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Research Article

Age-Related Changes of Elements in Human Posterior Intercostal Arteries and Their Observation by Scanning Electron Microscopy

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ABSTRACT

To elucidate changes of the posterior intercostal artery (PIA) with aging, the authors investigated age-related changes of elements in the PIAs by direct chemical analysis and endothelial changes by scanning electron microscopy. Used subjects consisted of 24 men and 6 women who ranged in age from 41 to 89 years (mean age, 70.6±11.8 years). The left fifth PIA was dissected out and the proximal site was used for element analysis and for observation by scanning electron microscopy. The PIA samples were incinerated with nitric acid and perchloric acid, and element contents were determined by inductively coupled plasma-atomic emission spectrometry. It was found that the Ca content tended to increase slightly with aging, but the other six elements, P, S, Mg, Zn, Fe, and Na, did not change significantly in the PIA with aging. To elucidate whether calcification occurred in the PIAs in old age, the relationships among seven element contents and both the mass ratios of Ca/P and Mg/Ca were examined in the PIAs. Although significant correlations were found between Ca and either P or Mg contents, no significant correlation was found between P and Mg contents in the PIAs. The average mass ratio (mg/mg) of Ca/P was high, being 4.02±1.99 in the PIAs. The results indicated that calcification hardly occurred in the PIA in old age. The inner surfaces of the PIAs were observed by scanning electron microscopy. Endothelial lining was partial detached from the basement in many areas. It was indicated that a significant damage to the endothelium occurred frequently in the proximal site of the PIA in old age.

INTRODUCTION

Coronary artery bypass grafting is one of the most common major operations in the world. At present, both the internal thoracic and radial arteries are mainly used for coronary artery bypass grafts [1-6]. There has been a tendency toward increased use of arterial conduits. Strategies to increase the use of arterial conduit include the use of other arterial conduits apart from the internal thoracic and radial arteries.

There are a few histological reports [7-9] on the posterior intercostal artery (PIA) to use for coronary artery bypass grafting. Van Son et al. [7] first found that the PIA as well as the internal thoracic artery was composed of an elastic artery. Thereafter, similar findings were reported by Unlu et al. [8] and Reddy et al. [9]. To examine age-related changes of the PIA, Reddy et al. [9] divided the fifth PIA samples from

cadavers into three age groups of 19-40 years, 41-60 years, and over 61 years, and studied them by histological method. They reported that age-related pathological changes like intimal thickening or atherosclerosis were not observed in the PIA between 19 and 60 years old, but mild intimal thickening was found in 50% over 61 years old, and that no calcification of the tunica media was found in the PIA samples studied. However, little work had been done to study age-related changes of the PIA from a viewpoint of elements. Therefore, the authors investigated age-related changes of elements in the PIA by direct chemical analysis and endothelial changes by scanning electron microscopy.

MATERIALS AND METHODS

Sampling

Thirty cadavers as shown in Table 1 were used in the present study. The subjects consisted of 24 men and 6 women who ranged in age from 41 to 89 years (mean age, 70.6±11.8 years). In each cadaver, the left fifth PIA which was surgically suitable for coronary artery bypass graft [10], was dissected out. The extent of the dissection was 5 cm in length from its origin. The most proximal site of the PIA, about 1 cm long, was used for element analysis and the next proximal site of the PIA, about 1 cm long, was used for observation by scanning electron microscopy. The present study was approved by our institutional ethics committee.

