Decreases of Calcium, Phosphorus, Zinc and Iron in the Aortic and Pulmonary Valves of Pig with Development

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ABSTRACT

To elucidate compositional changes of the cardiac valves with development, the authors investigated changes of elements in the four cardiac valves of pig with development and the relationships among elements. The four cardiac valves of the aortic, pulmonary, mitral and tricuspid valves as well as the left and right coronary arteries were resected from 16 pigs, ranging in age from 2 mo to 5 mo. The element content was determined by inductively-coupled plasma-atomic emission spectrometry (ICP-AES). It was found that Ca, P, Zn and Fe decreased significantly with development in both the aortic and pulmonary valves, with one exception, but Ca, P, Zn and Fe did not decrease significantly with development in the mitral and tricuspid valves, except for Ca in the mitral valve. In both the left and right coronary arteries, Ca, P, Zn and Fe decreased significantly with development.

Regarding the relationships among elements, significant direct correlations were found among Ca, P, S, Mg and Zn in both the aortic and pulmonary valves, with two exceptions in the pulmonary valve. In contrast, no significant correlations were found among Ca, P, S, Mg and Zn in the mitral and tricuspid valves, except for Ca and Zn in the mitral and tricuspid valves. In both the left and right coronary arteries, significant direct correlations were found among Ca, P, S, Mg and Zn. With respect to the relationships among elements, the aortic and pulmonary valves were similar to the coronary arteries, but the mitral and

tricuspid valves were not similar to the coronary arteries.

Taken the relationships among elements into consideration, Ca decreased together with P and Zn in the aortic valve with development and Ca decreased together with only P in the pulmonary valve with development.

Key words: Cardiac valve; Coronary artery; Calcium; Phosphorus; Zinc; Iron; Pig; Development

INTRODUCTION

Clinical and pathological studies revealed that calcification occurred frequently in both the aortic and mitral valves (Simon and Liu, 1954; Pomerance, 1970), but it occurred rarely in the tricuspid and pulmonary valves (Cooksey et al., 1970). The authors previously investigated age-related changes of elements in the four cardiac valves of Japanese (Tohno, et al., 1999; Tohno, et al., 2000) and Thai (Ohnishi et al., 2003) and found that the accumulation of Ca and P with aging was highest in the aortic valve and decreased in the order of the mitral, pulmonary and tricuspid valves. Recently, the authors (Menetti et al., 2005) investigated age-related changes of elements in the cardiac valves of rhesus and Japanese monkeys and found that the contents of Ca, P, S and Zn were high in all of the four cardiac valves of the infant monkey, especially-high in the aortic and pulmonary valves, but decreased rapidly in all of the four cardiac valve of the monkey with development. Furthermore, the accumulation of Ca and P did not occur in any cardiac valves of the monkey at an advanced age. However, it is uncertain whether the decrease of Ca, P, S and Zn with development occurs in only the monkey or in other species. Therefore, the authors investigated changes of elements in the four cardiac valves with development, using pig and found that Ca, P, Zn and Fe decreased significantly in both the aortic and pulmonary valves with development, but they did not decrease significantly in the mitral and tricuspid valves with development.

MATERIALS AND METHODS

Sampling

The hearts were resected from 16 pigs bred in Thailand, ranging in age from 2 mo to 5 mo. The hearts were fixed with 10% formalin and stored in 10% formalin until use. The four cardiac valves of the aortic, pulmonary, mitral and tricuspid valves and both the left and right coronary arteries were resected from the hearts.

Determination of Elements

The samples were washed thoroughly with distilled water and dried at 80°C for 16 h. After 1 ml nitric acid was added, the mixtures were heated at 100°C for 2 h. After the addition of 0.5 ml 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 filtered through the filter paper (No. 7; Toyo Roshi Co., Osaka, Japan).

The resulting filtrates were analyzed with an inductively-coupled plasma-atomic emission spectrometer (ICPS-7510; Shimadzu Co., 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 amount of element was expressed on a dry-weight basis.

