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Age-related changes of elements in the human sinoatrial nodes

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Abstract

Introduction

To elucidate compositional changes of the sinoatrial node (SAN) with aging, the authors investigated age-related changes of elements in the SANs of Thai subjects with a wide range of ages.

Materials and methods

The Thai subjects consisted of 37 men and 36 women, ranging in age from 35 to 91 years (average age=67.713.3 years). The samples of the SANs were obtained by dissecting the tissue perpendicular to the terminal crest. After incinerating with nitric acid and perchloric acid, element contents were determined by inductively coupled plasma-atomic emission spectrometry.

Results

Although the P content tended to decrease in the SANs with aging, there were no significant correlations between age and seven element contents, such as Ca, P, S, Mg, Zn, Fe, and Na, in the SANs. However, on the examination separated into the men and women's SANs, a significant inverse correlation was found between age and P content only in the men's SANs, but it was not found in the women's ones.

Conclusion

The P content of tissue is mostly determined by the nucleic acid content (DNA and RNA) and the phospholipid content of the tissue. Nucleic acids in the cell nucleus and the cytosol and phospholipids in the cell membrane are indicators of metabolically active cells. Taking into consideration, it is reasonable to presume that the P content indicates the active cell density in the SAN, i.e. the number of active cells per volume. Therefore, there is a possibility that the active cell density of the SAN decreases significantly in men with aging. Regarding the relationships among elements, extremely or very significant direct correlations were found among the Ca, P, S, and Mg contents in the SANs.

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Introduction

The sinoatrial node (SAN) is the cardiac pacemaker tissue, which must perform over a lifetime. Aging increases vagal predominance and suppresses the cardiac functions[1,2]. Furthermore, the fibrous and fatty infiltration of the cardiac conduction system appears at over 40 years of age[3,4].

The authors[5] previously investigated age-related changes of elements in the SANs of Japanese who ranged in age from 65 to 102 years and obtained the finding that there were no significant changes of seven element contents, Ca, P, S, Mg, Zn, Fe, and Na, at very old age. In contrast, in the SANs of monkeys which ranged in age from one month to 30 years, it was found that the Ca and P contents decreased significantly in the SANs of monkeys with aging[6].

Therefore, the authors investigated age-related changes of elements in the SANs of Thai subjects with a wide range of ages and found that the P content decreased significantly in the SANs of men with aging, but it did not decrease significantly in ones of women.

Materials and Methods

Sampling

Thai cadavers were treated by injection of a mixture of 26% methanol, 14% glycerin, 3% phenol, 14% formalin, 0.34 M potassium nitrate, and 14 mM arsenic oxide through the femoral artery[7]. The Thai subjects consisted of 37 men and 36 women, ranging in age from 35 to 91 years (average age=67.713.3 years). The samples of the SANs (6-8 mm in width) were obtained by dissecting the tissue perpendicular to the terminal crest according to the method of Yanagawa and Nakajima[8].

Determination of Elements

The samples of the SANs were washed thoroughly with distilled water and were dried at 80C for 16 h. One millilitre concentrated nitric acid was added to the dry samples, and the mixtures were heated at 100C for 2 h. After the addition of 0.5 ml concentrated perchloric acid, they were heated at 100C for an additional 2 h. 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). The resulting filtrates were analysed by inductively coupled plasma-atomic emission spectrometry (ICPS-7500; Shimadzu, Kyoto, Japan)[9].

The conditions were as follows: 1.2 kW of power from a radiofrequency generator, a plasma argon flow rate of 1.2 l/min, a cooling gas flow of 14 l/min, a carrier gas flow of 1.0 l/min, an entrance slit of 20 m, an exit slit of 30 m, a height of observation of 15 mm, and an integration time lapse of 5 s. Specially prepared standard solutions of Ca, Mg, Zn, Fe, and Na for atomic absorption spectrometry and phosphate and sulphate ions for ion chromatography were purchased from Wako Pure Chem. Ind. (Osaka, Japan) and were used as standard solutions.

The detection limits of elements were determined to be 100 ng/ml for Ca, 50 ng/ml for P, S, Mg, and Na, and 25 ng/ml for Zn and Fe, respectively, from the standards. The element amount was expressed on a dry-weight basis.

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Histological Observation

The samples of the SANs were fixed with 10% formalin, dehydrated with ethanol, and embedded in paraffin. Sections of 5-m thick were prepared and stained with haematoxylin and eosin. They were observed under light microscopy.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Pearson's correlation was used to investigate the association between parameters. An unpaired Student's t test was used to analyse differences between groups. A p value of less than 0.05 was considered to be significant. Data were expressed as the meanstandard deviation.

