Neoplasms in Purebred Boxer Dogs following Long-Term Administration of N-Methyl-N-nitrosourea 1

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ABSTRACT

The carcinogenicity of N-methyl-N-nitrosourea (MNU) has been demonstrated in several species for a variety of tissues, particularly the nervous system. Boxer dogs, a breed with a high incidence of spontaneous tumors (including brain tumors), were exposed to MNU to determine their susceptibility to MNU and to compare the induced tumors with the types of tumors reported in the literature.

Twenty purebred Boxer dogs were given weekly i.v. injections of MNU, 5 mg/kg, for 36 weeks and were observed for approximately 3 years for the development of neoplasias. Nineteen of 20 dogs developed neoplasms 19 to 36.5 months after the first injection. The mean latent period was 30 ± 5 (S.D.) months. All tumor-bearing dogs had 2 or more neoplasias, including metastases, and 14 dogs had tumors of more than 1 tumor type. Malignant neurinomas of the small intestine were present in 11 dogs, and poorly differentiated sarcomas of the small intestine were found in 6 dogs. Metastasis occurred to liver, spleen, lymph nodes, lung, and adrenal. Two cases of malignant neurinoma developed in the heart, and 1 each developed in the stomach and colon. Two well-differentiated neurinomas occurred in the small intestine, and 1 each occurred in a spinal root, the stomach, and the colon.

The high incidence rate, young age of development, and unique tumor types clearly distinguished the tumors from spontaneous tumors of Boxers. Although the lack of brain involvement was unexplained, MNU had a neurooncogenic effect on the peripheral nerves of the gastrointestinal tract.

INTRODUCTION

The neurooncogenicity of MNU 4 in rats was first described by Druckrey et al. in 1964 (4) during a systematic investigation of the carcinogenic potential of 65 nitroso compounds (5). The susceptibility of rats to induction of nervous system tumors varied with the dose of MNU, route of administration, and strain of rat (17). Under certain conditions up to a 100% incidence of neurogenic tumors was achieved (16). A similar but less pronounced predilection for neuroectodermal tissues in rabbits was demonstrated (7, 13). Mice and dogs also developed tumors of the nervous system following repeated i.v. injections of MNU; however, the incidence was lower (3, 14, 15, 19). No nervous system tumors were reported in 13 of 43 necropsied nonhuman primates exposed to MNU and observed for up to 8 years (1). The oncogenicity of MNU was not exclusively neurotropic, however, since tumors were induced in many other organs with different dose schedules and routes of administration (8, 13, 17-19).

The use of dogs in carcinogenesis studies provides valuable comparative information that may assist in the recognition of environmental exposure to carcinogens. Dogs live in close contact with man, share his environment, and may be exposed to the same carcinogens. Secondly, dogs are often kept for the duration of their natural life span, during which time spontaneous tumors develop. Thirdly, similar types of tumors develop in dogs and humans at comparable incidence rates. By contrasting spontaneous tumors with those obtained in canine carcinogenicity studies, more accurate extrapolation to man may be obtained. Nevertheless, widespread usage of dogs in carcinogenesis studies is limited by the cost of maintaining them for long periods and by the amount of space required to house sufficient numbers of animals.

Boxer dogs have a significantly higher incidence of spontaneous neoplasia than do other breeds (2, 6). Brain tumors in particular are most commonly found in older brachycephalic dogs [e.g., Boxers and Boston terriers (11, 12)]. Since MNU is capable of inducing tumors of the nervous system and other tissues, purebred Boxer dogs were utilized to compare tumors induced with MNU to those reported in the literature.

MATERIALS AND METHODS

Carcinogen. Prior to each injection MNU, 5 mg/ml (K & K Laboratories, Plainville, N. Y.), was freshly dissolved in 0.15 M sodium chloride solution containing 6.7% phosphate-citrate buffer (pH 4.2). Between preparations the MNU was stored in a desiccator at −20°C. Stability and purity of the MNU were checked periodically by an UV spectrophotometer at 231 nm (5), assuming an E 1% 100 of 5.888 for a 1 M solution.

