Case Report

Histiocytic Sarcoma in a Cynomolgus Macaque (*Macaca fascicularis*) Fed with a High-Fat Diet

Tomomi Soshin¹, Kenji Adachi¹, Shigeo Suzuki², Koichi Kanisawa², Shuji Hayashi¹, Atsuhiko Kato¹, and Masami Suzuki¹

¹Fuji Gotemba Research Laboratories, Chugai Pharmaceutical Co., Ltd., 1–135 Komakado, Gotemba-shi, Shizuoka 412–8513, Japan

²Chugai Research Institute for Medical Science, Inc., Shizuoka 412–8513, Japan

Abstract: Histiocytic sarcoma is characterized by a malignant invasive proliferation of atypical cells showing morphologic and immunophenotypic features similar to those of mature tissue histiocytes. We report a case of histiocytic sarcoma in 11-year-old male cynomolgus macaque fed with a high-fat diet for 6 years. Invasive proliferation of neoplastic cells was localized in systemic organs such as liver, spleen, aorta, kidney, lung, peritonea, and mesenterium. Diffuse cellular foci of invasive proliferation were mainly observed in the peri/intra vascular area, sinus and serous membrane frequently accompanying arteriosclerosis. The tumor cells showed a round to spindle or polygonal shape with abundant foamy to vacuolated cytoplasm. Hemophagocytosis was noted infrequently. In immunohistochemical examinations, some medium to large-sized histiocytic atypical cells were positive for histiocytic markers such as CD68 and Lysozyme. Our case raises the possibility that histiocytic proliferation might be related to hypercholesterolemia and atherosclerosis. This is the first case report of histiocytic sarcoma in non-human primates. (J Toxicol Pathol 2008; 21: 69–72)

Key words: histiocytic sarcoma, high-fat diet, atherosclerosis, cynomolgus macaque

Histiocytic sarcoma in human is characterized by malignant invasive proliferation of atypical cells showing morphologic and immunophenotypic features similar to those of mature tissue histiocytes^{1,2}. Morphologically, tumor cells proliferate invasively in a diffuse non-cohesive pattern. The individual cells are usually large and round to oval in shape with foamy cytoplasm. Hemophagocytosis is occasionally seen. The cells are usually positive for histiocytic markers such as CD68, lysozyme, CD11c, and CD14. Histiocytic sarcoma is a rare disease in human³, and there has been no report on malignant proliferative histiocytic disorder in non-human primates. In this report, we describe a case of histiocytic sarcoma in an 11-year-old male cynomolgus macaque (*Macaca fascicularis*) fed with a high-fat diet.

The animal was imported from China, and had been fed with a high-fat diet (CMK-2 with added 2% cholesterol and 6% corn oil, CLEA Japan, Inc., Tokyo, Japan) for 6 years. After initiation of the high-fat diet, body weight increased to

6.6 kg from 3.9 kg and total serum cholesterol varied within the range of 250–630 mg/dL (reference range; 191–365 mg/dL). The animal showed decrease of food consumption one month before necropsy, and developed profound anorexia losing body weight from 5.3 kg to 4.8 kg in the last 2 weeks. A poor prognosis was judged, and the animal was euthanized by exsanguination under anesthesia by isoflurane inhalation after intramuscular injection of ketamine hydrochloride and atropine sulfate. In serum chemistry at necropsy, increase of total cholesterol (535 mg/dL) and low-density lipoprotein (925 IU/L; reference range; 85–129 IU/L) was noted. The animal used in this study was treated in accordance with the ethical guidelines for animal care, handling, and termination followed at Chugai Pharmaceutical Co., Ltd. These guidelines meet the generally accepted international criteria for human treatment, sparing animals needless pain and suffering, and ensure that experiments conducted are of actual scientific benefit to mankind.

