Original Article

Serum Salusin-α Levels Are Decreased and Correlated Negatively with Carotid Atherosclerosis in Essential Hypertensive Patients

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Salusin-α is a new bioactive peptide with mild hypotensive and bradycardic effects. Our recent study showed that salusin-α suppresses foam cell formation in human monocyte-derived macrophages by down-regulating acyl-CoA:cholesterol acyltransferase-1, contributing to its anti-atherosclerotic effect. To clarify the clinical implications of salusin-α in hypertension and its complications, we examined the relationship between serum salusin-α levels and carotid atherosclerosis in hypertensive patients. The intima-media thickness (IMT) and plaque score in the carotid artery, blood pressure, serum levels of salusin-α, and atherosclerotic parameters were determined in 70 patients with essential hypertension and in 20 normotensive controls. There were no significant differences in age, gender, body mass index, fasting plasma glucose level, or serum levels of high-sensitive C-reactive protein, high- or low-density lipoprotein (LDL) cholesterol, small dense LDL, triglycerides, lipoprotein(a), or insulin between the two groups. Serum salusin-α levels were significantly lower in hypertensive patients than in normotensive controls. The plasma urotensin-II level, maximal IMT, plaque score, systolic and diastolic blood pressure, and homeostasis model assessment for insulin resistance (HOMA-IR) were significantly greater in hypertensive patients than in normotensive controls. In all subjects, maximal IMT was significantly correlated with age, systolic blood pressure, LDL cholesterol, urotensin-II, salusin-α, and HOMA-IR. Forward stepwise multiple linear regression analysis revealed that salusin-α levels had a significantly independent and negative association with maximal IMT. Serum salusin-α levels were significantly lower in accordance with the severity of plaque score. Our results suggest that the decrease in serum salusin-α, an anti-atherogenic peptide, may be associated with carotid atherosclerosis in hypertensive patients. (*Hypertens Res* 2008; 31: 463–468)

***Key Words*: salusin-α, hypertension, atherosclerosis, intima-media thickness, plaque score**

Introduction

Salusins are a new class of bioactive peptides discovered by bioinformatics analyses of a full-length cDNA library (*1*). Shichiri *et al*. recently identified and characterized two related peptides of 28 and 20 amino acids, which they designated salusin-α and salusin-β, respectively (*1*). These pep-

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tides are thought to be biosynthesized from preprosalusin, an alternative-splicing product of the torsion dystonia–related gene (TOR2A), after frameshift reading and digestion at dibasic amino acids (*1*). Salusins are expressed and synthesized ubiquitously within human tissues, including the vasculature, central nervous system, and the kidneys, and salusin-α is additionally present in human plasma and urine (*1*, *2*). Salusin-β rapidly induces hypotension, bradycardia, and cardiac dysfunction by a cholinergic mechanism (*3*). Salusin-β also stimulates human macrophage foam cell formation (*4*), proliferation of vascular smooth muscle cells (VSMCs) and fibroblasts (*1*), and cardiomyocyte growth and anti-apoptosis (*5*, *6*). In contrast, salusin-α has a mild hypotensive effect (*1*), and suppresses human foam cell formation by down-regulation of acyl-CoA:cholesterol acyltransferase-1 (ACAT-1), which stores cholesterol ester (CE) converted from free cholesterol in macrophages (*4*). Serum salusin-α levels are significantly decreased in acute coronary syndrome (ACS) patients as compared with healthy volunteers and are lower in accordance with the severity of coronary atherosclerotic lesions among ACS patients. In coronary atherosclerotic lesions of ACS patients, the level of expression of salusin-α is lower than that of salusin-β (*4*). However, the significance of salusin-α in hypertension and its complications remains unclear.

The present study was performed to examine the relationship between the serum salusin-α level and blood pressure or carotid atherosclerosis, as assessed from the intima-media thickness (IMT) and plaque on B-mode ultrasonography, in patients with essential hypertension. We compared the relationship of salusin-α to increased carotid IMT with those of urotensin-II, the most potent vasoconstrictor identified to date (*7*), and other atherogenic factors.