Results

The samples of the SANs were obtained by dissecting the tissue perpendicular to the terminal crest and were confirmed by means of histological observation. Figure 1 shows a typical image of the SAN.

Table 1 indicates the average contents of seven elements in the SANs. In the SANs, major elements were Ca and S, and minor elements were P, Mg, Zn, Fe, and Na.

Age-Related Changes of Elements in the SANs

Figure 2 shows age-related changes of element contents in the SANs. The correlation coefficients between age and seven element contents were estimated to be 0.058 (p=0.625) for Ca, 0.130 (p=0.275) for P, 0.106 (p=0.375) for S, 0.025 (p=0.832) for Mg, 0.002 (p=0.985) for Zn, 0.085 (p=0.473) for Fe, and 0.084 (p=0.482) for Na. Although the P content tended to decrease in the SANs with aging, no significant correlations were found between age and seven element contents in the SANs.

Relationships among Elements in the SANs

The relationships among elements were examined in the SANs using Pearson's correlation. Figure 3 shows the relationships between the Ca and other six element contents in the SANs. The correlation coefficients were estimated to be 0.319 (p=0.006) between the Ca and P contents, 0.649 (p<0.0001) between the Ca and S contents, 0.949 (p<0.0001) between the Ca and Mg contents, 0.665

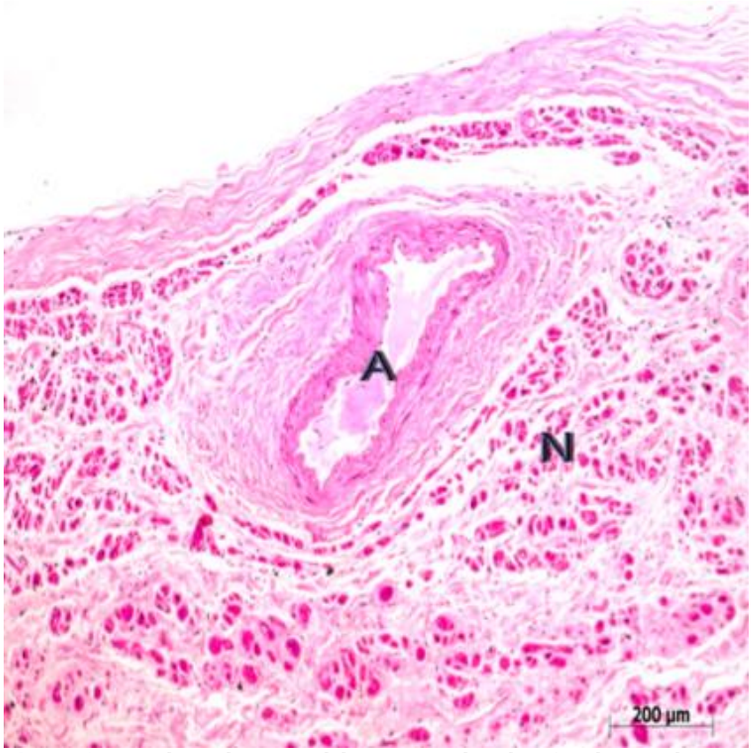

Figure 1: Histological image of the sinoatrial nodes. A indicates the sinoatrial nodal artery and N indicates the sinoatrial nodal cells.

