Case Report

A case of spontaneous myocardial necrosis and cerebral ischemic lesions in a laboratory beagle dog

Kohei Matsushita¹, Yukari Kohara¹, Yuko Ito¹, Tsuyoshi Yoshikawa¹, Makoto Sato¹, Keisuke Kitaura¹, and Satoshi Matsumoto¹*

¹ Department of Toxicology, Drug Safety Research Center, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawachi-cho, Tokushima City, Tokushima 771-0192, Japan

Abstract: A beagle dog treated with saline as a control animal in a preclinical study was euthanized due to sudden systemic deterioration. On histopathological examination, contraction band necrosis of myocardial cells was observed widely in the left ventricular wall, including the papillary muscle and apex, and observed slightly in the ventricular septum and left atrium. In the brain, necrosis was observed in neurons and glia of the cerebral cortex, hippocampal pyramidal cells, glial cells of the rostral commissure and Purkinje cells of the cerebellar vermis. It is highly probable that the marked systemic deterioration was caused by cardiac dysfunction due to the spontaneous contraction band necrosis of the myocardial cells, although the pathogenesis of the myocardial lesions remains unclear. Given the distribution of neuronal necrosis in the brain, it is likely that these lesions resulted from the ischemia responsible for acute cardiac failure. (DOI: 10.1293/tox.2015-0024; J Toxicol Pathol 2015; 28: 233–236)

Key words: contraction band necrosis, ischemic brain lesion, spontaneous lesion, beagle dog

Unexpected sudden death of laboratory beagles is a very rare event in toxicological research. The present case was euthanized due to sudden systemic deterioration, possibly due to acute cardiac failure. On histopathological examination, contraction band necrosis of myocardial cells in the heart and ischemic changes in the neurons and glia in the brain were observed.

A 20-month-old female beagle dog (Covance Research Products Inc., Denver, PA, U.S.A) had been treated with intramuscular saline (0.2 mL/kg) once a week as a control in a 52-week toxicological study. The animal protocol was reviewed and approved by the Institutional Animal Care and Ethics Committee of Otsuka Pharmaceutical Co., Ltd. In the morning of day 356 (week 51), the dog was found in a lateral position with lacrimation, eye discharge and palpebral congestion. Because it soon fell into a deep unconscious state with convulsions, it was humanely euthanized by exsanguination from the carotid artery under deep anesthesia immediately after blood sampling for hematological and blood biochemical examinations.

Hematological and blood biochemical examinations conducted immediately before necropsy revealed marked changes in several parameters, although no abnormal changes were found before dosing or at weeks 26, 39 and 51. An increase in white blood cells (WBCs; neutrophils and monocytes), hemoglobin, hematocrit, red blood cells (RBCs), and prolonged PT and APTT was evident in the hematological examination (Supplementary Table 1: online only), whereas a decrease in blood glucose level and increase in CPK, LDH, AST, ALT, ALP, blood urea nitrogen, serum creatinine, P, Na and Cl was evident in the blood biochemical examination (Supplementary Table 2: online only).

At necropsy, multiple red foci were scattered on the endocardial surface of the left ventricle, ranging from less than 1 mm in diameter to 5 × 2 mm in size. Other findings included pale brown foci on the mucosal surface of the gallbladder, black-red bile in the gallbladder, red discoloration of the conjunctiva and oral cavity, eye discharge, small size of the mesenteric lymph nodes, red foci in the synovial membrane of the stifle joint capsule and black foci on the lungs.

Histopathological examinations were conducted on
the following organs and tissues: liver, gallbladder, kidneys, thymus, mandibular lymph nodes, medial retropharyngeal lymph nodes, mesenteric lymph nodes, popliteal lymph nodes, spleen, heart, aorta, lungs and bronchi, trachea, larynx, esophagus, submaxillary glands, sublingual glands, parotid glands, zygomatic glands, tongue, stomach, duodenum, jejunum, ileum comprising Peyer’s patch, cecum, colon, rectum, pancreas, urinary bladder, ureters, ovaries, oviducts, uterus, uterine cervix, vagina, pituitary gland, thyroid glands, parathyroid glands, adrenal glands, skin, mammary glands, skeletal muscle (brachial biceps), brain, spinal cord (thoracic), sciatic nerve, optic nerve, lacrimal glands, injection site (skeletal muscles of the thigh and rump), sternum and femur with marrow, stifle joint (articular capsule, femoral trochlea) and eyeballs. In the heart, contraction band necrosis of myocardial cells was observed widely in the left ventricular wall, including the apex and papillary muscle (Fig. 1a). Less severe contraction band necrosis was also observed in the ventricular septum and left atrial wall. Although slight hemorrhage and neutrophil infiltration accompanied these lesions (Fig. 1b and 1c), regeneration of myocardial cells was not observed. Coagulation

