Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Levels as a Biomarker of Acute Intracerebral Hemorrhage

Takashi Inoue, MD, PhD,* Tomohisa Ishida, MD,* Tomoo Inoue, MD, PhD,* Atsushi Saito, MD, PhD,* Masayuki Ezura, MD, PhD,* Hiroshi Uenohara, MD, PhD,* Miki Fujimura, MD, PhD,† Kenichi Sato, MD, PhD,† Toshiki Endo, MD,† Shunsuke Omodaka, MD, PhD,† Hidenori Endo, MD, PhD,‡ Kuniyasu Niizuma, MD, PhD,‡ and Teiji Tominaga, MD, PhD‡

Background: Correct diagnosis of cerebral stroke type, hemorrhagic or ischemic, is essential in the early stage to establish the optimum treatment. The diagnosis is mainly determined based on imaging studies. Other more available diagnostic methods are desirable, such as blood sample examination. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is very important in vascular dysfunction induced by oxidized low-density lipoprotein, including cell apoptosis. The present study evaluated LOX-1 as a biomarker for cerebral stroke. *Subjects and Methods:* Patients with newly diagnosed stroke were prospectively enrolled between February and July 2014. LOX-1 serum values were measured twice, within 24 hours and 2 months after the onset. *Results:* A total of 16 patients were enrolled; 7 patients with intracerebral hemorrhage (ICH) and 9 patients with cerebral infarction. Median LOX-1 values of patients with ICH and infarction in the acute phase were 1825.8 and 593.9 pg/mL, respectively, significantly higher in patients with ICH than in patients with infarction ($P < .0001$). *Conclusion:* LOX-1 serum level has potential as a biomarker of ICH.

Key Words: Biomarker—Diagnosis—intracerebral hemorrhage—lectin-like oxidized low-density lipoprotein receptor-1

Subject Terms: Diagnostic testing—Intracranial hemorrhage

Introduction

Stroke is the third most common cause of death in developed countries, and the most common cause of disability worldwide. Ischemic stroke is the most common type, with approximately 13% of strokes caused by hypertensive intracerebral hemorrhage (ICH). Correct diagnosis of cerebral stroke type as hemorrhagic or ischemic is essential in the early stage. Patients with ICH should be treated with antihypertensive therapy, whereas patients with infarction require fibrinolytic therapy. Neuroimaging is the main approach to establish the diagnosis. Computed tomography (CT) is the best method to diagnose ICH, and magnetic resonance (MR) imaging is valuable to identify infarction. However, neuroimaging, especially MR imaging, is not always available in the clinical situation. Only comprehensive stroke centers have neuroimaging facilities with full time availability, whereas some local hospitals only have day time availability. Therefore, other diagnostic methods should be developed, such as blood sample examination, to diagnose the causes of stroke.

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is apparently very important in vascular

From the *Department of Neurosurgery, Sendai Medical Center, Sendai, Japan; †Department of Neurosurgery, Kohnan Hospital, Sendai, Japan; and ‡Department of Neurosurgery, Tohoku University Graduate School of Medicine, Sendai, Japan.

Received August 20, 2018; revision received October 11, 2018; accepted October 22, 2018.

Disclosure: None.

Sources of Funding: This work was supported by Grants-in-Aid for Scientific Research (KAKENHI No. 24592112) provided by the Japan Society for the Promotion of Science.

Address correspondence to Takashi Inoue, MD, PhD, Department of Neurosurgery, Sendai Medical Center, 2-8-8 Miyagino, Miyagino-ku, Sendai, Miyagi 983-8520, Japan. E-mail: tainoue@snh.go.jp.

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.10.027

dysfunction induced by oxidized low-density lipoprotein (LDL), including cell apoptosis.[1-5] Circulating LOX-1 levels are significantly elevated during the acute stage of coronary syndrome.[6] LOX-1 or oxidized LDL is reported to be a sensitive and specific biomarker of acute coronary syndrome.[7,8] LOX-1 is expressed not only in the coronary artery but also in carotid plaque and the intracerebral artery.[9-11] Therefore, we hypothesized that circulating LOX-1 level is elevated in the acute stage of cerebral stroke, and may be useful to diagnose the type of stroke.

