Annals of Clinical Anatomy

Research Article
Published: 25 Apr, 2018

Calcification Scarcely Occurs in Human Atrioventricular Nodal Arteries in Old Age

Yoshiyuki Tohno[1], Setsuko Tohno[1], Takeshi Minami[2], Pasuk Mahakkanukrauh[1,3,4] and Noppadol Phasukdee[1]*

[1]Department of Anatomy, Faculty of Medicine, Chiang Mai University, Thailand

[2]Laboratory of Environmental Biology, Department of Life Science, Faculty of Science and Technology Kinki University, Japan

[3]Forensic Osteology Center, Faculty of Medicine, Chiang Mai University, Thailand

[4]Excellence in Osteology Research and Training Center (ORTC), Chiang Mai University, Thailand

Abstract

To elucidate age-related changes of the Atrioventricular Nodal (AVN) artery, the authors investigated age-related changes of elements in the AVN artery by direct chemical analysis. In addition, the effects of different arterial origins, arterial sizes, and genders on element accumulation were investigated in the AVN arteries. Sixty-two formalin-fixed adult Thai hearts were dissected, and the following two types of the AVN artery were found: The first type was a single AVN artery arising from the Right Coronary Artery (RCA). The second type was a single AVN artery arising from the terminal part of the Left Circumflex Artery (LCX). For element analysis, both 55 and 7 AVN arteries arising from the RCA and the LCX, respectively, were used. After the arteries were incinerated with nitric acid and perchloric acid, element contents were determined by inductively coupled plasma-atomic emission spectrometry. It was found that the Zn content decreased significantly in the AVN arteries with aging, but six element contents such as Ca, P, S, Mg, Fe, and Na did not change significantly with aging. Regarding the relationships among seven elements in the AVN arteries, extremely significant direct correlations were found both between Ca and Mg contents and between P and S contents, and a significant direct correlation was found between S and Mg contents. However, no significant correlation was found between Ca and P contents in the AVN arteries. To examine an effect of the different arterial origins on element accumulation, the AVN arteries were separated into the RCA and the LCX groups by the arterial origin and age-related changes of element contents were compared between two groups. It was found that there were no significant differences between the RCA and LCX groups in age-related changes of Ca and P contents. No gender differences and effect of arterial size were found in age-related changes of Ca and P contents in the AVN arteries. To elucidate whether calcification occurred in the AVN arteries in old age, both the mass ratios of Ca/P and Mg/Ca were estimated in the AVN arteries. The mass ratio of Ca/P increased progressively in the AVN arteries with Ca increase, being not constant. The mass ratio of Mg/Ca decreased gradually in the AVN arteries with Ca increase, but the average mass ratio of Mg/Ca was moderate, being 8.9% ± 0.9%. These results indicated that calcification scarcely occurred in the AVN arteries in old age, independently of the arterial origin, arterial size and gender.

Keywords: Atrioventricular nodal artery; Coronary artery; Calcium; Phosphorus; Magnesium; Aging

OPEN ACCESS

Correspondence:
Yoshiyuki Tohno, Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand, Tel: 66-53-935312; Fax: 66-53-935304;
E-mail: yoshiyuki.t@cmu.ac.th

Received Date: *27 Mar 2018*
Accepted Date: *18 Apr 2018*
Published Date: *25 Apr 2018*

Citation:
Tohno Y, Tohno S, Minami T, Mahakkanukrauh P, Phasukdee N. Calcification Scarcely Occurs in Human Atrioventricular Nodal Arteries in Old Age. Ann Clin Anat. 2018; 1(1): 1002.

Introduction

The conduction system of the heart is supplied by the Sinoatrial Nodal (SAN) artery, the Atrioventricular Nodal (AVN) artery, the first septal branch of the Left Anterior Descending artery (LAD), and the posterior descending branch of the Right Coronary Artery (RCA). These arteries ensure adequate blood supply to maintain the electrical properties in the heart. It is well known that a high accumulation of Ca and P occurs in the proximal sites of the LAD, Left Coronary Artery (LCA), RCA, and Left Circumflex Artery (LCX) with aging [1-3]. For example, Montenegro and Eggen [1] studied the distribution of atherosclerotic lesions along the axis of the coronary artery (topography) and the relationship of topography to concepts of pathogenesis and reported that a higher prevalence of atherosclerotic lesions in isolated coronary arteries occurred from the first to second centimeters of both the LAD and the RCA, with a decrease in prevalence from 3 cm onward in the LAD and 5 cm onward in the RCA.

Table 1: Subjects used in the present study.