Determination of elements

The arterial samples were washed thoroughly with distilled water and were dried at 95^{0}C for 16 h. After 1 ml concentrated nitric acid was added to the dry samples to incinerate, the mixtures were heated at 100^{0}C for 2 h. After the addition of 0.5 ml concentrated perchloric acid, they were heated at 100^{0}C for an additional 2 h [11]. The samples were adjusted to a volume of 10 ml by adding ultrapure water and were filtered through filter paper (no. 7; Toyo Roshi, Osaka, Japan). Seven elements of Ca, P, S, Mg, Zn, Fe, and Na were selected for measurement because of the following reasons: Both Ca and P are directly correlated with Mg on calcification [12]; smooth muscles containing S decrease on atherosclerosis [13]; Zn [14] and Fe [15] are related to atherosclerosis; and Na is an important cation. The resulting filtrates were analyzed by inductively coupled plasma-atomic emission spectrometry (iCAP 7400 ICP-OES Duo; Thermo Fisher Scientific Japan Inc., Kanagawa, Japan). The conditions were as follows: 1.15 kW from the radiofrequency forward power, an auxiliary gas flow rate of 0.5 l/min, a nebulizer gas flow rate of 0.55 l/min, a coolant gas flow rate of 12 l/min, a purge gas flow rate of 3.2 l/min, and an exposure time of 10 s. Especially prepared standard solutions of Ca, Mg, Zn, Fe, and Na for atomic absorption spectrometry and phosphate and sulfate ions for ion chromatography were purchased from Wako Pure Chem. Ind. (Osaka, Japan) and were used as standard solutions. The measurement of elements was performed at a fixed wavelength of 588.995 nm for Na, 393.366 nm for Ca, 279.553 nm for Mg, 259.940 nm for Fe, 213.856 nm for Zn, 180.731 nm for S, and 177.495 nm for P. The amount of elements was expressed on a dry weight basis.

Table 1: Subjects Used in the Present Study.

Age (Years)	Sex	Cause of Death
41	M	Hepatitis
55	M	Heart attack
56	W	Liver cancer
56	M	Sepsis
58	W	Breast cancer
59	M	Sepsis
60	W	Paresis
62	M	Stroke
63	M	Sepsis
64	M	Sepsis
66	M	Pneumonia
68	M	Acute heart failure
70	M	Kidney failure and complication
70	M	Airway obstruction
70	M	Heart and kidney failures with hypertension
71	M	Diabetes mellitus
72	W	Debility
74	M	Abnormal bone marrow
76	M	Heart failure
76	M	Acute stroke
76	M	Pneumonia
77	M	Coronary artery disease
78	M	Sepsis
80	M	Lung cancer
84	M	Senility
85	M	Senility
87	W	Pneumonia
88	W	Senility
88	M	Senility
89	M	Coronary artery disease

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Scanning electron microscopy

The arterial samples were carefully dissected longitudinally. The samples were post-fixed in 2% osmium tetroxide, 0.1 M phosphate buffer (pH 7.4) for 1 h to 2 h and were rinsed in the phosphate buffer. The samples were then dehydrated in a series of graded ethanol (50% to 100%) for 15 min. After critical point drying, the samples were rendered conductive by sputtering them with gold. The coated samples were examined by scanning electron microscopy (JSM-6610LV; JEOL, Tokyo) operated at 15 kV.

STATISTICAL ANALYSIS

Statistical analyses were performed using the GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). Pearson's correlation was used to investigate the association between parameters. A two-tailed unpaired Student's *t* test was used to analyze differences between groups. A *p* value of less than 0.05 was considered to be significant. Data were expressed as the mean±standard deviation.

