Age-Related Changes of Calcium in the Coronary Arteries of Thai, Japanese and Monkeys

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ABSTRACT

To examine whether there were differences between different races and between different species in regard to age-related changes of elements in the coronary arteries, the authors investigated age-related changes of Ca in the left coronary arteries of Thai, Japanese and monkeys by direct chemical analysis. After ordinary dissections at Chiang Mai University and Nara Medical University were finished, the left coronary arteries were resected from the subjects. The anterior interventricular branch was used as the left coronary artery. The anterior interventricular branches were also resected from rhesus and Japanese monkeys, bred at Primate Research Institute, Kyoto University. After ashing of the arteries with nitric acid and perchloric acid, the Ca content was determined by inductively-coupled plasma-atomic emission spectrometry. In the left coronary arteries of Thai, the average content of Ca increased progressively from the forties to the seventies. In the left coronary arteries of Japanese, the average content of Ca increased remarkably in the seventies and thereafter increased in the nineties. The average content of Ca in the seventies was two times higher in the left coronary arteries of Thai than in those of Japanese. The Ca accumulation in the left coronary arteries of Thai occurred at least 10 years earlier in comparison with Japanese. In contrast, the Ca accumulation hardly occurred in the left coronary arteries of rhesus and Japanese monkeys at old age.

Key words: Coronary artery, Atherosclerosis, Calcium, Aging, Human, Monkey

INTRODUCTION

To elucidate compositional changes of the arteries with aging, the authors investigated age-related changes of elements in almost all of the arteries of human (Tohno, Y. et al., 1996; Araki and Tohno, 1996; Tohno, S. et al., 1997a,b; Tohno, Y. et al., 1997; Tohno, S. and Y. Tohno, 1998; Tohno, S. et al., 1998; Tohno, S. et al., 1999; Masuda et al., 1999; Tohno, S. et al., 2001c; Tohno, Y. et al., 2001a,b,c,d,e; Tohno, S. et al., 2002; Azuma et al., 2003; Tohno, S. et al., 2004; Mahakkanukrauh, et al., 2005; Tohno, Y. et al., 2006; Ongkana, et al., 2007; Prieto, et al., 2007; Tohno, Y. et al., 2008) and monkeys (Tohno, S. et al., 2001a,b; Tohno, S. et al., 2003; Tohno, S. et al., 2005). It is established that with regard to the human arteries, there are the following two types of arteries: The first type is one that a significant accumulation of Ca and P occurs with aging, whereas the second type is one that an accumulation of Ca and P hardly occurs with aging (Tohno, S. and Y. Tohno, 1998). The thoracic and abdominal aortas and the basilar, coronary, common carotid, splenic, common iliac, internal iliac, external iliac, uterine, internal pudendal, femoral, popliteal, posterior tibial and dorsalis pedis arteries belong to the first type, whereas the internal thoracic, cerebral, pulmonary, axillary, brachial, radial, ulnar and obturator arteries belong to the second type.

In the present paper, the authors focus on the left coronary arteries in which a high accumulation of Ca and P occurs with aging and describe the differences between different races and between different species in regard to age-related changes of Ca in the left coronary arteries.

MATERIALS AND METHODS

Sampling of Arteries

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 (Tohno, Y. et al., 2001b). Japanese cadavers were treated by injection of a mixture of 36% ethanol, 13% glycerin, 6% phenol and 6% formalin through the femoral artery (Tohno, Y. et al., 1985). After ordinary dissections by medical students at Chiang Mai University and Nara Medical University were finished, the left coronary arteries were resected from the subjects.

Rhesus and Japanese monkeys were bred in Primate Research Institute, Kyoto University. Monkeys were pretreated with an intramuscular injection of ketamine hydrochloride (10 mg/kg) and were deeply anesthetized by intravenous administration of pentobarbital sodium (Nembutal, 30 mg/kg). They were then perfused through the left ventricle with 0.5 L of ice-cold saline containing 2 mL (2,300 units) of heparin sodium, followed by 1-2 L of ice-cold fixative consisting of 2% paraformaldehyde and 0.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4) (Tohno, S. et al., 2001a). After the perfusion, the left coronary arteries were resected from the monkeys.

The anterior interventricular branch of the left coronary artery was used as the left coronary artery in the present study.

Determination of Elements

The samples of the coronary arteries were washed thoroughly with distilled water and were dried at 80? for 16 h. After 1 mL conc. nitric acid was added to the dry samples, the mixtures were heated at 100°C for 2 h. After the addition of 0.5 mL conc. perchloric acid, they were heated at 100°C 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 analyzed with an inductively-coupled plasma-atomic emission spectrometer (ICPS-7510; Shimadzu, Kyoto, Japan) (Tohno, Y. et al., 1996). The conditions were 1.2 kW of power from a radio-frequency 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 μm, an exit slit of 30 μm, a height of observation of 15 mm and an integration time lapse of 5 s. The element amount was expressed on a dry-weight basis.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism version 3.0 (GraphPad Software Inc., San Diego, CA, USA). Pearson's correlation was used to investigate the association between parameters. A p-value of less than 0.05 was considered to be statistically significant. Data were expressed as the mean±standard deviation.