Methods

Subjects

A total of 90 subjects (77 male, 13 female; aged 40–91 years) were consecutively enrolled in this study. The subjects included 70 patients referred to our outpatient hospital for hypertension and 20 gender- and age-matched normotensive volunteers (14 healthy persons, 3 mildly hyperlipidemic patients, and 3 mildly diabetic patients) without antihypertensive therapy or a history of hypertension. Hypertension was diagnosed as a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg on repeated measurement in the morning, according to Japanese Society of Hypertension Guidelines (*8*), or by current medical treatment for hypertension. Forty-eight patients were taking antihypertensive medication: calcium channel blockers (26%), angiotensin II type 1 (AT_1) receptor blockers (21%), angiotensin-converting enzyme (ACE) inhibitors (12%), diuretics (7%), α- or β-blockers (0%), or a combination of calcium channel blockers and AT_1 receptor blockers (34%). Possible causes of secondary hypertension were excluded based on the results of thorough clinical and laboratory investigations. Patients with angiographically proven coronary artery disease and heart failure were excluded from the study, because we have found decreased levels of serum salusin-α in patients with these diseases. This study was conducted in accordance with the principles of the Helsinki Declaration and was approved by the local institutional review committee. Informed consent for participation in this investigation was obtained from all subjects.

Carotid Ultrasonography

Carotid atherosclerosis was evaluated by high-resolution B-mode ultrasonography using a 7.5-MHz linear-array transducer (SSA-770A; Toshiba Corp., Tokyo, Japan). The maximal IMT and plaque score, as indices of carotid atherosclerosis, were determined by three trained operators blinded to the subjects' clinical records. All ultrasound images were obtained with the subject in the supine position with the neck mildly extended and rotated to the contralateral side, and measurement of IMT and plaque score was performed on the frozen frame, perpendicular to the vascular walls by scanning bilateral common and internal carotid arteries at the time of examination. In each subject, the maximal and mean IMT values were obtained by IMT measurements in 6 sites of the far walls in the bilateral carotid arteries excluding plaque. The upper normal limit of IMT was 1.0 mm, and the lesions with a focal IMT ≥1.1 mm were defined as plaques (*9*). The plaque score was calculated by summing all plaque thickness measurements in both carotid arteries (*9*). The severity of carotid atherosclerosis was graded by plaque score as follows: none, 0.0; mild, 1.1–5.0; moderate, 5.1–10.0; and severe, ≥10.1 (*9*).

Assays

Blood samples were collected in the morning after an overnight fast. Plasma glucose levels and serum levels of triglycerides and low-density lipoprotein (LDL) cholesterol were analyzed by enzymatic methods using automated techniques (*9*). Serum levels of small dense LDL and high-density lipoprotein (HDL) cholesterol were determined by the precipitation method (*10*). Serum levels of lipoprotein(a), insulin, and high-sensitive C-reactive protein (hs-CRP) were determined by the latex agglutination method, enzyme immunoassay, and latex-enhanced immunonephelometric assay, respectively (*9*). Plasma urotensin-II levels and serum salusin-α levels were measured by enzyme-linked immunosorbent assay and radioimmunoassay, respectively, as described previously (*2*, *9*).

Statistical Analysis

Results are expressed as the means±SEM for continuous variables and as frequencies for categorical variables. Differ-

Table 1. Clinical Characteristics of Hypertensive Patients and Normotensive Controls

	Hypertensive (n=70)	Normotensive (n=20)	p value
Age (years)	63±2	61±3	0.5365
Male (%)	89	75	0.1245
Body mass index (kg/m^2)	24±1	23±1	0.0930
Smoking habit (%)	34	35	0.7618
Hyperlipidemia (%)	21	15	0.3891
Diabetes mellitus (%)	17	15	0.5619
Statin therapy (%)	13	10	0.5397
Fibrate therapy (%)	7	5	0.5994
Insulin therapy (%)	0	0	1.0000
Systolic blood pressure (mmHg)	139±2	122±1	<0.0001
Mean blood pressure (mmHg)	97±2	87±1	0.0007
Diastolic blood pressure (mmHg)	77±2	70±2	0.0154

Table 2. Laboratory Data of Hypertensive Patients and Normotensive Controls

	Hypertensive (n=70)	Normotensive (n=20)	p value
HDL cholesterol (mg/dL)	48±2	44±3	0.1669
LDL cholesterol (mg/dL)	118±4	98±5	0.0045
Small dense LDL (mg/dL)	32±2	31±4	0.7895
Triglycerides (mg/dL)	129±11	113±16	0.4334
Lipoprotein(a) (mg/dL)	26±3	21±4	0.3615
Hs-CRP (mg/dL)	0.25±0.09	0.30±0.09	0.6998
Insulin (μU/mL)	8.3±1.3	4.6±0.6	0.0878
Fasting plasma glucose (mg/dL)	101±3	92±3	0.0997
HOMA-IR	2.5±0.4	1.2±0.2	0.0477

HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hs-CRP, high-sensitive C-reactive protein; HOMA-IR, homeostasis model assessment for insulin resistance.

ences in continuous variables between hypertensive patients and normotensive controls were assessed by Student's unpaired t-test. The χ^2 test was used for categorical data. Pearson's correlation coefficient was used to analyze relationships between maximal IMT and other continuous variables. Values of $p<0.05$ were taken to indicate statistical significance.

Results

The clinical characteristics and laboratory data of the 70 patients with essential hypertension and 20 normotensive controls are listed in Tables 1 and 2. The mean age, gender, body mass index, prevalence of a smoking habit, hyperlipidemia, and diabetes mellitus, and prevalence of therapy using statins, fibrates, and insulin did not differ significantly between the two groups (Table 1). The average systolic, mean, and diastolic blood pressure (Table 1), maximal IMT (Fig. 1A), mean IMT (Fig. 1B), plaque score (Fig. 1C), plasma urotensin-II levels (Fig. 1D), LDL cholesterol levels (Table 2), and homeostasis model assessment for insulin resistance (HOMA-IR, Table 2) were significantly greater in hypertensive patients than in normotensive controls. Serum salusin-α levels were significantly lower in hypertensive patients than in normotensive controls (Fig. 1E). There were no significant differences in serum levels of HDL cholesterol, small dense LDL, triglycerides, lipoprotein(a), hs-CRP, insulin, or fasting plasma glucose between the two groups (Table 2).

In all subjects, maximal IMT showed significant positive correlations with age, systolic blood pressure, LDL cholesterol, urotensin-II, and HOMA-IR (Table 3) and negative correlations with salusin-α (Fig. 2A). Forward stepwise multiple linear regression analysis revealed that salusin-α, urotensin-II, LDL cholesterol, HOMA-IR, and systolic blood pressure had significant independent associations with maximal IMT. Serum salusin-α levels were significantly lower in accordance with the severity of plaque score (Fig. 2B).

Discussion

The results of the present study indicated that patients with essential hypertension showed decreased serum levels of salusin-α and more severe carotid atherosclerosis as assessed

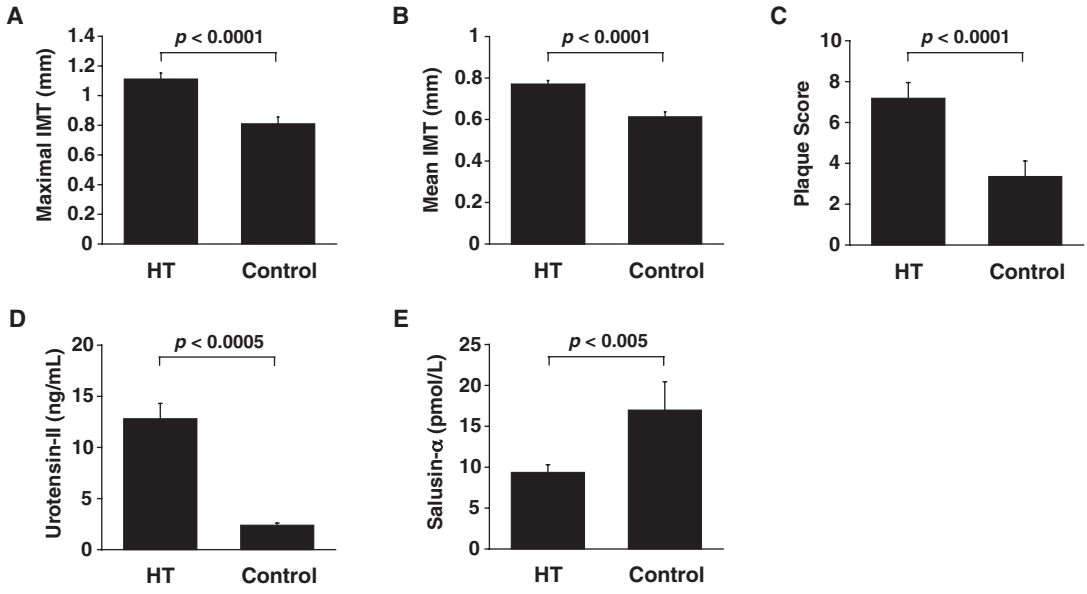

Fig. 1. *Comparisons of maximal intima-media thickness (IMT) (A), mean IMT (B), and plaque score (C) in the carotid artery, plasma urotensin-II levels (D), and serum salusin-α levels (E) between 70 hypertensive (HT) patients and 20 normotensive controls.*