The present study evaluated the use of LOX-1 as a biomarker for cerebral stroke.

Subjects and Methods

Patients

Patients were prospectively enrolled for this study between February and July 2014. The inclusion criteria were newly diagnosed as acute (within 24 hours from onset) stroke, follow up for more than 2 months, and age 20-80 years. The diagnosis of hemorrhagic or ischemic stroke was based on the findings of CT or MR imaging. The study protocol was approved by the local ethical committee (RIN-25-68, January 16, 2014), and written informed consent was obtained before entering the study.

LOX-1

LOX-1 was measured with enzyme immunoassay, commercially available from NK Medico Co., Ltd. (Tokyo, Japan). LOX-1 values were estimated twice, within 24 hours and 2 months (±5 days) after the onset.

Statistical Analysis

Data are expressed as the median and range. Bivariate associations between LOX-1 values of hemorrhagic and ischemic stroke were assessed using analysis of variance. Differences were considered significant at probability values of less than .05.

Results

A total of 16 patients were enrolled, 7 with ICH and 9 with cerebral infarction. Clinical characteristics are shown in Table 1. The median ages of patients with ICH and infarction were 65 (49-77) and 63 (37-78) years, respectively, showing no statistical difference. Median LOX-1 values of patients with ICH and infarction in the acute phase were 1825.8 (1034.3-2130) and 593.9 (305.6-778.6) pg/mL, respectively (Fig 1 upper). The median LOX-1 value was significantly higher in patients with ICH than in patients with infarction ($P < .0001$). Median LOX-1 values in patients with ICH and infarction after 2 months were 620.6 (461.1-1065.1) and 563.4 (360.8-791.4) pg/mL, respectively, showing no significant difference (Fig 1 lower). Median LDL values in patients with ICH and infarction in the acute phase were 113 (100-164) and 115 (68-159) mg/dL, respectively. Those at 2 months later were 123 (87-189) and 117 (59-167) mg/dL, respectively. Median of initial blood pressure of patients with ICH and infarction was 157/100 (135-209/72-125) and 146/95 (123-182/72-107) mmHg, respectively, with no significant difference. Past history of hypertension, arterial fibrillation, disorder of carbohydrate metabolism, and dyslipidemia showed no significant differences between patients with ICH and infarction.

Discussion

The LOX-1 serum level was significantly elevated in patients with ICH compared with patients with infarction in the acute phase. LOX-1 is a sensitive biomarker of early acute coronary syndrome.[6] The present results indicate that LOX-1 also has potential as a diagnostic biomarker of ICH, and could be very useful for the diagnosis of stroke in facilities without access to neuroimaging equipment.

The reasons for elevated LOX-1 level in patients with ICH are difficult to ascertain, but several possibilities can be considered. LOX-1 is a scavenger receptor present primarily on vascular endothelial cells.[5] Oxidized LDL caused by the intracellular production of reactive oxygen species will increase through binding to the LOX-1 receptor.[12,13] Patients with hemorrhagic or ischemic stroke could potentially express LOX-1 on their endothelial cells. However, immediate destruction of brain vessels caused by hematoma formation in the brain after ICH would result in outflow of LOX-1 into the brain venous systems. In contrast, gradual damage of the vessel walls by cerebral infarction would result in slower release of LOX-1. The LOX-1 serum level at 2 months after onset did not show significant differences between patients with ICH and infarction, suggesting that patients had similar levels of LOX-1 before suffering stroke.