RESULTS

Age-Related Changes of Ca in the Left Coronary Arteries of Thai

Thai subjects consisted of 63 men and 28 women, ranging in age from 24 to 87 years (average age=61.4±12.5 years). Figure 1a shows age-related changes of the Ca content in the left coronary arteries of Thai. The correlation coefficient was estimated to be 0.350 (p=0.0007) between age and Ca content in the left coronary arteries of Thai. An extremely significant direct correlation was found between age and Ca content in the left coronary arteries of Thai. Figure 2 shows the average content of Ca in the left coronary arteries of Thai and Japanese by age group. The average content of Ca increased progressively from the forties to the seventies in the left coronary arteries of Thai. The average content of Ca in the left coronary arteries in the seventies corresponded to about 7-fold the amount of that in the forties.

Age-Related Changes of Ca in the Left Coronary Arteries of Japanese

Japanese subjects consisted of 29 men and 23 women, ranging in age from 55 to 94 years (average age=79.4±10.1 years). Figure 1b shows age-related changes of the Ca content in the left coronary arteries of Japanese. The correlation coefficient

was estimated to be 0.352 (p=0.010) between age and Ca content, indicating that there was a significant direct correlation between age and Ca content. In the left coronary arteries of Japanese, the average content of Ca increased remarkably in the seventies and thereafter increased in the nineties (Fig. 2).

The average content of Ca in the seventies was two times higher in the left coronary arteries of Thai than in those of Japanese. The accumulation of Ca in the left coronary arteries of Thai occurred at least 10 years earlier in comparison with Japanese.

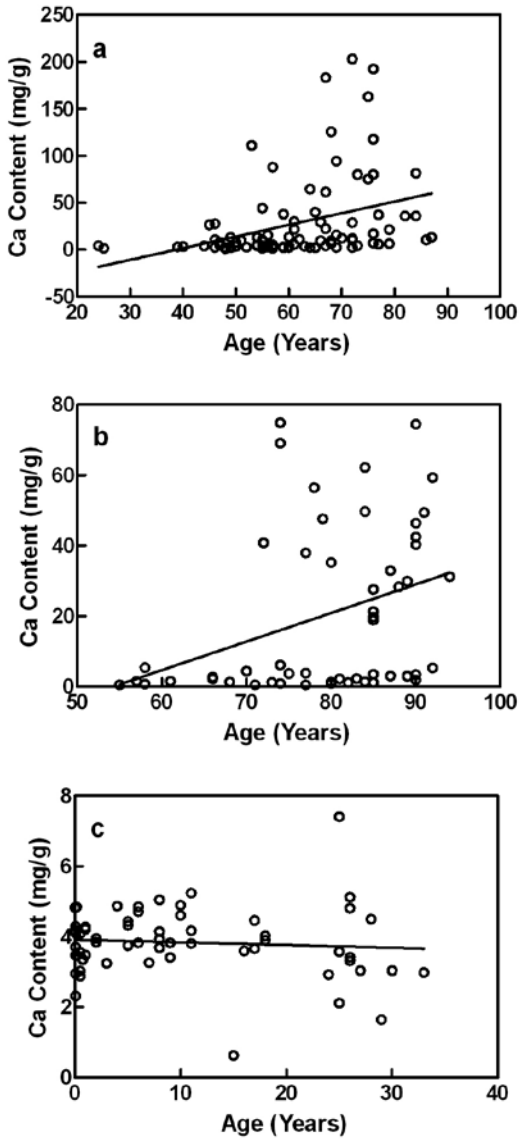

Figure 1. Age-related changes of the Ca content in the left coronary arteries of Thai (a), Japanese (b) and monkeys (c)

Age-Related Changes of Ca in the Left Coronary Arteries of Monkeys

Monkey subjects consisted of 38 rhesus and 23 Japanese monkeys. Rhesus monkeys were composed of 16 males, 16 females, and 6 unknown, whereas Japanese monkeys were composed of 9 males and 14 females. The monkeys ranged in age from newborn to 33 years (average age=9.8±10.0 years). Rhesus and Japanese monkeys were treated as one monkey group in the present study.

Figure 1c shows age-related changes of the Ca content in the left coronary arteries of monkeys. All of the Ca content was less than 8 mg/g in the left coronary arteries of rhesus and Japanese monkeys. The correlation coefficient was estimated to be -0.199 (p=0.125) between age and Ca content in the left coronary arteries of monkeys. No significant correlation was found between age and Ca content in the left coronary arteries of monkeys.