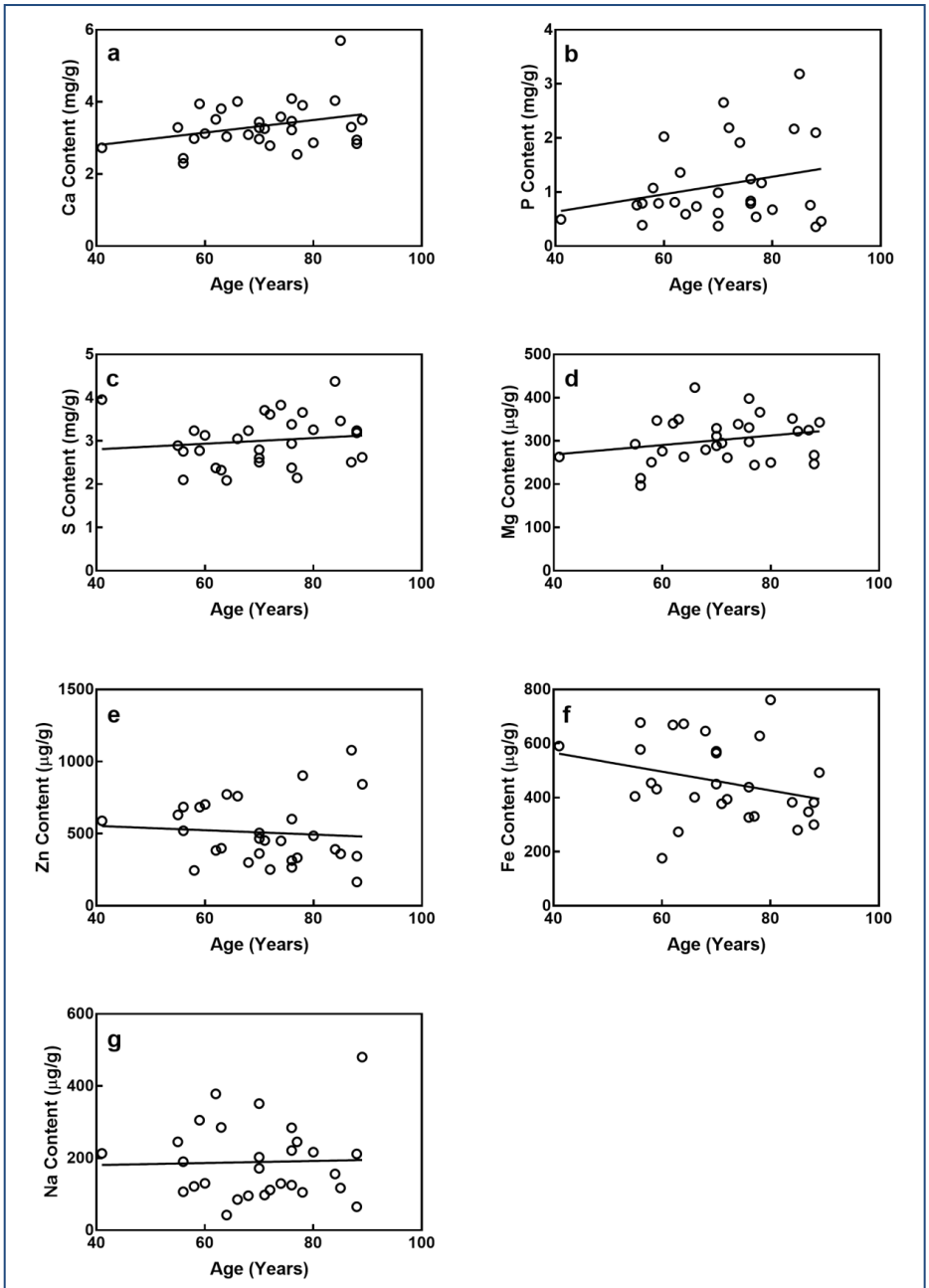

Figure 1: Age-related changes of Ca (a), P (b), S (c), Mg (d), Zn (e), Fe (f), and Na (g) contents in the PIAs.

RESULTS

Table 1 indicates the ages, sexes, and causes of deaths of the 30 subjects used in the present study.

Age-related changes of elements in the PIAs

Figure 1 shows age-related changes of seven element contents in the PIAs studied. The correlation coefficients between age and element contents were estimated to be 0.315 (p=0.090) for Ca, 0.259 (p=0.175) for P, 0.131 (p=0.490) for S, 0.247 (p=0.189) for Mg, -0.081 (p=0.672) for Zn, -0.282 (p=0.138) for Fe, and 0.032 (p=0.868) for Na. No significant correlations were found between age and seven element contents in the PIAs. The pattern of distribution for the seven element contents did not change significantly in the PIA with aging. However, the Ca content tended to increase slightly with aging.