Age (Years)	Sex	Cause of death
36	M	Epilepsy
36	W	Lymphoma
38	M	Strangulation
40	M	Hepatic cancer
47	M	Pneumonia
50	W	Hepatic cancer
51	M	Leukemia
52	M	Gunshot wound
53	W	Chronic renal failure
55	M	Intracerebral hemorrhage
57	M	Alcoholism
57	M	Hypertension
57	W	Cerebrovascular disease
57	W	Lung cancer
60	M	Urinary tract infection
63	M	Bile duct cancer
63	W	Intracerebral hemorrhage
64	M	Myocardial infarction
65	M	Acute natural illness
66	M	Intracerebral hemorrhage
66	M	Liver cirrhosis
67	M	Senility
67	M	Rectal cancer
67	W	Heart failure
69	W	Intracerebral hemorrhage
70	M	Traumatic head injury
72	M	Acute myocardial infarction
72	W	Aspiration pneumonia
73	M	Cardiac hypertrophy
73	M	Pneumonia
73	M	Septicemia
75	M	Senility
75	W	Senility
76	W	Pneumonia
76	W	Cerebrovascular disease
78	M	Hepatic cancer
78	M	Intracerebral hemorrhage
78	M	Hyperglycemic crisis
78	M	Tracheal obstruction
78	W	Senility
78	W	Septicemia
79	M	Pneumonia
79	W	Intestinal cancer
79	M	Senility
80	W	Senility
80	W	Cardiac arrest
81	M	Senility
81	W	Senility
83	M	Septicemia
83	W	Cerebrovascular disease
83	W	Infected emphysema
83	M	Septicemia
84	W	Senility
85	M	Septicemia
86	W	Pneumonia
87	W	Heart failure
88	M	Respiratory tract infection
88	W	Myocardial infarction
89	M	Pneumonia
92	M	Heart failure
93	W	COPD and pneumonia
94	W	Pneumonia

Note: M and W indicate man and woman.

Table 2: Incidence of the AVN artery with the Ca content more than 5.0 mg/g.

Age group (years)	Incidence (%)
30s (n=3)	0 (0/3)
40s (n=2)	50.0 (1/2)
50s (n=9)	44.4 (4/9)
60s (n=11)	18.2 (2/11)
70s (n=19)	31.6 (6/19)
80s (n=15)	26.7 (4/15)
90s (n=3)	33.3 (1/3)

Note: The numbers of cases are indicated in parentheses.

There are a few reports on age-related changes of the AVN artery [4-6]. Velican et al. [4,5] investigated histological changes of the vessels supplying the conduction system of the heart including the AVN artery, with aging. They reported that in the healthy subjects, intimal thickening of the AVN artery started to occur in the 30s of the subjects and then increased in the 40s and the 50s of the subjects. However, changes of the AVN artery in old age had not yet been studied. Therefore, the authors investigated age-related changes of the AVN artery using adult Thai hearts (of the subjects between 36 and 94 years of age) from a viewpoint of elements. It was found that calcification scarcely occurred in the AVN arteries in old age, independently of the arterial origin, arterial size, and gender.

Materials and Methods

Dissection of the AVN arteries

The research was carried out on 62 adult Thai hearts (of subjects between 36 and 94 years of age: average age, 70.7 ± 14.5 years) received from the Department of Anatomy, Faculty of Medicine, Chiang Mai University. The perivascular fatty tissue was carefully removed, where necessary, to visualize the epicardial course of the coronary arteries. The AVN arteries were carefully dissected in the hearts. The AVN artery arising from the distal RCA penetrated into the posterior interatrial septum base at the level of heart crux, and then coursed forwards and upwards, to be directly related to the upper angle of the Posterior Septal Space (PSS) until its apex. The AVN artery also

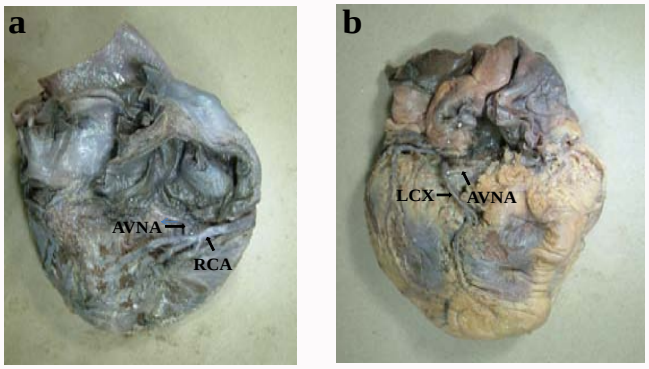

Figure 1: Posterior views of the hearts with type 1 (a) and type 2 (b) of the AVN artery. AVNA atrioventricular nodal artery; LCX left circumflex artery; RCA right coronary artery.