LOX-1 level previously reported to have elevated in acute stroke patients.[14] They investigated 377 patients with stroke, and reported that serum LOX-1 level in stroke patients were significantly higher than those in controls. Among subtypes of ischemic stroke, median LOX-1 levels in atherothrombotic brain infarction only were significantly higher than those in controls. Compared with our present study, they enrolled much larger number of patients and compared with control group. Although we evaluated smaller number of patients, we recruited all patients prospectively. The selection bias is lower in prospective study than retrospective one. And another thing compared with Yokota's report, we collected the blood sample within 24 hours in all cases. That

Table 1. *Clinical characteristics and oxidized-LDL serum values of patients*

Case no.	Sex	Age (y)	Diagnosis	LOX-1 value (pg/mL)		LDL value (mg/dL)		Initial BP (mmHg)		Past history			
				Within 24 h	After 2 mo	Within 24 h	After 2 mo	Upper	Lower	HT	af	DM	DL
1	M	49	ICH	1825.8	1065.1	119	123	153	103	Y	N	N	N
2	M	59	ICH	2127.8	851.83	113	97	198	125	Y	N	N	N
3	F	64	ICH	2130	ne	124	127	150	91	Y	N	N	N
4	F	65	ICH	1327.2	461.1	100	189	209	112	Y	N	Y	Y
5	F	74	ICH	1124.2	551.28	107	106	135	72	Y	N	N	N
6	F	76	ICH	2029.1	ne	100	87	197	93	Y	N	N	N
7	M	77	ICH	1034.3	620.56	164	129	157	100	Y	N	N	Y
8	M	37	infarction	481.13	512.74	127	167	123	72	N	N	N	Y
9	F	49	infarction	593.87	434.29	118	81	182	96	Y	N	Y	Y
10	M	57	infarction	664.94	791.43	159	125	146	96	Y	Y	N	Y
11	F	63	infarction	715.51	614.12	108	112	167	107	Y	N	Y	Y
12	M	63	infarction	305.63	ne	87	119	150	95	Y	N	N	N
13	M	67	infarction	486.28	ne	68	59	145	87	Y	N	Y	N
14	M	75	infarction	501.65	754.91	121	135	164	98	Y	N	N	N
15	M	78	infarction	732.01	ne	114	117	131	92	Y	Y	N	N
16	M	78	infarction	778.56	360.77	115	82	140	81	Y	N	Y	Y

Abbreviations: af, arterial fibrillation; BP, blood pressure; DL, dyslipidemia; DM, disorder of carbohydrate metabolism; HT, hypertension; ICH, intracerebral hemorrhage; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; N, no; ne, not examined; Y, yes.

