TITLE:
Angiomatous hyperplasia in the heart of a young rat

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ABSTRACT

This case report describes angiomatous hyperplasia in the heart which is an unusual location in a young male Sprague-Dawley rat in a short-term toxicity study. Histologically, the lesion was characterized by blood-filled vascular channels of variable diameter lined by a thin wall and surrounded by a thin fibrous stroma and minimal lympho-plasmacytic and neutrophilic infiltrate in the apex of the heart. Immunohistopathology using CD31 confirmed the blood vessel origin, and using Ki67 confirmed low cell-proliferative activity in the vascular endothelial cells. To the authors’ knowledge, this is the first report of spontaneous angiomatous hyperplasia in the heart of a young rat.

KEYWORDS

Rat; heart; angiomatous; hyperplasia; immunohistochemistry; vascular.
CASE REPORT

Angiomatous hyperplasia is an infrequent background finding in mice and rats and generally observed in the long-term toxicity studies (Historical control database of Covance Laboratories, United Kingdom). It is most commonly observed in the mesenteric lymph node [CD-1 mice (0.15% in males, 0.30% in females) and Han-Wistar rat (1.07% in males, 0.99% in females)] as well as in the uterus of rats and mice. In the Han-Wistar rat, angiomatous hyperplasia has been reported in mandibular lymph node (0.08% in males) and spleen (Historical control database of Covance Laboratories, United Kingdom).

Angiomatous hyperplasia has also been seen as an induced change in the subcutis of mice with peroxisome proliferator-activated receptors (PPARs).

This vascular anomaly also described as hemangiomatous hyperplasia, is often considered part of biological continuum leading to hemangiomas and hemangiosarcomas which are fairly common in mice and rats.

Angiomatous hyperplasia is characterized by localized proliferation of thin-walled vascular spaces lined by normal flattened endothelial cells lacking nuclear atypia or mitoses. The increased capillaries and other vascular structures are often blood-filled, uniform or variable in size, and associated with varying increase in adjacent extracellular matrix. This localized change generally does not distort adjacent normal tissue and may be seen coincidentally with vascular neoplasia. We report here angiomatous hyperplasia in an unusual location, the heart, observed incidentally in a Sprague-Dawley rat from a toxicity study. Up to our knowledge, this is the first report of spontaneous angiomatous hyperplasia in the heart of a young rat.

The animal, supplied by Charles River Laboratories (Margate, UK) and housed at Covance Laboratories (Harrogate, UK), was a male rat, aged 11 to 12 weeks old at necropsy and used as a negative control which received water by oral administration once daily in a 4-week oral toxicity study. The study was performed in accordance with the 2013 consolidated version of the Animals (Scientific Procedures) Act 1986 and after approval by the local Animal Welfare and Ethical Review Body. It was housed in a group of 5 animals, in a controlled environment: 20 to 24°C temperature range, 45 to 65% humidity range with 15 -20 air changes/hour and a photoperiod of 12 hours nominal. It had ad libitum access to 5LF2 EU Rodent Diet and water bottles. The animal was observed twice daily for health monitoring, once weekly and on the day of terminal necropsy for clinical observations, once daily at 1 hour post dose and on the day of terminal necropsy for post dosing signs, once weekly for body weight, and at least once weekly for food consumption. Measurements of hematology
(hemoglobin concentration, hemoglobin distribution width, red blood cell count, red cell distribution width, packed cell volume, platelet count, reticulocytes, platelet crit, mean cell volume, mean platelet volume, mean cell hemoglobin, platelet distribution width, mean cell hemoglobin concentration, Total and differential white cell count), coagulation (prothrombin, activated partial thromboplastin time, fibrinogen), serum chemistry (aspartate aminotransferase, total protein, alanine aminotransferase, albumin, alkaline phosphatase, globulin, triglyceride, albumin/globulin ratio, sodium, total cholesterol, potassium, glucose, calcium, urea, inorganic phosphate, total bilirubin, chloride, creatinine), and urinalysis (volume, colour, turbidity, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, microscopy of sediment) were performed on week 4.

Necropsy was performed and full tissue list was collected in 10% neutral buffered formalin. All tissues, including the heart, were embedded in paraffin wax, sectioned at nominal 5 μm and stained with hematoxylin and eosin (H&E). Sections of the heart were used for immunohistochemistry and both primary antibodies were sourced from Abcam® and Thermoscientific (CD31 endothelial marker ab28364 and Ki67 cellular proliferation marker PA5-19462): polyclonal rabbit anti-CD31 primary antibody (concentration: 1µg/ml for 1 hour at 37ºC) with Discovery OmniMap anti-rabbit HRP DAB detection (Roche 760-4311); and polyclonal rabbit anti-Ki67 primary antibody (concentration: 0.5ug/ml for 32mins at 37ºC). Heat induced epitope retrieval was performed using CC1 at 95ºC for 64 minutes. Immunohistochemistry was performed using the Ventana Discovery ULTRA Automated Stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). Positive and negative controls were examined. Pictures from the entire lesion were taken at 400x in order for pathologists to evaluate Ki67 immunolabeling, count cells and calculate the index.