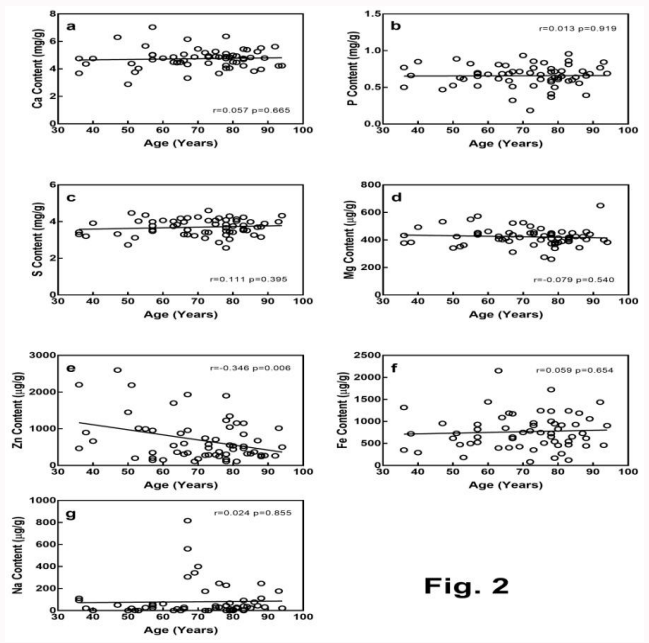

Figure 2: Age-related changes of Ca (a), P (b), S (c), Mg (d), Zn (e), Fe (f), and Na (g) contents in the AVN arteries. The AVN arteries consisted of 55 and 7 AVN arteries arising from the RCA and the LCX, respectively.

arose from the terminal part of the LCX. The artery ran through the fat-filled space located at the inferior wall of the right atrium. The diameter of the AVN artery was measured by the precise electronic caliper. For element analysis, the 62 AVN arteries arising from the RCA and the LCX were used in the present study.

Determination of elements

The arterial samples were washed thoroughly with distilled water and were dried at 95°C for 16 h. After 1 ml concentrated nitric acid was added to the dry samples to incinerate, the mixtures were heated at 100°C for 2 h. After the addition of 0.5 ml concentrated perchloric acid, they were heated at 100°C for an additional 2 h [7]. 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 [8]; smooth muscles containing S decrease on atherosclerosis [9]; both Zn [10] and Fe

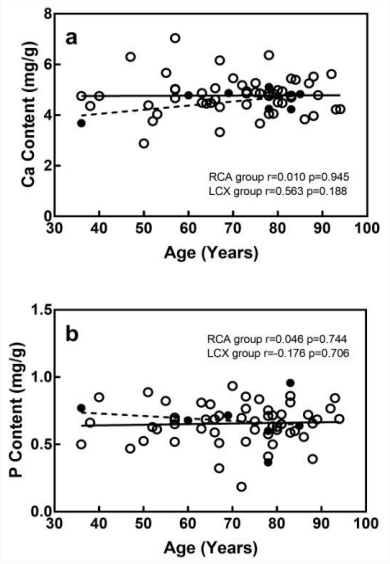

Figure 3: Age-related changes of Ca (a) and P (b) contents in the RCA and LCX groups of the AVN arteries which arise from the RCA and the LCX, respectively. The open and solid circles indicate the RCA and LCX groups of the AVN arteries, respectively. The straight and dotted lines of trend with age indicate the RCA and LCX groups of the AVN arteries, respectively.

[11] 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 Chemical Industries (Osaka, Japan) and were used as standard solutions. The measurement of elements was performed at a fixed wave length 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.

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 1h 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. It was analyzed whether significant differences were found between two slopes and between two intercepts of the regression lines. The two-tailed unpaired student *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.

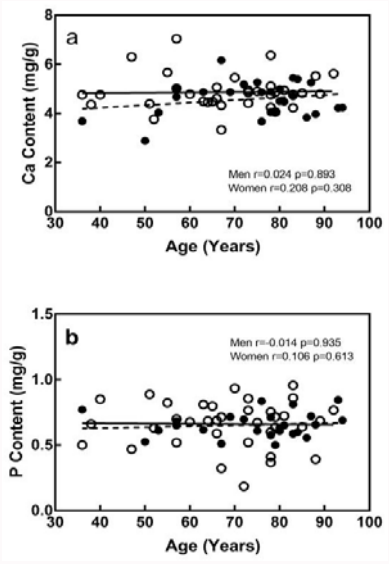

Figure 4: Age-related changes of Ca (a) and P (b) contents in men and women's AVN arteries arising from the RCA and the LCX. The open and solid circles indicate men and women's AVN arteries, respectively. The straight and dotted lines of trend with age indicate men and women's AVN arteries, respectively.

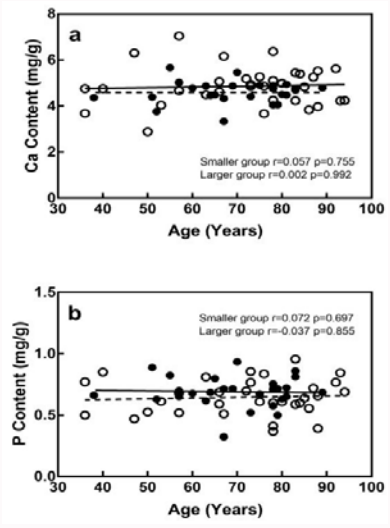

Figure 5: Age-related changes of Ca (a) and P (b) contents in the smaller group less than 1.0 mm of the diameter and the larger group more than 1.0 mm of the diameter in the AVN arteries. The open and solid circles indicate smaller and larger groups. The straight and dotted lines of trend with age indicate smaller and larger groups.

