Experimental Myocardial Infarction with Cartilaginous and Osseous Metaplasia in SHR and WKY Rats

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Abstract: Myocardial infarction was induced by ligation of the coronary artery in fifteen 32-week-old male spontaneously hypertensive rats (SHR) and fifteen 32-week-old male normotensive Wistar-Kyoto rats (WKY). Age-matched male SHR (n=6) and WKY (n=7) without the operation were used as non-infarcted control rats. Four weeks after the operation, cartilaginous metaplasia and osseous metaplasia were observed histopathologically in all SHR and 13 of the 15 WKY with myocardial infarction, but not in the control rats. The mean rates of infarction to the heart and the mean areas of metaplasia per rat showing metaplasia were greater in SHR than in WKY. The metaplastic areas were well correlated with the infarct percentage in SHR. It is therefore considered that extensive myocardial infarction is associated with the pathogenesis of cartilaginous and osseous metaplasia.

Key words: bone, cartilage, metaplasia, myocardial infarction

Metaplasia is well known as an adaptive response to a new environment. Cartilaginous metaplasia and osseous metaplasia are frequently found in soft tissues, and either fibroblasts or non-differentiated mesenchymal cells are thought to be transformed into chondroblasts and osteoblasts [8]. These types of metaplasia in the heart have been reported to occur rarely in human myocardial infarction [12], but are experimentally inducible at a low incidence in birds [16]. Moreover, they have been provoked by either aging or allylamine treatment at an incidence of 22–33% in the rat heart [3, 4, 11], but no detailed study has been reported on cartilaginous and osseous metaplasia in myocardial infarction in rats.

In this study, we performed coronary artery ligation in spontaneously hypertensive rats (SHR) and normo-

tensive Wistar-Kyoto rats (WKY), and investigated histopathologically the occurrence of cartilaginous and osseous metaplasia in myocardial infarction.

Animals and experimental induction of myocardial infarction: Fifteen 32-week-old males of both SHR and WKY strains (Kowa Tokyo Research Laboratories) were used for experimental myocardial infarction. Non-operated 32-week-old males of SHR (n=6) and WKY (n=7) were used as control animals. Room temperature and relative humidity were controlled at 21–25 °C and 50–70%, respectively, and lighting was controlled to give a 12-hr light-dark cycle. All animals had free access to tap water and pellets (CE-2, CLEA JAPAN, Inc.). Under ether anesthesia, left-sided thoracotomy was performed and the left coronary artery was ligated.

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Histopathological preparation: Four weeks after the operation, under pentobarbital anesthesia, the heart was excised and the right ventricle was incised. A catheter was inserted into the left ventricular cavity in retrograde fashion through the aorta, and the atrioventricular groove was ligated. The left ventricle was perfused with 10% neutral buffered formalin at a constant pressure of 5 mm Hg. After fixation, the left ventricle was cut from the atrium and the right ventricle, and routinely processed for paraffin embedding. Three serial cross sections were cut from each site located at 1, 3 and 5 mm from apex to base. Two sections at each site were stained with hematoxylin-eosin (HE) and azan, respectively, for light microscopic observation. To demonstrate cartilage and bone tissues, the remaining sections were stained with either toluidine blue or Dahl's method for calcium (alizarin red S method).

Morphometry of metaplasia and infarction: In HE sections, cartilage and bone tissues were histopathologically identified by the following features: flat or oval cells in prominent lacunae were embedded within a homologous basophilic matrix, and flat or irregularly shaped cells in the lacunae were embedded within a homologous eosinophilic matrix, respectively [7, 9, 18]. The area of metaplasia and the proportion of the infarcted area to the transverse area of the heart

were measured by means of a computerized image analyzer (LA-555, PIAS Co. Ltd.). HE- and azan-stained specimens from each site were available for identification of metaplastic and infarcted areas, respectively. The correlation between the area of metaplasia and the infarcted percentage of the heart was examined in infarcted rats.

