N^{ε}-(carboxymethyl)lysine Concentration in Debris from Carotid Artery Stenting Correlates Independently with Signal Intensity on T1-Weighted Black-Blood Magnetic Resonance Images

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Background and Purpose: Because magnetic resonance imaging (MRI) focuses on the morphological characteristics of carotid artery plaques, its diagnostic value with respect to plaque vulnerability is limited. We examined the correlation between N^{ε}-(carboxymethyl)lysine (CML), a main chemical structure of advanced glycation end-products, and the vulnerability of plaques visualized on MRI scans. *Materials and Methods:* We enrolled 43 patients who had undergone carotid artery stenting (CAS) for carotid artery stenosis; all underwent MRI studies, including black-blood MRI and diffusion-weighted imaging (DWI). The signal intensity ratio (SIR) of plaques to adjacent sternocleidomastoid muscle (P/M) on T1- and T2-weighted images (T1WI, T2WI) was calculated. Protein samples were extracted from debris trapped by a filter device. The concentrations of CML and myeloperoxidase (MPO) were measured by solid-phase enzyme-linked immunosorbent assay. *Results:* The patients were classified into 2 groups based on their SIR-P/M on T1WI and T2WI scans. We observed a higher incidence of post-CAS DWI lesions in patients with a higher than a lower SIR-P/M on T1WI; the CML and MPO concentrations in their CAS debris were also higher. No such differences were seen in patients with a higher or lower SIR-P/M on T2WI scans. The concentration of CML in CAS debris correlated independently with the SIR-P/M on T1WI of the carotid plaques, and was related to the concentration of MPO in CAS debris. *Conclusions:* Our findings suggest CML as a candidate molecular imaging probe for the identification of vulnerable plaques. **Key Words:** Nε-(carboxymethyl)lysine—carotid artery stenting—debris—vulnerable plaque—myeloperoxidase—magnetic resonance imaging.

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Introduction

The benefits of carotid artery stenting (CAS) depend on the risk of procedural neurologic complications. Diffusion-weighted magnetic resonance imaging (DWI) findings may help predict post-CAS cerebral embolism.[1] Elsewhere we documented that statin therapy for dyslipidemia decreased the incidence of multiple new DWI lesions after CAS,[2] suggesting that certain carotid plaque characteristics predispose to the development of new ischemic DWI lesions after CAS.

Black-blood MRI (BB-MRI) is widely used to characterize the components of carotid artery plaques. Plaques with high signal intensity (SI) on T1-weighted images (T1WI) are thought to reflect complex atheromas mainly comprised of a lipid core and associated hemorrhage[3] whereas plaques with high SI on T2-weighted images (T2WI) have been suggested to contain large amounts of extracellular matrix.[4] However, because BB-MRI is based on the morphological characteristics of plaques, its diagnostic value is limited.

Molecular imaging techniques that target various aspects of vulnerable plaques such as inflammation, neoangiogenesis, and apoptosis have been developed.[5,6] N^{ε}-(carboxymethyl)lysine (CML), a main chemical structure of advanced glycation end-products (AGE), accumulates in human atherosclerotic lesions and contributes to atherogenesis.[7,8] CML, formed exclusively in atheromatous plaques through Anderson's pathway induced by the reaction of myeloperoxidase (MPO) released from neutrophils and by activating macrophages that infiltrate vulnerable atheromatous plaques,[9] may be a candidate molecular imaging probe for vulnerable plaques.

As few studies have addressed the effects of CML accumulation on carotid artery plaque images, we investigated the correlation between pre-CAS BB-MRI findings and CML in debris captured by a CAS filter device.

Materials and Methods

Study Protocol

We registered 75 patients with severe carotid artery stenosis treated by CAS at Fukuoka University Chikushi Hospital between April 2014 and July 2015. CAS was as described elsewhere.[2] All patients who underwent carotid pre- and post-CAS MRI studies were eligible for inclusion in this study. Their clinical characteristics and debris trapped by embolic protection filter devices (SPIDER FX 6.0 mm; Covidien, Plymouth, MN) were collected. The research protocol was approved by the Ethics Committee of Fukuoka University Chikushi Hospital and all included patients provided their written informed consent for participation in this study.

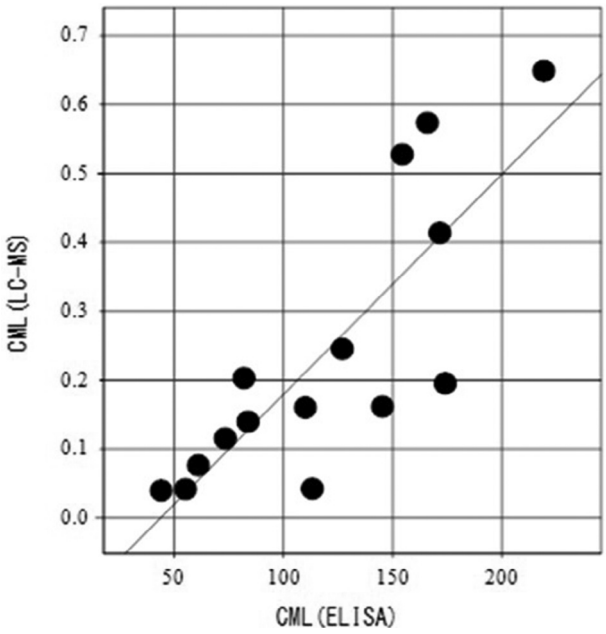

Figure 1. *Graph of CML levels in CAS debris measured by solid-phase ELISA and LC-MS/MS. Best-fit regression line:* $CML_{LC\text{-}MS/MS}$ *(mmol/mol lysine) = −.139 + .0032*CML_{ELISA} *(ng/mg protein). X intercept: 43.7 ng/mg protein, r = .818 (95% confidence interval: .5253-.9373),* $P < .001$, $n = 15$. *Abbreviations: CAS, carotid artery stenting; CML,* N^{ε}*-(carboxymethyl)lysine; ELISA, enzyme-linked immunosorbent assay; LC-MS/MS, liquid chromatography-tandem mass spectrometry.*

Patient Selection

Of the 75 patients, we excluded 29 (38.7%) because the protein concentration in debris extracts was too low for solid-phase enzyme-linked immunosorbent assay (ELISA). Another 3 patients were excluded because the CML level yielded by liquid chromatography-tandem mass spectrometry (LC-MS/MS) could not be assessed (Fig 1). Consequently, our study population consisted of 43 patients: 35 men and 8 women. Their clinical characteristics are listed in Table 1.

Clinical Data

In patients who had undergone CAS, we recorded the age, sex, symptomatic or asymptomatic status (presence or absence of symptoms related to carotid artery stenosis within 6 months of CAS), hypertension (blood pressure ≥140/90 mm Hg measured repeatedly, or use of hypotensive drugs), diabetes mellitus (HbA1c >6.5%, fasting blood glucose >126 mg/dL, or use of insulin or oral glucose inhibitors), dyslipidemia (fasting serum low-density lipoprotein ≥140 mg/dL, triglyceride ≥150 mg/dL, high-density lipoprotein <40 mg/dL, or use of statins), and new postoperative ischemic DWI lesions.

CAS Procedures

The indication for CAS was symptomatic or asymptomatic carotid artery stenosis greater than or equal to 50% or