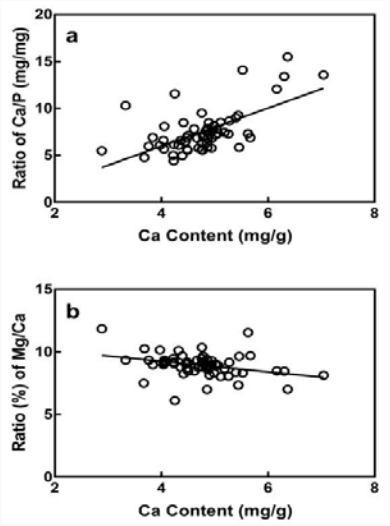

Figure 6: Changes of mass ratios of Ca/P (a) and Mg/Ca (b) in the AVN arteries. The mass ratios of Ca/P and of Mg/Ca are plotted against the Ca content.

Results

Table 1 indicates ages, sexes, and causes of deaths of the 62 subjects used in the present study.

AVN arteries

The following two types of the AVN artery were found in 62 adult hearts (Figure 1): The first type was a single AVN artery arising from the RCA (Figure 1a). It arose from the distal RCA, penetrated into the posterior interatrial septum base at the level of heart crux, and then coursed forwards and upwards, to be directly related to the upper angle of the PSS until its apex. The second type was a single AVN artery arising from the LCX (Figure 1b). It arose from the terminal part of the LCX and ran through the fat-filled space located at the inferior wall of the right atrium. The incidences of types 1 and 2 were 88.7% (55/62) and 11.3% (7/62), respectively.

Measurements on the AVN arteries

The mean diameters of the AVN arteries arising from the RCA and the LCX were 1.01 mm ± 0.30 mm (n=55) and 0.76 mm ± 0.27 mm (n=7), respectively. The mean diameter of the AVN arteries arising from the RCA was significantly larger than that from the LCX (p=0.046).

Age-related changes of elements in the AVN arteries

The 62 AVN arteries arising from both the RCA and the LCX were used for the present study. Figure 2 shows age-related changes of seven element contents in the AVN arteries. The correlation coefficients between age and element contents were estimated to be 0.057 (p=0.665) for Ca, 0.013 (p=0.919) for P, 0.111 (p=0.395) for S, -0.079 (p=0.540) for Mg, -0.346 (p=0.006) for Zn, 0.059 (p=0.654) for Fe, and 0.024 (p=0.855) for Na. A very significant inverse correlation was found between age and Zn content in the AVN arteries, but no significant correlations were found between age and the other element contents such as Ca, P, S, Mg, Fe, and Na.

The average contents of seven elements were 4.746 mg/g ± 0.724 mg/g for Ca, 0.658 mg/g ± 0.149 mg/g for P, 3.700 mg/g ± 0.446 mg/g for S, 423.3 μg/g ± 65.74 μg/g for Mg, 687.7 μg/g ± 579.2 μg/g for Zn, 767.9 μg/g ± 399.3 μg/g for Fe, and 80.45 μg/g ± 146.0 μg/g for Na. The average content of Ca was highest, and it decreased in the order of S, Fe, Zn, P, Mg, and Na.

Table 2 indicates the incidence of the AVN artery with the Ca content more than 5.0 mg/g, which is not contained in a normal artery [12]. The incidence of the AVN artery with the Ca content more than 5.0 mg/g was 50% and 44% in the 40s and the 50s, respectively and did not increase in the age groups older than the 50s. The mean incidence was 29.6% between the 50s and the 80s of the subjects.

Relationships among seven element contents in the AVN arteries

The relationships among seven element contents were examined

in the AVN arteries described above. Table 3 lists the relationships among seven element contents in the AVN arteries. Extremely significant direct correlations were found both between Ca and Mg contents and between P and S contents, and a significant direct correlation was found between S and Mg contents in the AVN arteries. However, no significant correlation was found between Ca and P contents in the AVN arteries. As the Ca content increased in the artery, the Mg content increased simultaneously in the artery, but the P content did not increase.

Age-related changes of elements in the two different AVN arteries

The AVN arteries were separated into the RCA (n=55) and the LCX (n=7) groups by the arterial origin. The RCA group ranged in age from 36 to 94 years (average age=70.8 ± 14.3 years), whereas the LCX group ranged in age from 36 to 85 years (average age=69.9 ± 17.2 years). The two groups were separately analyzed to examine whether age-related changes of elements were different between two groups.

Figure 3 shows age-related changes of Ca and P contents in both the RCA and the LCX groups. The correlation coefficients between age and Ca content were estimated to be 0.010 (p=0.945) in the RCA group and 0.563 (p=0.188) in the LCX group (Figure 3a). The Ca content did not change significantly in two groups with aging. The correlation coefficients between age and P content were estimated to be 0.046 (p=0.744) in the RCA group and -0.176 (p=0.706) in the LCX group (Figure 3b). No significant correlations were found between age and either Ca or P content in two groups.

