Short Communication

NEPHRON

Nephron 2001;89:455–458

Accepted: December 7, 2000

Soluble Osteopontin and Vascular Calcification in Hemodialysis Patients

Kosaku Nitta[a] Tsutomu Ishizuka[a] Shigeru Horita[a] Tetsuo Hayashi[a] Akiko Ajiro[a] Keiko Uchida[a] Kazuho Honda[a] Takashi Oba[b] Akira Kawashima[a] Wako Yumura[a] Takashi Kabaya[b] Takashi Akiba[a] Hiroshi Nihei[a]

[a]Department of Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, and [b]Dialysis Unit, Minami Senju Hospital, Tokyo, Japan

Key Words

Hemodialysis · Vascular calcification · Osteopontin · CT scan

Abstract

Background: Vascular calcification often occurs in patients with uremia. As osteopontin (OPN) is not only involved in the physiological but also the pathological calcification of tissues, OPN may be associated with the pathogenesis of aortic calcification in hemodialysis (HD) patients. ***Methods:*** We examined the expression of OPN in atherosclerotic aortas of HD patients. In addition, we performed a prospective longitudinal study by using CT scans to detect aortic calcifications and by measuring the plasma OPN concentration by ELISA in HD patients (20 men, 16 women; mean age 55.2 ± 21.3 years) and in healthy volunteers (18 men, 17 women; mean age 54.0 ± 13.2 years). ***Results:*** By immunohistochemical staining, OPN was abundantly localized in atherosclerotic plaques of HD patients. The macrophages surrounding the atheromatous plaques were identified as the OPN-expressing cells. We furthermore found that the concentration of soluble plasma OPN was significantly higher in HD patients as compared with the concentrations in age-matched healthy volunteers (837.3 ± 443.2 vs. 315.1 ± 117.4 ng/ml, $p < 0.01$). The OPN concentration was positively correlated with the aortic calcification index in HD patients ($r = 0.749$, $p < 0.01$). ***Conclusion:*** These data suggest that OPN, secreted by macrophages, plays a role in the calcification of atheromatous plaques in HD patients.

Introduction

Osteopontin (OPN) is a secreted phosphoprotein originally described as a noncollagenous protein in the bone matrix, but was more recently observed in several nonmineralizing tissues, including the kidneys and arteries [1]. OPN is a low-affinity, high-capacity, calcium-binding protein that can bind tightly to hydroxyapatite, the primary constituent of mineralized bone. In addition, OPN contains an arginine-glycine-aspartate motif that is similar to the motifs found in many cell-matrix adhesion molecules, including fibronectin, thrombospondin, and vitronectin [2]. OPN has recently been reported to exist as a novel component of the human atherosclerotic plaques found in association with calcified deposits [3–5], suggesting that OPN may be an important mediator of arterial neointima formation as well as dystrophic calcification.

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0028–2766/01/0894–0455$17.50/0

Accessible online at:
www.karger.com/journals/nef

Kosaku Nitta, MD
Department of Medicine, Kidney Center
Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku
Tokyo 162-8666 (Japan)
Tel. +81 3 3353 8111, ext. 39112 4, Fax +81 3 3356 0293, E-Mail nitta@kc.twmu.ac.jp

Vascular calcification, which has been described in diabetics and the elderly, is frequently seen in patients with uremia. Goldsmith et al. [6] reported that vascular calcification steadily becomes more prevalent with the onset of hemodialysis (HD) in 39% of the patients. In 92% of the patients, however, vascular calcification begins to become more prevalent after an average HD duration of 16 years, with a mean onset of 9.7 years after the initiation of HD. As well as becoming more prevalent, the calcification becomes progressively severe in most patients. We previously reported [7] that calcification of the aorta in HD patients appears earlier than in control subjects, but that no correlations were found between the grade of aortic calcification, the age of the patients, and the duration of HD. Even though an abnormal calcium metabolism seems to be associated with vascular calcification in patients with uremia, the precise mechanism has not been elucidated.