by maximal IMT and plaque score compared with normotensive controls. Despite the reduction of serum salusin-α in essential hypertensives, however, there was no direct correlation between serum salusin-α level and blood pressure. These observations suggest that serum salusin-α may not be under the direct control of physiological blood pressure changes. Furthermore, salusin-α *per se* may play a relatively minor role in regulating blood pressure. Salusin-α is known to exert mild hypotensive effects, but its mechanism of action remains unknown. On the other hand, salusin-β, which is theoretically synthesized concomitantly with salusin-α mainly in the vascular endothelium and kidneys, causes potent hypotension without a vasodilator effect, and this effect is probably mediated at least in part *via* the activation of the parasympathetic nervous system (*1*). Hypertension is known to shift the autonomic balance toward the sympathetic dominant state. It is therefore likely that the hypotensive effect of salusin-α may be masked in hypertension. Further, serum salusin-α levels may be reduced in hypertension, unlike the serum levels of urotensin-II, a similar peptide produced in endothelial cells (*7*). Modulators for the synthesis, release, and degradation of salusin-α still remain to be elucidated (*11*). In addition, the influence of antihypertensive drugs on serum salusin-α levels has not yet been determined. Patients taking catecholamines, α- or β-blockers, and tranquilizers were excluded from this study, since these drugs may have affected the results by influencing the autonomic nervous system. Further studies are required to determine the association between serum salusin-α levels and the balance of sympathetic and parasympathetic tone.

The results of the present study also revealed a significant negative correlation between serum salusin-α levels and the severity of carotid atherosclerosis. Further, serum salusin-α levels were negatively correlated with maximal IMT and the severity of plaque score. The case showing the maximal value of salusin-α in Fig. 2A was a 91-year-old woman without carotid atherosclerosis, ischemic heart disease, or stroke, suggesting that this high salusin-α level may reflect intact vascular conditions. Our preliminary studies also suggested that serum salusin-α levels are independent of age, atherosclerotic markers, smoking, and exercise. Thus, decreased serum salusin-α levels may predict carotid atherosclerosis. Further studies should be performed to establish the overall usefulness of this peptide as a possible indicator of clinical atherosclerosis.

Macrophage foam0007 cell formation characterized by CE accumulation, a key event in the development of atherosclerosis, is modulated by various molecules such as scavenger receptors (cholesterol influx), ACAT-1 (storage CE converted from free cholesterol), and ATP-binding cassette transporter A1 (ABCA1, cholesterol efflux) (*12*). Salusin-α suppresses acetylated LDL-induced foam cell formation in primary cultured human monocyte-derived macrophages by down-regulation of ACAT-1 without affecting class A scavenger receptor or ABCA1 (*4*). Serum salusin-α levels were shown to be significantly decreased in 60 patients with ACS as compared with 53 healthy volunteers, and were lower in accordance with the severity of coronary atherosclerotic lesions among ACS patients. Salusin-α and -β are generally considered to be biosynthesized from a common precursor

Table 3. Correlations of Maximal Intima-Media Thickness (IMT) in the Carotid Artery in All Subjects

	r	95% confidence interval	*p* value
Age (years)	0.293	0.076–0.484	0.0092
Systolic blood pressure (mmHg)	0.338	0.125–0.522	0.0025
LDL cholesterol (mg/dL)	0.226	0.004–0.427	0.0466
Urotensin-II (ng/mL)	0.377	0.186–0.541	0.0002
HOMA-IR	0.478	0.274–0.641	<0.0001

LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance.

