Case Report

Inflammation of the cardiac coronary artery in ICR mice

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Abstract: Inflammation of the cardiac coronary artery in ICR mice is occasionally observed in toxicity studies; however, this has not been well explored histologically. Herein, we investigated the detailed histology of the associated lesions in 6–8-week-old ICR mice. Coronary artery inflammation in the right ventricular wall was observed in 10 of 142 mice (7.0%). Histopathological examination revealed hypertrophy of the vascular smooth muscle cells and perivascular infiltration of macrophages in mild cases. In moderate to marked cases, single-cell necrosis of vascular smooth muscle cells, hemorrhage of the tunica media, and fibrinoid necrosis of the vessel wall were observed, in addition to the changes seen in mild cases. Electron microscopic examination of moderate cases revealed a discontinuous internal elastic lamina suggestive of rupture, and vascular smooth muscle cells beneath the elastic lamina showed degeneration and necrosis. These findings suggest that the lesions developed as a rupture of the internal elastic lamina and necrosis of vascular smooth muscle cells, while leaked plasma components caused vascular and perivascular inflammation. In ICR mice, dystrophic calcinosis (DCC) is known to occur rarely in the right ventricle. DCC is defined as focal calcification in necrotic myocardial fibers, while dystrophic calcinosis (DCC) is known to occur rarely in the right ventricle. DCC is defined as focal calcification in necrotic myocardial fibers, and the pathogenesis of which is considered to involve ectopic calcification. Since calcification was not observed in any part of the heart, including the inflammation region, the pathophysiology of cardiac arterial inflammation seen in our ICR mice was considered to differ from that of DCC. (DOI: 10.1293/tox.2022-0022; J Toxicol Pathol 2022; 35: 345–348)

Key words: inflammation, coronary artery, dystrophic cardiac calcinosis, heart, mouse

Inflammation of the cardiac coronary artery in Cr:CD1(ICR) mice is occasionally observed in toxicity studies. However, the related lesions have not been investigated and reported in detail. In this study, we investigated the histology of this type of lesion in 6–8-week-old ICR mice in detail, using light and transmission electron microscopy, and attempted to elucidate the morphological characteristics of the vascular changes.

We collected the hearts of untreated, 0.5% methylcellulose solution-treated, and saline-treated ICR mice (n=142, 122 males and 20 females; The Jackson Laboratory Japan, Inc., Yokohama, Japan) used in toxicity studies at Daiichi Sankyo Co., Ltd. (Tokyo, Japan), from 2016 to 2020. The study was approved by the Ethics Review Committee for Animal Experimentation of Daiichi Sankyo Co., Ltd. (Tokyo, Japan) and was performed in accordance with the guidelines of the Animal Care and Use Committee of Daiichy Sankyo Co., Ltd., and in compliance with legal and guidelines regulations relating animal welfare, including the Standards Relating to the Care and Management of Experimental Animals (Notification No. 6 of the Prime Minister’s Office, Japan; March 27, 1980) and the Guidelines for Animal Experimentation (Japanese Association for Laboratory Animal Science; May 22, 1987).

Animals were housed in individual or pair-breeding cages in an animal study room with a controlled temperature of 20–26°C, humidity of 30–70%, and a 12-h light (150–300 lx) and 12-h dark cycle. Certified pellet diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were provided ad libitum.

