

Case Report

A Cardiac Rhabdomyoma in a Guinea Pig

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Abstract: A guinea pig (9-week-old) that had been placed in a control group for a pharmacological test was found to have a single nodule on the surface of the right ventricular wall. In a transverse section of the heart after fixation, a whitish mass was found that extended from the subendocardium to the subepicardium of the right ventricular wall. Histopathological examination revealed a spongy network consisting of vacuolated spaces in the myocardium of the right ventricle extending to the myocardium and subepicardium of the right atrium. The vacuolated space was PAS-positive. Immunohistochemical examinations revealed that the lesions contained striated fibers that were positive for anti-desmin and anti-myoglobin. Electron micrographs revealed the lesions resulting in affected striated muscle fibers and accumulations of many glycogen granules. Based on the findings, the lesions were diagnosed as a cardiac rhabdomyoma. This is the first report of application of immunohistochemical examinations to diagnosis of cardiac rhabdomyoma in the guinea pig. (*J Toxicol Pathol* 2010; 23: 107–110)

Key words: cardiac rhabdomyoma, guinea pig, histopathology, immunohistochemical examination

Cardiac rhabdomyoma is a lesion characterized by the presence of a number of vacuolated myocardial cells containing glycogen that has often been reported in the guinea pig and dog^{1–7}. In most guinea pig cases, cardiac rhabdomyomas appear as multiple small lesions that cannot be detected by gross examination. Under a microscope, the lesions interdigitate with the surrounding normal myocardial fibers^{1–6}. In the present study, we report a rare case of cardiac rhabdomyoma in a guinea pig, in which the lesion was relatively large and not interspersed with myocardial fibers, and immunohistochemical examinations of the cardiac rhabdomyoma in the guinea pig.

A 5-week-old male guinea pig (Std: Hartley) was purchased from Japan SLC, Inc. (Shizuoka, Japan). After an acclimation period of 7 days, the animal was allocated to the control group of a pharmacological test for 3 weeks. During the study, no abnormalities were observed in its general conditions, body weight or food consumption. At the time of necropsy, the animal was 9-week-old and its weight was 561 g. A single large nodule was observed on the surface of the right ventricular wall. The heart was fixed in phosphate buffered 10% formalin solution, embedded in paraffin and sectioned at 4 μ m. Tissue sections were stained with hematoxylin and eosin (H&E), the periodic acid-Schiff

(PAS) reaction, Masson's trichrome (MT) and phosphotungstic acid hematoxylin (PTAH). Serial sections were obtained from the nodule and immunohistochemically stained with anti-myoglobin (LVC), anti-desmin (Dako Japan, Kyoto, Japan), anti-S-100 protein (LVC), anti-proliferating cell nuclear antigen (PCNA, DAKO) and anti-smooth muscle actin (SMA, DAKO). Pieces of samples in phosphate buffered 10% formalin solution were fixed in 70% Karnovsky's solution, postfixed in 1% OsO₄ and processed for electron microscopy.

In the gross pathology of the whole heart, a single nodule (approximately 5 × 5 mm in diameter) was observed on the surface of the right ventricle (Fig. 1A). No similar lesions were found in the other organs. In transverse sections of the heart after fixation, a whitish mass was grossly observed in the right ventricular wall, projecting to both the endocardial and epicardial sides (Fig. 1B). On the endocardial side, the mass was indistinctly outlined from normal muscular tissue. No gross lesion was observed except in the right ventricular wall.