Statistical Analysis

Statistical analyses were performed by using the GraphPad Prism Version 3.0 (GraphPad Software Inc., San Diego, CA, USA). A p-value of < 0.05 was considered to be significant. Data were expressed as the mean ± standard deviation.

RESULTS

Pigs used in the present study consisted of nine males and six females (unknown one), ranging in age from two mo to five mo.

Age-Related Changes of Elements in the Cardiac Valves

Figure 1 shows age-related changes of Ca in the four cardiac valves. The correlation coefficients between age and Ca content were estimated to be -0.506 (p = 0.046) in the aortic valve (Fig. 1A), -0.370 (p = 0.158) in the pulmonary valve (Fig. 1B), -0.594 (p = 0.015) in the mitral valve (Fig. 1C) and -0.486 (p = 0.066) in the tricuspid valve (Fig. 1D). Significant inverse correlations were found between age and Ca content in both the aortic and mitral valves, but no significant correlations were found between them in the pulmonary and tricuspid valves.

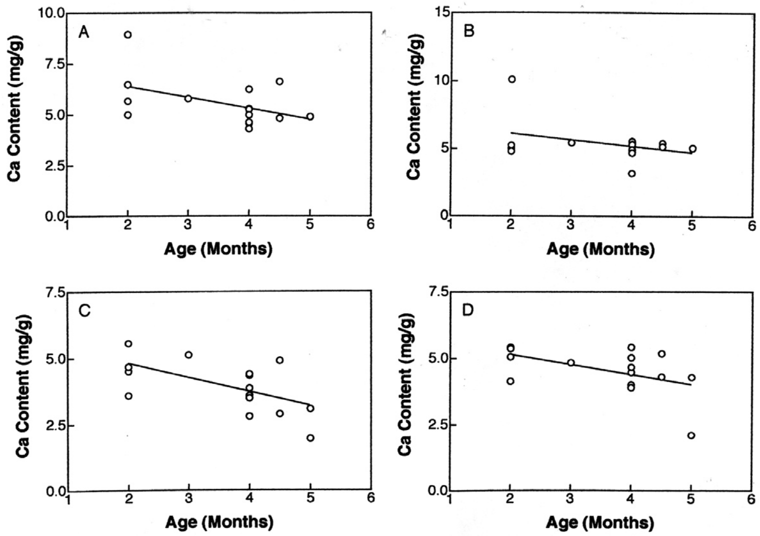


Figure 1. Age-related changes of Ca in the aortic valve (A), pulmonary valve (B), mitral valve (C) and tricuspid valve (D).

Figure 2 shows age-related changes of P in the four cardiac valves. The correlation coefficients between age and P content were estimated to be -0.664 (p = 0.005) in the aortic valve (Fig. 2A), -0.535 (p = 0.033) in the pulmonary valve (Fig. 2B), -0.097 (p = 0.720) in the mitral valve (Fig. 2C) and -0.149 (p = 0.597) in the tricuspid valve (Fig. 2D). A very significant inverse correlation was found between age and P content in the aortic valve, and a significant inverse correlation was found between them in the pulmonary valve. However, no significant correlations were found between them in the mitral and tricuspid valves.