The collected hearts were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). The heart was evaluated in a vertical section, including the bilateral arteries and ventricles. Portions of the 10% neutral-buffered, formalin-fixed tissue specimens from three heart samples, with or without inflammation of the coronary arteries, were cut into 1-mm3 cubes, re-fixed in 2.5% glutaraldehyde, and post-fixed in 1% OsO4 for 2 h. These specimens were then dehydrated through a series of increasing concentrations of alcohol and embedded in epoxy resin. Semi-thin sections stained with toluidine blue were prepared to assess the distribution of the coronary arteries and the pathological grade of the lesions. Ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined using an H-7500 transmission electron microscope (Hitachi High-Technologies Cor-
Inflammation of the coronary arteries was observed in the right ventricular wall, especially at the root of the right coronary artery, in 10 of the 142 mice (8/122 males and 2/20 females; 7.0% of all animals). In addition, one of these animals displayed a lesion in the right auricle, while another displayed a lesion in the left ventricular wall (Fig. 1). In most cases (n=7) with lesions, only a single lesion was observed, and the largest number of lesions in a single animal was three. According to histopathological examination by HE staining, the coronary artery was classified as either normal (Fig. 2) or one of three grades of inflammation: mild (6 cases), moderate (2 cases), or marked (2 cases). In mild cases, hypertrophy of vascular smooth muscle cells and slight infiltration of macrophages in the perivascular connective tissue were typical features (Fig. 3). In moderate cases, single-cell necrosis of the vascular smooth muscle cells and leakage of red blood cells in the tunica media were observed, in addition to the changes seen in mild cases (Fig. 4). In marked cases, fibrinoid necrosis of the tunica media was observed with degeneration and necrosis of vascular smooth muscle cells and hypertrophy of endothelial cells (Fig. 5). Macrophage infiltration and fibrosis were observed in the perivascular connective tissue, accompanied by mild degeneration of adjacent myocytes. Electron microscopy of the right coronary artery in moderate cases revealed discontinuous internal elastic lamina suggestive of rupture, whereas the normal case showed continuous internal elastic lamina (normal, Fig. 6; mild, Fig. 7). In moderate cases, the thickness of the internal elastic lamina was uneven, thinned, or partly eliminated, and plasma leakage was observed between the internal elastic lamina and vascular smooth muscle cells. Vascular smooth muscle cells beneath the ruptured internal elastic lamina showed degeneration and necrosis, with increased lysosomes and swelling of the rough endoplasmic reticulum. No abnormal changes were observed in the endothelial cells of the tested specimens.

In the present study, inflammation of the coronary arteries in ICR mice was observed mainly in the right ventricular wall in 10/144 animals (7.0%). Our investigation revealed that the lesion develops with rupture of the elastic lamina and degeneration and necrosis of vascular smooth muscle cells, which result in the leakage of plasma components into the tunica media and surrounding connective tissues. Eventually, these changes induce infiltration of inflammatory cells and fibrinoid necrosis of the vascular wall, which were observed in marked cases. This histopathological sequence differs from that of mouse dystrophic cardiac calcinosis (DCC), a lesion characterized by calcification of the epicardium and adjacent myocardium\(^1\)\(^,\)\(^2\). In DCC, calcification often occurs in the right ventricle, which may also be

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**Fig. 1.** Distribution of coronary artery inflammation seen in ICR mice. Ten of 144 animals presented lesions, which were mostly localized in the right ventricular wall. Black dots indicate the inflammation region of animals with a single lesion (seven animals). Blue, green, and red dots indicate the inflammation region of animals with multiple lesions (three animals; same colors indicate same animals), respectively. IVS: intraventricular septum; LA: left auricle; LV: left ventricular wall; RA: right auricle; RV: right ventricular wall.

**Fig. 2.** Normal histology of right coronary artery in an ICR mouse. Hematoxylin and eosin (HE) staining. Bar=100 μm.

**Fig. 3.** Mild case of coronary artery inflammation in an ICR mouse showing hypertrophy of vascular smooth muscle cells and infiltration of macrophages in the perivascular connective tissue. Hematoxylin and eosin (HE) staining. Bar=100 μm.
seen to a smaller extent in the coronary arterial wall. In the early stages of DCC, basophilic microgranules, which are swollen mitochondria with calcific deposition, are observed within the cytoplasm of degenerating myofibers. Since such findings were not observed anywhere in the heart, including the region of inflammation, inflammation of the coronary arteries in ICR mice was considered to have a different pathophysiology than that in DCC. However, in cases of dystrophic calcification caused by fibrinoid necrosis followed by inflammation, differentiation from DCC in the coronary artery is difficult. Degeneration of the myocytes surrounding the coronary artery with inflammation, as in the present cases, may also lead to dystrophic calcification, which could be confused with DCC.

