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Serum carboxy-terminal telopeptide of type I collagen (ICTP) as a surrogate marker for vulnerable plaques in atherosclerotic patients: a pilot study

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Abstract

Evaluation of atherosclerotic plaques depends on invasive intravascular ultrasonography (IVUS). Carboxy-terminal telopeptide of type I collagen (ICTP) is produced by matrix metalloproteinase (MMP)-dependent digestion of type I collagen. Because vulnerable plaques are rich in type I collagen and MMPs from macrophages, we examined the association between serum ICTP and coronary plaques in patients with coronary disease. We recruited 46 men and 17 women without renal failure or bone diseases affecting serum ICTP, who underwent coronary IVUS. Serum ICTP levels were higher in patients with coronary plaques containing more than 10% necrotic core area than in patients with less than 10% necrotic core area. A positive correlation was found between serum ICTP and necrotic core area. Only serum ICTP was positively correlated with necrotic core area by multivariate analysis ($p<0.05$). These results suggest that serum ICTP can be used as a non-invasive marker of vulnerable plaques in atherosclerotic patients.

(148 words)

Introduction

Acute coronary syndrome aggravates morbidity and mortality, causing an increase in the risk of further events despite the use of percutaneous coronary intervention [1]. Many previous studies have demonstrated that acute coronary occlusion develops at the site of a vulnerable atherosclerotic plaque with a lipid-rich necrotic core [2,3]. Therefore, if there is a way to screen such vulnerable plaque lesions, preventive measures can be implemented before the development of acute coronary syndrome.

Intravascular ultrasound-virtual histology (IVUS-VH) uses radiofrequency analysis of ultrasound backscatter signals to overcome the limitations of grayscale IVUS by providing a more detailed plaque morphology, and is able to provide in vivo plaque analysis [4]. Thus, this procedure can directly detect vulnerable plaques in patients who are susceptible to develop acute coronary syndrome. However, IVUS involves invasive procedures with the risk of vascular complications.

Because atherosclerotic lesions, are rich in type I collagen, and because macrophages producing matrix metalloproteinases (MMPs) invade into these lesions, type I collagen degradation products by MMPs may be increased in these lesions. C-terminal telopeptide of type I collagen (ICTP) is an MMP-dependent degradation product of type I collagen, and serum ICTP level has been used for a marker of destructive bone lesions by metastatic cancers [5]. In contrast to the other bone resorption markers such as type I cross-linked C-telopeptide (CTX) and type I cross-linked N-telopeptide (NTX), ICTP is not produced by cathepsin K cleavage and is not elevated by osteoclastic bone resorption [6]. Thus, the present study was undertaken to examine whether serum ICTP level can be used as a non-invasive

predictive marker for vulnerable atherosclerotic plaques.

Patients and Methods

We recruited 46 men and 17 women (50 to 87 years, mean age 67.6) who were admitted to Takamatsu City Hospital and Tokushima University Hospital due to coronary symptoms, and underwent coronary angiography with IVUS from September 2005 to September 2007. The study was approved by institutional review board of the University of Tokushima Hospital. Written informed consent was obtained from all the patients who presented with acute coronary syndrome before patients underwent diagnostic coronary angiography. Among those patients, we randomly recruited only those who had at least one coronary plaque with 50 to 75% stenosis to this prospective study. IVUS-VH of the coronary plaque was performed at the most severe stenosis in each patient. Patients with renal dysfunction, metabolic bone diseases or thyroid diseases that affect serum ICTP levels were excluded. All patients were evaluated for coronary risk factors including dyslipidemia, sex, current smoking, diabetes mellitus or hypertension. Blood samples were taken before undergoing IVUS study after overnight fasting, and parameters of coronary risk factors or surrogate marker of vulnerable plaques were evaluated, including HbA1c (NGSP), triglyceride (TG), low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, lipoprotein(a) (Lp(a)), high sensitivity C-reactive protein (hs-CRP) and ICTP. Serum ICTP was measured by a two-antibody Telopeptide ICTP radioimmunoassay kit (Orion Diagnostica, Espoo, Finland). The normal range of serum ICTP was 1.8 to 5.0 ng/ml [7]. As bone turnover markers, serum NTX and bone-specific alkaline phosphatase (BSAP)

were measured in comparison with ICTP. Data were expressed as means +/- SD. Statistical significance was determined using paired Student's t-test and analyses were performed by SPSS statistics 17.0 for Macintosh. A *p* value of less than 0.05 was considered to be statistically significant.

