Expression of Cyclophilin A in Coronary Artery Plaque with Intraplaque Hemorrhage Is More Frequent in Deceased Patients Who Had Impaired Kidney Function

Mai Nakai, MD, Aiko Shimokado, MD, Takashi Kubo, MD, Yosuke Katayama, MD, Tsuyoshi Nishiguchi, MD, Manabu Kashiwagi, MD, Kunihiro Shimamura, MD, Yasutsugu Shiono, MD, Akio Kuroi, MD, Takashi Yamano, MD, Takashi Tanimoto, MD, Yoshiki Matsuo, MD, Hironori Kitabata, MD, Yasushi Ino, MD, Tomoyuki Yamaguchi, MD, Atsushi Tanaka, MD, Takeshi Hozumi, MD and Takashi Akasaka, MD

Summary

Patients with impaired kidney function have a high frequency of intraplaque hemorrhage (IPH) in their coronary arteries. Levels of cyclophilin A (CyPA), an indirect matrix metalloproteinase inducer, are increased in deceased patients who had impaired kidney function. In this study, we have examined the relationship between IPH and CyPA.

We examined 47 samples of coronary plaque from 27 cadavers with coronary stenosis. These sections, all with > 50% coronary stenosis, were stained with an antibody against CyPA and the expression of CyPA was semi-quantified. Cadavers and plaques were classified into one of two groups depending on the presence or absence of IPH. IPH was defined as the presence of red blood cells stained with hematoxylin and eosin (HE) indicative of overt acute hemorrhage.

In an individual analysis, estimation of glomerular filtration rate (eGFR) in the IPH group was significantly lower than that in the non-IPH group (P = 0.002). In a histological analysis, the percentage of stained area of CyPA in the IPH group was significantly higher than that in the non-IPH group (P < 0.0001).

IPH was associated with a significantly higher expression of CyPA in this study. In addition, patients with IPH in their coronary arteries had significantly impaired kidney function.

Key words: Coronary atherosclerosis

Impaired kidney function is associated with a high incidence of coronary artery disease (CAD) due to multiple factors including diabetes, hypertension, platelet and endothelial dysfunction, oxidative stress, and pH change. Furthermore, previous autopsy studies have shown that advanced coronary atherosclerosis is prevalent in cadavers with impaired kidney function. Intraplaque hemorrhage (IPH) is common in advanced coronary atherosclerotic lesions. It is known to promote plaque progression, and thus has been suggested as an influence on plaque vulnerability. Cadavers of patients who had suffered from impaired kidney function very frequently have IPH in their coronary arteries, but the underlying mechanisms require elucidation.

Serum cyclophilin A (CyPA) levels are increased in patients with impaired kidney function or CAD. In histological analyses, it was found that CyPA abundantly expresses intracellular protein and is contained within coronary atheroma. Secretion of CyPA is induced from vascular smooth muscle cells by oxidative stress. The chemotactic activity of CyPA is partly mediated by binding with clusters of differentiation 147 (CD147) receptor. Recently, a growing body of research has suggested CyPA and CD147 involvement in key processes of kidney disease pathologies. CD147 induces the production of several matrix metalloproteinases (MMPs) which are involved in atherosclerosis and plaque vulnerability. MMP-9 serum levels were reported to be significantly higher in patients with recent IPH. IPH is therefore possibly related to patients with chronic kidney disease (CKD) via CyPA. The aim of the present study was to examine the relationships between IPH and renal function and between IPH and CyPA in cadavers.

Methods

Study population: The study population is shown in Figure 1. Between November 2011 and May 2016, 122 coronary arteries (5 left main coronary, 43 left anterior descending, 37 left circumflex, and 37 right coronary artery) were examined.

From the Department of Cardiovascular Medicine, Wakayama Medical University, Wakayama, Japan.

Address for correspondence: Takashi Kubo, MD, Department of Cardiovascular Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama, 641-8510, Japan. E-mail: takakubo@wakayama-med.ac.jp

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ies) were collected from 46 human hearts acquired during autopsy (85% men, mean age 69 years) at Wakayama Medical University Hospital. In 4 cases (9%) the cause of death was ischemic heart disease, and in 9 cases (20%) there was a history of coronary artery disease. Hypertension, type 2 diabetes mellitus, and a history of smoking were documented in 17 (37%), 13 (28%), and 7 (15%) cadavers, respectively. For all cadavers, eGFR was calculated with the modification of diet in renal disease (MDRD) formula and expressed in mL/minute/1.73 m². If the value of serum creatinine was < 0.06 mmol/L, it was rounded up to 0.06 mmol/L when eGFR was calculated. This study complies with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Wakayama Medical University. Written informed consent was obtained from the family members of all deceased patients.

