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Contrasting effects of stanniocalcin-related polypeptides on macrophage foam cell formation and vascular smooth muscle cell migration

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ABSTRACT

Stanniocalcin (STC) is a calcium- and phosphate-regulating hormone secreted by the corpuscles of Stannius, an endocrine gland of bony fish. Its human homologues, STC1 and STC2 showing 34% amino acid identity each other, are expressed in a variety of human tissues. To clarify their roles in atherosclerosis, we investigated the effects of their full-length proteins, STC1(18–247) and STC2(25–302), and STC2-derived fragment peptides, STC2(80–100) and STC2(85–99), on inflammatory responses in human umbilical vein endothelial cells (HUVECs), human macrophage foam cell formation, the migration and proliferation of human aortic smooth muscle cells (HASMCs) and the extracellular matrix expression. All these polypeptides suppressed lipopolysaccharide-induced expressions of interleukin-6, monocyte chemotactic protein-1, and intercellular adhesion molecule-1 in HUVECs. Oxidized low-density lipoprotein-induced foam cell formation was significantly decreased by STC1(18–247) and increased by STC2(80–100) and STC2(85–99), but not STC2(25–302), in human macrophages. Expression of acyl-CoA:cholesterol acyltransferase-1 (ACAT1) was significantly suppressed by STC1(18–247) but stimulated by STC2(80–100) and STC2(85–99). Expression of ATP-binding cassette transporter A1 was significantly stimulated by STC1(18–247). Neither STC1(18–247) nor STC2-derived peptides significantly affected CD36 expression in human macrophages or HASMC proliferation. STC2(80–100) and STC2(85–99) significantly increased HASMC migration, whereas STC1(18–247) significantly suppressed the angiotensin II-induced HASMC migration. Expressions of collagen-1, fibronectin, matrix metalloproteinase-2, and elastin were mostly unchanged with the exception of fibronectin up-regulation by STC2(80–100). Our results demonstrated the contrasting effects of STC1 and STC2-derived peptides on human macrophage foam cell formation associated with ACAT1 expression and on HASMC migration. Thus, STC-related polypeptides could serve as a novel therapeutic target for atherosclerosis.

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1. Introduction

Atherosclerosis is a pathological injury-to-response process that is initiated by early inflammatory responses of vascular endothelial cells (ECs) [28]. Endothelial inflammation is characterized by increased production of pro-inflammatory cytokines and adhesion molecules, such as interleukin-6 (IL6), monocyte chemotactic protein-1 (MCP1), intercellular adhesion molecule-1 (ICAM1), vascular adhesion molecule-1 (VCAM1), and E-selectin in ECs, and monocyte adhesion and infiltration into the neointima lesion, followed by oxidized low-density lipoprotein (oxLDL)-induced transformation of macrophages into foam cells [28]. Accumulation of cholesterol ester (CE) in macrophages is a hallmark of foam cell formation [38]. This accumulation depends on the balance between the uptake of oxLDL via CD36 and the efflux of free cholesterol (FC) controlled by ATP-binding cassette transporter A1 (ABCA1) [38]. To protect the cells from the toxicity caused by excessive FC accumulation, the FC is esterified to CE by acyl-CoA:cholesterol acyltransferase-1 (ACAT1) [38]. Apart from accumulation of macrophage-derived foam cells, the migration and proliferation of vascular smooth muscle cells (VSMCs) and

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their production of extracellular matrix (ECM) components, such as collagen-1, fibronectin, matrix metalloproteinase-2 (MMP2), and elastin, contribute to the progression of the atherosclerotic plaque [28].

Stanniocalcin (STC) is a glycoprotein hormone secreted by the corpuscle of Stannius, an endocrine gland of bony fish [31]. This hormone plays a key role in calcium and phosphate homeostasis [7]. Its human homologues, STC1 (247 amino acids) and STC2 (302 amino acids), have been currently treated in the fields of endocrinology and cardiovascular research [2]. Human STC2 has 34% amino acid identity to both human STC1 and eel STC [2,7]. Both human STCs are expressed in a variety of human tissues, such as heart, kidneys, and blood [7]. STC1 is expressed in human cardiovascular system [23], and stimulates angiogenesis and prevents cerebral ischemia and renal ischemia/reperfusion injury [8,21,40]. STC1 suppresses the migration of monocytes/macrophages and ECs [1,9,41], reactive oxygen species production in macrophages and ECs [24,32], and tumor necrosis factor (TNF)-α-induced permeability in human coronary artery ECs [3]. A recent report showed the expression of STC1 in macrophages and ECs within coronary atherosclerotic lesions in patients with acute myocardial infarction and stable angina [15]. These findings suggest that STC1 has potential atheroprotective effects. While, STC2 is a hypoxia-inducible factor-1 target gene that promotes cell proliferation in hypoxia [14], and inhibits hyperphosphatemia-induced calcification in aortic VSMCs [29]. Overexpression of STC2 reduced atherosclerosis in hypercholesterolemic mice [27]. However, the molecular mechanisms that STCs could modulate atherosclerosis remain unclear.

