Case Reports

FIVE CASES OF SPONTANEOUS HEMANGIOENDOTHELIAL CELL TUMOR OF THE HEART IN B6C3F₁ MICE

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Abstract: Spontaneous hemangioendothelial cell tumor of the heart was found in five of 4,160 male and 3,910 female B6C3F₁ (C57BL/6CrSlc×C3H/HeSlc) mice which were used in chronic toxicology and carcinogenicity studies at the An-Pyo Center. Two cases were diagnosed histologically as benign hemangioma and the other three cases were diagnosed as hemangiosarcoma. Hemangioendothelioma of the bone marrow, liver, and spleen were also observed in two out of three cases with hemangiosarcoma of the heart, and these cases were recognized as systemic, multiple vascular cell tumor of B6C3F₁ mice. The tumor cells were identified as hemangioendothelial cells by immunohistochemical techniques and lectin binding patterns. The finding of five cases of spontaneous hemangioendothelial cell tumor of the heart in this report is considered to be extremely rare. (J Toxicol Pathol 7: 249~254, 1994)

Key words: Hemangioma, Hemangiosarcoma, Heart, Spontaneous, B6C3F₁ mice

Introduction

Hemangioendothelial cell tumor occurs spontaneously either as a single lesion or multiple lesions in B6C3F₁ mice. These tumors are known to develop in a variety of organs including the spleen, liver, bone marrow and subcutis tissue^{5,22,23}. Spontaneous cardiac vascular endothelial cell tumors in rodents, an extremely rare finding, were reported in a rat by Schardein (1968) *et al.*, and in a mouse by Ligeti (1984) *et al.*^{1,6,7}. Also, a few cases of vascular tumor of the heart have been reported to be induced by chemicals including 1, 3-butadiene^{13,19}, 3-methylcholanthrene⁶, captafol⁹ and urethane⁸ or by irradiation^{2,6,7}. Heman-

giosarcoma of the heart in human and dogs has been suggested to occur occasionally in the right atrium and show distal metastasis^{10-12,15,20}. Hemangioendothelial cell tumor of the heart in other animals was either not known or reported as extremely rare case.

Materials and Methods

Spontaneous hemangioendothelial cell tumor of the heart was found in five (3 males and 2 females) of 4,160 male and 3,910 female B6C3F₁ (C57BL/6CrSlc×C3H/HeSlc) mice which were used in chronic toxicology and carcinogenicity studies at the An-Pyo Center. Animals were housed individually in suspended stainless steel wire-mesh cages in light-, humidity- and temperature-controlled rooms. Free access to food and tap water was provided. Hematological determinations were conducted in all five cases. These animals were sacrificed or killed in a moribund

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condition between Weeks 90 and 104 of the studies. Two cases of hemangioendothelial cell tumor were observed in control animals and another three were observed in the experimental groups administered with test substances. No lesions attributable to the administration of the test substances were found in the heart of the animals in the experimental groups.

Following complete gross postmortem examination, selected organs were harvested, measured, weighed, and fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, cut and stained with hematoxylin and eosin.

Sections of the heart from all cases of hemangioendothelial cell tumor were stained immunohistochemically for vimentin, von Willebrand factor (factor VIII related antigen), and human endothelial cell (CD31) antigens. The standard labelled streptavidin biotin technique was used to identify endothelial cells using a DAKO LSAB kit³. Commercially available mouse anti-swine vimentin monoclonal antibody, rabbit anti-human von Willebrand factor (factor VIII-related antigen) polyclonal antibodies, and mouse anti-human endothelial cell (CD31) monoclonal antibody¹⁶, diluted 1 : 10, 1 : 200, and 1 : 40, respectively, were used (DAKO, JAPAN). The primary

antibodies were omitted in negative controls.

The following lectins were stained immunohistochemically using the standard labelled streptavidin biotin technique: *Ulex europaeus agglutinin I* (UEA-1), *Wheat germ agglutinin* (WGA) and *Concanavalin A* (Con-A) (DAKO, JAPAN)^{4,18}. Elimination of lectin-binding capacity was performed by incubating each lectin with the corresponding inhibiting sugars to determine the specificity of lectin binding.

Results

The spontaneously occurring cardiac endothelial cell tumors in B6C3F₁ mice were diagnosed as benign hemangioma in two cases (1 male and 1 female) and hemangiosarcoma in three cases (2 males and 1 female) (Table 1).

In hematological examinations, one case of hemangiosarcoma revealed decreases in hematocrit, hemoglobin level, red blood cell, and platelet counts. Atypical red blood cells were also observed in this case.

Macroscopically, reddish nodule of the heart, 3×5 mm in size, was observed in one case of hemangiosarcoma. No macroscopic lesion of the heart was found in the other cases.

Table 1. Clinical History and Pathological Diagnosis of the Cases of Cardiac Hemangioendothelial Tumor in B6C3F₁ Mice

[Case]	A :	B :	C :	D :	E :
Age (weeks)	109	109	109	109	95
Sex	male	female	male	male	female
Classify	S	S	S	S	M
	(S : Schedule sacrificed M : Moribund sacrificed)				
Administration	Dose	Dose	Control	Dose	Control
[Histological site]					
case A :	Interventricular septum (left ventricular site)				
case B :	Left ventricular wall				
case C :	Interventricular septum (~posterior wall)				
case D :	Apex				
case E :	Interventricular septum (~right ventricular wall)				
[Histological diagnosis]					
case A :	Hemangioma				
case B :	Hemangioma				
case C :	Hemangiosarcoma				
case D :	Hemangiosarcoma				
case E :	Hemangiosarcoma				

Histologically, the tumors were found in the interventricular septum in three cases (1 benign hemangioma and 2 hemangiosarcoma). Other tumors were found in the left ventricular wall in one case (benign hemangioma), and at the apex in one case (hemangiosarcoma) (Table 1).