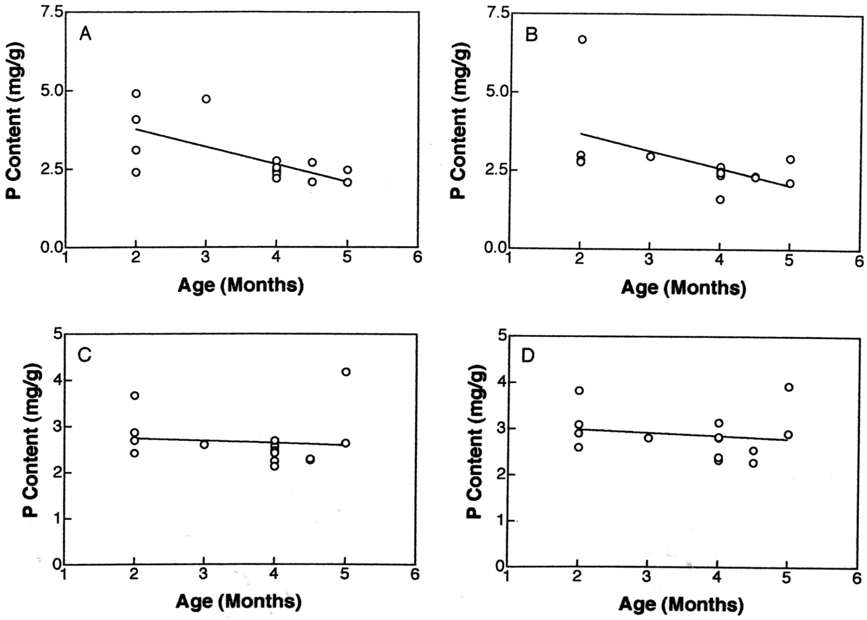


Figure 2. Age-related changes of P in the aortic valve (A), pulmonary valve (B), mitral valve (C) and tricuspid valve (D).

Age-related changes of Zn in the four cardiac valves are shown in Fig. 3. The correlation coefficients between age and Zn content were estimated to be -0.779 (p = 0.0004) in the aortic valve (Fig. 3A), -0.682 (p = 0.004) in the pulmonary valve (Fig. 3B), -0.463 (p = 0.071) in the mitral valve (Fig. 3C) and -0.411 (p = 0.128) in the tricuspid valve (Fig. 3D). An extremely significant inverse correlation was found between age and Zn content in the aortic valve, and a very significant inverse correlation was found between them in the pulmonary valve. However, no significant correlations were found between age and Zn content in the mitral and tricuspid valves.

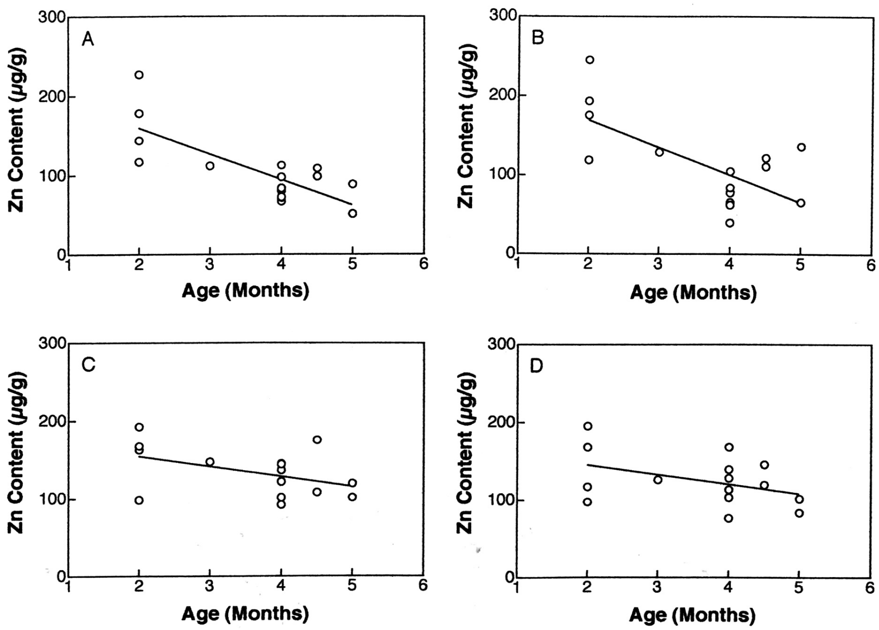


Figure 3. Age-related changes of Zn in the aortic valve (A), pulmonary valve (B), mitral valve (C) and tricuspid valve (D).