![Fig. 1](image)

**Table 1.** Data for Body Weights of the Present Case and Other Control Female Animals

|               | Week 1   | Week 25  | Week 49  | Week 51 (necropsy) |
|---------------|----------|----------|----------|-------------------|
| Body weight (kg) | Present case | 8.0      | 8.9      | 9.2               | 7.8               |
|               | Control animals | 8.0 ± 0.8 | 9.2 ± 1.0 | 10.0 ± 1.0 | -                |

*Control female animals excluding the present case (n=3). Values are shown as the mean ± SD.

![Histopathological features of the dog's heart](image)
necrosis of myocardial cells was not detected at any site in
the heart. The distribution of focal endocardial hemorrhages
in the left ventricle corresponded to that of the red foci ob-
erved at necropsy. No particular changes were observed in
the right ventricle and atrium. Very slight hemorrhage was
observed in the tunica media of the ascending aorta, right
and left coronary arteries and intramural coronary arteries.
These vascular alterations were not accompanied by any
other lesions, such as intimal thickening, medial degenera-
tion, thrombus formation, adventitial edema and changes of
the valves.

The brain exhibited multiple lesions containing ne-
crotic neurons and glia (Fig. 2). Necrosis of cortical neu-
rons and glia was accompanied by a vacuolated neuropil and
mild neutrophil infiltration. In the hippocampal formation,
necrosis of hippocampal pyramidal cells and dentate gyrus
granule cells was observed. Glial cells with nuclear pykno-
sis also were observed in the rostral commissure, and spo-
radic necrosis of Purkinje cells was seen in the cerebellar
vermis. Necrotic neurons were characterized by acidophilic
and shrunken cytoplasm, pyknotic nuclei and loss of Nissl
granules. No gliosis or microglial reactions were found in
any of these brain lesions.

Mild hemorrhagic pneumonia was detected in the cau-
dal lobes of both the right and left lung, which showed slight
neutrophils infiltration with no evidence of infectious dis-
ease, such as bacterial clumps. No other findings, such as
hemosiderin-laden macrophages, thrombus formation (or
thrombosis), or embolization were not detected in the lung.
Hemorrhage was also seen in the gall bladder mucosa, syn-
ovaial membrane of the stifle joint capsule, and every lymph
node examined. Atrophic changes were observed in the thy-
mus and systemic lymph nodes.

In the present case, it is highly probable that the rapid
systemic deterioration was caused by acute cardiac dysfunction resulting from widespread contraction band necrosis. Sudden death in dogs can occur as a result of acute cardiac failure indicative of myocardial necrosis or myocarditis due to infectious, nutritional, toxic or congenital diseases. However, no such symptoms were observed in the present case. Contraction band necrosis due to hypercontraction and rupture of myocardial cells is the hallmark change following ischemia–reperfusion insult. In the present case, it is unlikely that hemorrhage observed in the tunica media of several coronary arteries caused the ischemic condition in the heart because these vascular alterations were very slight, and no other signs of disturbed cardiac microcirculation were observed. Thus, it was indicated that the contraction band necrosis was not caused by ischemia–reperfusion insult. Central nervous system insults, including subarachnoid hemorrhage, cerebral infarction and head trauma, can also induce contraction band necrosis of myocardial cells due to catecholamine toxicity mediated by sympathetic hyperstimulation, leading to sudden death. However, it is more likely that the neuronal lesions observed in the present case were secondary to acute cardiac failure. Although the pathogenesis of contraction band necrosis of myocardial cells in the present case remains unclear, it was probably a peracute or acute lesion judging from the mild hemorrhage and neutrophil infiltration and the lack of myocardial cell regeneration. Certain brain regions are highly vulnerable to global ischemia, including the cerebral cortex, hippocampus, caudate nucleus, and cerebellar Purkinje cells, resulting in a specific pattern of neuronal anatomical lesions. In the present case, the distribution of necrotic neurons and glia showed substantial overlap with those regions known to be most vulnerable to ischemic insult. Given the widespread contraction band necrosis of myocardial cells, necrosis of neurons and glia was most likely the result of the ischemia responsible for acute cardiac failure. On the other hand, the data on hematological and blood biochemical examinations indicated hemoconcentration and hypoglycemia in the present case. These conditions could be possibly related to the development of lesions of the heart or brain.