In the histopathological examinations, a spongy network was observed from the subepicardial tissue to the subendocardial tissue in the right ventricular wall (Fig. 2A). Microscopically, it appeared as a round focus, and its area was approximately 10.5 mm² (approximately 5 × 3 mm in diameter). The spongy network consisted of vacuolated spaces of various sizes, and small vacuolated spaces were often observed in the subepicardial tissue. The vacuolated spaces containing chromatic, enlarged and oval nuclei were surrounded by fibers as shown by blue staining with MT

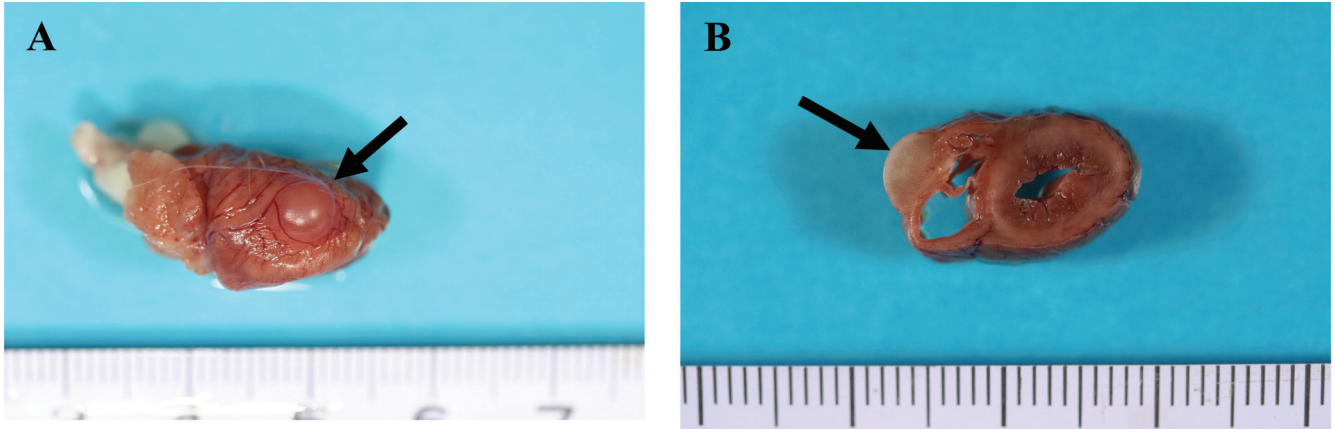


Fig. 1.