Figure 4 shows age-related changes of Fe in the four cardiac valves. The correlation coefficients between age and Fe content were estimated to be -0.579 ($p = 0.019$) in the aortic valve (Fig. 4A), -0.675 ($p = 0.004$) in the pulmonary valve (Fig. 4B), -0.029 ($p = 0.916$) in the mitral valve (Fig. 4C) and -0.113 ($p = 0.688$) in the tricuspid valve (Fig. 4D). Although no significant correlations were found between age and Fe content in the mitral and tricuspid valves, significant and very significant inverse correlations were found between them in the aortic and pulmonary valves, respectively.

No significant correlations were found between age and other element contents, such as S, Mg and Na in the four cardiac valves, with one exception that a significant inverse correlation was found between age and Na content in the aortic valve ($p = 0.019$).

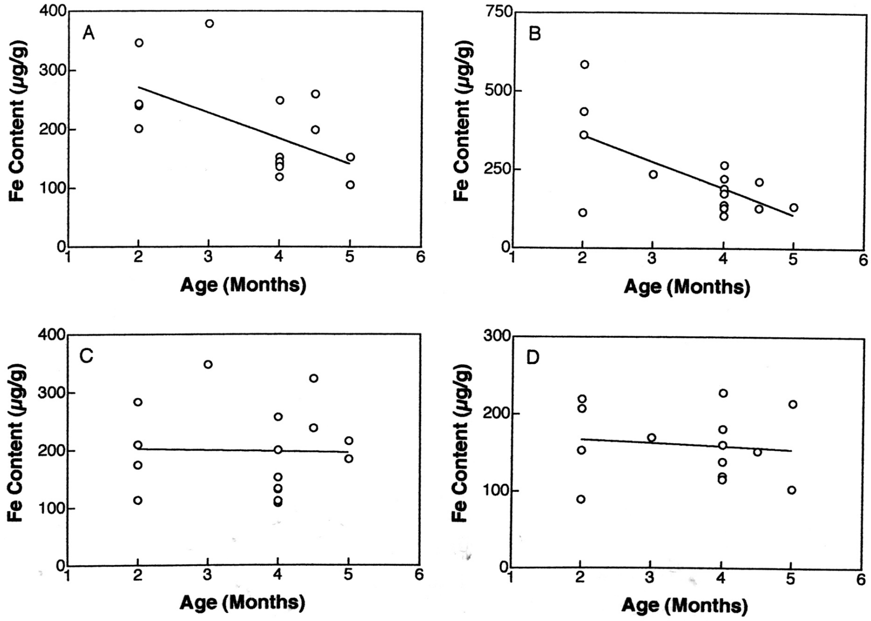


Figure 4. Age-related changes of Fe in the aortic valve (A), pulmonary valve (B), mitral valve (C) and tricuspid valve (D).

Age-Related Changes of Elements in the Coronary Artery

Age-related changes of Ca, P, Zn and Fe in the left coronary artery are shown in Fig. 5. The correlation coefficients between age and element content were estimated to be -0.633 ($p = 0.009$) for Ca (Fig. 5A), -0.582 ($p = 0.018$) for P (Fig. 5B), -0.676 ($p = 0.004$) for Zn (Fig. 5C) and -0.648 ($p = 0.007$) for Fe (Fig. 5D). In the left coronary artery, very significant inverse correlations were found between age and element contents, such as Ca, Zn and Fe, and a significant inverse correlation was found between age and P content.

With regard to S, Mg and Na, no significant correlations were found between age and the element content in the left coronary artery.

Furthermore, with regard to age-related changes of elements, similar results were also obtained in the right coronary artery.