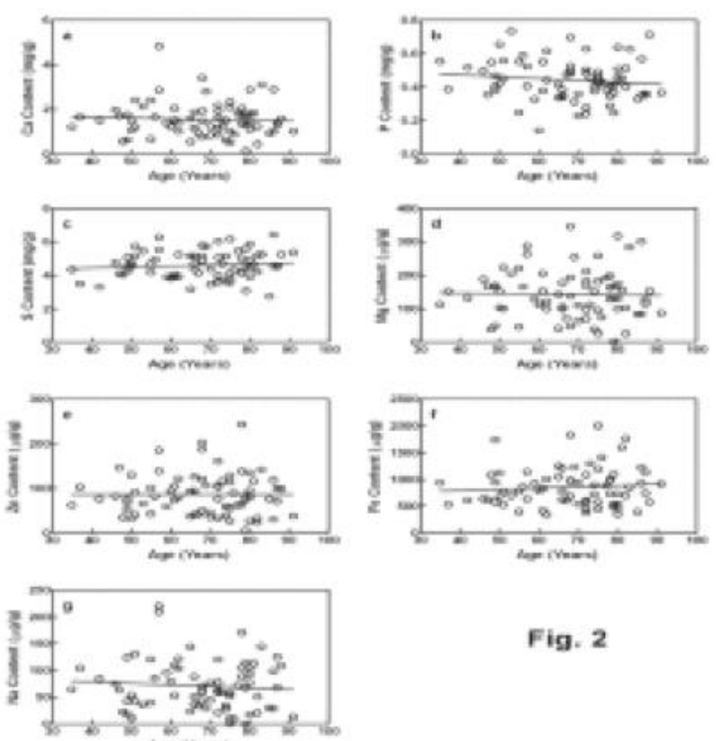

Figure 2: Age-related changes of the Ca (a), P (b), S (c), Mg (d), Zn (e), Fe (f), and Na (g) content in the sinoatrial nodes.

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(p<0.0001) between the Ca and Zn contents, 0.262 (p=0.025) between the Ca and Fe contents, and 0.601 (p<0.0001) between the Ca and Na contents in the SANs. Extremely significant direct correlations were found between the Ca and S, Mg, Zn, or Na contents in the SANs and a very significant direct correlation was found between the Ca and P contents. In contrast, a significant inverse correlation was found between the Ca and Fe contents in the SANs.

Likewise, the relationships among the P, S, Mg, Zn, Fe, and Na contents were examined in the SANs. The results are summarized in Table 2. Extremely significant direct correlations were found between the P and S contents, between the Mg and S, Zn, or Na contents, and between the Zn and Na contents.

Very significant direct correlations were found between the P and Mg contents and between the S and either Zn or Na contents. In addition, a significant inverse correlation was found between the Mg and Fe contents. Therefore, there were extremely or very significant direct correlations among the Ca, P, S, and Mg contents in the SANs.

Gender Differences in the Element Content of the SANs

The Thai subjects consisted of 37 men and 36 women. The ages of the men subjects ranged from 42 to 91 years (average age=69.812.4 years) and of the women subjects from 35 to 88 years (average age=65.514.1 years).

Age-related changes of seven element contents were examined separately in the SANs of men and women. Figure 4 shows age-related changes of the P content in the SANs of both men and women. The correlation coefficients between age and P content in the SANs were estimated to be 0.410 (p=0.012) in men and 0.042 (p=0.809) in women. A significant inverse correlation was found between age and P content in the men's SANs, but it was not found in the women's ones. The regression equation for men is: y = 0.0037x + 0.7188. The regression equation for women is: y = 0.0004x + 0.3980.

The analysis of the regression lines between age and P content in Figure 4 showed that no significant differences were found between the two slops (p=0.055) and between the two intercepts (p=0.114) of the regression lines for men and women.

Regarding Ca, S, Mg, Zn, Fe, and Na, no significant correlations were found between age and six element contents in the SANs of both men and women, because p values were higher than 0.05.

Table 1: Average Contents of Elements in the Sinoatrial Nodes.

Element	Average Content (mg/g)
Ca	1.559±0.770
P	0.442±0.119
S	4.611±0.801
Mg	0.143±0.073
Zn	0.086±0.046
Fe	0.855±0.365
Na	0.071±0.046