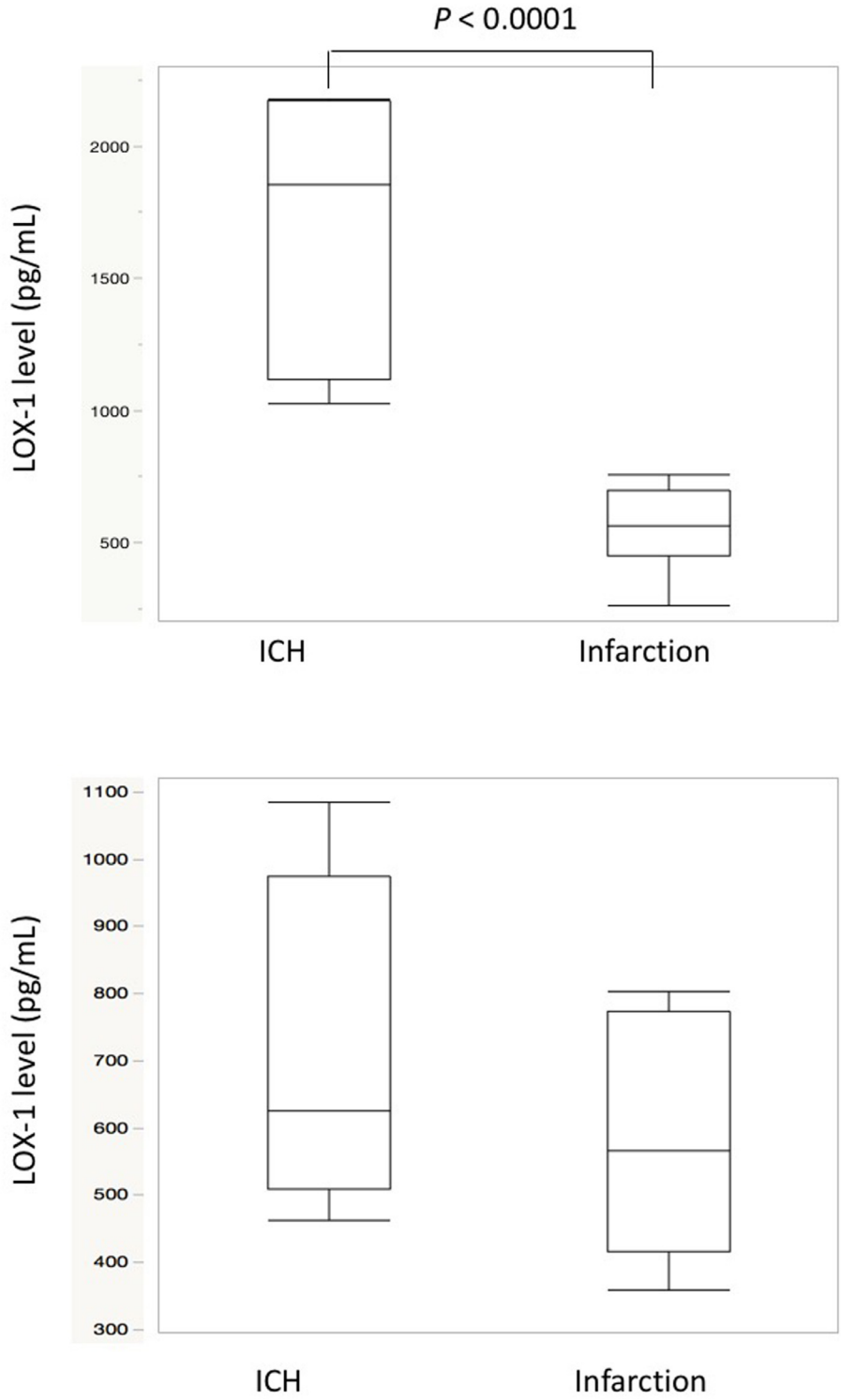

Figure 1. *Upper: Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) serum levels in patients with intracerebral hemorrhage (ICH) and infarction in the acute phase. LOX-1 serum level was significantly higher in patients with ICH than in patients with infarction* (P < .0001). *Lower: LOX-1 levels after 2 months showing no difference between the 2 groups.*

may be one of the reasons why ischemic patients in our study did not show high level of LOX1. We consider that the LOX-1 level of ischemic stroke patients increases more slowly than hemorrhagic stroke patients.

Hemorrhagic and ischemic stroke are difficult to distinguish based on initial blood pressure or past history, such as hypertension, arterial fibrillation, disorder of carbohydrate metabolism, and dyslipidemia which are known

major risk factors for atherosclerosis.[15] The atherosclerosis is likely to promote both hemorrhagic and ischemic stroke. Although we investigated the power of diagnosis of initial blood pressure, symptom, or past histories, there were no statistical significance between hemorrhagic and ischemic stroke. Acute ischemic stroke easily diagnoses using MR imaging, but it was sometimes difficult with CT scan. We did not investigate the usefulness against CT scan. There are some clinical impacts if LOX-1 could diagnose earlier and easily than CT scan.

