The Production of Hemangioendothelialsarcoma in Rats by Feeding 5-Acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine


SUMMARY

Thirty-five female Sprague-Dawley weanling rats were fed 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine for 37 weeks. All 32 rats surviving 28 weeks or more developed hemangioendothelialsarcomas at one or more sites. These unusual tumors were located in the livers of 31 rats, in the mesentery of 30 rats, and in the lungs of 5 rats. Nine rats developed alveolar carcinomas, and 1 rat developed a benign fibroadenoma of the mammary gland. The distribution of tumors observed following the administration of this nitrofuran derivative was surprisingly different from that observed after feeding N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide, a potent urinary bladder carcinogen for the rat.

INTRODUCTION

It was reported by Stein et al. (8) and Morris et al. (5) that certain nitrofuran derivatives, when fed to rats, induced neoplasms of the mammary gland, kidney, intestine, and other tissues. Ertürk et al. (2) observed that the oral administration of N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide to female rats was followed by the appearance of gross urinary bladder carcinomas in 29 of the 30 animals evaluated.

When 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine, a nitrofuran derivative, was fed to rats, all 32 rats surviving 28 weeks or more developed hemangioendothelialsarcomas (malignant hemangioendotheliomas) at one or more sites. A description of this unusual experimental vascular sarcoma is the subject of this report.

MATERIALS AND METHODS

5-Acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine was prepared from 5-chloromethyl-3-(5-nitro-2-furyl)-1,2,4-oxadiazole in a two-step synthesis involving a ring expansion to an oxadiazole. Fifteen grams of 5-chloromethyl-3-(5-nitro-2-furyl)-1,2,4-oxadiazole were suspended in 100 ml of liquid ammonia, were cooled to -65°C (acetone-dry ice bath), and were stirred for 2 hr. The solution was then allowed to reach -33°C. The yellow product was collected on a sintered glass funnel and thoroughly washed with water. The yield of the 5-amino-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine was 12.3 grams (90%). It was recrystallized from N,N-dimethylformamide or nitromethane, m.p. 220–222°C (dec).

Calculated for C_{9}H_{8}N_{4}O_{5}: C, 42.86; H, 3.20; N, 22.22
Found: C, 42.70; H, 3.30; N, 22.36

The amino compound was stirred in 60 ml of acetic anhydride at 55°C for 36 hr. The reaction mixture was then cooled in an ice bath, and the cream-colored 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine was collected by filtration. After washing with water and recrystallization from boiling ethanol, the product (98% yield) melting at 202–204°C was used in the feeding experiment.

Calculated for C_{9}H_{6}N_{4}O_{4}: C, 40.00; H, 2.88; N, 26.66
Found: C, 40.05; H, 2.93; N, 26.82

Seventy female weanling Sprague-Dawley rats weighing from 51 to 76 gm were fed powdered Wayne Lab-Blox (Allied Mills, Inc., Chicago, Illinois). Half of the animals received 0.189% of 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine (Chart 1) mixed with the diet for the first 21 weeks. Thereafter, the dosage of the chemical was reduced to 0.15% because of its growth retarding effects on the treated animals. Because of the sudden death of several rats from massive internal hemorrhage, the experiment was terminated at the end of the 37th week. The rats were weighed frequently, and food consumption was evaluated biweekly (Chart 2). Control of respiratory infections in the rat colony was achieved by an injection of Bicillin

Chart 1. Structure of a new vascular carcinogen, 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine.
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300

• GROWTH OF CONTROL RATS

GROWTH OF RATS FED TEST CHEMICAL

•—• CUMULATIVE DOSE OF TEST CHEMICAL

0 10 15 20 25 30 35 40

Chart 2. Growth curves of control and test rats and cumulative dosage of 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine.

Long-Acting (Wyeth Laboratories, Inc., Phila., Pa.) once each month (2).