Macroscopically, yellow-whitish nodules were sporadically observed in the endoceliac and intrathoracic organs as follows: liver (18 mm in maximal diameter, multiple), spleen (splenic hilum, 4 mm in maximal diameter), kidney (mainly renal hilus, 5 mm in maximal diameter), peritonea, aorta (thoracic and abdominal), and lung. In the abdominal cavity, peritoneal adhesion with adrenal glands, intestinal adhesion and thickening of renal

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Mailing address: Tomomi Soshin, Safety Assessment Department, Fuji Gotemba Research Laboratories, Chugai Pharmaceutical Co., Ltd., 1–135 Komakado, Gotemba-shi, Shizuoka 412–8513, Japan
TEL: 81-550-87-6374 FAX: 81-550-87-6383
E-mail: soshintmm@chugai-pharm.co.jp

and testicular capsule were observed. In the thoracic wall, adhesion of lung to the thoracic wall was found. Increase of pleural and peritoneal effusion was also observed. No abnormal changes were found in systemic lymph nodes. Striated or circular white thickened areas were observed in intima of thoracic and abdominal aorta, especially in vascular bifurcation. Nodular-thickening areas were also observed in the skin of four limbs.

For histopathological examination, systemic organs and tissues were fixed in 20% neutral-buffered formalin and embedded in paraffin. The sections were prepared and stained with hematoxylin and eosin (HE). For immunohistochemical analysis, antibodies against CD68 (KP-1; monoclonal mouse anti-human CD68 specific to macrophages and histiocytes; DakoCytomation, Carpinteria, CA, USA), lysozyme (polyclonal rabbit anti-

human lysozyme; DakoCytomation), CD3 (polyclonal rabbit anti-human CD3 specific to T lymphocytes; DakoCytomation), CD20cy (monoclonal mouse anti-human CD20cy specific to B lymphocytes; DakoCytomation), CK8/18 (monoclonal mouse anti-human CK8/18 reacting with hepatocytes; DakoCytomation) and CK19 (monoclonal mouse anti-human CK19 reacting with for epithelia of bile ducts; DakoCytomation) were used as the primary antibodies. Immunohistochemical staining was performed according to the labeled streptavidin-biotin (LSAB) method with the Dako LSAB2 kit (DakoCytomation). The immunoreaction was visualized by the peroxidase-diaminobenzidin reaction. The sections were finally counterstained with hematoxylin.

In the histopathological examination, multiple invasive proliferations of neoplastic cells were observed in the systemic organs that showed macroscopic nodule, thickened capsule, or adhesion. In the abdominal cavity, neoplastic infiltrations were prominent in liver, spleen, abdominal aorta, mesenteric artery, mesenterium, mesenteric lymph node, renal artery, testicular artery, and they were also observed in the adrenal gland, kidney, urinary bladder, testis, epididymis, rectum, abdominal wall, and also in the pancreas, stomach, duodenum and sciatic nerve, but only slightly. In the thoracic cavity, infiltrations were observed in the thoracic aorta, lung, and also in the esophagus, but only slightly. The infiltrates were diffusing non-adhesive and invasive without strong external compression, and normal architecture was relatively preserved. In the neoplastic foci, necrosis was not found.

In each organ, the neoplastic cells showed periarterial, perivascular, intravascular, sinusoidal or subserous proliferating patterns. In the liver, neoplastic cells were observed in the central vein, capsule, Glisson's sheath and in large numbers in the sinusoidal capillary (Fig. 1). In the macroscopic yellow-whitish nodules, tumor cells infiltrated mainly the sinusoidal capillary, and vascular invasion was also observed. In the infiltrated areas, lobular architecture was relatively preserved and atrophied hepatic cords were observed, and thickened capsule with occasional hypertrophy and proliferation of mesothelial cells were noted. In the spleen, invasive proliferations of neoplastic cells were observed predominantly in vessel and interstitium

around the splenic artery in the capsule of the splenic hilum (Fig. 2).