The average contents of seven elements were 3.335±0.656 mg/g for Ca, 1.132±0.752 mg/g for P, 3.006±0.588 mg/g for S, 302.3±52.54 μg/g for Mg, 508.5±219.8 μg/g for Zn, 459.9±148.3 μg/g for Fe, and 189.4±102.6 μg/g for Na. The average content of Ca was highest and it decreased in the order of S, P, Zn, Fe, Mg, and Na in the PIAs.

The incidence of the PIA with the Ca content more than 5 mg/g, which is not contained in a normal artery [16], was examined. Such an artery was found in one out of seven cases in the 80s of the subjects, but it was not found in the 50s, 60s, and 70s of the subjects. The incidence of such an artery was 3.3% in all the subjects studied.

Relationships among seven element contents in the PIAs

To examine whether calcification occurred, the relationships among seven element contents were examined in the PIAs. Table 2 lists the relationships among seven element contents in the PIAs. An extremely significant direct correlation was found between Ca and Mg contents and a very significant direct correlation was found between P and S contents. A significant direct correlation was found between Ca and P contents, whereas significant inverse correlations were found between P and either Fe or Na contents and between S and Na contents. However, no significant correlation was found between P and Mg contents.

Table 2: Relationships among Seven Element Contents in the PIAs.

Element	Correlation Coefficient and p Value					
	P	S	Mg	Zn	Fe	Na
Ca	0.462 (0.012)	0.232 (0.217)	0.746 (<0.0001)	0.105 (0.583)	−0.356 (0.058)	0.063 (0.746)
P		0.559 (0.002)	0.114 (0.556)	− 0.335 (0.075)	−0.444 (0.018)	−0.431 (0.022)
S			0.117 (0.539)	−0.209 (0.268)	−0.120 (0.536)	−0.394 (0.034)
Mg				0.293 (0.117)	−0.269 (0.158)	0.226 (0.240)
Zn					0.130 (0.500)	0.097 (0.618)
Fe						−0.091 (0.639)

p Values are indicated in parentheses.

Mass ratios of Ca/P and Mg/Ca in the PIAs

To elucidate whether calcification occurred in the PIAs in old age, both the mass ratios of Ca/P and Mg/Ca were investigated in the PIAs. Figure 2 shows changes of both the mass ratios of Ca/P and Mg/Ca in the PIAs as a function of Ca content. The correlation coefficient was estimated to be -0.185 (p=0.337) between the mass ratio of Ca/P and Ca content (Figure 2a). The mass ratio of Ca/P did not change significantly with Ca increase. The average mass ratio (mg/mg) of Ca/P was high in the PIAs, being 4.02±1.99.

Regarding the mass ratio (%) of Mg/Ca, the correlation coefficient was estimated to be -0.389 (p=0.034) between the mass ratio of Mg/Ca and Ca content (Figure 2b). The mass ratio of Mg/Ca decreased significantly and gradually in the PIAs with Ca increase. The average mass ratio (%) of Mg/Ca was moderate in the PIAs, being 9.13±0.82%.