IVUS-VH displays the reconstructed color-coded tissue map of plaque composition superimposed on cross-sectional images of the coronary artery (Volcano Therapeutics, Inc., Rancho Cordova, California). After baseline angiography, a 3.2-F, 30-MHz Ultrasound Imaging catheter (Boston Scientific Scimed Inc., Maple Grove, Minnesota) was placed distal to the target lesion. The catheter tip was subsequently pulled back using a motorized pullback system (Volcano Therapeutics, Inc.). Using this technology, coronary artery plaques at the section of most severe luminal obstruction were divided into fibrotic tissue, fibro-fatty tissue, necrotic core and dense calcium. Necrotic core area is composed of a mixture of lipid-like dead cells, foam cells and trapped blood cells. In this area, most of any real structure is lost, with some areas containing microcalcification as a byproduct from the dead cells, and friable areas next to sharp calcification become sites of gross instability and rupture [4]. Because a vulnerable plaque is reported to have more than 10% necrotic core without evidence of a fibrous cap [8], we selected the 10% area of necrotic cores as a cut-off to define vulnerable plaques.

Results

Necrotic core area was 0 to 38% of the sections of the most severe stenosis in each patient (mean 16.1+- 8.9%). There were 40 patients with more than 10% necrotic core

area in coronary plaque, and the remaining 23 patients showed less than 10% necrotic core area. Fibrotic tissue occupied 60.6+-13.2%, fibrofatty tissue 14.5+-11.4%, and dense calcium 8.9+-9.6% of the total section area. Figure 1 shows IVUS-VH images of coronary lesions with more than 10% necrotic core (A) or without vulnerable plaque (B).

We divided patients into those with 10% or more necrotic core and with less than 10% necrotic core. There was no significant difference between the two groups in age, current smoking, hypertension, diabetes mellitus, dyslipidemia, HbA1c, TG, LDL-C, HDL-C, Lp(a) and hs-CRP. Among bone turnover markers, only serum ICTP was significantly higher in the group with 10% or more necrotic core (Supplementary Table). Serum ICTP levels of age-matched control subjects (n=63) without atherosclerotic lesions by carotid ultrasonography were significantly lower (3.17 +/- 1.20 ng/ml) than those of the patients in this study (7.22 +/- 10.10 ng/ml) (p<0.005). Serum ICTP showed no significant relationship with either BSAP (p=0.147) or NTX (p=0.228).

There was a significant positive correlation between serum ICTP and necrotic core area by regression analysis before and after logarithmic transformation (Figure 1C, 1D), while no significant correlation was found between hs-CRP and necrotic core area (p=0.241). Furthermore, multivariate analysis after logarithmic transformation revealed that only ICTP was independently and positively correlated with necrotic core area (Table 1). These results demonstrate that serum ICTP can be a serum surrogate marker for the presence of vulnerable atherosclerotic plaque lesions.

Discussion

Coronary vulnerable plaques cause acute coronary syndrome, including unstable angina and acute myocardial infarction. However, characterization and evaluation of coronary plaques depends on invasive examinations such as IVUS and fiberscope. Atherosclerotic lesions are rich in type I collagen and macrophages producing MMPs [9,10], and the level of serum MMPs is significantly higher in patients with ruptured plaque [11]. Histological examination demonstrated that activated MMPs are overexpressed at the atherosclerotic plaques, and suggested that the focal expression of activated MMPs may promote destabilization and complication of atherosclerotic plaque [12]. Destabilization of vulnerable plaques by angiotensin II is mediated by angiotensin II-induced increase in the expression and activation of MMP8 and MMP13, with increased type I collagen breakdown [13]. ICTP is an MMP-dependent degradation product of type I collagen, while other type I collagen degradation products used as osteoclastic bone resorption markers are formed by cathepsin K-dependent degradation. In the present study, there was a significant correlation between serum ICTP and necrotic core area, and only serum ICTP, but not other bone turnover markers, was significantly correlated with the necrotic core area. Although metastatic bone lesions of cancer causes an increase in serum ICTP by increased MMPs-dependent type I collagen degradation in the bone, none of the participants showed cancer metastasis to the bone. Thus, it is likely that the source of serum ICTP in these patients is not from bone but from atherosclerotic plaques. In fact, only serum ICTP showed a significant correlation with the necrotic core area of coronary artery directly evaluated by IVUS-VH. Thus, serum ICTP can be used as a non-invasive surrogate marker for the

presence and severity of vulnerable plaques in patients without cancer metastasis to bone.

