/dL)             | 195 (41)        | 201 (31) | 0.04  | 193 (43)        | 203 (29) | 0.03  |
| HDL-C (mg/dL)          | 51 (14)         | 59 (16) | <0.001 | 56 (16)        | 61 (16) | 0.02  |
| hs-CRP (mg/dL)         | 0.10 (0.04, 0.25) | - |       | 0.10 (0.05, 0.25) | - |       |
| Cigarette smoking (%)  | 30              | 19      | 0.003 | 17              | 18      | 0.87  |
| Hypertension (%)       | 79              | 33      | <0.001 | 92              | 36      | <0.001 |
| Dyslipidemia (%)       | 52              | 30      | <0.001 | 34              | 31      | 0.55  |
| Diabetes mellitus (%)  | 31              | 10      | <0.001 | 28              | 12      | 0.002 |
| NIHSS score            | 4 (2, 7)        | -       |       | 12 (5, 18)      | -       |       |

BMI means body mass index. TC means total cholesterol. HDL-C means HDL cholesterol. hs-CRP means high sensitive-C reactive protein. sLOX-1 means soluble LOX-1. High soluble LOX-1 (sLOX-1) level was defined as 1177 ng/L (corresponding of the 80th percentile of all stroke patients) or more. In sLOX-1, hs-CRP, and NIHSS score, median (inter-quartile range) are shown. Data are mean (standard deviation) unless noted otherwise.

Aim

We aimed to cross-sectionally examine the serum sLOX-1 levels in acute stroke patients compared with age- and sex-matched healthy controls according to stroke subtype.

Methods

Subjects

We subsequently enrolled 377 patients with stroke (251 men and 126 women; 40–79 years old), 250 with ischemic stroke and 127 with intracerebral hemorrhage (ICH), who were admitted to the National Cerebral and Cardiovascular Center (NCVC) within 3 days after the onset from August 2008 to August 2010.

As controls, we randomly selected age- and sex-matched subjects without a past history of cardiovascular disease according to stroke subtype. Controls were randomly selected from the participants of the Suita study, which is a community-based cohort study conducted by NCVC, ongoing since 1989. Control subjects were from among those who had visited NCVC for follow-up survey from April 2006 to December 2007. The details of the Suita study were previously described\(^7\).

This study was approved by the Institutional Research and Ethics Committee of NCVC, Suita, Japan. Informed consent was obtained from all participants.
subtype, which were compared with age- and sex-matched controls and adjusted for age, cigarette smoking, body mass index, hypertension, diabetes, and dyslipidemia. High sLOX-1 level was defined as 1177 ng/L (corresponding to the 80th percentile of all stroke patients) or more. A p value of <0.05 was considered to indicate statistical significance.

## Results

Median values of serum sLOX-1 in patients with acute stroke were significantly higher than those in

### Statistical Analysis

All analyses were conducted using SAS version 9.3 (SAS Institute, Cary, California, USA). Inter-quartile ranges of sLOX-1 levels were shown with associated p values using the Wilcoxon signed rank sum test for inter-group comparison. For other continuous variables, means and standard deviations were shown with p values using paired t-test. Proportions were compared between groups using McNemar’s test. In addition, conditional and unconditional logistic regression analyses were used to calculate odds ratios and 95% confidence intervals for high sLOX-1 levels by each stroke subtype, which were compared with age- and sex-matched controls and adjusted for age, cigarette smoking, body mass index, hypertension, diabetes, and dyslipidemia. High sLOX-1 level was defined as 1177 ng/L (corresponding to the 80th percentile of all stroke patients) or more. A p value of <0.05 was considered to indicate statistical significance.

### Table 2. Comparison of soluble LOX-1 (sLOX-1) levels between stroke cases and age-matched controls by subtypes of ischemic stroke