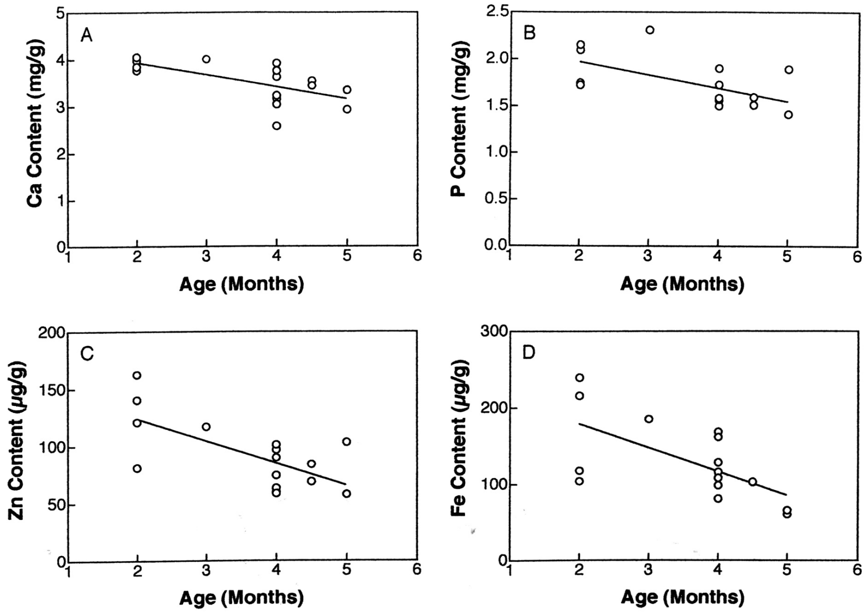
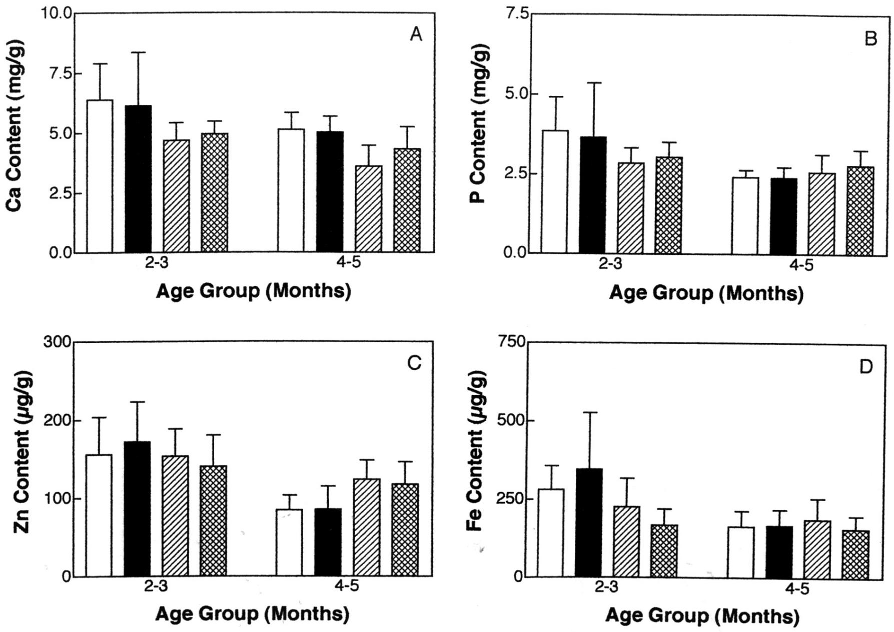


Figure 5. Age-related changes of Ca (A), P (B), Zn (C) and Fe (D) in the left coronary artery.

Comparison in the Average Content of Elements by Age Group

Figure 6A shows the average content of Ca in the four cardiac valves at the ages of 2-3 mo and 4-5 mo. The average content of Ca decreased to 80-90% in all of the four cardiac valves of 4-5 mo in comparison with that of 2-3 mo. As shown in Fig. 6B, the average content of P decreased to about 65% in both the aortic and pulmonary valves of 4-5 mo in comparison with that of 2-3 mo, but it hardly decreased in the mitral and tricuspid valves of 4-5 mo. Likewise, the average content of Zn and Fe decreased to 50-60% in both the aortic and pulmonary valves of 4-5 mo in comparison with that of 2-3 mo, and it decreased to 80-90% in the mitral



and tricuspid valves of 4-5 mo (Figs. 6C and 6D).