The individual neoplastic cells were round to spindle or polygonal in shape with abundant foamy to vacuolated cytoplasm (Fig. 3). These cells had variable proportions with eccentric, horseshoe- or kidney-shaped nuclei with an eosinophilic region near the nucleus. Mono- or multi-nucleated giant cells were frequently found. The chromatin of nuclei showed clear or vesicular appearance, and mitotic figures were frequently observed. Erythrophagocytosis and foamy phagocytic cells were occasionally found (Fig. 4). In the immunohistochemical examination, some of the medium to large sized histiocytic atypical cells showed immunoreactivity for CD68 and lysozyme (Fig. 5). On the other hand, all the histiocytic atypical cells were negative for CD3, CD20cy, CK8/18 and CK19.

From the above mentioned morphological and immunohistochemical features of multiple, invasive proliferations of histiocytic atypical cells, a diagnosis of histiocytic sarcoma was made.

In the WHO classification of human sarcomas, a histiocytic neoplasm is listed as a histiocytic sarcoma. Histiocytic sarcoma is thought to be the rarest of haematopoietic and lymphoid neoplasms. The commonest sites of involvement are the lymph node, skin, and the gastrointestinal tract, and sometimes multiple sites are involved. In diagnosis, CD68, a membrane antigen of lysozyme, is one of the key markers for histiocytic sarcoma. However, CD68 and lysozyme are not a definitive diagnostic index because reactivity to lysozyme decreases with phagocytosis due to loss of lysosomal granules. It is considered that reactivity to lysozyme differed among tumor cells in the present case. In our case of histiocytic sarcoma, the proliferation pattern, morphological features and immunophenotype of neoplastic cells were similar to those described in the WHO classification of human sarcomas, although neoplastic lesions were mainly localized in the liver, spleen, kidney, peritonea, aorta and lung.

In addition to the neoplastic lesions, changes related to the high-fat diet were also observed. Large- to medium-sized systemic arteries showed atherosclerosis at heterogeneous levels frequently accompanying the neoplastic infiltration. In those arteries, thickening of intima

- Fig. 1.** Liver of the cynomolgus macaque. Invasive proliferations of histiocytic atypical cells are noted in the sinusoidal capillary and central vein (asterisk). In the neoplastic proliferation area, lobular architecture is relatively well preserved, and atrophied hepatic cords are observed. HE. $\times 40$.
- Fig. 2.** Spleen of the cynomolgus macaque. Histiocytic atypical cells are observed predominantly in vessel and interstitium around the splenic artery (SA) in the capsule of the splenic hilum (asterisks). Proliferation foci of histiocytic atypical cells are also noted in red pulp (arrow heads). HE. $\times 20$.
- Fig. 3.** Liver of the cynomolgus macaque. The individual neoplastic cells are round to spindle or polygonal in shape with abundant foamy to vacuolated cytoplasm. These cells have variable proportion with eccentric, horseshoe- or kidney-shaped nuclei with clear or vesicular chromatin. Mono- or multi-nucleated giant cells are also seen (arrow heads). HE. $\times 400$.
- Fig. 4.** Spleen of the cynomolgus macaque. Erythrophagocytosis (arrow heads) and foamy phagocytic cells are occasionally found in vessel around the splenic hilum. HE. $\times 400$.
- Fig. 5.** Spleen of the cynomolgus macaque. Some of the medium to large-sized histiocytic atypical cells are positive for CD68. Labeled streptavidin-biotin method, counterstained by hematoxylin. $\times 400$.

with foamy macrophage accumulation was observed. Infiltration and accumulation of lipid containing large macrophages were found in bone marrow and subcutis of skin. In the liver, proliferation of foamy Kupffer cell and fatty change of hepatocytes were observed. There have been reports of hypercholesterolemic xanthoma in human, and xanthoma occurred in cynomolgus monkeys fed a high-fat diet^{4–6}. These results imply an association between hypercholesterolemia and histiocytic proliferation. In the present case xanthoma was observed in the bone marrow and subcutis. However, there has been no report associating hypercholesterolemia with malignant histiocytic proliferation in either humans or non-human primates. Our case raises the possibility that histiocytic proliferation might be related to hypercholesterolemia and atherosclerosis.

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