Kupffer Cell Sarcoma in Rats After Exposure to Small Doses of Dimethylnitrosamine and N-2-Acetylaminofluorene During Hepatic Regeneration

P. Chopra, M.D., A. Manga, M.Sc., and N. C. Nayak, M.D.

ABSTRACT—During experiments to study the evolution of hepatocellular carcinoma in adult male Wistar rats by exposing regenerating livers to the action of small doses of dimethylnitrosamine (DMN) or N-2-acetylaminofluorene (AAF), several primary sarcomas of the liver were incidentally observed. The morphology and behavior of the tumors suggest their origin from Kupffer’s cells. Kupffer cell sarcomas occurred more frequently when 70% hepatectomy was used as the regenerative stimulus. None of the 36 animals treated with AAF alone and 2 of the 38 rats given DMN only had this tumor. —J Natl Cancer Inst 62: 1089-1095, 1979.

Tumors arising from the littoral cells of the hepatic sinusoids are rare. Most are considered to have endothelial cell origin, and they have been called angiosarcoma, hemangiosarcoma, hemangiendothelioma, and primary hepatic sarcoma. They have also been interchangeably called reticulosarcoma and KCS, though currently available evidence indicates that phagocytic Kupffer’s cell and the endothelial cell are distinctly different in their morphologic and enzymatic makeup (1-7).

Angiosarcomas of the liver, identical to those seen in man (8-10), have been reported in animals after prolonged administration of various chemical carcinogens, important among which are DMN and DENA (11-18).

Although development of vascular tumors in the liver is well documented for several species, there are only a few reports on experimentally induced KCS. Gillman and Hallowes (19, 20) observed “reticulendothelial” tumors in the livers of rats after repeated injections of trypan blue. Recently, Evans and Grasso (unpublished observations) claimed to have induced KCS in the liver by administration of DMN and DENA.

During the study on HCC induced in rats with one or two small doses of DMN or with three small doses of AAF administered during hepatic regeneration, we encountered several sarcomas that we believe to have arisen from Kupffer’s cells. We describe here our findings to support this histogenesis of the tumors.

Two different carcinogens during or immediately preceding hepatic regeneration.

Two carcinogens, DMN and AAF, and two regenerative stimuli, partial hepatectomy (21) and carbon tetrachloride (CCl4) intoxication, were used in various combinations (table 1).

Groups of animals were killed at 1½, 3, 4, 10, 20, 25, 30, 40, 50, 60, and 70 weeks after administration of a carcinogen. The animals were anesthetized lightly with ether and exsanguinated by cutting the heart. The entire liver was removed and was carefully examined first for the presence of any macroscopic tumor or nodule.

Small pieces of liver from grossly visible lesions and elsewhere were cut into 1-mm squares and processed for electron microscopic study. After fixation for 2 hours in 1% osmium tetroxide in Veronal buffer, the pieces were dehydrated through ascending grades of alcohol and embedded in Epon. Sections were cut in a Reichter ultratome, stained with uranyl acetate and lead hydroxide, and examined under a Philips 300 electron microscope operated at 60-80 kV. Thick sections (1 μm) from each block were stained with 0.2% toluidine blue and examined under the light microscope.

Slices of fresh liver and other tissues were fixed in 10% buffered Formalin and processed by the usual techniques; cut paraffin sections, 5-μ thick, were stained routinely with H & E, PAS and pyronin methyl green stainings were also done. Frozen sections of Formalin-fixed material were stained with Oil Red O for neutral fat.

RESULTS

The incidences of sarcomas in the Wistar rats of different groups are shown in table 2. Because tumors, either sarcoma or HCC, did not appear before 30 weeks, the various incidence figures were calculated from among rats examined only from that interval.

MATERIALS AND METHODS

Sixteen adult male Wistar rats that developed KCS in the liver form the basis of this report. These were among a total of 680 animals examined for the evolution of HCC, which was the original objective of the study. The original design of the experiment is summarized in table 1. Observations were made at intervals between 1½ and 70 weeks, after exposure to
Changes in the Liver

Gross features.—Tumor-bearing livers showed irregular or rounded gray-white patches from 1 to 5 mm. These patches were distributed throughout the liver with intervening areas of normal-looking parenchyma but generally did not rise above the surface of liver. At times gray-white elevated nodules, 1-2 cm in diameter, were also encountered. On cut surface, all these foci had a homogeneous gray-white appearance with occasional areas of hemorrhage and necrosis.