The first hemangioendothelial sarcoma was detected in a rat that died suddenly in the 28th week of feeding. Postmortem examination demonstrated a massive intraperitoneal hemorrhage from a large tumor located in the mesentery. Thereafter, all animals were observed twice daily by inspection of conjunctiva, mucosa, and feces for evidence of hemorrhage. At autopsy, all gross lesions and specimens of mammary glands, liver, lungs, kidney, adrenal, spleen, uterus, ovary, gastrointestinal tract with mesentery, muscle, and bone marrow (ribs, vertebra, and sternum) were fixed in a mixture of 95% ethanol (2 liters), formalin (0.6 liter), acetic acid (0.1 liter), and water (4 liters). Urinary bladders were inflated with Bouin’s solution. All tissues were stained with hematoxylin and eosin.

RESULTS

All rats that survived for more than 28 weeks developed hemangioendothelial sarcomas at one or more sites as a result of ingesting 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine. Some toxicity of the chemical was evident by the retardation of growth of the test animals compared with the controls (Chart 2). At the termination of the experiment the weight differential was highly statistically significant ($P < 0.01$). The average animal ingested 4.3 gm of the chemical in 28 weeks and 5.5 gm in 37 weeks (Chart 2).

The rats were palpated periodically to ascertain the presence of mammary tumors and were observed closely for anemia and internal or gastrointestinal hemorrhage. The temporal sequence of development of hemangioendothelial sarcomas in the mesentery, liver, and lungs is presented in Chart 3. The initial hemangioendothelial sarcomas were present only in the mesentery and were usually located near the junction of the mesentery and the small intestine (duodenum and jejunum). Hemangioendothelial sarcomas were first detected in the liver at the 30th week and in the lung at the 31st week (Chart 3). The first alveolar carcinoma observed appeared at the 35th week.

Massive blood clots located on the surfaces of the abdominal viscera were present in those animals in which the tumors caused intraperitoneal or gastrointestinal hemorrhage. In many cases the point of hemorrhage could be determined in a tumor mass located either in the mesentery or liver. The tumors from which hemorrhages occurred varied from the size of a pinpoint to 5 cm in diameter. Tumors arising in the mesentery were frequently multiple in origin, and the associated mesenteric arteries and veins were dilated, reddish-brown cords. The liver hemangioendothelial sarcomas were also variable in size and...
were detectable just under Glisson's capsule as well as deep in the different lobes of the organ. The lung tumors varied in their gross appearance from hemorrhagic to whitish-grey in color. The hemangioendothelial sarcomas were located in the liver (31 rats), mesentery (30 rats), and lungs (5 rats). Alveolar carcinomas were present in the lungs of 9 rats, and 1 fibroadenoma of the mammary gland was present. In the 35 control rats, 1 mammary fibro adenoma was also found, so it seems reasonable to conclude that the single benign mammary tumor found in the rats fed the nitrofuran derivative was not chemically induced.

Hemangioendothelial sarcomas occurring in the mesentery, liver, or lung originated in the endothelial cells (Figs. 1—6) and formed masses partially or completely occluding the vascular lumen, or they infiltrated through the vascular wall and formed perivascular masses (Figs. 1—6). The cells comprising the infiltrating hemangioendothelial sarcomas varied in type and size (Figs. 5—7). Some cells contained large, vesicular nuclei and a considerable amount of cytoplasms, while other cells contained smaller nuclei (Figs. 3, 7). Uniformly sized, younger-appearing endothelial cells contained a remarkably dense chromatin network in their pycnotic nuclei (Figs. 7—9). Nuclei containing mitotic figures were frequently observed (Figs. 3, 6, 8), and often 1 to 5 mitotic figures were present in a high-power microscopic field. Some vascular tumors which appeared grossly as large, round, nodular, dark-brown masses containing large amounts of blood appeared histologically to be cavernous hemangiomas (Fig. 10). Both the hemangioendothelial sarcomas and cavernous hemangiomas exhibited varying degrees of invasion of adjacent intestinal walls. The typical appearance of the pulmonary alveolar carcinomas observed is present in Figs. 11 and 12.