The average content of Ca in the left coronary arteries of monkeys was 3.88±0.79 mg/g at below 20 years of age and 3.68±1.49 mg/g at more than 20 years of age, respectively. It is generally said that the age of 20 years in rhesus and Japanese monkeys corresponds to the age of 60 years in human (Tohno, S. et al., 2001a). The average content of Ca did not increase in the left coronary arteries of monkeys at old age and decreased by 13% in the left coronary arteries of monkeys more than 20 years of age in comparison with that below 20 years of age.

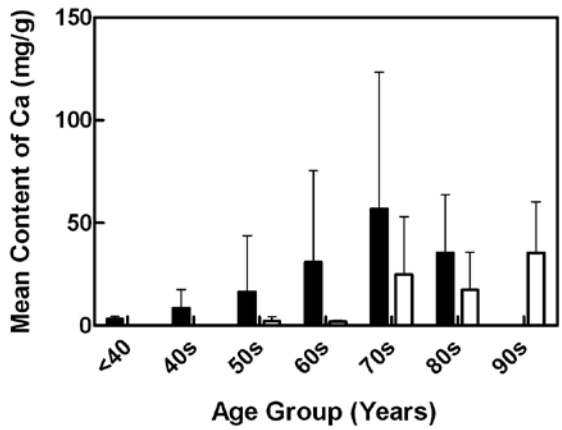

Figure 2. Comparison in the average content of Ca in the left coronary arteries of Thai (shaded bar) and Japanese (open bar) by age group.

Comparison in the Ca Content of the Left Coronary Arteries Among Thai, Japanese and Monkeys

Figure 3 shows age-related changes of the Ca content in the left coronary arteries of Thai, Japanese and monkeys. It is clear that a higher accumulation of Ca occurred in the left coronary arteries of the Thai at old age.

The average content of Ca was 41.32±53.67 and 21.98±23.67 mg/g in the left coronary arteries of Thai and Japanese more than 60 years of age, respectively,

whereas it was 3.68±1.49 mg/g in the left coronary arteries of monkeys more than 20 years of age. The average content of Ca in the left coronary arteries of Thai and Japanese corresponded to 11- and 6-fold the amount of that in the left coronary arteries of monkeys, respectively.

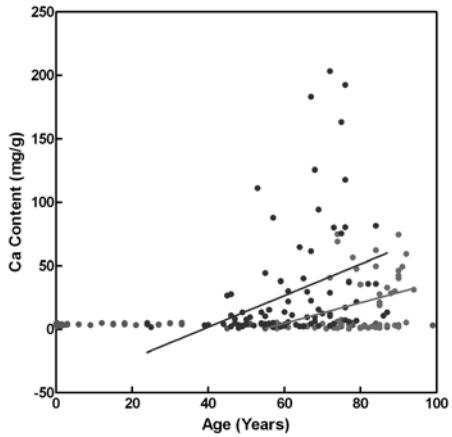

Figure 3. Age-related changes of the Ca content in the left coronary arteries of Thai (blue solid circle), Japanese (red solid circle) and monkeys (brown solid circle). The age of monkey is multiplied by three.

Table 1 indicates the incidence of the left coronary arteries of Thai and Japanese with the Ca content more than 10 mg/g, which is not contained in a normal artery. In Thai, the incidence of the left coronary artery with the high Ca content was 31% in the forties, 74% in the seventies and 100% in the eighties. In Japanese, the incidence of the left coronary artery with the high Ca content was 0% in the sixties, 43% in the seventies and 70% in the nineties. As far as the seventies and eighties are concerned, the incidences of the left coronary artery of Thai with the high Ca content were about two times higher in comparison with Japanese.

Table 1. Incidence of the Left Coronary Arteries of Thai and Japanese with the Ca Content Higher Than 10 mg/g.

Age Group (Years)	Incidence (%)	
(Years)	Thai	Japanese
<40	0 (0/3)	NA
40s	30.8 (4/13)	NA
50s	30.8 (8/26)	0 (0/4)
60s	52.0 (13/25)	0 (0/4)
70s	73.7 (14/19)	42.9 (6/14)
80s	100.0 (5/5)	50.0 (10/20)
90s	NA	70.0 (7/10)

Note: The numbers of cases are indicated in parentheses.
NA indicates that the specimen was not analyzed.

DISCUSSION

The present study revealed that a high accumulation of Ca occurred at least 10 years earlier in the left coronary arteries of Thai than in those of Japanese.

Regarding the coronary artery of the Thai, Hirst et al., (1962), using the coding technique of Gore and Tejada (1957), studied the atherosclerotic lesion in 203 unselected autopsies in Bangkok. There was a marked similarity in the progression and mean percent of intimal surface between these cases and a series of random cases in Los Angeles. However, the extent of involvement of the coronary arteries in Bangkok between the fourth and eighth decades averaged only one-half of that found in Los Angeles.