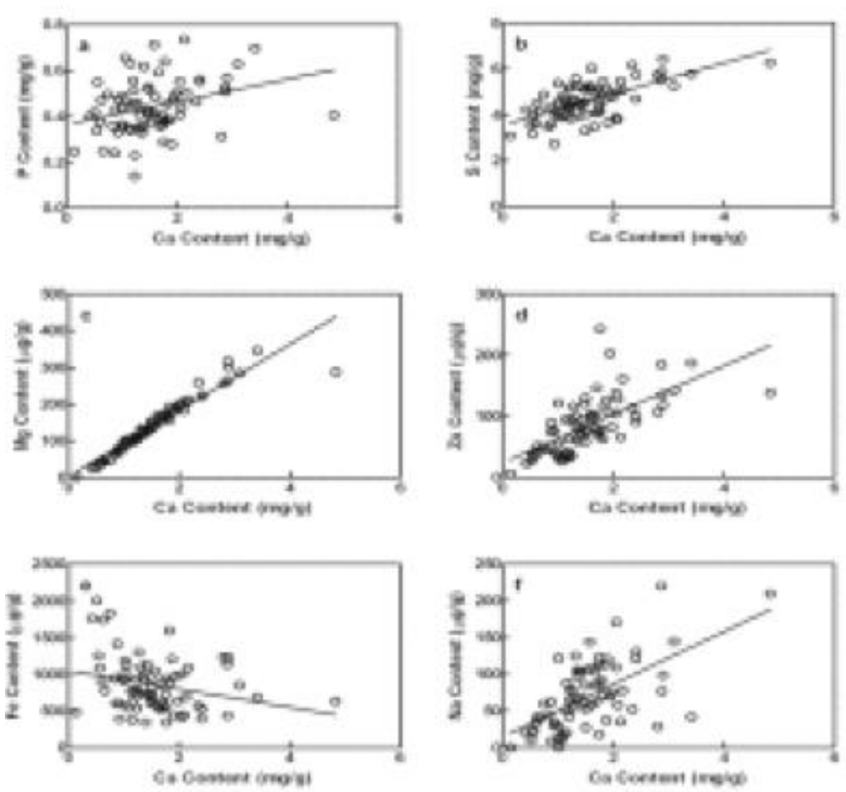

Figure 3: Relationships between the Ca and P (a), S (b), Mg (c), Zn (d), Fe (e), or Na (f) contents in the sinoatrial nodes.

Table 3 indicates the average contents of P in the SANs of both men and women by age group. With regard to the P content of the SANs, a significant gender difference was found in the 50s of the subjects, but it was not found in the 60s, the 70s, and the 80s of the subjects. The average content of P in the SANs in the 50s of the subjects was significantly higher in men than in women. Therefore, such a gender difference in the P content as monkeys[10] was not found in the SANs of Thais.

Discussion

The present study revealed that although the P content tended to decrease in the SANs of Thais with aging, no significant correlations were found between age and the seven element contents in the SANs of Thais.

The authors[5] previously investigated age-related changes of elements in the SANs of Japanese who ranged in age from 65 to 102 years and obtained the finding that the seven element contents including P did not change significantly in the SANs of Japanese at very old age. In addition, the authors[6] investigated

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Table 2: Relationships among Elements in the Sinoatrial Nodes.

Element	Correlation Coefficient and *p* Value					
	P	S	Mg	Zn	Fe	Na
Ca	0.319 (0.006)	0.649 (<0.0001)	0.949 (<0.0001)	0.665 (<0.0001)	–0.262 (0.025)	0.601 (<0.0001)
P		0.428 (0.0002)	0.369 (0.001)	0.136 (0.250)	–0.051 (0.667)	0.081 (0.493)
S			0.667 (<0.0001)	0.354 (0.002)	0.036 (0.762)	0.313 (0.007)
Mg				0.700 (<0.0001)	–0.294 (0.012)	0.526 (<0.0001)
Zn					–0.103 (0.388)	0.510 (<0.0001)
Fe						–0.177 (0.134)

Note: *p* Values are indicated in parentheses.

age-related changes of elements in the SANs of monkeys which ranged in age from 27 days to 30 years and found that the P and Ca contents decreased significantly in the SANs of monkeys with aging. It is well known that P-containing phosphate has five major functions: (1) it is a part of large molecules or molecular assemblies, e.g. DNA, RNA and membranes, (2) it acts as a carrier of substrates, e.g. ATP, (3) it acts as a signalling device in the cytoplasm, e.g. in cAMP, (4) it acts as a reversible chemical modification of proteins, and (5) it is a constituent of biominerals[11].

The P content of tissue is mostly determined by the nucleic acid content (DNA and RNA) and the phospholipid content of the tissue. Nucleic acids in the cell nucleus and the cytosol and phospholipids in the cell membrane are indicators of metabolically active cells[12].

Taking these into consideration, it is reasonable to presume that the P content indicates the active cell density in the SAN, i.e. the number of active cells per volume. Therefore, there is a possibility that the active cell density of the SANs decreases significantly in monkeys and tends to decrease in Thais with aging.

There are several histological studies[3,4,13,14,15,16,17] on the SAN. Shiraishi et al.[13] investigated compositional changes of the SAN in 26 persons ranging in age from 3 months to 89 years using a digital colour image analyser and reported that although the actual volume of SA nodal cells increased during growth, the total volume and volume percentage of the SA nodal cells virtually decreased during aging, whereas the volume of fibrous

Table 3: Comparison in the P Content in the Sinoatrial Nodes of Men and Women by Age Group.

Age Group (Years)	Men	Women
< 50	0.490±0.076	0.441±0.079
50s	0.591±0.117	0.419±0.118
60s	0.464±0.118	0.403±0.148
70s	0.406±0.078	0.374±0.123
80s	0.466±0.113	0.492±0.136

connective tissues in the SAN remained unchanged.