Light microscopic features.—The tumor showed a consistent pattern in almost all the animals. Sheets of tumor cells were diffusely and extensively infiltrating the liver sinusoids throughout the lobule and interlobular areas. The tumor cells were diffusely and extensively infiltrating the liver sinusoids throughout the lobule and interlobular areas. The tumor cells were large, irregularly round, oval, or rarely elongated. The cell membrane was invariably ruffled and showed several small finger-like processes (figs. 7-9). The nucleus was oval or round, having a relatively smooth surface, large hyperchromatic nuclei to multinucleated giant types (fig. 4). In such instances mitoses were frequent. Despite the bizarre appearance of tumor cells, their sinusoidal location was well evident in many places. In none of the instances was an attempt at formation of vascular spaces or slits by tumor cells observed, though focal areas of sinusoidal dilatation and congestion were present.

Electron microscopic features.—Tumor cells of varying shapes and sizes lined or filled the sinusoids and could be clearly distinguished from the neighboring hepatocytes that presented characteristic appearances (figs. 6, 7). The tumor cells were large, irregularly round, oval, or rarely elongated. The cell membrane was invariably ruffled and showed several small finger-like processes (figs. 7-9). The nucleus was oval or irregularly shaped, sometimes with indentations of the nuclear membrane (figs. 6, 7, 9). The nuclear chromatin was clumped at the membrane and elsewhere, and nucleoli were often prominent (figs. 6-8). The cytoplasm contained few mitochondria (figs. 7, 8), many profiles of RER (figs. 6-9), large number of free ribosomes (figs. 7, 8), and several dense bodies (fig. 9). Vacuoles resulting from autophagia (fig. 8) and crystalline structures (fig. 9) were also frequently encountered. In contrast, the endothelial cells appeared flattened and elongated, having a relatively smooth surface, large nucleus, and a thin rim of cytoplasm that contained several vesicles. Mitochondria, dense bodies, RFC profiles, and free ribosomes were fewer in number (fig. 10). (The only organ studied by electron microscopy was the liver.)
Changes in the Other Organs

Gross features.—In 1 rat of group III (AAF-hepatectomy), the spleen on its external surface showed a gray-white, firm nodule measuring 2 mm in diameter. A segment of the small intestine was enveloped by a gray-white tumor mass. Except for some congestion, the lungs were not remarkable.

Light microscopic features.—In 7 of the 13 rats for which the lungs were examined, multiple tumor emboli were present in the blood vessels (fig. 11). The tumor cells morphologically resembled those in the liver. The lymphoid tissue in the lung revealed hyperplasia, particularly marked in the AAF-treated animals. Reticuloendothelial cells in the spleen showed moderate-to-severe hyperplasia with development of an identical tumor as in the liver in one case. Sections from the tumor in the intestine also showed similar features to those seen in the liver and spleen.

DISCUSSION

The morphology, behavior, and evolution of tumors described here are quite different from those of HCC that developed in many animals in our experiments (Manga A, et al: Manuscript in preparation). In fact, in some animals HCC and KCS coexisted. The occurrence of HCC was preceded by a series of hepatocytic alterations often evolving in a definite sequence. Also, in KCS cells as well as in HCC cells, several structural and biochemical characteristics typical of the hepatocyte were clearly evident. However, the tumor cells in sarcomas appeared entirely different from hepatocytes and showed both topographic and morphologic features typical of Kupffer's cells.

Several recent studies have helped to bring out clear distinctions between Kupffer’s cell and the endothelial cell, the most important feature being endogenous peroxidase activity that is absent in the endothelial cell but abundant in Kupffer’s cell (4-7, 22, 23). In addition, the active phagocytic property and several ultrastructural characteristics like a ruffled cell membrane, relative preponderance of RER, free ribosomes and dense bodies, and paucity of smooth endoplasmic reticulum and paucity of vacuoles help to differentiate Kupffer’s cell from the endothelial cell (1, 3, 5, 6, 23). The tumors described here were incidentally discovered during a study designed for experimental induction of HCC; as such, endogenous peroxidase activity could not be assessed by histochemical techniques. However, the tumor cells showed evidence of phagocytosis, namely, intracytoplasmic inclusions of erythrocytes, nuclear material, and crystalloid structures, as well as many structural features attributed to Kupffer’s cells. The tumor cells grew along the sinusoids or filled these spaces, with the latter action resulting in pressure atrophy of the intervening hepatic cell plates. Also, the invasion of hepatic vein tributaries and the spreading to the lung through pulmonary arterial circulation by these cells were not uncommon happenings. The occurrence of HCC alone, though much more frequent in these experimental conditions, was not associated with vascular invasion. We believe that this selective vascular invasion by KCS was most certainly due to the intravascular origin of this tumor. The existence of tumor nodules in spleen and intestine in 1 animal is difficult to explain. The possibility of either the tumor’s spreading from the liver or the tumor’s occurring multifocally cannot be excluded. It is well known that in chemically induced tumors more than one organ may be involved.

