A case report of ventricular septal defect in an adult Sprague Dawley rat

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Abstract: A 20-week-old male Sprague Dawley rat noted with decreased body weight, dyspnea, and anorexia beginning 2 days before death was necropsied in the recovery period of a sub-chronic toxicity study. The heart was severely enlarged (30 × 20 × 20 mm), 3–4 times larger than normal, with an approximately 6 mm wide defect in the upper, membranous portion of the ventricular septum. Both ventricles measured 4 mm in thickness, and the right ventricle was 4 times thicker than normal. According to a microscopic examination, the myocardial fibers were severely hypertrophic in the right ventricle and mildly hypertrophic in the left ventricle and septum. Myocardial vacuolation, focal hemorrhages with hemosiderin-laden macrophages, myocardial necrosis, focal fibrosis, hyalinized myocardial fibers, and multifocal adhesive pericarditis were also present. This is the first report concerning severe ventricular septal defects in an adult Sprague Dawley rat with a detailed histopathological examination. (DOI: 10.1293/tox.2016-0073; J Toxicol Pathol 2017; 30: 327–332)

Key words: congenital heart anomaly, histopathology, spontaneous, Sprague Dawley rat, ventricular septal defect

A ventricular septal defect (VSD) indicates failure of complete development of the interventricular septum that allows shunting of blood between the ventricles1. The defect more commonly occurs in the upper part of the interventricular septum resulting from fusion failure of the membranous and muscular components1–3.

VSD is present not only in humans but also in many animals such as horses, ruminants, pigs, dogs, cats, and laboratory animals1. Rats, mice, and rabbits have been used as treatment-induced VSD models for evaluation of human congenital heart disease4–7, which can be induced experimentally by agents that delay fusion of the cardiac septae. These include various chemicals, such as trypan blue, salicylate2, dimethadione8, and trimethadione2; hypoxia; deficiencies of nutrients, such as vitamin A and folic acid; and nutritional excesses, such as vitamin A and copper excess2.

Spontaneous cardiac VSD has been reported in IS/Kyo9 and Sprague Dawley (SD) rats10, specifically in fetal rats with a small VSD. To our knowledge, there are no reports of VSD in adult SD rats with detailed histopathological findings. Our report describes pathological findings of VSD in an adult SD rat with a severe defect in the ventricular septum.

A 20-week-old male Crl:CD(SD) rat was used as a control, in which the vehicle was administered by gavage, in a toxicity study for 13 weeks followed by a 4-week recovery period. This animal was supplied by Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The animal was housed and cared for according to the principles outlined in the guidelines for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our laboratory.

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3.5 mm for the right ventricle, septum, and left ventricle, respectively (Table 1). On the reverse plane, the heart had an approximately 6 mm wide defect in the upper, membranous portion and a 6 mm thick wall in the muscle portion of the ventricular septum (Fig. 2). The left and right ventricular walls measured 4 mm each in thickness, and the right ventricular wall was 4 times thicker than normal (Table 1). The left atrium was severely dilated (12 mm in diameter in the histological section). The lungs showed a dark reddish change due to chronic congestion with a large amount of serosanguineous fluid (approximately 20 mL) in the thoracic cavity. Perithymic lymph nodes showing a dark reddish change were 2 to 3 times larger than normal. The liver was severely enlarged and showed a dark reddish change. The abdominal cavity contained serosanguineous fluid (approximately 1.5 mL). There were no lesions in the vertebral bones at autopsy.

Microscopic findings: The myocardial fibers were severely hypertrophic, 4 to 5 times larger than normal, in the right ventricle (Fig. 3) and mildly hypertrophic, 1.5 to 2 times larger than normal, in the left ventricle and septum (Fig. 4). Myocardial vacuolation was seen in the all chambers; however, both the atrium (Fig. 5) and right ventricle (Fig. 3) were more severe than the others. The dilated left atrium was mildly congested and had mild hemorrhages with small numbers of hemosiderin-laden macrophages (Fig. 5). The severity of hemorrhage and macrophage infiltration in the right atrium was less than that in the left atrium. In the septum, focal myocardial necrosis with inflammatory cells infiltration (Fig. 6) and hyalinized myocardial fibers (Fig. 7) were recognized. Although the myocardial vacuolation was mild in the left ventricle, small foci of myocardial necrosis (Fig. 8) and fibrosis were found sporadically. Multifocal adhesive epicarditis was also present with mild edema (Fig. 9). No coronary artery disease or myocardial infarction was found. In the lung, the alveolar septa were thickened due to large number of erythrocytes in the dilated capillaries or due to edema with small amounts of inflammatory cell infiltration. The alveolar spaces contained large numbers of hemosiderin-laden macrophages (heart failure cells) (Fig. 10). The liver showed severe centrilobular congestion with hepatocellular degeneration/necrosis and fibrosis indicative of passive venous congestion (Fig. 11). There was also moderate to severe congestion of the parathympic lymph node, spleen, and adrenal inner cortex. Interstitial edema was seen in the pancreas, prostate, and dermis of the whole