Inoue et al.[14] examined histologically the SAN cells of Japanese ranging in age from 20 to 86 years and reported that the number of nodal cells decreased with aging and the collagen fibres increased with aging. Luo et al.[15] investigated age-related changes of the SAN area in 106 persons ranging in age from 2 days to 83 years by computerized microimage analysis and reported that the area of nodal cells decreased after 40 years, whereas the area of the interstitial tissues in the SAN increased progressively with aging. These histological studies[3,4,13,14,15,16,17] demonstrate that the number of the SAN cells decreases with aging and the collagen fibres in the SAN increase with aging. The present study on the SAN of Thais is compatible with these histological studies[3,4,13,14,15,16,17]. Using monkey subjects with a

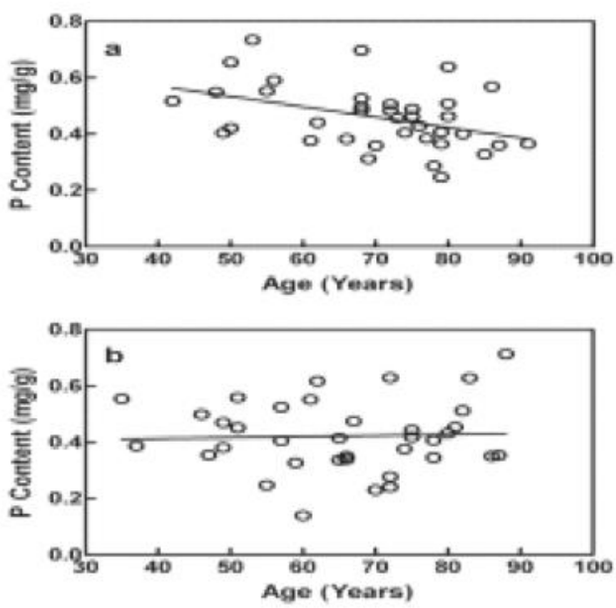

Figure 4: Age-related changes of the P content in the sinoatrial nodes of men (a) and women (b). The regression equation for men is: y = 0.0037x + 0.7188. The regression equation for women is: y = 0.0004x + 0.3980.

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wide range of ages, the authors[10] investigated whether there were any gender differences in elements of the SAN, right atrial wall, left ventricular wall, mitral valve, and left coronary artery and found that there were significant gender differences in the P content of all the SAN, right atrial wall, and left ventricular wall, but not in the mitral valve and left coronary artery.

The P content in all the SAN, right atrial wall, and left ventricular wall of monkeys was significantly higher in females than in males. In the present study, the P content decreased significantly in the SANs of men with aging, but it did not change significantly in the SANs of women with aging. In the case of the SAN of Thais, such a gender difference in the P content as monkeys was not found.

The monkeys used in these studies[6,10] were bred in the Primate Research Institute, Kyoto University and fed on Nihon Clea Cho of an adequate diet under control. In humans, their diet, life style, and living environment are very different. The results that the P content varied widely in the SANs of Thais may be attributed to the significant differences of individuals.

The present study revealed that there were extremely or very significant direct correlations among the Ca, P, S, and Mg contents in the SANs. A similar finding was previously obtained in the SANs of Japanese[5].

It is known that regarding the relationships among elements, there were significant direct or inverse correlations among the Ca, P, S, and Mg contents in most of the arteries examined[18]. In the arteries, significant direct correlations were found among the Ca, P, and Mg contents, whereas significant inverse correlations were found between the S and Ca, P, or Mg contents. On accumulation of Ca, P, and Mg in the arteries (arteriosclerosis), the smooth muscle cells decrease in the arteries[19,20]. The muscle cell has a large quantity of proteins such as myosin and actin containing S. The S content of the muscle cell is mostly determined by such proteins as myosin and actin. Therefore, as the Ca, P, and Mg contents increase in the artery, the S content decreases reversely in the artery.

On the other hand, there were significant direct correlations among the Ca, P, S, and Mg contents in the SANs. Significant direct correlations were found between the S and Ca, P, or Mg contents in the SANs. As the cell density decreases in the SAN with aging, the protein amount of myosin and actin decreases in the SAN. Therefore, it is thought that as the P content decreases in the SAN, the S content decreases simultaneously in the SAN.

