Case Report

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

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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 palpebrae 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. No remarkable changes in general condition were observed until the day before euthanasia.

Although body weight and food consumption of the aforementioned beagle were similar to other control female beagles a few weeks before euthanasia, body weight measured immediately before necropsy (7.4 kg) showed a marked decrease compared with that at week 49 (9.2 kg) (Table 1). Moreover, no food intake was recorded for 2 days before euthanasia. No abnormalities were detected on an electrocardiogram before dosing or at weeks 1, 26 and 39.

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 (day 352). 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

| Table 1. Data for Body Weights of the Present Case and Other Control Female Animals |
|---------------------------------|-------|-------|-------|--------|
| Body weight (kg) | Present case | Week 1 | Week 25 | Week 49 |
| Control animals* | 8.0 ± 0.8 | 9.2 ± 1.0 | 10.0 ± 1.0 |
| Week 51 (necropsy) | 7.8 |

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

Fig. 1. Histopathological features of the dog’s heart. Transverse hypercontraction bands spanning myofibers were extensive in the left ventricle (a). Slight hemorrhage (b) and neutrophil infiltration (c) accompanied contraction band necrosis. Hematoxylin and eosin staining.
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 observed 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 degeneration, thrombus formation, adventitial edema and changes of the valves.

The brain exhibited multiple lesions containing necrotic neurons and glia (Fig. 2). Necrosis of cortical neurons 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 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 caudal lobes of both the right and left lung, which showed slight neutrophils infiltration with no evidence of infectious disease, 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, synovial membrane of the stifle joint capsule, and every lymph node examined. Atrophic changes were observed in the thymus 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 rupture. Although no such symptoms were observed in the present case, it was probably a peracute or acute lesion judging from the mild hemorrhage and neutrophil infiltration and the lack of myocardial cell rupture. 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 rupture. Although no such symptoms were observed in the present case, it was probably a peracute or acute lesion judging from the mild hemorrhage and neutrophil infiltration and the lack of myocardial cell rupture. However, it is more likely that the neuronal lesions observed in the present case were secondary to acute cardiac failure.

References