Cartilaginous metaplasia and osseous metaplasia were found in all infarcted SHR and 13 of the 15 infarcted WKY, but not in non-infarcted control animals. These metaplastic changes were located within an infarction zone of papillary muscle and sub-endocardium near the apex (Fig. 1). Histologically, cartilaginous matrix containing cartilage cells within lacuna exhibited metachromasia following toluidine blue stain and was partially calcified. Chondroblasts and fibroblasts were located around cartilaginous tissues (Fig. 2). Osseous tissue was composed of osteocytes and a homologous eosinophilic matrix that indicated calcium deposition (Fig. 3). Osteoblasts and osteoclasts were recognized around osseous tissues in some sections. Generally, cartilage and osseous tissues were next to each other, and some of them showed transformation from cartilage into bone.

Total areas of cartilaginous and osseous tissues were 30.29 mm² and 4.12 mm² in SHR, and 4.44 mm² and

Fig. 1. Myocardial infarct zone in SHR, showing cartilaginous and osseous metaplasia. HE. $\times 27$.

Fig. 2. Magnified view of Fig. 1, showing cartilaginous tissue. HE. $\times 93$.

Fig. 3. High power view of Fig. 1, showing osseous metaplasia. HE. $\times 93$.

0.10 mm² in WKY, respectively (Table 1). The mean areas of cartilaginous and osseous metaplasia per rat showing metaplasia were greater in SHR, 2.02 mm² and 0.27 mm², than in WKY, 0.34 mm² and 0.01 mm², respectively. The proportion of the infarcted area ranged from 40 to 90% and averaged 62.6% in SHR. In contrast, it ranged from 25 to 40% and averaged

32.8% in WKY. A definite correlation ($r=0.82$) between the metaplastic area and the infarct percentage was found only in SHR.

Experimental myocardial infarction in rats has been studied histopathologically by many investigators [1, 5, 10, 17, 20], but no mention at all has been made of cartilaginous or osseous metaplasia. In this study, meta-

Table 1. Area of metaplastic tissues and percentage of myocardial infarction in infarcted or non-infarcted SHR and WKY

Treatment	Strain	Incidence	Area (mm ²)			Mean percentage of infarct area (%)
			Cartilage	Bone	Total	
Non-infarcted	SHR	0/6 ^a	0	0	0	0
Non-infarcted	WKY	0/7	0	0	0	0
Infarcted	SHR	15/15	30.29	4.12	34.41	62.6
Infarcted	WKY	13/15	4.44	0.10	4.54	32.8

^a: Numerators and denominators indicate numbers of rats showing metaplasia and numbers of rats being observed, respectively.

plasia was observed in the hearts of SHR and WKY with myocardial infarction, but not in those of controls. In WKY, the proportion of the infarcted area and incidence of metaplasia were lower than in SHR. Furthermore, the metaplastic areas were well correlated with the percentage infarcted area in SHR. It is therefore conceivable that the occurrence of these types of metaplasia was related to myocardial infarct size.

Myocardial-ischemic rats derived from SHR easily develop myocardial infarction as a result of lipid deposition and thrombosis in the coronary artery [21], but no metaplasia in cartilage or bone has been reported in these rats. In this study, metaplasia was observed in both WKY and SHR, although the incidence and severity of metaplasia in WKY were less than in SHR. It is therefore considered that physical effects such as ligation of the coronary artery, but not specifically SHR, might contribute to the pathogenesis of the metaplasia.

It is possible that chemical mediators [19] such as TGF- β [15] and compressive stress [2], which are prerequisites for differentiation from undifferentiated mesenchymal stem cells to cartilage cells and osseous cells, might be related to the severity of metaplasia. It is also known that TGF- β increases in the myocardial infarction zone [6], and that SHR and WKY exhibit different responses to TGF- β [13]. Furthermore, spontaneous cartilage tissue is known to occur at the base of the non-coronary semilunar valve in rats, and mechanical force at this site is thought to spread to the cartilage tissues [14]. Such mechanical forces as hypertension and excessive wall stress on the myocardial wall are thought to be great in SHR, and might play important roles in the development of the metaplastic area.

In conclusion, it is considered that the existence of extensive myocardial infarction is associated with the

occurrence of metaplasia and that special factors associated with SHR, including hypertension, may have some relation to the development of metaplasia. Further studies should be pursued to clarify whether the response to chemical mediators and pressure overload caused by hypertension contribute to the severity of metaplasia and its high incidence in SHR.

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