| Atherothrombotic brain infarction | Cardioembolic stroke |
|-----------------------------------|----------------------|
| **Number** | **Case** | **Control** | **p** | **Case** | **Control** | **p** |
| Number | 43 | 43 | – | 59 | 59 | – |
| Men (%) | 86 | 86 | – | 47 | 47 | – |
| Age, years | 69.1 (7.5) | 68.5 (8.0) | – | 67.9 (7.4) | 66.9 (7.9) | – |
| BMI (kg/m²) | 23.6 (3.2) | 22.7 (3.5) | 0.22 | 23.4 (3.2) | 22.6 (2.8) | 0.33 |
| sLOX-1 (ng/L) | 641 (429, 1302) | 496 (337, 781) | 0.02 | 442 (255, 840) | 462 (333, 652) | 0.46 |
| sLOX-1 ≥ 1177 ng/L (%) | 28 | 5 | 0.01 | 19 | 7 | 0.07 |
| TC (mg/dL) | 202 (45) | 193 (33) | 0.27 | 189 (36) | 210 (27) | <0.001 |
| HDL-C (mg/dL) | 48 (14) | 61 (16) | <0.001 | 54 (15) | 58 (16) | 0.08 |
| hs-CRP (mg/dL) | 0.13 (0.05, 0.34) | – | – | 0.13 (0.04, 0.41) | – | – |
| Cigarette Smoking | 40 | 28 | 0.25 | 15 | 14 | 0.8 |
| Hypertension (%) | 91 | 33 | <0.001 | 68 | 24 | <0.001 |
| Dyslipidemia (%) | 74 | 23 | <0.001 | 39 | 36 | 0.68 |
| Diabetes mellitus (%) | 42 | 12 | <0.001 | 20 | 7 | 0.03 |
| NIHSS score | 4 (2, 6) | – | – | 8 (3, 19) | – | – |

| Lacunar infarction | Other types of infarction |
|--------------------|---------------------------|
| **Number** | **Case** | **Control** | **p** | **Case** | **Control** | **p** |
| Number | 56 | 56 | – | 92 | 92 | – |
| Men (%) | 77 | 77 | – | 72 | 72 | – |
| Age, years | 66.1 (8.7) | 65.7 (9.1) | – | 66.7 (9.7) | 65.9 (9.8) | – |
| BMI (kg/m²) | 23.5 (3.2) | 22.9 (2.7) | 0.33 | 22.9 (3.5) | 23.3 (3.1) | 0.48 |
| sLOX-1 (ng/L) | 529 (341, 743) | 558 (302, 850) | 0.67 | 526 (312, 919) | 463 (312, 705) | 0.07 |
| sLOX-1 ≥ 1177 ng/L (%) | 9 | 5 | 0.48 | 18 | 5 | 0.01 |
| TC (mg/dL) | 197 (43) | 196 (27) | 0.87 | 195 (40) | 203 (33) | 0.11 |
| HDL-C (mg/dL) | 52 (14) | 54 (13) | 0.41 | 51 (13) | 62 (17) | <0.001 |
| hs-CRP (mg/dL) | 0.09 (0.05, 0.20) | – | – | 0.09 (0.03, 0.21) | – | – |
| Cigarette Smoking | 30 | 21 | 0.25 | 34 | 16 | 0.004 |
| Hypertension (%) | 80 | 38 | <0.001 | 80 | 37 | <0.001 |
| Dyslipidemia (%) | 48 | 29 | 0.04 | 52 | 30 | 0.003 |
| Diabetes mellitus (%) | 27 | 9 | 0.01 | 36 | 11 | <0.001 |
| NIHSS score | 3 (2, 5) | – | – | 3 (2, 6) | – | – |

BMI means body mass index. TC means total cholesterol. HDL-C means HDL cholesterol. hs-CRP means high sensitive-C reactive protein. sLOX-1 means soluble LOX-1. High soluble LOX-1 (sLOX-1) level was defined as 1177 ng/L (corresponding of the 80th percentile of all stroke patients) or more. In sLOX-1, hs-CRP, and NIHSS score, median (inter-quartile range) are shown.

Data are mean (SD) unless noted otherwise.
artery as the onset of acute coronary syndrome; therefore, high levels of sLOX-1 in patients with ABI may indicate atherogenic reactions as the underlying mechanism for the onset of ABI. 

In this study, more than 90% of patients with ICH had hypertension. Up-regulation of LOX-1 expression in the cortex of spontaneously hypertensive rats was implicated to induce neuronal apoptosis\(^1\)\(^\text{3}\). In contrast, the contribution of LOX-1 to hypertensive ICH has not been clarified. Colocalization of LOX-1 and matrix metalloproteinases were reported in a patient with ruptured and unruptured multiple dissections of the middle cerebral artery\(^1\)\(^\text{4}\), and extremely high sLOX-1 levels were shown to be present in patients with acute aortic dissection\(^1\)\(^\text{5}\). We reported that cultured bovine aortic endothelial cells and Chinese hamster ovary cells expressing bovine LOX-1 bound and phagocytosed aged red blood cells and dead cells, apart from oxLDL as a ligand for LOX-1\(^1\)\(^\text{6}\). In addition, the binding of LOX-1 ligands including oxLDL and CRP usually up-regulates the expression of LOX-1. These findings suggest that LOX-1 would bind red blood cells of ruptured hematoma in the brain tissues after the onset of ICH, causing the up-regulation of sLOX-1 as well as LOX-1 expression in the present study.