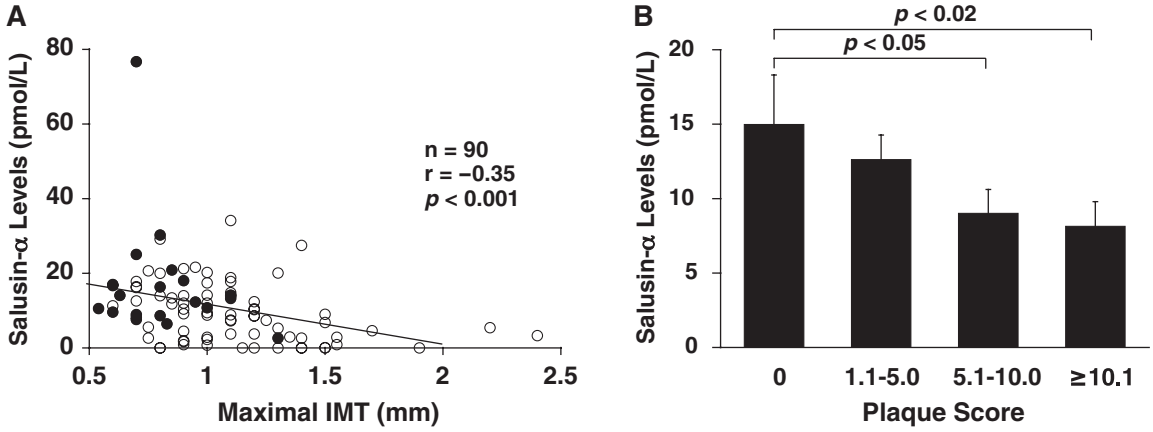

Fig. 2. *Relationships between serum salusin-α levels and maximal IMT (A) and the severity of plaque score (B) in all subjects. ○, 70 hypertensive patients; ●, 20 normotensive controls.*

peptide, the so-called preprosalusin, in the same tissues. However, the level of expression of salusin-α is lower than that of salusin-β in coronary atherosclerotic lesions of ACS patients (*4*). These data suggest altered translational or post-translational regulation of salusin-α and salusin-β in the pathophysiology of atherosclerosis, and thus further investigations are required to establish their contrasting roles; salusin-α acts as an anti-atherogenic factor, while salusin-β acts as an atherogenic factor.

There are several potential limitations of the present study. Some of the patients in both the hypertensive and normotensive groups had hyperlipidemia and were receiving statins/fibrates, which are known to exert pleiotropic anti-atherosclerosis effects *via* anti-inflammatory effects, anti-VSMC proliferation effects, and atherosclerotic plaque stabilization in addition to their lipid-lowering effects (*13*, *14*). The baseline levels of serum LDL cholesterol in hypertensive patients were higher than those in the normotensive subjects. Although the precise influence of such differences on carotid atherosclerosis as well as on salusin-α levels are unclear, the direct effects of these drugs on our conclusions may be limited, as there were no differences in the prevalence of the use of statins and fibrates between the two populations. Another limitation of this study was related to the variety of antihypertensive drugs prescribed for our hypertensive patients. Among these, AT_1 receptor blockers and ACE inhibitors have been reported to reduce carotid IMT in hypertensive patients (*15*, *16*). However, in our study, most patients taking ACE inhibitors showed increased rather than reduced IMT. Thus, the difference in carotid IMT between the hypertensive and normotensive groups may have been underestimated in the present study. Results from the comparison in subjects who had no antihypertensive, anti-lipidemic, or diabetes treatment showed that the 15 hypertensive patients had lower salusin-α levels (8.6±1.9 *vs*. 15.9±2.3 pmol/L, $p<0.05$) and higher maximal IMT (1.17±0.11 *vs*. 0.75±0.06 mm, $p<0.02$) than did the 7 normotensive controls, which was consistent with the results described above.

Salusin-β, a more potent hemodynamic regulator synthesized concomitantly with salusin-α, should be taken into account when interpreting the results of the present study. However, it remains unknown whether these molecules are secreted concomitantly into the circulation to regulate hemodynamics and atherosclerosis under patho-physiological conditions. Measurements of the salusin-β concentration in biological fluids are still unavailable due to technical difficulties related to the unexpected physical properties of this peptide (*17*). Further studies will be needed to evaluate the correlation between serum salusin-β level and the development of carotid atherosclerosis in the same subjects.

In conclusion, our results demonstrated the decrease in serum salusin-α levels in patients with essential hypertension, suggesting that it's decrease plays a role in the development and progression of atherosclerosis. Our results suggest that salusin-α may be a novel therapeutic candidate for the treatment of atherosclerosis.

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