High sensitivity CRP has been widely used as a marker of vulnerable plaques [14], but can be affected by various factors including inflammation. In the present study, there was a wide variation in serum hs-CRP, and no significant correlation was found between hs-CRP and necrotic core area. Serum BSAP was reported to show a correlation with vascular stiffness [15]. However, the relationship between serum BSAP and atherosclerotic plaques is unclear, and no significant correlation was observed between serum BSAP and necrotic core area.

Our study has limitations. First, this is a cross-sectional study with relatively small sample size, and longitudinal data on serum ICTP and necrotic core area before and after medical interventions were not obtained. Second, we could assess only the section with most severe coronary plaque in each patient. ICTP can be released not only from severe coronary plaques but also from vulnerable plaques in other areas. Further studies will be necessary to delineate patients and conditions in which serum ICTP can be used as a surrogate marker for the development of acute coronary syndrome.

In conclusion, serum ICTP is correlated with necrotic core area of coronary plaque lesions in patients with coronary heart disease, and can be used as a non-invasive surrogate marker for vulnerable plaque lesions in atherosclerotic patients.

(1,491 words)

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Table 1. Multivariate analysis of the correlation of various risk factors with necrotic core area.

	Coeffeicient	SE	T value	95%CI	*p* value
Sex	-4.68	2.96	1.578	-10.629 - 1.277	0.120
Age	0.01	0.10	0.059	-0.201 - 0.213	0.952
Diabetes mellitus	-0.18	2.33	0.076	-4.861 - 4.505	0.939
Hypertension	-2.80	2.48	1.129	-7.793 - 2.184	0.263
Dyslipidemia	-3.55	2.42	1.464	-8.418 – 1.321	0.148
Serum TG	0.00	0.02	0.059	-0.043 - 0.046	0.952
Serum LDL-C	-0.03	0.04	0.936	-0.108 - 0.039	0.351
Serum HDL-C	-0.03	0.08	0.396	-0.197 - 0.132	0.693
Serm Lp(a)	0.35	1.22	0.288	-2.104 – 2.808	0.775
Serum ICTP	4.40	1.71	2.569	0.969 – 7.839	0.012

TG; triglyceride, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, Lp(a); lipoprotein (a), ICTP; C-terminal telopeptide of type I collagen

Figure legends

Figure 1. Images of atherosclerotic coronary lesions using intravascular ultrasound-virtual histology (IVUS-VH), and the relationship between serum ICTP and necrotic core area.

Green area represents fibrous tissue, yellow area fibro-fatty plaque, red area necrotic core, and white area represents dense calcium. (A) An IVUS-VH section of atheroma lesion with 25% necrotic core area, representing unstable atheromatous lesion. (B) A section of atheroma lesion with 4% necrotic core, representing stable atheromatous lesion. (C) Regression analysis of the relationship between serum ICTP and necrotic core area (%). Positive correlation was observed between serum ICTP and necrotic core area ($p<0.03$). (D) Regression analysis of relationship between logarithmic transformed serum ICTP and necrotic core area ($p<0.02$).