The present study has several limitations. First, changes in sLOX-1 levels before and after the onset of stroke have not been examined because this is a cross-sectional study. Second, variation in sLOX-1 levels could be large, and the power to estimate the differences may not be adequate because of the small sample size. Further examinations with a large number of cases are required to clarify the role of sLOX-1 in each type of ischemic stroke. Third, delay in blood sampling in the present study could underestimate the

## Discussion

This is the first study to be shown that serum sLOX-1 concentrations in patients with acute stroke were higher than age- and sex-matched controls. 

LOX-1 is primarily expressed in endothelial cells, and several studies have revealed that it is also expressed in macrophages and smooth muscle cells\(^\text{10}\). Cellular uptake of oxLDL via LOX-1 by macrophage and smooth muscle cells was demonstrated to be involved in atherogenic reactions, such as apoptosis, and expression of matrix metalloproteinases\(^\text{4, 11}\). Elevated levels of sLOX-1 are considered to reflect the increased expression of LOX-1, and it was suggested that high levels of sLOX-1 could be a biomarker for vulnerability of atherosclerotic plaques\(^\text{6}\). Peak levels of sLOX-1 in patients with acute coronary syndrome were reported to occur within one day after admission to hospital\(^\text{6}\). In the present study, significant increases in serum sLOX-1 levels were observed in patients with ABI compared with those in controls. Ogata \textit{et al}\(^\text{12}\) showed that the rupture of an atheromatous plaque can cause thrombotic occlusion of a stenotic internal carotid artery as the onset of acute coronary syndrome; therefore, high levels of sLOX-1 in patients with ABI may indicate atherogenic reactions as the underlying mechanism for the onset of ABI.

In this study, more than 90% of patients with ICH had hypertension. Up-regulation of LOX-1 expression in the cortex of spontaneously hypertensive rats was implicated to induce neuronal apoptosis\(^1\)\(^\text{3}\). In contrast, the contribution of LOX-1 to hypertensive ICH has not been clarified. Colocalization of LOX-1 and matrix metalloproteinases were reported in a patient with ruptured and unruptured multiple dissections of the middle cerebral artery\(^1\)\(^\text{4}\), and extremely high sLOX-1 levels were shown to be present in patients with acute aortic dissection\(^1\)\(^\text{5}\). We reported that cultured bovine aortic endothelial cells and Chinese hamster ovary cells expressing bovine LOX-1 bound and phagocytosed aged red blood cells and dead cells, apart from oxLDL as a ligand for LOX-1\(^1\)\(^\text{6}\). In addition, the binding of LOX-1 ligands including oxLDL and CRP usually up-regulates the expression of LOX-1. These findings suggest that LOX-1 would bind red blood cells of ruptured hematoma in the brain tissues after the onset of ICH, causing the up-regulation of sLOX-1 as well as LOX-1 expression in the present study.

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| Table 3. Adjusted odds ratios for high soluble LOX-1 (sLOX-1) level in stroke patients compared to control subjects |
|-----------------|-----------------|-----------------|
|                  | Conditional Logistic | Unconditional Logistic |
|                  | Odds ratio | 95% CI          | Odds ratio | 95% CI          |
| All brain infarction (n = 250) |          |                  |          |                  |
| Model 1          | 3.34      | 1.73-6.44        | 3.77      | 2.01-7.09        |
| Model 2          | 3.28      | 1.68-6.39        | 3.67      | 1.94-6.94        |
| Model 3          | 11.32     | 2.17-59.18       | 3.80      | 1.86-7.74        |
| Intracerebral hemorrhage (n = 127) |          |                  |          |                  |
| Model 1          | 4.29      | 1.79-10.25       | 4.26      | 1.93-9.39        |
| Model 2          | 5.20      | 1.87-14.45       | 4.20      | 1.90-9.26        |
| Model 3          | 19.30     | 2.12-175.52      | 5.97      | 2.13-16.77       |

Model 1: adjusted for age
Model 2: adjusted for Model 1 + body mass index, cigarette smoking
Model 3: adjusted for model 2 + hypertension, diabetes and dyslipidemia

High soluble LOX-1 (sLOX-1) level was defined as 1177 ng/L (corresponding to the 80th percentile of all stroke patients) or more.
levels of sLOX-1 because peak levels of sLOX-1 in acute coronary syndrome were reported within one day after admission.

**Conclusion**

The present study showed that acute stroke was associated with high levels of sLOX-1 compared with age- and sex-matched controls. High levels of sLOX-1 can be useful as biomarkers for acute stroke. Further studies are required to clarify the contribution of sLOX-1 to the pathogenesis of stroke.

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**Possible Conflict of Interests**

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