The analysis of the regression lines between age and Ca content in Figure 3a showed that no significant differences were found between the two slopes (p=0.403) and between the two intercepts (p=0.431) of the regression lines for the RCA and the LCX groups. The analysis of the regression lines between age and P content in Figure 3b showed that no significant differences were found between the two slopes (p=0.558) and between the two intercepts (p=0.750) of the regression lines for the RCA and the LCX groups. Therefore, no significant differences were found in age-related changes of Ca and P contents between the two groups.

Gender differences in Ca and P contents of the AVN arteries

Men's samples consisted of 31 and 5 AVN arteries arising from the RCA and the LCX, respectively. The average age of men's subjects was 68.3 ± 14.5 years. Women's samples consisted of 24 and 2 AVN arteries arising from the RCA and the LCX, respectively. The average age of women's subjects was 74.0 ± 14.1 years.

Figure 4 shows age-related changes of Ca and P contents in men and women's AVN arteries. The correlation coefficients between age and Ca content were estimated to be 0.024 (p=0.893) in men's AVN arteries and 0.208 (p=0.308) in women's AVN arteries (Figure 4a). The correlation coefficients between age and P content were estimated to be -0.014 (p=0.935) in men's AVN arteries and 0.106 (p=0.613) in women's AVN arteries (Figure 4b). No significant correlations were found between age and either Ca or P content in men and women's ones. Both the Ca and P contents did not increase significantly in men and women's AVN arteries with aging.

The analysis of the regression lines between age and Ca content in Figure 4a showed that no significant differences were found between the two slopes (p=0.488) and between the two intercepts (p=0.111) of the regression lines for men and women's AVN arteries. The analysis of the regression lines between age and P content in Figure 4b showed that no significant differences were found between the two slopes (p=0.757) and between the two intercepts (p=0.871) of the regression lines for men and women's AVN arteries. Therefore, no gender differences were found in the AVN arteries with regard to age-related changes of Ca and P contents.

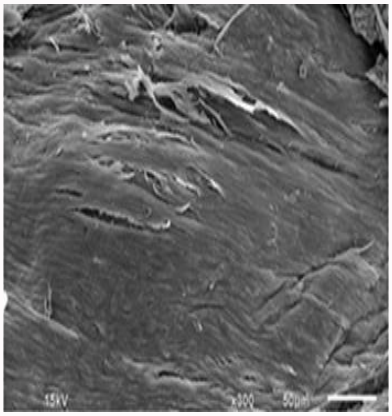

Figure 7: Observation of the inner surface of the AVN artery from 82-year-old man by scanning electron microscopy. Scale bar: 50 μm.

Effect of arterial size on age-related changes of Ca and P contents

To examine an effect of arterial size on age-related changes of elements, the AVN arteries were separated into the smaller group (n=34) less than 1.0 mm of the diameter and the larger group (n=28) more than 1.0 mm of the diameter. The smaller group ranged in age from 36 to 94 years (average age=71.5 ± 16.5 years), whereas the larger group ranged in age from 38 to 89 years (average age=69.3 ± 12.3 years). The two groups were separately analyzed to examine whether age-related changes of Ca and P contents were different between smaller and larger groups.

Figure 5 shows age-related changes of Ca and P contents in both the smaller and larger groups. The correlation coefficients between age and Ca content were estimated to be 0.057 (p=0.755) in the smaller group and 0.002 (p=0.992) in the larger group (Figure 5a). No significant correlations were found between age and Ca content in two groups. The correlation coefficients between age and P content were estimated to be 0.072 (p=0.697) in the smaller group and -0.037 (p=0.855) in the larger group (Figure 5b). No significant correlations were found between age and P content in two groups.

The analysis of the regression lines between age and Ca content in Figure 5a showed that no significant differences were found between the two slopes (p=0.839) and between the two intercepts (p=0.170) of the regression lines for the smaller and larger groups. The analysis of the regression lines between age and P content in Figure 5b showed that no significant differences were found between the two slopes (p=0.710) and between the two intercepts (p=0.226) of the regression lines for the smaller and larger groups. Therefore, no significant differences were found in age-related changes of Ca and P contents between the smaller and larger groups.

Mass ratios of Ca/P and Mg/Ca in the AVN arteries

To elucidate whether calcification occurred in the AVN arteries in old age, both the mass ratios of Ca/P and Mg/Ca were investigated in 62 AVN arteries arising from both the RCA and the LCX.

Table 3: Relationships among seven element contents in the AVN arteries.

Element	Correlation coefficient and *p* value					
	P	S	Mg	Zn	Fe	Na
Ca	-0.080 (0.546)	0.033 (0.802)	0.773 (<0.0001)	-0.141 (0.279)	0.111 (0.402)	-0.109 (0.408)
P		0.591 (<0.0001)	0.158 (0.224)	-0.005 (0.973)	0.074 (0.574)	-0.015 (0.911)
S			0.290 (0.023)	0.141 (0.280)	-0.020 (0.881)	-0.076 (0.561)
Mg				-0.123 (0.340)	0.082 (0.534)	-0.004 (0.974)
Zn					-0.026 (0.847)	0.032 (0.806)
Fe						-0.037 (0.780)

p Values are indicated in parentheses.