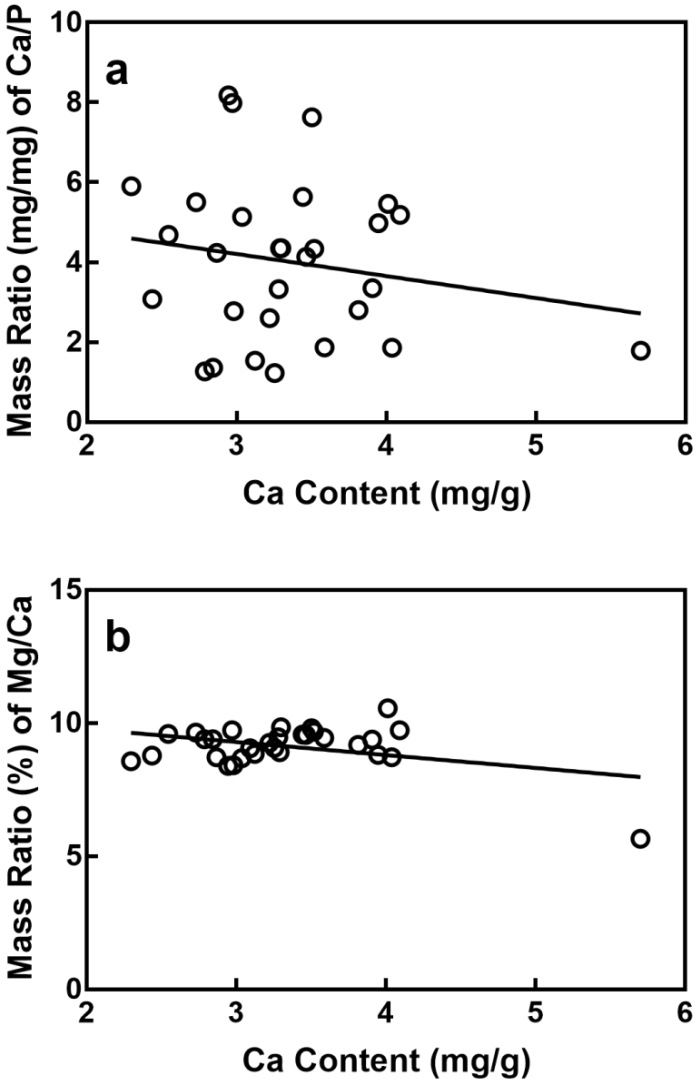

Figure 2: Changes of mass ratios of Ca/P (a) and Mg/Ca (b) in the PIAs. The mass ratios of Ca/P and Mg/Ca are plotted against the Ca content.

Observation of inner surfaces of the PIAs by scanning electron microscopy

To examine the endothelial changes of the PIA in old age, the inner surfaces of the PIAs were observed by scanning electron microscopy. The proximal sites of the PIAs from eight men and two women were used. Figure 3 shows the inner surfaces of the PIAs from a 66-year-old man (a), a 76-year-old man (b), and an 87-year-old woman (c). The proximal sites of these PIAs contained 4.01, 3.47, and 3.30 mg/g of Ca, respectively, as determined by inductively coupled plasma-atomic emission spectrometry. Endothelial lining was partially detached from the basement in many areas (Figure 3a,c). There was a defect in the surface that exposed the underlying connective tissue. As shown in Figure 3b, the detached endothelial lining was less in the PIA from a 76-year-old man compared with those from a 66-year-old man (a) and an 87-year-old woman (c). These results indicated that a significant damage to the endothelium occurred frequently in the proximal site of the PIA in old age.

DISCUSSION

There are a few histological reports [7-9] on the PIA to use for coronary artery bypass grafting. Van Son et al. [7] first found that the PIA as well as the internal thoracic artery was composed of an elastic artery. Thereafter, similar findings were reported by Unlu et al. [8] and Reddy et al. [9]. To examine age-related changes of the PIA, Reddy et al. [9] divided the PIA samples from cadavers into three age groups of 19-40 years, 41-60 years, and over 61 years and studied the PIA by histological method. They reported that age-related pathological changes like intimal thickening or atherosclerosis were not observed in the PIA below 60 years old, but mild intimal thickening was found in 50% over 61 years old, and that no calcification of the tunica media was found in the PIA samples studied.