Animals. Twenty purebred Boxer dogs were purchased from private breeders, acclimatized to the laboratory environment, and immunized against canine distemper, infectious canine hepatitis, and leptospirosis. They were maintained in individual cages until several months after the last administration of carcinogen. Thereafter, they were housed in

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4 The abbreviation used is: MNU, N-methyl-N-nitrosourea.

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groups of 2 to 3 in indoor-outdoor runs. They were observed daily, fed Purina dog chow, and provided with water ad libitum.

**Experimental Procedure.** Ten male and 8 female dogs received their first injections of MNU at 4 months of age. Two additional males, which were older when purchased, began receiving MNU at 10 months of age. MNU was administered via 5-mg/kg i.v. injections in the cephalic vein each week for 36 weeks. The dogs were weighed before feeding on treatment days for calculation of the appropriate dose.

**Necropsy and Histopathology.** A complete necropsy was performed on each dog that died or was killed when moribund. Lesions were recorded, and representative samples of major organs and all lesions were fixed in neutral buffered 10% formalin. Paraffin-embedded sections were cut at 6 μm and stained with hematoxylin and eosin. Selected tumors were stained by Masson's trichrome and Wilder's reticulin methods.

**RESULTS**

Nineteen of 20 Boxer dogs developed neoplasms within 19 to 36.5 months of the first injection of MNU. The average latent period was 30 ± 5 (S.D.) months, with a median of 30 months. One dog was removed from the study after 23 months because of illness resulting from a 10-cm organized abscess in the s.c. tissue near the right shoulder. No neoplasms were detected in this animal. Most dogs appeared in good health throughout the observation period. The duration of clinical illness was generally short, and several dogs died suddenly. Those with a longer clinical course were euthanized when moribund. One dog was killed at 36.5 months to terminate the experiment. Advanced neoplastic lesions were present at necropsy, although the dog was clinically healthy at the time of euthanasia.

The types and locations of the tumors are shown in Table 1. All 19 tumor-bearing dogs had 2 or more neoplasms, including metastatic tumors, and 14 dogs had tumors of more than 1 tumor type.

**Neurinomas.** A neurinoma developed in the left C5 spinal root of 1 dog (Fig. 1). It compressed the spinal cord, resulting in paralysis of the left foreleg and paraplegia of the rear legs. Neurinomas in the gastrointestinal tract occurred as firm white nodules in the wall of the stomach, small intestine, and colon. Histologically, they were well differentiated, encapsulated, and did not metastasize (Fig. 2).

**Malignant Neurinomas.** The small intestine was the most common site for malignant neurinomas. Thirteen malignant neurinomas occurred in 11 dogs. They began as nodular outgrowths in the wall of the small intestine (Figs. 3 and 4). Growth extended outwardly and laterally before the lumen was compromised. Obstruction did not occur even with the largest tumors. Ulceration of the mucosa was common in larger neoplasms, and occasionally perforation occurred, initiating acute peritonitis. In such cases adhesions of the omentum and visceral organs were present. Small lesions were firm, white, and nodular. When the mucosa was ulcerated, the tissue appeared tan to dark gray. Yellow patches of necrosis were scattered throughout the tissue.

Malignant neurinomas of the small intestine frequently metastasized to the liver (Figs. 5 and 6). The metastatic tumors were randomly scattered in all lobes and ranged in size from a few mm to masses that replaced a whole lobe. The nodules were round, firm, and white to pink. Larger masses contained irregular areas of necrosis and cysts up to 2 mm in diameter.

The spleen was also a site of metastasis of malignant neurinomas (Fig. 7). The metastases ranged from small invasive nodules to large masses 10 to 15 cm in diameter, which replaced nearly the entire spleen. They were white, cystic, and irregularly spherical or nodular and contained patches or streaks of necrosis.