Preparation of histology: The histological preparations were performed as previously reported. Briefly, the hearts were pressure-fixed in formalin and decalcified with ethylenediaminetetraacetic acid (EDTA) to maintain orientation and size. The surrounding soft tissues were dissected from each specimen. The left main coronary artery and the proximal and middle portions of the 3 coronary arteries were dissected from the surface of the heart. Distal portions and all branches were excluded. Parallel sections were serially sectioned along the longitudinal axis of the vessel at intervals of 3 mm. Following embedding in paraffin, the tissue blocks underwent whole-mount sectioning (4 μm sections) and staining with hematoxylin and eosin (HE) from the distal side of each 3-mm block. Each histopathology slide was digitized with a microscope at low magnification (× 1.25). The measurements of external elastic membrane area and luminal area were measured using ImageJ software (National Institutes of Health, Bethesda, MA). Sections with stenosis were examined. An area of cross-sectional luminal stenosis (1 - [luminal area/external elastic membrane area] × 100) ≥ 50% was diagnosed as stenosis.

Classification according to IPH: In the present study, we conducted two analyses: individual and histological studies. In the individual analysis, autopsy cases were classified into one of two groups according to the presence or absence of IPH in their coronary cross-sections. In the histological analysis, plaque was defined as serial atherosclerotic lesions in adjacent cross-sections. We analyzed 47 plaques and divided them into two groups according to the presence or absence of IPH in plaque (Figure 1). IPH was defined as the presence of red blood cells stained with HE indicating overt acute hemorrhage in coronary artery plaque.

Immunohistochemical study: We performed an immunohistochemical study using a peroxidase-based method with diaminobenzidine chromogen as previously described. Briefly, to retrieve the antigen, paraffin-embedded tissues were sectioned, deparaffinized, rehydrated and microwave-heated for 5 minutes using citrate buffer (0.01 M citric acid monohydrate, pH 6.0). Endogenous peroxidases were deleted by incubation with 3% hydrogen peroxide methanol for 30 minutes at room temperature and blocking was performed using 10% normal goat serum for 30 minutes at room temperature. The sections were incubated in primary antibody anti-CyPA (Abcam, catalog no. ab41684, Cambridge, MA, USA; 1:100 dilution) for 1 hour. EnVi-

Figure 1: Study design. In the individual analysis, 27 autopsy cases were analyzed from 46 autopsy cases. In the histological analysis, 47 plaques from 34 coronary arteries were included. We excluded 81 coronary arteries because the percentage of stenosis was < 50%. Autopsy cases and plaques were divided into two groups according to the presence or absence of IPH. IPH indicates intraplaque hemorrhage.
Statistical analysis: All statistical analyses were performed with JMP version 14.0 (SAS Institute, Cary, NC). Categorical data are reported as absolute values and performed with JMP version 14.0 (SAS Institute, Cary, NC). Statistical analysis: All statistical analyses were performed with JMP version 14.0 (SAS Institute, Cary, NC). Categorical data are reported as absolute values and compared by either Pearson’s chi-square test, or by Fisher’s exact test if an expected cell count was <5. Continuous variables are presented as the mean ± SD when normally distributed and otherwise as the median and interquartile range. Differences between groups were calculated using Student’s t test or Mann-Whitney’s U test for normal and skewed variables, respectively. The homogeneity of variances was tested using the Welch test. Values of P < 0.05 were considered statistically significant.

Results

Clinical demographics and individual analysis: Of the 46 autopsy cases examined, 27 had coronary stenosis. In our laboratory, the stented arteries could not be sectioned, so stented regions were excluded. Mean age was 69 years, 23 (85%) were men, and 20 (74%) had coronary artery disease. We harvested 27 human hearts and 47 plaques were studied (4 left main coronary, 16 left anterior descending, 10 left circumflex, and 17 right coronary arteries). Clinical characteristics are summarized in Table I. IHP was observed in 16/27 (59%) cadavers. The cause of death of the remaining 27 cases was ischemic heart disease in 2 cases (IHP group; n = 1, non-IHP group; n = 1). In the individual analysis, the rate of dyslipidemia in the IHP group was significantly higher than in the non-IHP group. Furthermore, estimations of glomerular filtration rate (eGFR) in the IHP group were significantly lower than in the non-IHP group (P = 0.002). There were no significant differences in age, gender, or prevalence of hypertension, diabetes mellitus, smoking, or cardiac history between the groups.