Figure 6. Comparison of the average contents of Ca (A), P (B), Zn (C) and Fe (D) in the four cardiac valves between 2-3 mo and 4-5 mo of age. The open, shaded, hatched and crossed bars indicate the aortic, pulmonary, mitral and tricuspid valves, respectively.

Table 1. Comparison in the Average Content of Elements in the Left Coronary Artery Between 2-3 and 4-5 Months.

Element	Average Content (mg/g)		Ratio
	2-3 Mo	4-5 Mo	4-5 Mo/2-3 Mo
Ca	3.929±0.120	3.327±0.389	0.85
P	2.009±0.257	1.625±0.156	0.81
S	4.842±0.521	4.564±0.383	0.94
Mg	0.290±0.022	0.261±0.026	0.90
Zn	0.125±0.078	0.078±0.018	0.63
Fe	0.173±0.060	0.108±0.035	0.63
Na	0.073±0.024	0.057±0.022	0.78

Table 1 shows the average content of elements in the left coronary artery of pig. The average content of Ca, P and Na decreased to about 80% in the left coronary artery of 4-5 mo, compared with that of 2-3 mo. Regarding Zn and Fe, the average content decreased to about 60% in the left coronary artery of 4-5 mo, compared with that of 2-3 mo. The average content of S and Mg decreased a little in the left coronary artery of 4-5 mo, compared with that of 2-3 mo.

Relationships Among Elements in the Cardiac Valves

Tables 2-5 indicate the relationships among elements in the four cardiac valves. In the aortic valve, extremely or very significant direct correlations were found among the contents of Ca, P, S, Mg and Zn (Table 2). Extremely or very significant direct correlations were also found among the contents of Ca, P, S and Mg in the pulmonary valve (Table 3). In addition, significant direct correlations were found between Zn and either P or Mg contents in the pulmonary valve. In contrast, no significant correlations were found among the contents of Ca, P, S and Mg in the mitral and tricuspid valves (Tables 4 and 5). With regard to the relationships among the contents of Ca, P, S and Mg, the aortic and pulmonary valves were similar to each other, but they were different from the mitral and tricuspid valves.

Table 2. Relationships Among Elements in the Aortic Valves.

Element	Correlation Coefficient and *p*-Value					
	P	**S**	**Mg**	**Zn**	**Fe**	**Na**
Ca	0.760	0.911	0.863	0.789	0.268	0.591
	(0.0006)	(<0.0001)	(<0.0001)	(0.0003)	(0.315)	(0.016)
P		0.636	0.760	0.715	0.510	0.569
		(0.008)	(0.0006)	(0.002)	(0.043)	(0.021)
S			0.902	0.773	0.337	0.701
			(<0.0001)	(0.0005)	(0.202)	(0.003)
Mg				0.753	0.600	0.486
				(0.0008)	(0.014)	(0.056)
Zn					0.403	0.690
					(0.121)	(0.003)
Fe						0.028
						(0.919)

Note: *p*-Values are indicated in parentheses.

Table 3. Relationships Among Elements in the Pulmonary Valves.

Element	**Correlation Coefficient and *p*-Value**					
	P	**S**	**Mg**	**Zn**	**Fe**	**Na**
Ca	0.946	0.980	0.647	0.444	0.490	0.923
	(<0.0001)	(<0.0001)	(0.007)	(0.085)	(0.054)	(<0.0001)
P		0.910	0.648	0.585	0.524	0.935
		(<0.0001)	(0.007)	(0.017)	(0.037)	(<0.0001)
S			0.710	0.473	0.495	0.870
			(0.002)	(0.064)	(0.051)	(<0.0001)
Mg				0.530	0.179	0.440
				(0.035)	(0.507)	(0.088)
Zn					0.669	0.373
					(0.005)	(0.155)
Fe						0.461
						(0.073)

Note: *p*-Values are indicated in parentheses.