To elucidate the manner of element accumulation in the arteries with aging, the authors [12,13,17] investigated age-related changes of elements in the arteries and found that when calcification occurred in the arteries, a significant accumulation of Ca, P, and Mg occurred simultaneously in the arteries and both the Ca and P contents were well correlated with the Mg content. In addition, the mass ratio of Ca/P was constant independently of Ca content, and the mass ratio of Mg/Ca was low [18]. In the PIAs studied in the present study, the following results were obtained: No Ca, P, and Mg contents increased significantly with aging, but the Ca content alone tended to increase slightly with aging. Although significant direct correlations were found both between Ca and P contents and between Ca and Mg contents, no significant direct correlation was found between P and Mg contents in the PIAs. The mass ratio of Ca/P did not change significantly with Ca increase. However, the average mass ratio of Ca/P was high, being 4.02±1.99 which was two times higher compared with that of calcified sites in the human thoracic aorta [12]. The mass ratio of Mg/Ca decreased significantly and gradually with Ca increase. The average mass ratio of Mg/Ca was moderate, being 9.13±0.82%. These results suggested that calcification hardly occurred in the PIA in old age. This finding was compatible with the finding of Reddy et al. [10].

The authors [19] previously investigated the Ca, P, S, and Mg contents in 19 kinds of Japanese arteries such as the thoracic and abdominal aortas, and coronary, common carotid, anterior,