Matsuda-Inoguchi et al., (2000) investigated the nutrient intakes of working women in Bangkok by the total food duplicate method and reported that although about a half of the women had insufficient energy intakes, lipid intakes such as vegetable oil, butter and lard were in excess in more than a half of the women. Yoshimoto et al., (1994) studied the association between socioeconomic changes and health status among 140 middle-aged subjects resident in an urban area of Chiang Mai province and reported that the women had significantly higher values of body fat percent and high-density lipoprotein cholesterol, and the men had significantly higher values of triglyceride and arteriosclerosis index. There is a possibility that the excess lipid intake is related to earlier and higher accumulation of Ca in the coronary artery of the Thai compared with that of the Japanese.

It is known that the accumulation of Ca and P does not occur uniformly in the left and right coronary arteries with aging and a higher accumulation of Ca and P occurs in the proximal sites of both the left and right coronary arteries compared with the distal sites (Azuma et al., 2003). The left and right coronary arteries ramify orthogonally from the ascending aorta and, in consequence, a mechanical (hemodynamic) stress occurs at the original sites of the coronary arteries (Fukushima et al., 2005). It is presumed that this difference might result from a difference in mechanical stress between the proximal and distal sites of the coronary arteries.

The authors previously investigated age-related changes of elements in the coronary artery in comparison with the ascending aorta, aortic valve and mitral valve in Thai and found that the Ca accumulation occurred earlier in the left coronary artery than in the ascending aorta, aortic valve and mitral valve and that with respect to the Ca, P, Mg and Na contents, the coronary artery correlated well with both the aortic valve and ascending aorta, especially with the aortic valve, but it did not correlate with the mitral valve.

To elucidate whether the accumulation of elements occurred simultaneously in the various arteries with aging, the authors investigated age-related changes of elements in the eight arteries, such as the thoracic and abdominal aortas and the coronary, common carotid, pulmonary, splenic, common iliac and uterine arteries. It was found that there were significant direct correlations in the contents of Ca, P, Mg, Zn, Fe and Na between the coronary and splenic arteries.

The present study revealed that Ca accumulation did not occur in the left coronary arteries of rhesus and Japanese monkeys at old age. There are several

pathological reports (Chawla et al., 1967; Kramsch and Hollander, 1968; Prathap and Lau, 1972; Prathap, 1973; Clarkson et al., 1985; Weingand, 1989; Cefalu and Wagner, 1997; Sukhova et al., 2002) on atherosclerosis of the coronary artery in nonhuman primates. Prathap (1973) and Prathap and Lau (1972) examined wild adult male cynomolgus monkeys and reported that fatty streaks in the aortic arch and thoracic aorta in 90% of these animals were observed and rare fibrous plaques without complicated lesions were seen. Chawla et al., (1967) reported that the incidence of naturally-occurring atherosclerosis in the coronary artery was low in rhesus monkeys. Our finding of the left coronary arteries of monkeys was consistent with the finding by Chawla et al., (1967).

The authors (Tohno, S. et al., 2001a) examined age-related changes of the Ca content in various arteries of Japanese monkeys and found that the Ca content increased progressively in the arteries such as the axillary, brachial, radial, subclavian, common carotid, common iliac and femoral arteries. In comparison between two groups of Japanese monkeys below and more than 20 years of age, the average content of Ca increased to two times higher in the thoracic aorta, common iliac, internal iliac, external iliac, common carotid, and subclavian arteries of more than 20 years of age in comparison with below 20 years of age. Regarding more than 20 years of age, the average content of Ca in the left coronary arteries of rhesus and Japanese monkeys was similar to that of the radial and ulnar arteries (Tohno, S., et al., 2001a), in which the average content of Ca was the lowest, within the limits of the arteries of Japanese monkeys analyzed by us.

Kramsch and Hollander (1968) were the first to describe diet-induced atherosclerosis of wild adult male cynomolgus monkeys. Clarkson et al., (1984, 1987) reported that atherosclerosis was induced in rhesus monkey by feeding with a cholesterol-containing diet and atherosclerotic lesions were more severe in the coronary arteries and at the carotid bifurcation. However, it is ambiguous whether the induction of atherosclerosis occurs in the coronary arteries of Japanese monkey by feeding with a cholesterol-containing diet.

It should be noted that atherosclerosis occurs frequently in the coronary arteries of Thai and Japanese, but it hardly occurs naturally in the left coronary arteries of rhesus and Japanese monkeys.

These results lead to the following conclusion. There are significant differences between different races and between different species in regard to age-related changes of the Ca content in the left coronary artery.

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