Two malignant neurinomas occurred in the heart (Fig. 8). Both were attached to the wall of the right side of the heart at the junction of the atrium and the ventricle. One extended into the lumen of the right ventricle, while the other was on

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### Table 1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of dogs affected</th>
<th>Location</th>
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<tr>
<td>Malignant neurinoma</td>
<td>11</td>
<td>Small intestine</td>
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<tr>
<td></td>
<td>6</td>
<td>Liver</td>
<td>M</td>
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<td></td>
<td>3</td>
<td>Spleen</td>
<td>M</td>
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<td></td>
<td>2</td>
<td>Heart</td>
<td>P</td>
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<td></td>
<td>1</td>
<td>Stomach</td>
<td>P</td>
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<td></td>
<td>1</td>
<td>Colon</td>
<td>P</td>
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<tr>
<td>Poorly differentiated sarcoma</td>
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<td>Small intestine</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td></td>
<td>2</td>
<td>Spleen</td>
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<tr>
<td></td>
<td>3</td>
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<tr>
<td>Fibrosarcoma</td>
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<td>Femur</td>
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³ P, primary; M, metastatic.
The histological appearance of malignant neurinomas of the small intestine varied from well differentiated to anaplastic. Growth appeared to originate between the muscularis mucosae and the muscularis interna or between the muscularis interna and the muscularis externa. Invasion into the mucosa occurred only in advanced lesions. The well-differentiated malignant neurinomas were characterized by interlacing bundles of spindloid cells with long slender nuclei arranged in palisades (Fig. 9). Collagen fibers were sparse, but reticulin fibers were abundant. Less-differentiated malignant neurinomas lacked palisades (Fig. 10), but other features, including reticulin formation, were retained (Fig. 11). Cell populations were moderately dense, and mitotic figures were abundant. Anaplastic neurinoma cells had sparse cytoplasm (Fig. 12). The nuclei were round to oval and hyperchromatic. Degenerative cysts containing small amounts of proteinaceous fluid were sometimes present, while reticulin fibers were sparse.

Metastatic malignant neurinomas of the liver (Fig. 13) and spleen were generally less differentiated than were the primary tumors but retained recognizable characteristics. One of the malignant neurinomas of the heart was moderately differentiated, while the other was anaplastic.

**Poorly Differentiated Sarcomas.** Poorly differentiated sarcomas of the small intestine were grossly indistinguishable from malignant neurinomas that metastasized to the liver and spleen. However, they lacked enough histological features to identify clearly a neurogenic origin. The tumors were composed of densely packed spindloid cells with round to oval nuclei, fibrillar cytoplasm, and indistinct cytoplasmic borders (Fig. 14). Collagen fibers were present, but reticulin fibers were lacking. Mitotic figures were abundant. In addition to hepatic and splenic metastases, there were metastases to mesenteric lymph nodes and the adrenal gland.

**Hemangiosarcomas and Hemangiomas.** Two dogs developed hemangiosarcomas of the heart. The tumors were dark red and cystic and were located in the myocardium of the right ventricle, extending to the right atrium. Metastasis occurred to the lungs, kidneys, and adrenal gland. A hemangiosarcoma developed in the subumbular region of the abdomen of 1 dog and metastasized to the kidneys and lungs. Another hemangiosarcoma occurred at the base of the dorsal arch of vertebra T4 and compressed the spinal cord. The spleen of 1 dog contained a primary hemangiosarcoma. Cavernous hemangiomas occurred in the skin of 3 dogs.

Histologically, the hemangiosarcomas contained numerous blood-filled channels lined with pump endothelial cells. Cavernous hemangiomas were well demarcated from surrounding tissue. The endothelium lining the large blood-filled spaces was flattened and mature.

**Malignant Lymphomas.** Two dogs developed cancers of the lymphoreticular system. In 1 animal the lesions were limited to massive involvement of the liver and spleen. Multiple round nodules with central necrosis were present in the liver. The spleen had a single, large, white, firm mass. The other dog had generalized enlargement of peripheral lymph nodes, which first became evident as a mass in the orbit. Both cases of malignant lymphoma were of reticulum-cell type.

**Carcinomas.** One dog had a yellow, soft, 4- x 3.5- x 2.5-cm carcinoma of the jejunum. The mucosal surface was ulcerated, and neoplastic cells invaded the muscular layer to the serosa. Metastases occurred in the liver as multiple raised nodules with central necrosis. One dog had multiple bronchiolar carcinomas up to 0.5 cm in diameter in all lobes of the lung; however, the diaphragmatic lobes were predominantly involved.