Figure 6 shows changes of both the mass ratios of Ca/P and Mg/Ca in the AVN arteries as a function of Ca content. The correlation coefficient was estimated to be 0.622 ($p<0.0001$) between the mass ratio of Ca/P and Ca content (Figure 6a). The mass ratio of Ca/P increased significantly and progressively with Ca increase, being not constant. The average mass ratio (mg/mg) of Ca/P was 7.53 ± 2.34 in the AVN arteries.

Regarding the mass ratio of Mg/Ca, the correlation coefficient was estimated to be -0.328 ($p=0.010$) between the mass ratio of Mg/Ca and Ca content (Figure 6b). The mass ratio of Mg/Ca decreased significantly and gradually in the AVN arteries with Ca increase. The average mass ratio (%) of Mg/Ca was 8.9% ± 0.9%.

Observation of inner surface of the AVN arteries by scanning electron microscopy

To examine the endothelial changes of the AVN artery in old age, the inner surface of the AVN arteries was observed by scanning electron microscopy. The used subjects were 76 and 82 year-old men.

Figure 7 shows the inner surface of the AVN artery from an 82-year-old man. Endothelial lining was partially detached from the basement in some areas. There was a defect in the surface that exposed the underlying connective tissue. A similar finding was also obtained in the AVN artery of a 76-year-old man.

Discussion

There are many reports [4-6,13-19] on intimal thickening of the arteries. Thoma [13] first described intimal thickening lacking the features of atherosclerosis or other disease processes in the human aorta. Wolkoff [14,15] showed that the tunica intima of the coronary artery consisted of only 1-2 cell layers in young children, increased 10-15 cell layers at 15 years of age, and then, reached 25-30 cell layers at 25-30 years of age. Many investigators have described similar intimal thickening in human coronary arteries [4-6,16-19]. Velican et al. [4] and Velican and Velican [5] investigated histological changes of the AVN arteries with aging and reported that in apparently healthy persons, about 1/4 of mature adults (46-55 years old) had intimal thickening in the AVN arteries. Kozlowski et al. [6] investigated histological changes of the AVN arteries with aging and reported that in normal subjects, about 1/2 of subjects (17-86 years old) had intimal thickening in the AVN arteries.

The vast literature on the intimal layer includes opinions which range from the concept that intimal thickening is an essentially "normal" structural change, to the concept that it is an early stage of atherosclerotic involvement [4,5,20,21]. It is generally agreed that intimal thickening is preconditioned (or underdeveloped) for the development of atherosclerosis.

Vascular calcification is a hallmark of atherosclerosis. On calcification, deposition of calcium orthophosphate first occurs in the artery. Thereafter, it progresses to amorphous calcium phosphate and then to calcium phosphate crystalline structures such as hydroxyapatite [22]. Calcification occurs in most, but not all, arteries in old age [23].

To elucidate the manner of element accumulation in the arteries with aging, Tohno et al. [8,9,24] 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 [25]. In the AVN arteries studied in the present study, the following results were obtained: No Ca, P, and Mg contents increased significantly with aging. No significant direct correlations were found among the Ca, P, and Mg contents, except for an extremely significant direct correlation between Ca and Mg contents. The mass ratio of Ca/P increased significantly and progressively with Ca increase, being not constant. The average mass ratio of Mg/Ca was moderate, being 8.9% ± 0.9%. Therefore, it was suggested that calcification scarcely occurred in the AVN arteries arising from the RCA and the LCX in old age.

There are in vitro and animal studies of an effect of Mg on calcification [26-29]. These studies suggest that Mg may prevent plaque formation and calcification. In the present study, the Mg content was positively correlated with the Ca content in the AVN arteries. As the Ca content increased in the AVN artery, the Mg content also increased in the artery.

The present study revealed that the average content of P in the AVN arteries was very low, being 0.658 mg/g ± 0.149 mg/g. Tohno et al. [30] previously investigated the Ca, P, S, and Mg contents in 19 kinds of Japanese arteries such as the thoracic and abdominal aortas coronary, common carotid, anterior, middle and posterior cerebral, vertebral, basilar, internal thoracic, axillary, radial, truncus coeliacus, 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 mg/g ± 0.25 mg/g in the internal thoracic arteries (age range, 65-93 years; average age, 79.8 ± 8.0 years) and 0.85 mg/g ± 0.66 mg/g in the radial arteries (age range, 55-92 years; average age, 76.4 ± 10.8 years). These arteries are widely used for coronary artery bypass grafting [31,32]. It is recognized that atherosclerosis scarcely occurs in the internal thoracic artery in old age and occurs rarely in the radial artery [33]. Recently, the authors investigated age-related changes of elements in the SAN arteries and found that the average content of P was very low

in the SAN arteries of the subjects (age range, 36-94 years; average age, 70.6 ± 14.5 years), being 0.644 mg/g ± 0.259 mg/g [34]. All the AVN, SAN, internal thoracic, and radial arteries are characterized by a low average content of P. 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.