Table 4. Relationships Among Elements in the Mitral Valves.

Element	**Correlation Coefficient and *p*-Value**					
	P	**S**	**Mg**	**Zn**	**Fe**	**Na**
Ca	-0.133	-0.223	-0.382	0.720	0.107	-0.825
	(0.624)	(0.406)	(0.145)	(0.002)	(0.694)	(<0.0001)
P		0.342	0.335	0.016	0.155	0.532
		(0.195)	(0.205)	(0.955)	(0.567)	(0.034)
S			0.083	-0.102	0.471	0.362
			(0.761)	(0.707)	(0.066)	(0.168)
Mg				-0.245	0.297	0.519
				(0.362)	(0.264)	(0.039)
Zn					-0.0004	-0.513
					(0.999)	(0.042)
Fe						-0.023
						(0.933)

Note: *p*-Values are indicated in parentheses.

Table 5. Relationships Among Elements in the Tricuspid Valves.

Element	Correlation Coefficient and *p*-Value					
	P	**S**	**Mg**	**Zn**	**Fe**	**Na**
Ca	-0.133	-0.064	-0.434	0.519	-0.075	-0.823
	(0.637)	(0.820)	(0.106)	(0.047)	(0.790)	(0.0002)
P		0.383	0.475	-0.183	0.416	0.507
		(0.159)	(0.074)	(0.513)	(0.124)	(0.054)
S			0.296	0.102	0.425	0.396
			(0.285)	(0.719)	(0.115)	(0.144)
Mg				-0.381	0.023	0.645
				(0.162)	(0.935)	(0.009)
Zn					-0.007	-0.396
					(0.981)	(0.144)
Fe						0.325
						(0.238)

Note: *p*-Values are indicated in parentheses.

Table 6 indicates the relationships among elements in the left coronary artery. Extremely significant, very significant, or significant direct correlations were found among the contents of Ca, P, S, Mg and Zn in the left coronary artery. A similar finding was also obtained in the right coronary artery.

Table 6. Relationships Among Elements in the Left Coronary Arteries.

Element	Correlation Coefficient and *p*-Value					
	P	**S**	**Mg**	**Zn**	**Fe**	**Na**
Ca	0.695	0.548	0.771	0.795	0.634	0.534
	(0.003)	(0.028)	(0.0005)	(0.0002)	(0.008)	(0.033)
P		0.507	0.701	0.629	0.315	0.269
		(0.045)	(0.003)	(0.009)	(0.234)	(0.314)
S			0.717	0.577	0.583	0.168
			(0.002)	(0.019)	(0.018)	(0.534)
Mg				0.778	0.505	0.107
				(0.0004)	(0.046)	(0.694)
Zn					0.591	0.232
					(0.016)	(0.387)
Fe						0.523
						(0.038)

Note: *p*-Values are indicated in parentheses.

It is noteworthy that regarding the relationships among the contents of Ca, P, S and Mg, the aortic and pulmonary valves of the semilunar valves are similar to the coronary arteries, but the mitral and tricuspid valves of the atrioventricular valves are not similar to the coronary arteries (Table 7).

Table 7. Cardiac Valves and Coronary Arteries with Significant Correlation.

Element	**P**		**S**		**Mg**		**Zn**			**Fe**		**Na**		
Ca	AV	PV	AV	PV	AV	PV	AV	MV	TV	LC	RC	AV	PV	MV
	LC	RC	LC	RC	LC	RC	LC	RC				TV	LC	
P			AV	PV	AV	PV	AV	PV		AV	PV	AV	PV	
			LC	RC	LC	RC	LC	RC		RC		MV		
S					AV	PV	AV	LC		LC	RC	AV	PV	
					LC	RC	RC							
Mg							AV	PV		AV	LC	MV	TV	
							LC	RC		RC				
Zn										PV	LC	AV	MV	
Fe												LC		

Note: AV = aortic valve, PV = pulmonary valve, MV = mitral valve, TV = tricuspid valve, LC = left coronary artery, and RC = right coronary artery.