**Fibrosarcoma.** One dog developed a slow-growing neoplasm at the distal end of the left femur. It was clinically apparent for over 1 year before the dog was euthanized. Although the tumor did not metastasize, it was locally invasive and completely replaced the bone in the distal third of the femur. The tumor was white and hard and measured 9 x 5 x 6 cm. Histologically, it was composed of spindle cells with abundant fibrillar streaming cytoplasm. Extensive amounts of collagen but few mitoses were present.

**DISCUSSION**

During a mean latent period of 30 ± 5 (S.D.) months, malignant tumors developed in 95% of the Boxer dogs exposed to 36 weekly i.v. injections of MNU. All tumor-bearing dogs had more than 1 tumor, and 14 of 19 had 2 or more tumors of differing cell types. The degree of susceptibility of Boxer dogs to the carcinogenic effect of MNU was similar to that of other species. In contrast to naturally occurring tumors in the Boxer breed, 3 features were particularly noteworthy: (a) the tumor incidence was much greater; (b) the age at which tumors developed was markedly reduced; and (c) the most common experimental tumors did not correspond to tumors with the highest spontaneous incidence.

The precise incidence of neoplasia in the canine population is difficult to obtain. Each type of study has unique shortcomings in arriving at the actual incidence. A recent report presented an epidemiological analysis of canine neoplasia observed over a 12-year period in a veterinary hospital (2). The authors pointed out that the overall prevalence ratio of neoplasia of 4.2% might have been related to the fact that the hospital served as a referral center and that the interest of the faculty was in cancer research. The study was based on hospital accessions and as such could not consider the total canine population at risk. Thus, the reported prevalence rates may be higher than those that would occur if bias could be eliminated. Nevertheless, the information, particularly as it relates to Boxer dogs, provides interesting comparisons with the data on MNU-induced neoplasms. In the report by Cohen et al. (2), the highest age-specific neoplasia ratio rose progressively from 9.9/1000 (1%) in Boxers less than 1 year old to 366.7/1000 (37%) in Boxers 13 years old and over. The ratio for 3-year-old Boxers was 57.7/1000 or 6%. These data are in sharp contrast to the ratio of 19/20 in Boxers following exposure to MNU and surviving to a mean age of 34 ± 5 (S.D.) months. The experimentally induced tumors occurred at a
much younger age and in a greater proportion of animals at risk.

The most common tumors found by Cohen et al. in Boxer dogs in decreasing order of frequency were mastocytoma, lymphosarcoma, adenocarcinoma, osteosarcoma, epidermoid carcinoma, melanoma, and fibrosarcoma. Of these types of tumors, the frequency of mastocytoma, lymphosarcoma, and osteosarcoma was significantly higher in Boxers than it was in other breeds. However, only 2 of 20 Boxers exposed to MNU developed cancer of the lymphoreticular system, and neither mastocytoma nor osteosarcoma were induced. The most common tumors were malignant neurinomas and poorly differentiated sarcomas of the small intestine. Hemangiosarcomas, the next most frequently occurring tumor, were not among those listed in the report of Cohen et al. (2).

In the present study nonepithelial tumors of the intestinal walls presented a wide spectrum of histological appearance but were considered to represent different stages of the same basic process. They were classified as neurinomas, malignant neurinomas, or poorly differentiated sarcomas. The neurinomas were benign localized tumors without invasion of surrounding layers of tissue and without metastasis. Their origin from Schwann cells was readily apparent. Malignant neurinomas were more densely cellular, had a higher ratio of mitosis, invaded other layers of tissue, and metastasized to organs such as the liver and/or spleen. Included in this category were tumors similar to those previously described as "anaplastic neurinomas" in rats (10). The cells were more rounded, the nuclei were hyperchromatic, and microcystic degeneration was occasionally present. Both Antoni A and B type tissues were present in the tumors. Poorly differentiated sarcomas were present in the same locations but did not possess features indicative of a neurogenic origin. The cells were spindloid, but little collagen or reticulin was present. In general, these tumors were larger and metastasized more widely than did malignant neurinomas. Metastasis from malignant neurinomas occurred only to the liver and spleen, whereas poorly differentiated sarcomas metastasized to mesenteric lymph nodes and the adrenal gland as well.