Conclusion

The P content of tissue is mostly determined by the nucleic acid (DNA and RNA) and the phospholipid content of the tissue. Nucleic acids in the cell nucleus and the cytosol and phospholipids in the cell membrane are indicators of metabolically active cells.

Taking into consideration, it is reasonable to presume that the P content indicates the active cell density in the SAN, i.e. the number of active cells per volume. Therefore, there is a possibility that the active cell density of the SAN decreases significantly in men with aging. Regarding the relationships among elements, extremely or very significant direct correlations were found among the Ca, P, S, and Mg contents in the SANs.

References

1. Kuga K, Yamaguchi I, Sugishita Y. Age-related changes of sinus node function and autonomic regulation in subjects without sinus node diseaseassessment by pharmacologic autonomic blockade. Jpn Circ J. 1993; 57:760-768.
2. Crasset V, Mezzetti S, Antoine M, Linkowski P, Degaute JP, van de Borne P. Effects of aging and cardiac denervation on heart rate variability during sleep. Circulation. 2001; 103: 84-88.
3. Alings AMW, Abbas RF, Bouman LN. Age-related changes in structure and relative collagen contents of the human and feline sinoatrial node. A comparative study. Eur Heart J. 1995; 16: 1655-1667.
4. Song Y, Yao Q, Zhu J, Lou B, Liang S. Age-related variation in the interstitial tissues of the cardiac conduction system; and autopsy study of 230 Han Chinese. Forensic Sci Int. 1999; 104: 133-142.
5. Tohno Y, Tohno S, Satoh H, Minami T, Moriwake Y, Azuma C, et al. Compositional changes of human mitral valves with aging. Biol Trace Element Res. 2002, 220; 88: 203-213.
6. Satoh H, Tohno S, Azuma C, Minami T, Oishi T, Hayashi M, et al. Age-related attenuation in the elements in monkey sino-atrial node. Biol Trace Element Res. 2005; 107: 43-51.
7. Tohno Y, Tohno S, Mahakkanukrauh P, Vaidhayakarn P, Somsarp V, Minami T, et al. Simultaneous accumulation of magnesium with calcium and phosphorus in aorta and iliac arteries of Thai. Biol Trace Element Res. 2001; 84: 19-35.
8. Yanagawa N, Nakajima Y. A simple dissection method for the conduction system of the human heart. Anat Sci Ed. 2009; 2: 78-80.
9. 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 Element Res. 1996; 54: 23-31.
10. Tohno Y, Tohno S, Satoh H, Hayashi M, Oishi T, Minami T, et al. Gender differences in the phosphorus content of the sino-atrial nodes and other cardiac regions of monkeys. Biol Trace Element Res. 2011; 143: 871-881.
11. Frausta da Silva JJR, Williams RJP. The biological chemistry of the elements. Oxford: Clarendon Press; p436-452.
12. Oster O, Dahm M, Oelert H, Prellwitz W. Concentrations of some trace elements (Se, Zn, Cu, Fe, Mg, K) in blood and heart tissue of patients with coronary heart disease. Clin Chem. 1989; 35: 851-856.
13. Shiraishi I, Takamatsu T, Minamikawa T, Onouchi Z, Fujita S. Quantitative histological analysis of the human sinoatrial node during growth and aging. Circulation. 1992; 85: 2176-2184.

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14. Inoue S, Shinohara F, Niitani H, Gotoh K. A new method for the histological study of aging changes in the sinoatrial node. Jpn Heart J. 1986; 27: 653-660.
15. Luo B, Song Y, Zhu J, Liang S, Xie J, Jianhua X. Computerized microimage analysis of age-related changes of the human sinoatrial node. Forensic Sci Int. 1995; 75: 149-155.
16. Song Y, Laaksonen H, Saukko P, Toivonen S, Zhu J. Histopathological findings of cardiac conduction system of 150 Finns. Forensic Sci Int. 2001; 119: 310-317.
17. de Melo SR, de Souza RR, Mandarim-de-Lacerda CA. Stereologic study of the sinoatrial node of ratsage related changes. Biogerontol. 2002; 3: 383-390.
18. 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 Element Res. 2001; 82: 9-19.
19. Nemetschek-Gansler H, Weiss H, Wenisch HJC, Noetzel B, Pretzsch U. Intimal thickening of human femoral arteries with special regard to elastin. Part 1. Diffuse intimal thickening due to growth and age. Atherosclerosis. 1979; 34: 167-191.
20. Sawabe M. Vascular aging: from molecular mechanism to clinical significance. Geriatr Gerontol Int. 2010; 10 (Suppl 1): S213-S220.

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