There are many studies on the relationship between Ca and cell proliferation [35-39]. For example, Whitfield et al. [35] demonstrated that Ca positively controlled the proliferation of non-tumorigenic epithelial and mesenchymally derived bovine, human, and rodent cells in vitro. According to Velican et al. [4], the incidence of the AVN artery with intimal thickening was 28% in 51-55 years of age with regard to apparently healthy persons. According to Kozlowski et al. [6], the incidence of the AVN artery with intimal thickening was about 50% in the normal subjects ranging in age from 17 to 86 years (average age, 56 ± 14 years). In the present study, the incidence of the AVN artery with the Ca content more than 5.0 mg/g was 44.4% (4/9 cases) in the 50s of the subjects and 29.0% (18/62 cases) in all the subjects of 36-94 years of age. The incidence of the AVN artery with this Ca content appears to be similar to those by Velican et al. [4] and Kozlowski et al. [6]. Therefore, there is a possibility that this Ca increase may be related to intimal thickening in the AVN artery.

Conclusion

Calcification scarcely occurred in the AVN arteries in old age, independently of the arterial origin, arterial size and gender.

Acknowledgement

The authors wish to thank Ms. Thippawan Yasanga (Medical Science Research Equipment Center, Faculty of Medicine, Chiang Mai University) for observation by scanning electron microscopy.

References

1. Montenegro MR, Eggen DA. Topography of atherosclerosis in the coronary arteries. Lab Invest. 1968;18(5):586-93.
2. Azuma C, Tohno S, Mahakkanukrauh P, Tohno Y, Satoh H, Chomsung R, et al. Different accumulation of elements in the rami of the coronary arteries of Thai. Biol Trace Elem Res. 2003;95(3):211-8.
3. Tohno Y, Tohno S, Mahakkanukrauh P, Minami T, Sinthubua A, Suwannahoy P, et al. Accumulation of calcium and phosphorus in the coronary arteries of Thai subjects. Biol Trace Elem Res. 2012;145(3):275-82.
4. Velican D, Serban-Piriu G, Petrescu C, Velican C. Prevalence of thick intimas and of obstructive lesions in the vessels supplying the conduction system of the heart. Med Interne. 1989;27(3):197-208.
5. Velican C, Velican D. Study of coronary intimal thickening. Atherosclerosis. 1985;56(3):331-44.
6. Kozlowski D, Owerczuk A, Kozluk E, Grzybiak M, Adamowicz-Kornacka M, Walczak E, et al. Histologic evaluation of the atrioventricular nodal artery in healthy humans and in patients with conduction disturbances. Folia Morphol (Warsz). 2000;59(3):145-52.
7. Tohno Y, Tohno S, Minami T, Ichii M, Okazaki Y, Utsumi M, et al. Age-related changes of mineral contents in human thoracic aorta and in the cerebral artery. Biol Trace Elem Res. 1996;54(1):23-31.
8. Tohno S, Tohno Y, Minami T, Moriwake Y, Azuma C, Ohnishi Y. Elements of calcified sites in human thoracic aorta. Biol Trace Elem Res. 2002;86(1):23-30.
9. Tohno Y, Tohno S, Moriwake Y, Azuma C, Ohnishi Y, Minami T. Accumulation of calcium and phosphorus accompanied by increase of magnesium and decrease of sulfur in human arteries. Biol Trace Elem Res. 2001;82(1-3):9-19.
10. Little PJ, Bhattacharya R, Moreyra AE, Korichneva IL. Zinc and cardiovascular disease. Nutrition. 2010;26(11-12):1050-7.
11. Kraml P. The role of iron in the pathogenesis of atherosclerosis. Physiol Res. 2017;66(Suppl1):S55-67.
12. Tohno Y, Tohno S, Minami T, Utsumi M, Moriwake Y, Nishiwaki F, et al. Age-related changes of mineral contents in the human aorta and internal thoracic artery. Biol Trace Elem Res. 1998;61(2):219-26.
13. Thoma R. Uber die Abhangigkeit der Bindegewebsneubildung in der Arterienintima von den mechanischen Bedingungen des Blutumlaufes: Erste Mitteilung. Virchows Arch Pathol Anat Physiol. 1883;93:443-505.
14. Wolkoff K. Uber die histologische Struktur der Coronararterien des menschlichen Herzens. Virchows Arch Pathol Anat Physiol. 1923;241:42-58.
15. Wolkoff K. Uber die Altersveranderungen der Arterien bei Tieren. Virchows Arch. 1924;252:208-28.
16. Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W, Richardson M, et al. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the committee on vascular lesions of the council on arteriosclerosis, American heart association. Circulation. 1992;85(1):391-405.
17. Nakashima Y, Chen YX, Kinukawa N, Sueishi K. Distributions of diffuse intimal thickening in human arteries: preferential expression in atherosclerosis-prone arteries from an early age. Virchows Arch. 2002;441(3):279-88.
18. Nakashima Y, Wight TN, Sueishi K. Early atherosclerosis in humans: role of diffuse intimal thickening and extracellular matrix proteoglycans. Cardiovasc Res. 2008;79(1):14-23.
19. Subbotin VM. Excessive intimal hyperplasia in human coronary arteries before intimal lipid depositions is the initiation of coronary atherosclerosis and constitutes a therapeutic target. Drug Discov Today. 2016;21(10):1578-95.
20. Adams CW. Multiple factors in the pathogenesis of atherosclerosis. Guys Hosp Rep. 1963;112:222-53.
21. Duggal K, Tandon HD, Karmarkar MG, Ramalingaswami V. Diffuse intimal thickening of the aorta in India and its relation to atherosclerosis. J Pathol Bacteriol. 1966;92(1):49-56.
22. Nakahara T, Dweck MR, Narula N, Pisapia D, Narula J, Strauss HW. Coronary artery calcification: From mechanism to molecular imaging. JACC Cardiovasc Imaging. 2017;10(5):582-93.
23. Ohnishi Y, Tohno S, Mahakkanukrauh P, Tohno Y, Vaidhayakarn P, Azuma C, et al. Accumulation of elements in the arteries and cardiac valves of Thai with aging. Biol Trace Elem Res. 2003;96(1-3):71-92.
24. Tohno S, Tohno Y, Moriwake Y, Azuma C, Ohnishi Y, Minami T. Quantitative changes of calcium, phosphorus, and magnesium in common iliac arteries with aging. Biol Trace Elem Res. 2001;84(1-3):57-66.
25. Bigi A, Compostella L, Fichera AM, Foresti E, Gazzano M, Ripamonti A, et al. Structural and chemical characterization of inorganic deposits in calcified human mitral valve. J Inorg Biochem. 1988;34(2):75-82.
26. Kircelli F, Peter ME, Sevinc OE, Celenk FG, Yilmaz M, Steppan S, et al. Magnesium reduces calcification in bovine vascular smooth muscle cells in a dose-dependent manner. Nephrol Dial Transplant. 2012;27(2):514-21.
27. Louvet L, Buchel J, Steppan S, Passlick-Deetjen J, Massy ZA. Magnesium prevents phosphate-induced calcification in human aortic vascular smooth muscle cells. Nephrol Dial Transplant. 2013;28(4):869-78.
28. Bigi A, Foresti E, Gregorini R, Ripamonti A, Roveri N, Shah JS. The role of magnesium on the structure of biological apatites. Calcif Tissue Int.