Patients and Methods

The present study was designed to examine the relation of OPN to vascular calcification in 36 HD patients (20 men, 16 women; mean age 55.2 ± 21.3 years) with a mean duration of HD of 8.7 (range 1–22) years prior to the study. The underlying diseases were chronic glomerulonephritis in 19 patients and diabetic nephropathy in 17 patients. Thirty-five age- and sex-matched healthy individuals with a mean age of 54.0 ± 13.2 years served as controls. All patients and healthy subjects gave their informed consent to participate in this study. We performed a prospective longitudinal study using CT scans to detect aortic calcification as described previously [7]. After identification of the slice with the most extensive arteriosclerosis, the proportion of aortic circumference covered by calcification was quantified in terms of degree of total circumference and expressed as the aortic calcification index (ACI).

Blood was obtained from each patient before HD. The serum concentrations of calcium, phosphate, intact parathyroid hormone, hemoglobin, bicarbonate, urea, or creatinine were measured. The plasma OPN concentration was measured using a commercially available ELISA kit (Immuno-Biotechnology Laboratory, Gunma, Japan) according to the manufacturer's protocol.

Segments of the human aorta were obtained from 6 autopsy cases of HD patients. Tissue samples were fixed with 10% buffered formaldehyde and embedded in paraffin. Immunohistochemistry was carried out using serial sections (3 μm thick), as described previously [8]. The primary monoclonal antibodies used in this study were the mouse antihuman OPN antibody 10A16 (Immuno-Biotechnology Laboratory) and PG-M1 (DAKO, Glostrup, Denmark). PG-M1 recognizes CD68 which is specific to monocyte-macrophage lineage cells. Bindings of monoclonal antibodies was demonstrated using the Vectastain ABC kit (Vector Laboratories, Burlingame, Calif., USA).

The results are expressed as mean ± SD. The significance of differences between patients and controls was determined using Student's t test. The level of significance was $p < 0.05$.

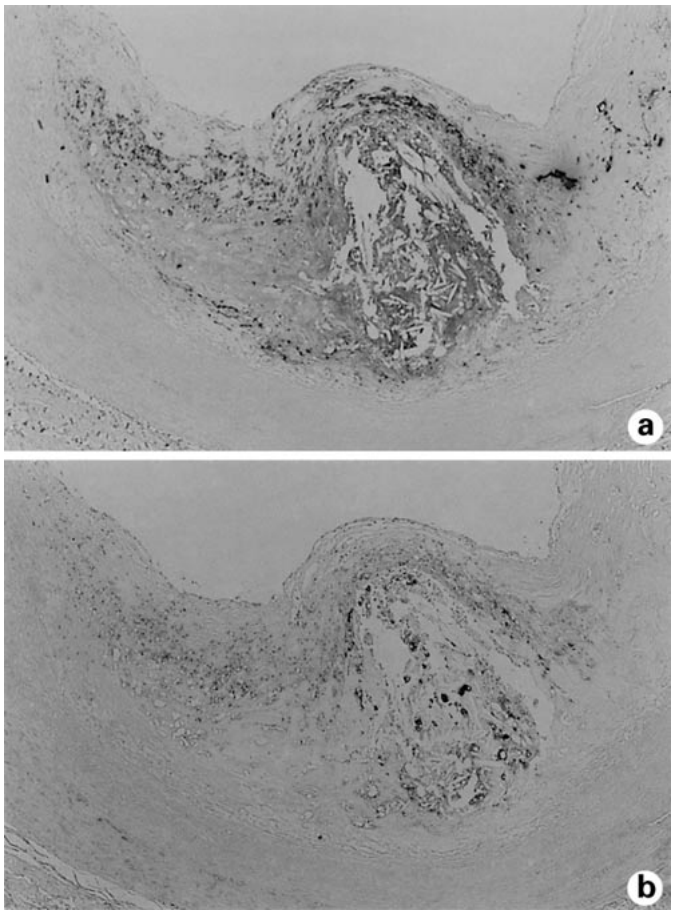

Fig. 1. Immunohistochemical localization of osteopontin (**a**) and macrophages (**b**) in aortic tissue with atheromatous lesions from a HD patient. × 200.