DISCUSSION

The present study revealed that Ca, P, Zn and Fe decreased significantly in both the aortic and pulmonary valves with development, with one exception. In contrast, Ca, P, Zn and Fe did not decrease significantly in the mitral and tricuspid valves with development, except for Ca in the mitral valve.

The authors (Menetti et al., 2005) investigated age-related changes of elements in the four cardiac valves of rhesus and Japanese monkeys and found that the Ca, P, S and Zn contents were high in all of the four cardiac valves of the infant monkey and thereafter decreased rapidly with development. The finding that the Ca, P and Zn contents decreased in both the aortic and pulmonary valves of the pig with development are consistent with the finding of those valves in the monkey. Although the S content tended to decrease in both the aortic and pulmonary valves of the pig with development, the decrease was not statistically significant. In the mitral and tricuspid valves of the pig, the Ca, P, S and Zn contents did not decrease significantly with development, except for Ca in the mitral valve.

In comparison of element contents in the cardiac valves between the pig below 3 mo and the monkey below 1 yr (Menetti et al., 2005), the average content of Ca is much higher in both the aortic and pulmonary valves of the monkey and it corresponds to 3-5 times the amounts of respective valves of the pig. Likewise, the average content of Ca is slightly higher in both the mitral and tricuspid valves of the monkey and it corresponds to 1.5-2 times the amounts of respective valves of the pig. Regarding P, the average content is a little higher in both the aortic and pulmonary valves of the pig, whereas it is reversely lower in the mitral and tricuspid valves of the pig.

In Japanese (Tohno, et al., 1999; Tohno, et al., 2000) and Thai (Ohnishi et al., 2003), the accumulation of Ca and P occurred remarkably in both the aortic and mitral valves at old age, especially in the aortic valve, whereas it hardly occurred in the tricuspid and pulmonary valves. Although the authors (Ohnishi et al., 2003) previously examined the four cardiac valves of Thai over 39 yr, we have not yet examined the cardiac valves of infant human. Therefore, it is necessary to examine whether Ca, P, S, Zn and Fe decrease in the cardiac valves of infant human with development.

With regard to the cardiac walls of rhesus and Japanese monkeys, it was found that Ca and P decreased significantly in all of the left and right atrial walls, the left and right ventricular walls, interatrial septum and interventricular septum with development, and S and Mg decreased only in the left and right ventricular walls with development (Tohno, et al., 2006). In the sino-atrial node of the monkey, it was found that Ca, P and Zn decreased significantly with development (Satoh et al., 2005).

It is well-known that the accumulation of Ca and P occurs remarkably in the human coronary artery at old age (Tohno, et al., 2002; Azuma et al., 2003). However, it is ambiguous whether Ca and P decrease in the human coronary artery with development, similar to the coronary artery of the pig.

Regarding the relationships among elements, significant direct correlations were found among Ca, P, S, Mg and Zn in both the aortic and pulmonary valves of the pig, with two exceptions. In the mitral and tricuspid valves of the pig, no significant correlations were found among Ca, P, S, Mg and Zn, with some exceptions. In all of the four cardiac valves of the monkey, significant direct correlations were found among Ca, P, S and Zn, with two exceptions. Significant direct correlations were found among Ca, P, S, Mg and Zn in the coronary arteries of the pig. Therefore, with respect to the relationships among Ca, P, S, Mg and Zn, the aortic and pulmonary valves of the pig and the monkey are similar to the coronary artery of the pig, but the mitral and tricuspid valves of the pig are not similar to the coronary artery of the pig.

In conclusion, Ca, P, Zn and Fe decreased significantly in both the aortic and pulmonary valves of the pig with development, but they did not decrease significantly in the mitral and tricuspid valves of the pig with development.

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