1992;50(5):439-44.

29. Hruby A, O'Donnell CJ, Jacques PF, Meigs JB, Hoffmann U, Mckeown NM. Magnesium intake is inversely associated with coronary artery calcification: the Framingham heart study. JACC Cardiovasc Imaging. 2014;7(1):59-69.

30. Tohno Y, Tohno S, Moriwake Y, Azuma C, Ohnishi Y, Minami T. Simultaneous accumulation of calcium, phosphorus, and magnesium in various human arteries. Biol Trace Elem Res. 2001;82(1-3):21-8.

31. Sajja LR, Mannam G. Internal thoracic artery: anatomical and biological characteristics revisited. Asian Cardiovasc Thorac Ann. 2015;23(1):88-99.

32. Baikoussis NG, Papakonstantinou NA, Apostolakis E. Radial artery as graft for coronary artery bypass surgery: advantages and disadvantages for its usage focused on structural and biological characteristics. J Cardiol. 2014;63(5):321-8.

33. Kaufer E, Factor SM, Frame R, Brodman RF. Pathology of the radial and internal thoracic arteries used as coronary artery bypass grafts. Ann Thorac Surg. 1997;63(4):1118-22.

34. Tohno Y, Tohno S, Quiggins R, Minami T, Mahakkanukrauh P. Scarce occurrence of calcification in human sinoatrial nodal arteries in old age. Biol Trace Elem Res. 2017.

35. Whitfield JF, Boynton AL, MacManus JP, Sikorska M, Tsang BK. The regulation of cell proliferation by calcium and cyclic AMP. Mol Cell Biochem. 1979;27(3):155-79.

36. Whitfield JF, Boynton AL, MacManus JP, Rixon RH, Sikorska M, Tsang B, et al. The roles of calcium and cyclic AMP in cell proliferation. Ann N Y Acad Sci. 1980;339:216-40.

37. Swierenga SH, Whitfield JF, Boynton AL, MacManus JP, Rixon RH, Sikorska M, et al. Regulation of proliferation of normal and neoplastic rat liver cells by calcium and cyclic AMP. Ann N Y Acad Sci. 1980;349(1):294-311.

38. Mailland M, Waelchli R, Ruat M, Boddeke HG, Seuwen K. Stimulation of cell proliferation by calcium and a calcimimetic compound. Endocrinology. 1997;138(9):3601-5.

39. Rodrigues MA, Gomes DA, Leite MF, Grant W, Zhang L, Lam W, et al. Nucleoplasmic calcium is required for cell proliferation. J Biol Chem. 2007;282(23):17061-8.