Results

Figure 1a shows the OPN expression in aortic atheromatous plaque of a HD patient. The OPN expression was greater in the atherosclerotic lesions with calcifications than in the putatively normal portions. Foam cells in atheromatous lesions could be either macrophages or smooth muscle derived cells. The OPN expression was related to the degree of atheromatous plaques in all aortic tissues studied. To determine what types of OPN-expressing cells were present, serial sections were stained with the monoclonal antibody PG-M1 which is specific to monocyte-macrophage lineage cells. As shown in figure 1b, macrophages detected by staining with PG-M1 were abundantly localized in the atherosclerotic lesions.

To further examine whether soluble OPN is detectable in the plasma of HD patients, we measured the OPN con-

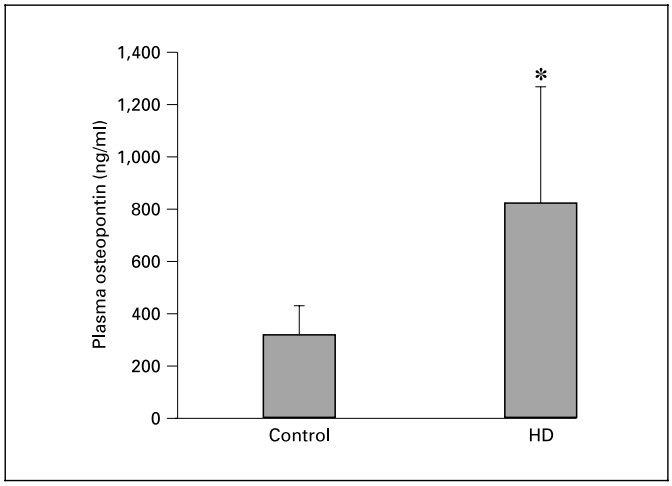

Fig. 2. Comparison of plasma osteopotin concentrations in healthy subjects (controls) and hemodialysis (HD) patients. * $p < 0.01$.

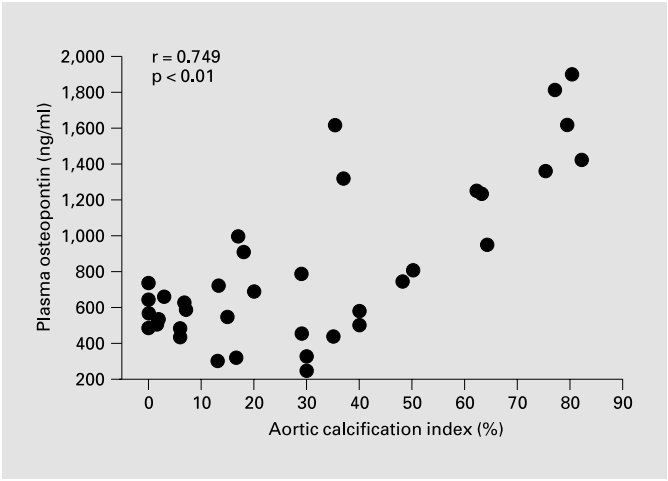

Fig. 3. Correlation between plasma osteopontin concentration and aortic calcification index in hemodialysis patients. n = 36.

centration using a commercially available ELISA kit. As compared with the plasma OPN concentrations in the healthy volunteers (315.1 ± 117.4 ng/ml), the concentrations in the age-matched HD patients were significantly higher (837.3 ± 443.2 ng/ml, $p < 0.01$; fig. 2). As shown in figure 3, the OPN concentration was positively correlated with the ACI in the HD patients ($r = 0.749$, $p < 0.01$). Moreover, the OPN concentration was not correlated with age, duration of dialysis, underlying diseases, blood pressure, or serum concentrations of calcium, phosphate, intact parathyroid hormone, hemoglobin, bicarbonate, urea, or creatinine (data not shown). A multivariable logistic regression analysis revealed that the plasma (OPN) concentration was independently associated with the ACI in HD patients.