In the two cases diagnosed as benign hemangioma, cavernous vascular proliferation lined by a single layer of endothelial cells with widened vascular spaces was found between the muscle fibers of the heart (Fig. 1). These endothelial cells were flat and sometime had large nuclei without atypia. Fibrous connective tissue in the stroma

was also observed.

In three cases diagnosed as hemangiosarcoma, proliferation of atypical endothelial cells which could be single- or multiple-layered in the heart was observed (Fig. 2). Vascularity which branched irregularly was formed to various degree in the tumor tissues. Widened vascular cavity was occasionally seen and associated with thrombus in severe cases. Large solid mass of atypical endothelial cells was formed within the multiple layer of these tumors. The tumor cells had abundant, spindle-shaped and eosinophilic cytoplasm, and round or oval enlarged nuclei; mitosis was fre-

Fig. 1. Benign hemangioma of the heart. Cavernous vascular proliferation lined by a single layer of endothelial cells with widened vascular spaces is seen between the muscle fibers. HE $\times 100$.

Fig. 2. Hemangiosarcoma of the heart. Proliferation of atypical endothelial cells in single or multiple layer can be seen. HE $\times 120$.

Table 2. Immunohistochemical Results of the Cases of Cardiac Hemangioendothelial Tumor in B6C3F₁ Mice

[Immunohistochemical results]					
Case	A	B	C	D	E
—Antigen—					
Vimentin	—	focal +	—	focal +	—
Factor VIII	+	+	focal +	focal +	focal +
CD31	focal +	+	focal +	focal +	+
—Lectin—					
UEA-1	—	focal +	focal +	focal +	focal +
Con-A	+	+	+	+	+
WGA	+	+	+	+	+

quently seen. Atypical endothelial cells proliferated invasively into the surrounding tissue between the muscle fibers and the border of the tumor was not clear. Among the cases of hemangiosarcoma, hemangioendotheliomas of the liver and bone marrow in one case, and of the spleen and bone marrow in another case were also observed. So, these cases were considered to be systemic multiple hemangioendothelial tumors.

Immunohistochemically, the endothelial cells of all cases of benign hemangioma and hemangiosarcoma showed positive staining, including focal positivity, with antibodies against human von Willebrand factor (Factor VIII related antigen) and human endothelial cell (CD31) antigen, and positive binding with lectins WGA and Con-A. The tumors of all cases showed focal positive staining with the antibody against vimentin. All cases of hemangiosarcoma and one case of benign hemangioma showed positive binding with UEA-1 lectin (Table 2).

Discussion

Almost all the hemangioendothelial cell tumors of the heart in mice reported in the literature were hemangiosarcomas induced by multi-organ carcinogens such as 1,3-butadiene^{13,19}, 3-methylcholanthrene⁶, captafol⁹, urethane⁸, or irradiation^{2,6,7}. Spontaneous cardiac vascular endothelial cell tumors were reported only rarely in rodents^{6,7}. Therefore, the finding of five cases of spontaneous hemangioendothelial cell tumor of the heart in this report is thought to be extremely

rare.

Spontaneous hemangioendothelial cell tumors of the heart were observed in the septum ventricular wall in three out of five cases in this report. In human and dogs, hemangiosarcoma of the heart was suggested to occur in the right atrium^{10,11,12,15,20}. There was a clear difference in the site of occurrence between mice and these animals.

Hemangioendotheliomas of the bone marrow, liver, and the spleen were also observed simultaneously in two cases of hemangiosarcoma of the heart in this study. These two cases were considered to be systemic multiple hemangioendothelial tumors. Hemangioendothelial cell tumor was reported to show multicentric proliferation occasionally in human being and other animals^{17,21,23}. Also, multiple hemangioendothelial tumors are known to develop in a variety of organs including the spleen, liver, bone marrow, and subcutis tissue in B6C3F₁ mice^{5,22,23}. However, this report may be the first one which describes spontaneous multiple hemangioendothelial tumors including the heart in mice. Decreases in hematocrit, hemoglobin level, red blood cell, and platelet counts, and appearance of atypical red blood cells were reported in cases of multiple hemangioendothelial tumors in dogs and mice^{17,23}. Hemolysis originated in the tumor was thought to be a cause of these hematological changes in their reports. Similar hematological changes were found in one case of this study.

The origin of the tumor cells in this study was demonstrated to be hemangioendothelial cells by

immunohistochemical and lectin binding techniques. Hemangioendothelial cells in humans were reported to show positive staining with antibodies against vimentin, von Willebrand factor (factor VIII-related antigen), and CD31, and to bind strongly with lectin of UEA-1^{14,16}. The tumor cells in this study showed positive staining with antibodies against vimentin, human von Willebrand factor, and CD31. F. Gumkowski *et al.* reported on the heterogeneity of the normal mouse vascular endothelium by staining with lectin⁴. They suggested that all endothelial cells stain positively with Concanavalin A (Con-A), *Ricinus communis* agglutinin I (RCA-1), and *Wheat germ* agglutinin (WGA). The tumor cells of the heart in this study bound strongly with the lectins WGA and Con-A, as reported in the literature.

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