Although the detailed pathogenesis is unclear, the present case dog may have been under severe stress during several days before euthanasia because markedly decreased food consumption and body weight as well as atrophic changes in the lymphoid organs were observed. Respiratory symptoms were not observed in the present case, suggesting the hemorrhagic pneumonia likely did not contribute to the sudden systemic deterioration. Additionally, it is considered that other hemorrhagic lesions observed in systemic organs may not be responsible for the sudden systemic deterioration because they were very slight.

To the best of our knowledge, there are few reports of spontaneous fatal myocardial lesions in laboratory beagle dogs. Hoshiya et al. reported a case of sudden death possibly due to acute cardiac failure resulting from spontaneous myocardial contraction band necrosis, similar to the present case. However, in contrast to the present case, the beagle also had severe alterations in branches of the coronary artery, which may have induced contraction band necrosis of myocardial cells via disturbance of cardiac microcirculation. Furthermore, there were ischemic brain lesions following cardiac failure in the present case. Thus, this report is of interest because of the clinical and pathological characteristics.

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Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Hyun C, and Filippich L. Molecular genetics of sudden cardiac death in small animals - a review. Vet J. 171: 39–50. 2006. [Medline] [CrossRef]
2. James TN, and Drake EH. Sudden death in Doberman pinschers. Ann Intern Med. 68: 821–829. 1968. [Medline] [CrossRef]
3. Janus I, Noszczyk-Nowak A, Nowak M, Cepiel A, Ciaputa R, Paslawska U, Dzigieli P, and Jabłońska K. Myocarditis in dogs: etiology, clinical and histopathological features (11 cases: 2007-2013). Ir Vet J. 67: 28–35. 2014. [Medline] [CrossRef]
4. Mete A, and McDonough SP. Epidural coronary artery fibromuscular dysplasia, myocardial infarction and sudden death in a dog. J Comp Pathol. 144: 78–81. 2011. [Medline] [CrossRef]
5. Odin M, and Dubey JP. Sudden death associated with Neospora caninum myocarditis in a dog. J Am Vet Med Assoc. 203: 831–833. 1993. [Medline]
6. Miyazaki S, Fujihara H, Onodera T, Kihara Y, Matsuda M, Wu DJ, Nakamura Y, Kumada T, Sasayama S, Kawai C, and Hamashima Y. Quantitative analysis of contraction band and coagulation necrosis after ischemia and reperfusion in the porcine heart. Circulation. 75: 1074–1082. 1987. [Medline] [CrossRef]
7. García-Dorado D, Rodriguez-Sinovas A, and Ruiz-Meana M. Gap junction-mediated spread of cell injury and death during myocardial ischemia-reperfusion. Cardiovasc Res. 61: 386–401. 2004. [Medline] [CrossRef]
8. Klener R, Genote CE, Whalen DA Jr, and Jennings RB. Effect of a transient period of ischemia on myocardial cells. II. Fine structure during the first few minutes of reflow. Am J Pathol. 74: 399–422. 1974. [Medline]
9. Samuels MA. The brain-heart connection. Circulation. 116: 77–84. 2007. [Medline] [CrossRef]
10. Lee VH, Oh JK, Mullvagh SL, and Wijdicks EF. Mechanisms in neurogenic stress cardiomyopathy after aneurysmal subarachnoid hemorrhage. Neurocrit Care. 5: 243–249. 2006. [Medline] [CrossRef]
11. Auer RN, and Sutherland GR. Hypoxia and related conditions. In: Greenfield’s Neuropathology, 7th ed. DI Graham, and PL Lantos (eds). Hodder Arnold Publication, London. 233–264. 2002.
12. Hoshiya T, Tamura K, Nagatani M, Yamaguchi Y, and Okaniwa A. Cardiac lesions leading to sudden death in a beagle. J Toxicol Pathol. 13: 127–129. 2000. [CrossRef]