DCC is found sporadically in certain strains of aged mice. The genetic background, age, sex, and diet have been reported to influence the prevalence and severity of this type of lesion. DBA/2, C3N, Balb/c, and CBA mice are common strains exhibiting this lesion, which can arise even at the age of 6–7 weeks. However, cases in ICR mice are uncommon and the age of onset and progression in these mice have not been well documented. The coronary artery inflammation seen in the present study appears to be

Fig. 4. Moderate case of coronary artery inflammation in an ICR mouse showing single-cell necrosis of a vascular smooth muscle cell (arrow) and red blood cells suggesting medial hemorrhage (arrowhead). Hematoxylin and eosin (HE) staining. Bar=100 μm.

Fig. 5. Marked case of coronary artery inflammation in an ICR mouse showing fibrinoid necrosis of the tunica media, degeneration and necrosis of vascular smooth muscle cells, hypertrophy of endothelial cells, and perivascular infiltration of macrophages with fibrosis. Adjacent myocytes show mild degeneration (arrows). Hematoxylin and eosin (HE) staining. Bar=100 μm.

Fig. 6. Continuous internal elastic lamina (arrows) observed in a normal coronary artery of an ICR mouse. Transmission electron microscopy (TEM). Bar=10 μm.

Fig. 7. Discontinuous internal elastic lamina suggesting rupture (arrowheads) seen in the right coronary artery of an ICR mouse with a moderate lesion. The thickness of the internal elastic lamina is irregular, thinned, or partly eliminated, and plasma leakage is seen between the internal elastic lamina and vascular smooth muscle cells. Vascular smooth muscle cells below the rupture show degeneration and necrosis. Transmission electron microscopy (TEM). Bar=2 μm.
more common in ICR mice than in B6C3F1 and C57BL/6 mice (in-house data), although more information is needed about the incidence in other strains to clarify the differences across different strains. Spontaneous polyarteritis syndrome, which is known to occur in aged ICR mice, is also considered a differential diagnosis for this type of lesion. The most affected tissues in this syndrome are the arteries of the reproductive tract, salivary glands, kidneys, heart, thymus, testis, mesentery, and pancreas. The histological findings are characterized by marked fibrinoid necrosis of the arterial wall, with limited perivascular inflammation. The pathogenesis of this syndrome is thought to involve aberrant immunological processes such as circulating immunocomplexes or autoantibodies. Examination of the systemic organs will help differentiate between the present type of lesion and spontaneous polyarteritis syndrome. In the present cases, no abnormalities were observed in any arteries of tissues/organs, except for the coronary artery in mice examined systemically. This suggests that this type of lesion has a different pathogenesis than that of polyarteritis.

The coronary artery is classified as a medium-sized muscular artery that shows a continuous elastic lamina with pores of varying sizes. Electron microscopic observation in the present study revealed rupture of the internal elastic lamina in moderate cases, which is a common finding in vasculitis with various causes. These causes include autoimmune reactions, direct endothelial cell damage, or excessive hemodynamic changes; however, none of these were apparent in the present cases. Vascular smooth muscle cells are known to produce extracellular matrix components such as elastin, laminin, and type IV collagen. In the present cases, the dysfunction of these components may be accompanied by hypertrophy or degeneration-induced ruptures of the internal elastic lamina. However, further investigation is required to clarify the relationship between rupture of the internal elastic lamina and degeneration of vascular smooth muscle cells.

In conclusion, the present study revealed that inflammation of the coronary artery can arise even in young ICR mice and indicated that the pathophysiology of this lesion is likely to differ from that of other previously reported lesions.

Disclosure of Potential Conflicts of Interest: The authors declare that there are no conflicts of interest.

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