| Table 1. Free Wall Thickness of the Each Chamber and Septum Thickness (mm) |
|-----------------------------|-----------------------------|-----------------------------|
|                            | Embedding plane             | Reverse plane               |
|                            | RV  | Septum | LV  | RV  | Septum | LV  |
| VSD                        | 2.5 | 2      | 3.5 | 4   | 6      | 4   |
| Normal (13 w)              | 1   | 3      | 4   | 1   | 5      | 3.5 |

RV; right ventricle, LV; left ventricle. #patent portion of the interventricular septum. $non-patent portion of the interventricular septum.
body. Moderate hyperplasia of erythrocytic hematopoietic cells was seen in the bone marrow of the femur and sternum. Atrophy was noted in the thymus, mesenteric lymph node, and white pulp of the spleen.

This report described a VSD in an adult Crl:CD(SD) rat that was characterized macroscopically by a severe and wide defect of ventricular septa and microscopically by myocardial hypertrophy, vacuolation, necrosis with inflam-
VSD in an Adult SD Rat

Inflammation and hyalinization, and congestion and hemorrhage with hemosiderin-laden macrophages. It has been reported that histological examination of spontaneously occurring VSD in rats revealed no morphologic changes in the ventricular walls or any evidence of inflammation, fibrosis, or cytologic changes. In contrast, myocardial changes are seen in various diseases. Abnormal metabolic, structural, and functional changes were found accompanied by hypertrophy that occurs in hypertension, obesity, and heart valve disease, after infarction, or with cardiomyopathy. Myocardial fiber damage takes the form of a cytoplasmic alteration such as vacuolation, and the irreversible consequence of myocyte damage is necrosis. Necrosis is accompanied by a variable degree of inflammation and leads to cell loss and fibrosis in all areas. In degenerative myocardial disease, the lesion begins as coagulative necrosis of myocardial fibers, which become deeply eosinophilic and hyalinized with pyknotic nuclei. Hemosiderin-laden macrophages are seen in the myocardium following hemorrhage or following repair of myocardial cell damage. Although the changes in the present case do not seem to be specific or uniform in VSD rats, this study would yield new information on the detailed histological findings of VSD in adult rats.

VSD usually has a relatively benign clinical course in humans, either closing spontaneously or causing congestive heart failure treatable by surgery. In patients with large defects, however, symptoms of congestive heart failure become evident in the first few weeks of life, and infants show...
high pulmonary blood flow and high pulmonary artery pressures, poor growth, rapid/labored breathing, tachycardia, and diaphoresis. This case was apparently healthy and had no problems in terms of body weight gain until 3 days before death. It is uncertain why dyspnea and anorexia were noted and weight loss was recorded only 2 days before death.

The causes of congenital cardiovascular anomalies are varied. A great variety of environmental and genetic factors are probably responsible for this outcome in rats. The pathogenic mechanism of the lesions detected in this case is speculated about below. As the right ventricular pressure equals the left ventricular pressure postnatally by left-to-right shunt in VSD, the right ventricle is confronted with a large systolic and diastolic load, which is followed by pulmonary hypertension with hypertrophy of the pulmonary arterioles upon histopathology. The present case contained numerous hemosiderin-laden macrophages in the alveolar spaces, which indicated chronic passive congestion, though hypertrophy of the pulmonary arterioles could not be distinguished. The liver showed passive venous congestion, which denotes elevation of pressure in the hepatic veins and venules relative to the pressure in the portal venules caused by congestive heart failure as a result of congenital cardiac disease. These findings in this case are consistent with congenital cardiac disease (ventricular septal defect).

There is a surprising lack of information concerning the incidence of spontaneous VSD in these widely used Crl:CD(SD) rats. VSD in adult rats may be seen more frequently than has been recognized in the past. We would like to request that pathologists and lab technicians using small ruminants including Crl:CD(SD) rats pay more attention to not only necropsy but also to the process of making sections because VSD may be present even with no significant clinical histories.

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