Discussion

Vascular calcification, which is described in the elderly and in diabetics, is frequently seen in patients having uremia. In arteries, calcification has been reported to be positively correlated with heart disease and an increased risk of myocardial infarction and ischemic episodes in peripheral vascular disease. Importantly, the presence of calcifications in arteries has recently been found to be predictive of death and myocardial infarction in high-risk, asymptomatic patients [9]. The mechanisms regulating dystrophic calcification are not known. It is possible that the pathological calcification of blood vessels shares features with normal mineralization of bone and cartilage tissue. Recent morphological studies [3–5] have reported that OPN is abundant at sites of calcification in human atherosclerotic plaques and in calcified aortic valves, but is not found in normal arteries. However, the role of OPN in the pathogenesis of vascular calcification is not known in HD patients.

The present study has demonstrated that OPN is abundantly expressed in the atherosclerotic lesions of aortic tissues with calcification in HD patients. The OPN expression was related to the degree of atheromatous plaques. Immunohistochemistry showed that the OPN-expressing cells were macrophages surrounding the atheromatous plaques. We speculate that OPN, secreted by macrophages, is in part associated with the pathogenesis of aortic calcifications in HD patients. Previous studies reported that smooth muscle cells expressed OPN in human atherosclerotic plaques [3–5]. As OPN mRNA expressing smooth muscle cells decrease with the development of atherosclerosis, macrophages could be major OPN-expressing cells in advanced stage of atheromatous lesions in HD patients.

The plasma OPN concentrations in the HD patients were significantly higher than those in the age-matched healthy volunteers. The main production site of OPN is thought to be the vasculature and monocytes/macrophages. OPN is a novel component of human atherosclerotic plaques found in association with calcified deposits of aortas in HD patients. The present study is the first to report the correlation between increases in the soluble

plasma OPN concentration and the degree of aortic calcification in HD patients. Because OPN appears to be critically involved in the regulation of mineralization in atherosclerotic plaques, we believe that the increase in plasma OPN concentration results from an uremia-induced increase in OPN expression in the vasculature and macrophages of atherosclerotic plaques, possibly providing a link between OPN concentration and vascular calcification in HD patients.

References

1 Butler WT: The nature and significance of osteopontin. Connect Tissue Res 1989;23:123–136.
2 Oldberg A, Franzen A, Heinegard D: Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell-binding sequence. Proc Natl Acad Sci USA 1986;83:8819–8823.
3 Giachelli CM, Bae N, Almeida M, Denhardt DT, Alpers CE, Schwartz SM: Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. J Clin Invest 1993;92:1686–1696.
4 Hirota S, Imakita M, Kohri K, Ito A, Mori E, Adachi S, Kim HM, Kitamura Y, Yutani C, Nomura S: Expression of osteopontin messenger RNA by macrophages in atherosclerotic plaques. Am J Pathol 1993;143:1003–1008.
5 Ikeda T, Shirasawa T, Esaki Y, Yoshiki S, Hirokawa K: Osteopontin mRNA is expressed by smooth muscle-derived foam cells in human atherosclerotic lesions of the aorta. J Clin Invest 1993;92:2814–2820.
6 Goldsmith DJA, Covic A, Sambrock PA, Ackrill P: Vascular calcification in long-term haemodialysis patients in a single unit: A retrospective analysis. Nephron 1997;77:37–43.
7 Kabaya T, Nitta K, Kimura H, Kawashima A, Narusawa K, Nihei H: Increased aortic calcification index in hemodialysis patients. Nephron 1999;81:354–355.
8 Nitta K, Horita S, Honda K, Uchida K, Watanabe T, Nihei H, Nagata M: Glomerular expression of cell-cycle-regulatory proteins in human crescentic glomerulonephritis. Virchows Arch 1999;435:422–427.
9 Locker TH, Schwartz RS, Cotta CW, Hickman JR: Fluoroscopic coronary artery calcification and associated coronary disease in asymptomatic young men. J Am Coll Cardiol 1992;19:1167–1172.