No obvious or overt vasoformative tendency of the sarcomas described here was observed; this, coupled with the fact that ultrastructurally the tumor cells did not resemble endothelial cells, indicates that these tumors are not of endothelial origin (5, 17). Gillman and Hallowes (19, 20) described “reticuloendothelial” tumors existing in rats after repeated injections of trypan blue; these rats showed constituent cell morphology identical to that observed by us. Evans and Grasso (unpublished observations) recently reported the occurrence of KCS in rats administered DENA or DMN and likened the general characteristics of these tumors to reticulum cell sarcomas. We believe that the tumor cells found in the so-called reticulum cell sarcomas of animals (Evans and Grasso: Unpublished observations) are morphologically identical to those seen in our experiments.

We encountered these sarcomas mostly in rats that were exposed to the action of carcinogens during hepatic regeneration. Some tumors did develop in the control DMN-treated group, whereas none were seen in the control animals inoculated with AAF. Actively proliferating cells are particularly vulnerable to neoplastic transformation by chemical carcinogens (24). Following partial hepatectomy, proliferation of Kupffer’s cells comes after that of liver cells, attaining a peak at about 48 hours of surgery (22). Thus in our rats subjected to partial hepatectomy after three daily doses of AAF, the carcinogenic metabolite of this chemical produced in the hepatocyte is likely to have affected proliferating Kupffer’s cells, imparting a neoplastic potential to them. DMN would also have acted the same way on Kupffer’s cells proliferating as a result of partial hepatectomy or CCl4-induced injury. The toxicity of DMN to hepatocytes would by itself also induce some regeneration. Herein may lie the explanation of sarcomas in the animals of the control DMN-treated group. However, AAF has very little, if any, hepatotoxic action and, when given in three small doses without any other interference, would not induce sufficient regenerative activity for target action by the carcinogen.

Following liver injury, endothelial cells lining the hepatic sinusoids regenerate slowly after a lapse of several days (22). This is possibly why we failed to encounter angiosarcomas in our experiments in which the small amount of carcinogen administered would have been either trapped in the cells or eliminated from the body at the time when the endothelial cell remains vulnerable during division. We are now testing these hypotheses in planned experiments.
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FIGURE 1.—Tumor diffusely infiltrating sinusoids of liver. Hepatocytes are compressed by tumor cells. Hepatic vein at upper right corner shows tumor embolus. H & E. × 100. FIGURE 2.—Tumor cells growing along sinusoids. Nuclei are large and hyperchromatic. H & E. × 400. FIGURE 3.—Tumor cells growing along sinusoids and into portal tract. Intervening hepatocytes are seen. Toluidine blue on Epon-embedded, thick section. × 100. FIGURE 4.—Pleomorphic variant of tumor showing numerous multinucleated giant cells. H & E. × 100. FIGURE 5.—Monomorphic appearance of tumor cell showing round-to-oval vesicular nuclei and a fair amount of light-staining cytoplasm. Some cells have phagocytosed erythrocytes (arrows). H & E. × 400.
FIGURE 6.—Tumor cells seen within sinusoids (S) appear distinct from hepatocytes (H) forming cell plates. Electron micrograph. X 1,330.

FIGURE 7.—Tumorous Kupffer's cells (K) are closely packed and have an irregular cell membrane thrown into folds. Nuclei are large and oval with irregularly clumped chromatin. Cytoplasm contains prominent RER, numerous ribosomes, and a vacuole resulting from autophagia. S = sinusoids; H = hepatocytes. X 5,200. FIGURE 8.—Higher magnification of fig. 7 to show numerous free ribosomes in cytoplasm. RER, mitochondria, and a vacuole resulting from autophagia (arrow) are present. Note villose processes of cell margin. Hepatocyte is seen on lower left corner. K = tumorous Kupffer's cells. X 16,660
FIGURE 9.—Prominent undulating cell membrane of tumorous Kupffer's cell (K). Nucleus of one cell is indented. RER profiles, dense bodies, ribosomes, and a crystalline inclusion (arrow) are present in cytoplasm. Parts of hepatocytes (H) are seen on upper left and lower right corners. S = sinusoids. × 4,400. FIGURE 10.—Endothelial cell (E), with portions of tumorous Kupffer's cell (K), hepatocyte (H), and sinusoid (S). Endothelial cell has large nucleus, relatively smooth cell outlines, and thin rim of cytoplasm that shows prominent vacuoles (arrow). × 9,000. FIGURE 11.—Section of lung showing tumor embolus in branch of pulmonary artery. H & E. × 100