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Original Article

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 stoke 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[1], is a key molecule in the pathogenesis of atherosclerosis[2]. Basal expression of LOX-1 is very low and up-regulation of endothelial LOX-1 is induced via oxLDL in proatherogenic conditions[3, 4]. Several reports showed that high levels of soluble LOX-1 (sLOX-1) are generated through proteolytic cleavage of the extracellular domain of LOX-1[5] and that sLOX-1 can be used as a diagnostic biomarker of acute coronary syndrome[6]. 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.

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

	Ischemic stroke			Intracerebral hemorrhage		
	Case	Control	*P*	Case	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 ≥ 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.

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 ($n=56$), 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°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[9] but using mouse anti-human LOX-1 monoclonal antibody (MAB1798, R&D, Minneapolis, Minnesota, USA) instead of TS92.

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		
	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/m^2)	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
Diebetes mellitus (%)	42	12	<0.001	20	7	0.03
NIHSS score	4 (2, 6)	–	–	8 (3, 19)	–	–
	Lacunar infarction			Other types of infarction		
	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/m^2)	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
Diebetes 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.

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.

Results

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

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.

controls: 526 vs. 486 ng/L in ischemic stroke (p= 0.009) and 720 vs. 513 ng/L in ICH ($p<0.001$) (**Table 1**). In patients with ABI, the median sLOX-1 levels were significantly higher than those in controls: 641 vs. 496 ng/L (p=0.02) (**Table 2**). There were no significant differences between patients and controls in sLOX-1 levels in the other subtypes of ischemic stroke. Ischemic stroke and ICH were associated with high levels of sLOX-1 after adjusting for age, cigarette smoking, body mass index, hypertension, diabetes, and dyslipidemia (odds ratio, 3.80 in ischemic stroke; 5.97 in ICH) (**Table 3**).

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[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[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[6]. Peak levels of sLOX-1 in patients with acute coronary syndrome were reported to occur within one day after admission to hospital[6]. In the present study, significant increases in serum sLOX-1 levels were observed in patients with ABI compared with those in controls. Ogata *et al*[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[13]. 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[14], and extremely high sLOX-1 levels were shown to be present in patients with acute aortic dissection[15]. 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[16]. 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

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

T.S. received consultancy fee from NK medico.

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