The histopathology of other tumors, such as hemangiosarcomas, malignant lymphomas, bronchiolar adenocarcinomas, and the jejunal carcinoma, was similar to their respective spontaneously occurring counterparts. However, as pointed out above, the incidence was higher and the age of occurrence was lower.

In 1970 Warzok et al. (19) reported the induction of brain tumors in 4 or 10 mixed-breed dogs following monthly 20-mg/kg i.v. injections of MNU. In addition to the intracerebral sarcomas and glioblastomas multiforme, multiple sarcomas of the lungs, spleen, vertebrae, heart, and intestine and malignant hemangioendotheliomas of the heart and lungs were observed. The average latent period of the 6 dogs that died of neoplasms was 13 months. Four dogs died of other causes. Stavrou and Haglid (15) produced peripheral nervous system tumors of the heart, duodenum, and cauda equina in 3 of 6 dogs with a similar dose level and schedule. The latent period averaged 23 months.

The tumors produced by Stavrou and Haglid were similar to the Boxer tumors with respect to tumor type and location. In both studies peripheral nervous system tumors were induced in the small intestine, heart, and spinal roots; however, no tumors of the brain were induced as in the study of Warzok et al. (19). Latent periods varied widely among all studies. The dissimilarities in tumor type, location, and latent period may be due to differences in breed, initial age of treatment, dose level, and schedule of MNU administration. Such factors alter the incidence, type, location, and latency period of MNU induction in the rat (17). Our choice of the Boxer breed, which is naturally susceptible to brain tumors, did not ensure success in producing an autochthonous brain tumor model in dogs. Additional studies in dogs with modifications of experimental factors are needed.

REFERENCES

MNU Carcinogenesis in Boxer Dogs

Fig. 1. Neurinoma of Spinal Root C₇ protruding through the dorsal lamina of Vertebra C₇ (bottom center). Right, Vertebra T₁, spinous process.

Fig. 2. Well-differentiated neurinoma of the colon. Bundles of tumor cells are surrounded by connective tissue. Lymphocytes have infiltrated the tumor. H & E, × 65.

Fig. 3. Malignant neurinoma arising from the antimesenteric wall of the small intestine.

Fig. 4. Cross-section of the malignant neurinoma shown in Fig. 3. Nodular growth involved a portion of the intestinal wall without obstruction of the lumen. Scale is in mm.

Fig. 5. Liver with multiple metastases of the malignant neurinoma shown in Figs. 3 and 4.

Fig. 6. Cross-section of the liver shown in Fig. 5 revealing multiple metastases of various sizes throughout the hepatic parenchyma. Scale is in mm.

Fig. 7. Spleen with massive replacement by metastatic malignant neurinoma.

Fig. 8. Malignant neurinoma of the heart at the junction of the right atrium and ventricle.

Fig. 9. Well-differentiated portion of a malignant neurinoma of the small intestine. The cells are clustered together to form palisades. H & E, × 100.

Fig. 10. Malignant neurinoma of the small intestine. The cells are arranged in interlacing bundles. The nuclei are small, slender, and densely packed. H & E, × 100.

Fig. 11. Abundant reticulin fibers of the malignant neurinoma shown in Fig. 10. Wilder’s reticulin, × 250.

Fig. 12. Anaplastic (malignant) neurinoma of the intestine. The cells are arranged in streaming bundles, and the nuclei are hyperchromatic and round to oval. H & E, × 100.

Fig. 13. Metastasis of a malignant neurinoma (center) in the liver (lower right). H & E, × 250.

Fig. 14. Poorly differentiated sarcoma of the intestine. The cells are large with abundant fibrillar cytoplasm, large oval nuclei, and prominent nucleoli. H & E, × 250.
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