Histological analysis: In the histological analysis, IHP was observed in 25/47 (53%) plaques. Figure 2 shows a typical example of IHP stained with HE. Plaque characteristics with/without IHP are shown in Table II. CyPA expression was significantly increased in IHP (P < 0.0001). Figure 3 shows representative cross-sections of with/without IHP and the immunohistochemistry for CyPA. The expression of CyPA in coronary cross-sections was low in non-IHP (Figure 3B) and high in IHP (Figure 3D). The plaques were classified into fibrous, calcified and lipid subtypes. The association between IHP and plaque subtype was not always significant, but the percentage of stenosis was similar across the 3 groups.

Discussion

To the best of our knowledge, this is the first study to investigate the relationship between IHP and impaired kidney function via CyPA. In the present autopsy study, the kidney function of cadavers with IHP was significantly lower than that in cadavers without IHP. The per-
In the current study, IPH in coronary plaque was observed through the secretion of CyPA from smooth muscle. This suggests that atherosclerotic plaque rupture and destabilization is believed to induce extracellular CyPA, and promote atherosclerotic plaque growth and destabilization.

Previous studies have examined the relationship between IPH and impaired kidney function. Nakano, et al confirmed the relationship between impaired kidney function and IPH in coronary atherosclerosis and classified their subjects into 4 categories based on their eGFR. The study also highlighted that patients with impaired kidney function had IPH in their coronary arteries. Indeed, the percentage of area stained with CyPA was significantly higher in coronary plaque with IPH than in coronary plaque without IPH. CyPA may play an important role in the involvement of IPH in increasing plaque burden, while being an important mediator of platelet function by regulating integrin αIIbβ3 bidirectional signaling.

The present study has several limitations. First, there was a limited number of samples because of difficulties in specimen collection associated with post-mortem study. In particular, the relationship between renal function and CyPA in the non-IPH group should be examined to validate the relationship between impaired kidney function and CyPA, but the sample size was small and could not be shown. Our findings require validation by further studies with a larger number of cases. Second, the current study did not examine where the stents were implanted. Therefore, two patients who died from ischemic heart disease were excluded due to having less than 50% stenosis except for lesions treated by stents. Third, since the findings of IPH and CyPA expression were based on the histopathologic observation of specimens from autopsy, the healing process of IPH could not be considered in this study. The non-IPH group may include cases of healed IPH. Moreover, the non-IPH group was slightly younger than the IPH group, so IPH may have appeared over time. To avoid these limitations, only specimens with atherosclerosis were included in this study.

In conclusion, there appears to be an association between IPH, impaired kidney function and CyPA. IPH is known to involve atherosclerotic plaque growth and destabilization. Based on the various studies cited above, we hypothesized that CyPA is involved in the mechanism in which cadavers with impaired kidney function frequently had IPH in their coronary arteries. Indeed, the percentage of area stained with CyPA was significantly higher in coronary plaque with IPH than in coronary plaque without IPH. CyPA may play an important role in the involvement of IPH in increasing plaque burden, while being an important mediator of platelet function by regulating integrin αIIbβ3 bidirectional signaling.

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In conclusion, there appears to be an association between IPH, impaired kidney function and CyPA. IPH is more frequent among patients with impaired kidney function. Coronary plaque with IPH has higher expression of CyPA. Further studies should explore the mechanisms and therapeutic potential of CyPA in coronary IPH in patients with CKD.
Expression of CyPA in Coronary Plaque with IPH

Figure 3. Representative IPH sections and corresponding immunohistochemistry sections and positive staining of CyPA. A: A representative IPH (-) section (hematoxylin and eosin, 10x). B: The corresponding immunohistochemistry section and negative staining of CyPA (anti-CyPA, 10x). C: A representative IPH (+) section (hematoxylin and eosin, 10x). D: The corresponding section and positive staining of CyPA (anti-CyPA, 10x). CyPA indicates cyclophilin A; and IPH, intraplaque hemorrhage. Scale bars: 1 mm.

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Disclosure
Conflicts of interest: The authors declare that there are no conflicts of interest.

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