In the present study, we assessed the modulatory effects of STC1, STC2, and STC2-derived polypeptides on the inflammatory response in ECs, macrophage foam cell formation, and the migration, proliferation, and ECM production in VSMCs. All experiments were performed in vitro using human vascular cells in this study.

2. Material and methods

2.1. Peptides and reagents

Recombinant human full-length STC1 and STC2 proteins, designated as STC1(18–247) and STC2(25–302) excepting for signal peptide sequences, respectively, were purchased from ITSI Biosciences (Johnstown, PA, USA). Following STC2 fragment peptides, STC2(9–16), STC2(43–54), STC2(91–99), STC2(80–100), STC2(85–99), STC2(164–172), and STC2(265–276) were synthesized and HPLC-purified by SCRUM Inc. (Tokyo, Japan). Angiotensin II (AngII), lipopolysaccharide (LPS), and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA).

2.2. Human cell culture

This investigation was approved by the Ethics Committee of Tokyo University of Pharmacy and Life Sciences. Written informed consent was obtained from all participants before enrollment. Human peripheral mononuclear cells were isolated from the blood of 25 healthy volunteers. Monocytes purified using anti-CD14 antibody-conjugated magnetic microbeads (Miltenyi Biotec, Auburn, CA) were seeded onto 3.5-cm dishes (1×10^6 cells/1 ml/dish) for cholesterol esterification assay and immunoblotting analysis [13,19,34–36,39]. Cells were incubated at 37 °C, 95% humidity, and 5% CO_2 for 7 days in RPMI 1640 medium supplemented with 10% human serum, 0.05 mg/ml streptomycin, 50 U/ml penicillin, and the indicated concentrations of STC1(18–247), STC2(25–302), STC2(80–100), or STC2(85–99). The medium in each dish was replaced with fresh medium containing STCs every 3 days.

2.3. Cholesterol esterification assay

Human macrophages differentiated by 7-day culture with the indicated concentrations of STCs were incubated for 19 h with 50 μg/ml human oxLDL in the presence of 0.1 mM [^{3}H]oleate (PerkinElmer, Yokohama, Japan) conjugated with BSA [13,19,34]. Cellular lipids were extracted and the radioactivity of cholesterol [^{3}H]oleate was determined by thin-layer chromatography.

2.4. Migration assay

Human aortic smooth muscle cells (HASMCs; Lonza, Walkersville, MD, USA) at passage 7 were seeded onto 3.5-cm dishes (5×10^4 cells/1 ml/dish). Cells were incubated at 37 °C, 95% humidity, and 5% CO_2 for 7 h in smooth muscle cell basal medium (SmBM) supplemented with 0.5 ng/ml human epidermal growth factor (EGF), 5 μg/ml insulin, 2 ng/ml human fibroblast growth factor (FGF), 50 μg/ml gentamicin, 50 ng/ml amphotericin B, and 5% fetal bovine serum (FBS). Then, while HASMCs were incubated for 5 h in serum-free SmBM with the indicated concentrations of STC1(18–247), STC2(25–302), STC2(80–100), or STC2(85–99) in the presence or absence of AngII (500 nM), photographs of cells were taken at 10-min intervals [13,19,34]. The average migration distance of 10 cells randomly selected in each dish was measured using a BIOREVO BZ-9000 microscope (Keyence, Osaka, Japan) [13,19,34].

2.5. Proliferation assay

HASMCs at passage 5–10 were seeded onto 96-well plates (1×10^4 cells/100 μl/well) and incubated at 37 °C, 95% humid-

Table 1
Primer sequences used for RT-PCR.

Gene		Primer sequence (5′ → 3′)	Product size (bp)
IL6	Forward	ATGAACTCCTTCTCCACAAGCGC	628
	Reverse	GAAGAGCCCTCAGGCTGGACT	
MCP1	Forward	CAATAGGAAGATCTCAGTGC	189
	Reverse	GTGTTCAAGTCTTCGGAGTT	
ICAM1	Forward	CGACTGGACGACAGGGATTGT	29
	Reverse	ATTATGACTGCGGCTGCTACC	
VCAM1	Forward	TCCCTACCATTGAAGATACTGGAAA	146
	Reverse	GCTGACCAAGACGGTTGTATCTC	
E-selectin	Forward	CCTACAAGTCCTCTTGTGCCTTC	206
	Reverse	ACAGGCGAACTTGCACACA	
GAPDH	Forward	ACCACAGTCCATGCCATCAC	451
	Reverse	TCCACCACCCTGTTGCTGTA	

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