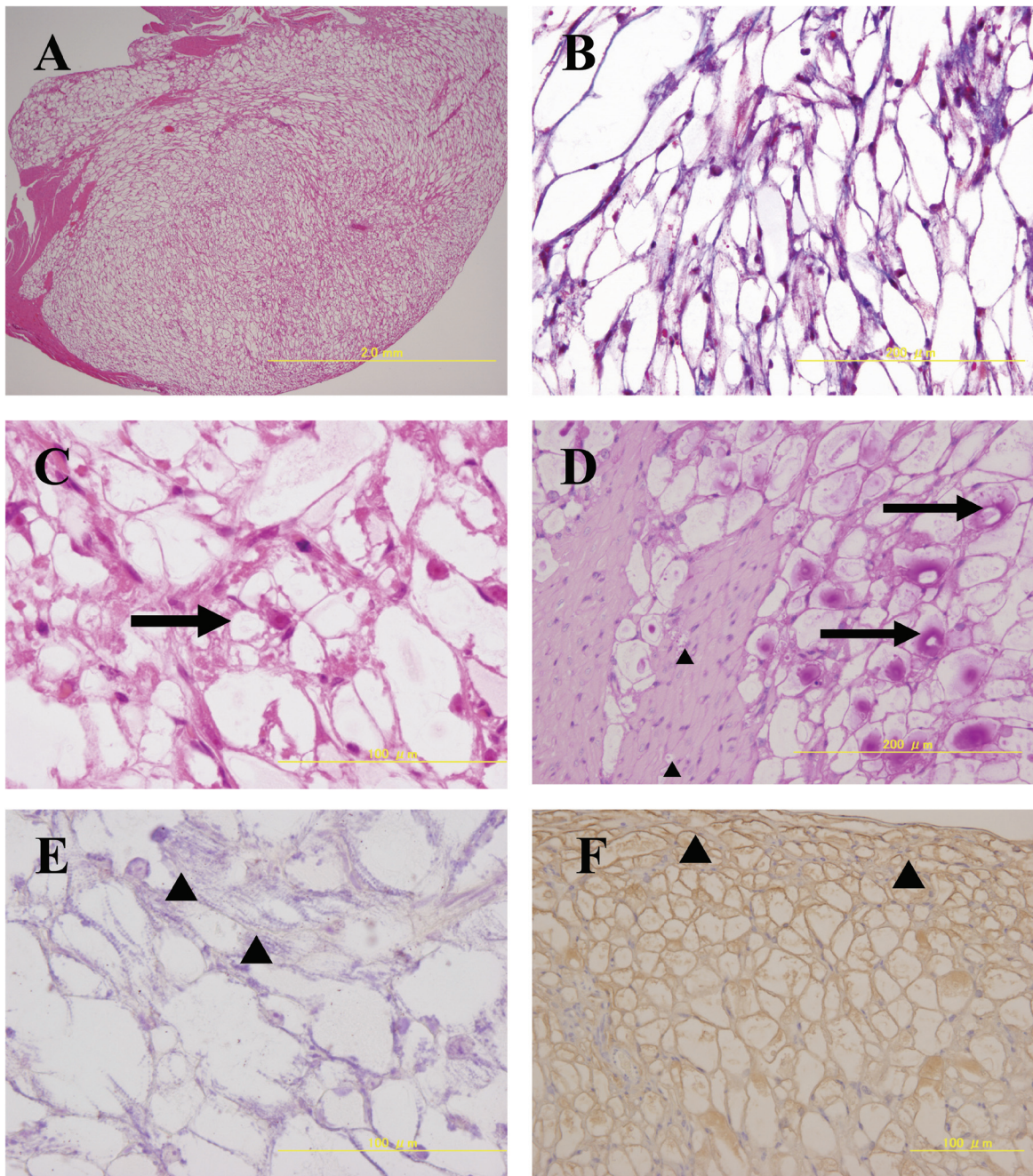


Fig. 2.

(Fig. 2B). The nucleus was often situated peripherally in the vacuolated spaces. In the vacuolated spaces with a centrally located nucleus, the space contained some smaller vacuoles that were connected by slender cytoplasmic threads to the cell wall (Fig. 2C). These cells were similar to a so-called “spider cell”, which had been previously reported in a human case⁸. Some vacuolated spaces were PAS-positive (Fig. 2D) and contained small granules and acidophilic bodies. The acidophilic bodies were stained red with MT and stained blue-purple with PTAH. Striated fibers, which were stained red with MT (Fig. 2B) and stained blue-purple with PTAH (Fig. 2E), were located peripherally in the vacuolated spaces. The striated muscle fibers were thin, dissociated or destroyed. Such lesions were mostly observed in small vacuolated spaces. The cardiac muscle fibers in the spongy network were atrophied, dissociated and slightly necrotic. No lesions were observed in the vessels in the spongy network. The striated muscle fibers surrounding the spongy network contained some small vacuoles containing PAS-positive granules (Fig. 2D). A lesion projected into the right atrium and the subepicardial tissue of the right atrial wall. No significant histopathological changes were observed except in right ventricular and atrial walls.

In the immunohistochemical examinations, the striated fibers were positive for anti-myoglobin (Fig. 2F) and anti-desmin and negative for anti-S-100 protein and anti-PCNA. The lesion had some positive areas for anti-SMA, except for the smooth muscle cells of the tunica media in the vessels. However, the acidophilic bodies were negative for all antibodies.

In the electron microscopic examinations, myofibrils with cross striations were often observed in the surroundings of vacuolated spaces in the subepicardial tissue. Dissociation, destruction and deformation occurred in the myofibrils. Furthermore, the affected myofibrils contained some areas of accumulation of glycogen granules. The glycogen granules were also observed in the vacuolated spaces (Fig. 3). The form of the glycogen granules was similar to those of β -type granules in a previous study⁵, and the size was approximately 25 nm in diameter.