There are some limitations in this study. First, the time required to measure LOX-1 serum level is several days in commercially available systems, which is not adequate for clinical applications. Stroke diagnosis must be confirmed within a few hours. LOX-1 could be measured by immune enzyme assay with monoclonal antibody,[16] which would take a few minutes for a qualitative assessment. Second, the present study did not estimate the exact source of LOX-1. We expected that LOX-1 originated from the endothelial cells of the brain vessels. Basic research is required to solve this question. Third, we did not have control group. So, we could not conclude the LOX-1 level at acute stage elevated or not exactly. LOX-1 level after 2 months did not show significant difference between hemorrhagic and ischemic stroke. We only mentioned the difference between these 2 situations. Fourthly, this study included a small number of patients. More cases are needed to evaluate the true value of LOX-1 as an acute stroke biomarker.

Conclusions

LOX-1 serum level has potential as a diagnostic biomarker of ICH. More basic and clinical research is planned.

References

1. Kataoka K, Hasegawa K, Sawamura T, et al. LOX-1 pathway affects the extent of myocardial ischemia-reperfusion injury. Biochem Biophys Res Commun 2003;300:656-660.
2. Kume N, Kita T. Apoptosis of vascular cells by oxidized LDL: involvement of caspases and LOX-1 and its implication in atherosclerotic plaque rupture. Circ Res 2004;94:269-270.
3. Kume N, Mitsuoka H, Hayashida K, et al. Soluble lectin-like oxidized low-density lipoprotein receptor-1 predicts prognosis after acute coronary syndrome—a pilot study. Circ J: Off J Jpn Circ Soc 2010;74:1399-1404.
4. Kume N, Mitsuoka H, Hayashida K, et al. Soluble lectin-like oxidized LDL receptor-1 (sLOX-1) as a sensitive and specific biomarker for acute coronary syndrome—comparison with other biomarkers. J Cardiol 2010;56:159-165.
5. Sawamura T, Kume N, Aoyama T, et al. An endothelial receptor for oxidized low-density lipoprotein. Nature 1997;386:73-77.
6. Hayashida K, Kume N, Murase T, et al. Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: a novel marker for early diagnosis. Circulation 2005;112:812-818.
7. Holvoet P, Lee DH, Steffes M, et al. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA 2008;299:2287-2293.
8. Holvoet P, Vanhaecke J, Janssens S, et al. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 1998;98:1487-1494.
9. Saito A, Fujimura M, Inoue T, et al. Relationship between lectin-like oxidized low-density lipoprotein receptor 1 expression and preoperative echogenic findings of vulnerable carotid plaque. Acta Neurochir 2010;152:589-595.
10. Saito A, Fujimura M, Inoue T, et al. Lectin-like oxidized low-density lipoprotein receptor 1 and matrix metalloproteinase expression in ruptured and unruptured multiple dissections of distal middle cerebral artery: case report. Acta Neurochir 2010;152:1235-1240.
11. Saito A, Shimizu H, Doi Y, et al. Immunoliposomal drug-delivery system targeting lectin-like oxidized low-density lipoprotein receptor-1 for carotid plaque lesions in rats. J Neurosurg 2011;115:720-727.
12. Cominacini L, Pasini AF, Garbin U, et al. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappaB through an increased production of intracellular reactive oxygen species. J Biol Chem 2000;275:12633-12638.
13. Cominacini L, Rigoni A, Pasini AF, et al. The binding of oxidized low density lipoprotein (ox-LDL) to ox-LDL receptor-1 reduces the intracellular concentration of nitric oxide in endothelial cells through an increased production of superoxide. J Biol Chem 2001;276:13750-13755.
14. Yokota C, Sawamura T, Watanabe M, et al. High levels of soluble lectin-like oxidized low-density lipoprotein receptor-1 in acute stroke: an age- and sex-matched cross-sectional study. J Atheroscler Thromb 2016;23:1222-1226.
15. Imamura T, Doi Y, Arima H, et al. LDL cholesterol and the development of stroke subtypes and coronary heart disease in a general Japanese population: the Hisayama study. Stroke; J Cereb Circ 2009;40:382-388.
16. Iwamoto S, Nishimichi N, Tateishi Y, et al. Generation and characterization of chicken monoclonal antibodies against human LOX-1. MAbs. 2009;1:357-363.