The animal had normal body weight at necropsy. No remarkable observations were recorded clinically. No significant clinical pathology findings were recorded. Macroscopically, no observation was recorded in the heart. Microscopically, the animal showed findings in tissues which were generally infrequent, of a minor nature and consistent with the usual pattern of findings in rat of this strain and age. However in the heart, a focal moderate proliferative lesion was present in the apex (Fig. 1). The finding was characterized by blood-filled vascular channels of variable diameter (60 to 300 μm in diameter) lined by a thin wall composed by a single layer of well-differentiated endothelium, an ill-defined and irregular layer of smooth muscle, and the adventitia. The lumen was distended by numerous erythrocytes, a few monocytes and polymorphonuclear cells in a proteinaceous serum. These channels could be either arterioles or venules according to their diameter and tunica media.4 They were surrounded by a thin fibrous stroma and minimal lympho-plasmacytic and neutrophilic infiltrate (Fig. 2). The endothelial cells were flattened with indistinct borders lining spaces filled by numerous
erythrocytes and a few lymphocytes, neutrophils and monocytes. They contained one oval to elongate nucleus with finely mottled to condensed chromatin and rarely one or two distinct nucleoli. The cytoplasm was eosinophilic, fibrillar and scarce. Anisokaryosis and anisocytosis were low. No mitoses were detected. There was no thrombus. A slight compression of the adjacent tissue by the distended vessels was noticed as well as minimal myocyte degeneration.

The result of CD31 immunostaining confirmed the blood vessel origin. The CD31 immunohistochemistry showed a membranous staining in the endothelial cells (Fig. 3). The result of Ki67 immunostaining indicated low proliferation of the lesion with an index of 4.86% positive endothelial cells. The endothelial cells showed a nuclear staining (Fig. 4). In conclusion, this lesion was compatible with angiomatous hyperplasia. The differential diagnoses include angiectasis, hemangioendothelial hyperplasia, hemangioma, hemangiosarcoma. Angiectasis usually presents dilated vascular spaces but not an increase number of vessels as seen in our case. Hemangioendothelial hyperplasia shows prominent proliferating endothelial cells which were not present in this case. The differentiation with hemangiomas or hemangiosarcomas can be more challenging. In this case, the absence of cellular atypia, pleiomorphism or mitoses and the low Ki67 index helped us exclude a tumor.

Our lesion was located along the myocardium of the apex and slightly compressed the myofibers. However, no major damage to the myocardial muscle was seen microscopically, which was consistent with the normal enzymatic creatinine result. Creatinine kinase is an important cytosolic enzyme involved in the muscle contraction process and is mainly found in skeletal muscle, cardiac muscle and brain. Therefore, an increase in serum creatinine kinase activity can be an indicator of degenerative or necrotizing muscle injury.

In the literature, similar histopathological changes are recorded under different, sometimes very broad terms but the approach of the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) project is now to preferentially use descriptive terms and call this lesion angiomatous hyperplasia. Also, it is often reported that the cause of the lesion is important. It could be developmental or induced by repair process. In our case, we were unable to determine that cause. However due to the young age of the animal, we speculate that it could be developmental.

A possible progression of the lesion over time into a tumor could not be investigated but knowing that angiomatous hyperplasia is usually part of biological continuum leading to hemangiomas and hemangiosarcomas certainly raise some concerns when finding this lesion in an unusual location like the heart. To our knowledge,
spontaneous hemangiomas and hemangiosarcomas in the mice and rat have been seen in a variety of tissues and only hemangiosarcomas have been reported in the heart at a low rate: 0.3% (1/369) in vehicle control female Harlan Sprague-Dawley rats, 0.2% (3/1354) in control male and 0.1% (1/1351) in control female B6C3F1 mice.7,8

In conclusion, this is the first report of angiomatous hyperplasia seen in the heart of a young Sprague-Dawley rat. The lesion was located at the apex and was not associated with a particular cause or with tumor formation. CD31 immunomarker was useful to confirm the blood vessel origin of the lesion.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
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Fig. 1. H&E. Local proliferation of blood-filled vascular channels with variable diameter (60 to 300 μm in diameter) noted in the apex of the heart.
Fig. 2. H&E. The vessels had a thin wall composed by a single layer of well-differentiated endothelium, an ill-defined and irregular layer of smooth muscle, and the adventitia. The lumen was distended by numerous erythrocytes, a few monocytes and polymophonuclear cells in a proteinaceous serum. The vessels were surrounded by a thin fibrous stroma and minimal lympho-plasmacytic and neutrophilic infiltrate.
Fig. 3. CD31. The CD31 immunohistochemistry showed a membranous staining in the endothelial cells.
Fig. 4. Ki67. The Ki67 immunohistochemistry showed a nuclear staining in rare endothelial cells.