**DISCUSSION**

Several, but not all, of the nitrofurans tested have demonstrated carcinogenic activity for the rat (2, 5, 6, 8). Structural alterations of nitrofuran derivatives can apparently significantly influence the site(s) of appearance of subsequent malignant neoplasms. Oral administration of formic acid 2-[(5-nitro-2-furyl)-2-thiazolyl] hydrazide to rats resulted in the appearance of carcinomas of the kidney, breast, liver, intestine, and ear duct, but no urinary bladder, vascular, or pulmonary neoplasms were found (5, 6, 8). When N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide was fed to rats, almost all animals developed urinary bladder carcinoma, and a few developed tumors of the renal pelvis and benign mammary tumors, but no vascular, hepatic, pulmonary, intestinal, or ear duct neoplasms were detected (2). The acetamido-oxadiazine nitrofuran derivative investigated in the present study surprisingly induced a 100% incidence of hemangioendothelial sarcomas and a lower incidence of alveolar carcinomas, but no urinary bladder, renal, mammary, intestinal, or ear duct neoplasms were found.

Hemangioendothelial sarcomas are rarely observed in humans (9) and have been infrequently produced in experimental animals (3). This seems unusual because vascular tissue is widely distributed and possesses a high regenerative potential. In mice, oral, i.v., or s.c. administration of 3-methylcholanthrene (10), o-aminoa zotoluene (1), or 9,10-dimethylbenz(a)anthracene (7) has been followed by the appearance of benign and malignant vascular neoplasms at local and distant sites in low incidence. In the rat, hemangiomas have been described following inoculation with polyoma virus (4) or after s.c. injection of 9,10-dimethylbenz(a)anthracene (3). The compound studied in the present experiment, 5-acetamido-3-(5-nitro-2-furyl)-6H-1,2,4-oxadiazine, is the first nitrofuran derivative which has been found to be a rat vascular carcinogen, and it appears to be one of the most effective carcinogens for this tissue and this species. The high incidence and relatively short latent period before the appearance of hemangioendothelial sarcomas produced by this compound make this carcinogen a potentially useful tool for studying the process of vascular carcinogenesis.

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**REFERENCES**

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Fig. 1. Mesentery: Very early stage of hemangioendothelialsarcoma. Endothelial cells are proliferating and migrating into perivascular tissues. Hematoxylin and eosin, X 50.

Fig. 2. Mesentery: Proliferating endothelial cells partially obliterating the arteriolar lumen. Hematoxylin and eosin, X 150.

Fig. 3. Mesentery: Proliferating endothelial cells forming a spongy hemangioendothelialsarcoma containing mitotic figures and blood elements. Hematoxylin and eosin, X 100.

Fig. 4. Mesentery: Proliferating endothelial cells forming a cellular hemangioendothelialsarcoma. Hematoxylin and eosin, X 150.

Fig. 5. Mesentery: An advanced hemangioendothelialsarcoma. The blood vessel is full of tumor tissue, the wall has been ruptured by migrating cells, and the surrounding tissues have been invaded. Hematoxylin and eosin, X 85.

Fig. 6. Liver: Hemangioendothelialsarcoma originating from a hepatic arteriole. Hematoxylin and eosin, X 110.

Fig. 7. Liver: A general view of an invasive type of hemangioendothelialsarcoma. Hematoxylin and eosin, X 85.

Fig. 8. Lung: Early stage of hemangioendothelialsarcoma arising from a peribronchiolar artery. Hematoxylin and eosin, X 110.

Fig. 9. Liver: An advanced hemangioendothelialsarcoma with polynucleated tumor cells showing tendency for blood vessel formation. Hematoxylin and eosin, X 110.

Fig. 10. Mesentery: Cavernous type of hemangioma. Hematoxylin and eosin, X 85.

Fig. 11. Lung: Alveolar carcinoma with mitoses. Hematoxylin and eosin, X 110.

Fig. 12. Lung: Alveolar carcinoma with mitoses. Hematoxylin and eosin, X 125.
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