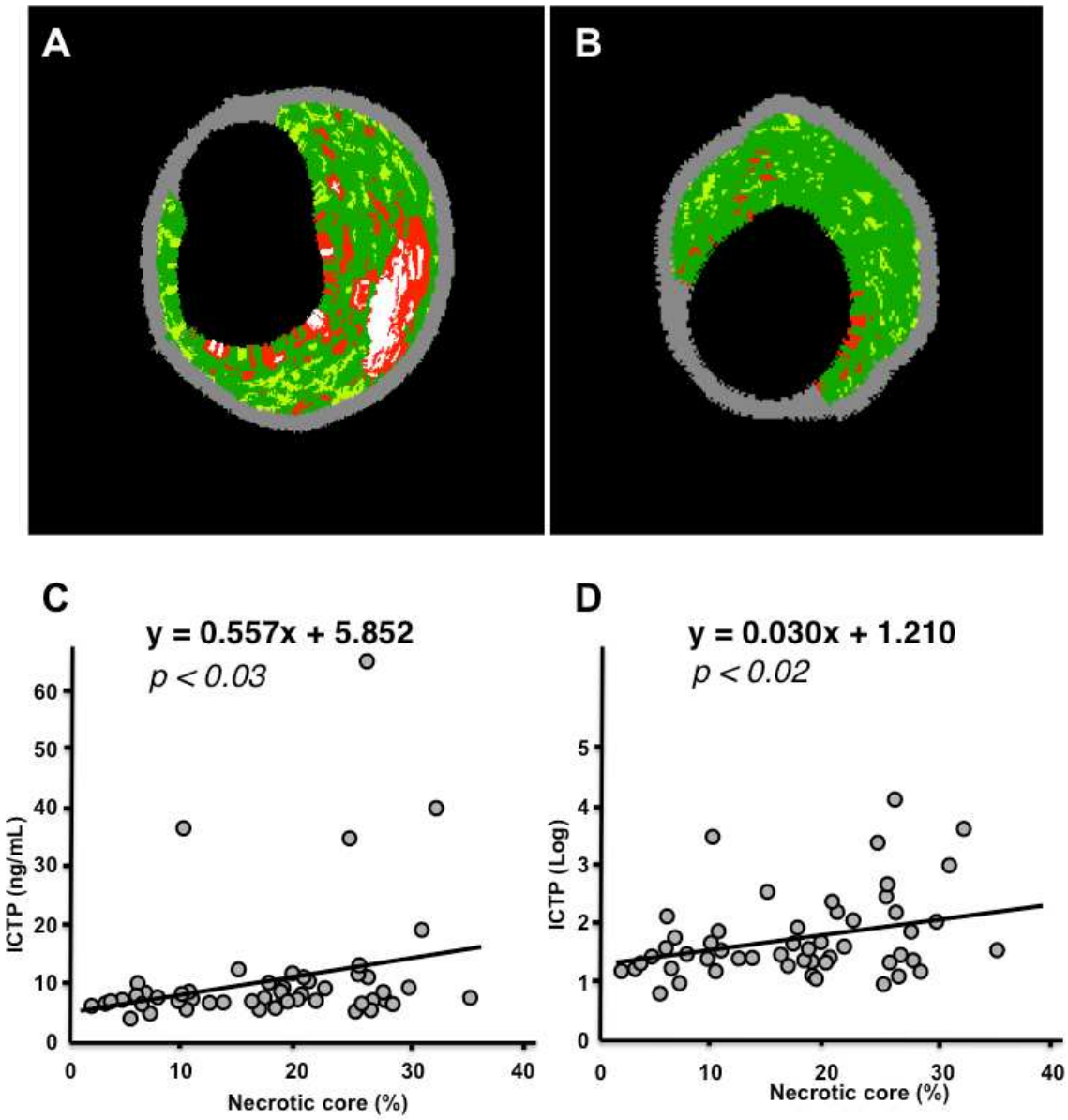
A
B
C
y = 0.557x + 5.852
p < 0.03
ICTP (ng/mL)
Necrotic core (%)
D
y = 0.030x + 1.210
p < 0.02
ICTP (Log)
Necrotic core (%)

Figure 1

Supplementaly Table. Comparison of clinical characteristics between patients with 10% or more and less than 10% necrotic core for atherosclerotic risk factors and bone turnover markers.

NC(%)	< 10	10≦	total	*p*-value
N	(23)	(40)	(63)	
Age	64.3+-9.9	69.5+-10.4	67.6+-10.4	
Current smoking	4	2	6	
Hypertension	18	25	43	
Diabetes mellitus	14	19	33	
Dyslipidemia	17	17	34	
HbA1c(%)	6.2+-1.4	6.2+-1.3	6.2+-1.4	0.995
TG(mg/dL)	128.7+-57.2	116.9+-56.0	120.8+-56.2	0.504
LDL-C(mg/dL)	128.2+-39.5	117.0+-26.4	123.2+-30.7	0.222
HDL-C(mg/dL)	47.1+-15.1	45.9+-14.8	46.1+-15.0	0.694
Lp(a) (mg/dL)	19.0+-16.7	29.2+-27.8	25.0+-24.7	0.172
Hs-CRP(mg/dL)	0.123+-0.169	0.439+-0.981	0.340+-0.820	0.241
BSAP(U/mL)	19.9+-5.9	23.5+-9.3	21.9+-8.2	0.147
NTX(nmolBCE/L)	13.2+-6.8	14.5+-5.4	14.4+-6.1	0.220
ICTP(ng/mL)	4.29+-1.12	8.96+-4.81	7.22+-10.10	<0.04

NC; necrotic core, TG; triglyceride, LDL-C; low-density lipoprotein cholesterol, HDL-C; high-density lipoprotein cholesterol, Lp(a); lipoprotein (a), Hs-CRP; high sensitivity C-reactive protein, BSAP; bone-specific alkaline phosphatase, NTX; type I cross-linked N-telopeptide, ICTP; C-terminal telopeptide of type I collagen