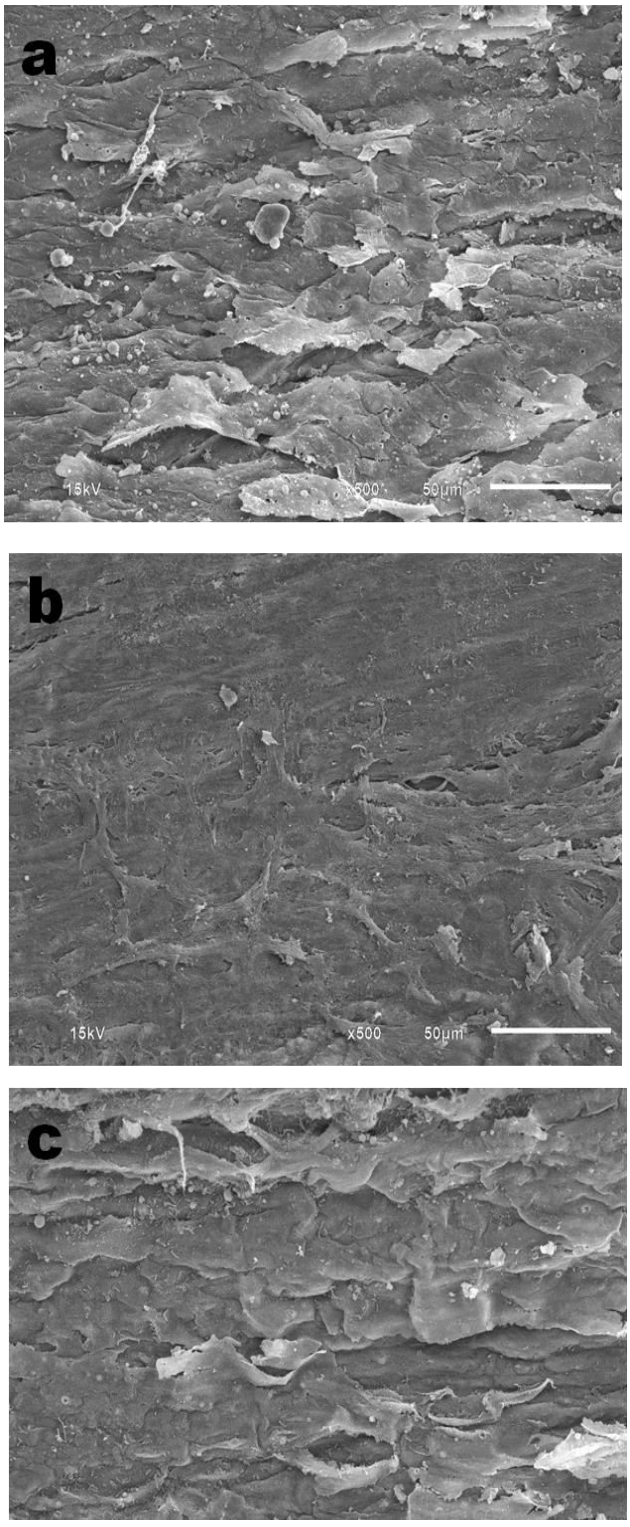

Figure 3: Observation of the inner surfaces of the PIAs from a 66 (a) and a 76 (b)-year-old men and an 87-year-old woman (c) by scanning electron microscopy. Scale bar: 50 μm.

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middle and posterior cerebral, vertebral, basilar, internal thoracic, axillary, radial, truncus celiacus, common, internal and external iliac, femoral, popliteal, and umbilical arteries. It was found that the average contents of P were very low in both the internal thoracic and the radial arteries, being 0.83±0.25 mg/g in the internal thoracic arteries (age range, 65-93 years; average age, 79.8±8.0 years) and 0.85±0.66 mg/g in the radial arteries (age range, 55-92 years; average age, 76.4±10.8 years). In the present study, the average content of P was 1.132±0.752 mg/g in the PIAs. The average content of P was slightly higher in the PIAs compared with those of the internal thoracic and radial arteries. The internal thoracic and radial arteries are widely used for coronary artery bypass grafting [4,5]. It is recognized that atherosclerosis scarcely occurs in the internal thoracic artery of the elastic artery in old age and occurs rarely in the radial artery of the muscular artery [1].

In the absence of calcification, the P content of tissue is mostly determined by the nucleic acid content (DNA and RNA) and the phospholipid content of tissue. Nucleic acids in the cell nucleus and the cytosol and phospholipids in the cell membrane are indicators of metabolically active cells [20]. Taking these into consideration, it is reasonable to presume that the P content in the artery indicates the active cell density, namely, the number of active cells per volume. Therefore, it is thought that the active cell density of the PIA does not change significantly with aging.

There are many studies on the relationship between Ca and cell proliferation [21-25]. For example, Whitfield et al. [21] demonstrated that Ca positively controlled the proliferation of non-tumorigenic epithelial and mesenchymally derived bovine, human, and rodent cells in vitro. According to Reddy et al. [9], the incidence of the PIA with mild intimal thickening was 50% (8/16 cases) between 61 and 83 years old. In the present study, the incidence of the PIA with the Ca content more than 5 mg/g was 0% (0/17 cases) between 62 and 80 years old. Such an artery was found in an 85-year-old man. There was a significant difference between the incidences of the PIA with mild intimal thickening and with the Ca content more than 5 mg/g. Therefore, it is thought that the PIA with mild intimal thickening does not contain the Ca content of over 5mg/g.

To our knowledge, there is no report observing endothelial changes of the PIA with aging by scanning electron microscopy. The authors previously observed the inner surfaces of the human left anterior descending (LAD) artery with myocardial bridge [26] and the human atrioventricular nodal artery [27] by scanning electron microscopy. Both the inner surface images of the PIAs from a 66-year-old man and an 87-year-old woman (Figure 3a, c) in the present study were similar to that of the segment proximal to the myocardial bridge in the LAD artery from an 85-year-old man, but they were not similar to the segment distal to the myocardial bridge. It is known that the former segment is vulnerable to atherosclerosis, whereas the latter segment is not vulnerable to atherosclerosis [28]. However, Reddy et al. [9] reported that atherosclerosis was not seen in the PIA below 83 years old. Taking into consideration, it is speculated that a significant damage to the endothelium in the PIA may not result in development into atherosclerosis.

CONCLUSION

Calcification hardly occurred in the PIA in old age, but a significant damage to the endothelium occurred frequently in the PIA in old age.

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