The histopathological and electron microscopic examinations revealed the presence of glycogen granules in some vacuolated spaces. In addition, the immunohistochemical and electron microscopic examinations revealed that the lesion resulting in cardiac muscle fibers. Based on the results, the cardiac lesions were diagnosed as rhabdomyoma, and the histopathological and electron

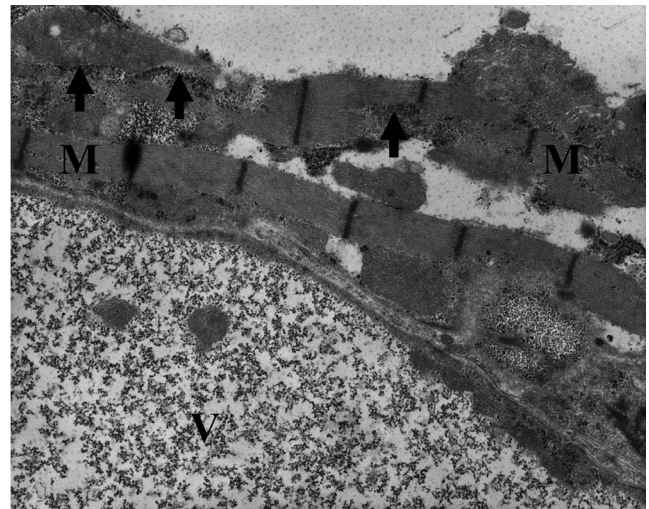


Fig. 3. Electron micrograph of the cardiac lesion. Myofibrils (M) with cross striations are observed in the surroundings of vacuolated space (V). The affected myofibrils with dissociation and destruction contained some areas of accumulation in glycogen granules (arrows). Many glycogen granules are accumulated in the vacuolated spaces. Magnification: $\times 9,000$.

microscopic features were similar to those of cardiac rhabdomyomas previously observed in the guinea pig¹⁻⁶. In most of the previous cases, the cardiac rhabdomyomas could not be seen easily with the naked eye. Moreover, Takahashi *et al.* observed small discrete lesions with total areas of 5–10 mm² or less and suggested that their locations were associated with the locations of Purkinje fibers⁵. Thus, the macroscopic and microscopic appearances of the present case were different from those of the previous cases. Therefore, the lesions in the present case may be unrelated to the location of Purkinje fibers. In the present case, some small vacuoles observed in the cardiac muscle fibers surrounding the spongy network appeared to be incipient lesions in the process of becoming vacuolated spaces. The spider-like cells might be structures in the process of becoming vacuolated spaces. In the immunohistochemical examinations, the spongy network included some anti-SMA positive areas. In injured cardiac tissue, activated myofibroblasts express anti-SMA⁹. The anti-SMA positive area in the present case was similar to those of a previous case⁹. The presence of anti-SMA positive areas might be

Fig. 1. Gross pathological features of the heart. A: Whole heart. A single nodule (arrow) is visible on the surface of the right heart. B: Transverse section of the heart after fixation. A whitish mass (arrow) is visible in the right ventricular wall.

Fig. 2. Histopathological features of the heart. A: Focal spongy network in the myocardium. HE. B: Vacuolated spaces are surrounded by fibers stained blue or red with MT stain. The striated fibers appear red with MT stain. MT. C: A spider-like cell (arrow) in the spongy network. HE. D: Some vacuolated spaces are PAS-positive (arrows). Small vacuoles containing PAS-positive granules (arrowheads) are visible in the muscle fibers surrounding the spongy network. PAS reaction. E: Striated fibers (arrowheads) around the vacuolated spaces. PTAH. F: Striated fibers positive for anti-myoglobin (arrowheads). Immunohistochemical staining with anti-myoglobin.

related to the presence of fibers, as shown by the blue staining with MT stain. As already mentioned, the present study reported both a rare case and immunohistochemical examinations of cardiac rhabdomyoma in a guinea pig.

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