A reproducible model of metastatic brain and ocular tumor by hematogenous inoculation of the VX2 tumor in rabbits

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A metastatic brain-tumor model has been developed in rabbits by infusing the VX2 carcinoma into the internal carotid artery to simulate hematogenous dissemination of tumor. In a series of 25 New Zealand White rabbits, multiple metastases arose in the hemisphere of 24 (96%) and in the eye of 22 (92%); in all instances ocular metastases were ipsilateral to the site of infusion. Ocular metastases were visible in the anterior chamber in 80% of animals 3 to 12 days after the infusion of VX2 tumor cell suspension. All rabbits deteriorated neurologically or died by Day 15 after the inoculation. Multiple metastases were demonstrated by magnetic resonance imaging as early as 5 to 7 days after infusion of the tumor cells and were confirmed at autopsy. This technique models hematogenous metastases to the brain and eye and is useful in evaluating the response of metastases to chemotherapy and radiation therapy directed to the brain and eye.

KEY WORDS • brain neoplasm • ocular neoplasm • VX2 tumor • magnetic resonance imaging • rabbit

VARIOUS animal brain-tumor models have been developed using direct implantation of tumor cells, viruses, or carcinogens into the brain. 1,3-8 Although these techniques are reproducible in large mammals (such as the dog or rabbit), they do not model the natural hematogenous spread of tumor metastases to the central nervous system (CNS). 1,3,4,7,10,11 Also, direct inoculation of tumor into the CNS is traumatic. The hemorrhage, inflammation, and breakdown of the blood-brain barrier resulting from implantation are not reliably distinguished from those due to the tumor itself, and preclude accurate assessment of tumor viability and growth by imaging modalities. 3 Ushio, et al., 12 infused Walker 256 carcinoma cells into the carotid artery of rats, producing hematogenous spread of cerebral metastases. However, most of the animals either died from or required treatment for overwhelming extracranial metastases.

This study was undertaken to develop a reproducible animal model of hematogenous spread of tumor metastases to the CNS. 6,12 The goal was to establish a model that would serve as a basis for investigation of the pathophysiology and response to therapy of CNS and ocular metastases.

Materials and Methods

All procedures were performed using sterile techniques. This study was approved by the animal care committee of the Clinical Center at the National Institutes of Health.

VX2 Tumor Preparation

The VX2 rabbit carcinoma was prepared in a manner reported previously. 3,6 The VX2 tumor was serially implanted in the thighs of carrier New Zealand White rabbits, each weighing 2.5 to 3.5 kg. After 14 to 21 days, the tumor was surgically removed and placed into calcium- and magnesium-free Hanks' balanced salt solution. 4 The tumor was then diced into 1-cm fragments and gently stirred for 2 hours at 37°C in 2 ml of 1% collagenase. The tumor suspension was passed twice

* Salt solution obtained from Whitaker MA Bioproducts, Walkersville, Maryland.
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through a sterile stainless steel wire mesh and then centrifuged at 100 G for 5 minutes. The resulting pellet of tumor cells was placed into RPMI 1640 medium with L-glutamine and 10% fetal bovine serum.† The final suspension contained approximately $10^7$ tumor cells/ml based upon a cell count of an aliquot of the suspension.

**Tumor Inoculation**

Twenty-five New Zealand White rabbits were anesthetized intramuscularly with ketamine, 25 mg/kg, and xylazine, 2 mg/kg. One percent xylocaine, 0.5 cc, was infused subcutaneously in the region of the femoral artery. A femoral artery cutdown was performed and a No. 3 French polyethylene catheter was inserted into the internal carotid artery (ICA) under fluoroscopic guidance. Heparin, 1 cc (1000 U/ml), was given intravenously through an ear vein. An ICA angiogram was obtained using 0.5 cc of Conray 60% (iothalamate meglumine 60%) as the contrast medium. A 3-cc aliquot of heparinized blood was then withdrawn from the artery and mixed with 0.15 cc of the prepared VX2 tumor cell suspension (approximately $10^6$ tumor cells by direct count) in 21 animals. Four animals were inoculated with $10^5$ tumor cells. The tumor cell suspension was infused over 1 minute into the ICA and was followed by a normal saline flush. The catheter was then removed and the animals' neurological and ocular status and food intake were monitored closely. Sixteen rabbits developed neurological symptoms 11 to 14 days postinfusion of tumor cells and were sacrificed with sodium pentobarbital, 60 mg/kg. These animals received an intravenous injection of Evans blue dye (2 mg/kg) prior to sacrifice so that areas of blood-ocular barrier and blood-brain barrier disruption could be demonstrated. Eight animals were found dead in the cage between Day 10 and Day 14. The brains and eyes of all the animals were fixed in 10% phosphate-buffered neutral formalin. After 7 days of fixation, 5-mm coronal sections of the brain were cut and submitted for paraffin embedding and sectioning. The eyes were submitted en bloc for embedding and sectioning. All pathological specimens were stained with hematoxylin and eosin and were reviewed by the pathologist (P.J.C.).

Magnetic resonance imaging (MRI) was performed in all animals 6 to 12 days after infusion of the VX2 tumor cells. Proton MRI was performed on a Picker superconducting magnetic system operating at 0.5 Tesla (21.3 MHz) using a Picker small-joint (saddle-shaped) surface coil.‡ The images were obtained using a 15-cm field of view and a slice thickness of 5 mm in the coronal and transaxial planes. Spin-echo (SE) pulse sequences were $T_1$-weighted, with an echo delay time (TE) of 40 msec and a repetition time (TR) of 550 msec (SE 550/40), and $T_2$-weighted, with a TE of 80 msec and a TR of 2000 msec (SE 2000/80). Four repetitions and 128 views were used. Images were reconstructed using a two-dimensional Fourier transformation algorithm with a $256 \times 256$-image matrix. Selected animals underwent serial MRI.

**Results**

Twenty-five rabbits were studied. Twenty-one were inoculated with $10^6$ VX2 tumor cells and four received $10^5$ tumor cells. All animals (except one of those receiving the smaller amount of inoculum) developed intracerebral metastases, resulting in a reproducibility rate of 96% (24 of 25 animals) overall and 100% for the larger inoculum dose. Ocular metastasis developed in 92% (22 of 24 animals), with one failure at each dose of tumor cells. Tumor implantation in the anterior ocular chamber was visible in 80% (19 of 24 animals), presenting as erythema or injection of the conjunctiva, sclera, or iris.

All rabbits that received the larger dose of inoculum ($10^6$ cells) were dead within 15 days. Thirteen of the 24 animals with metastases were sacrificed between Days 11 and 14 after manifesting inability to eat or neurological deterioration for 24 hours. Neurological deterioration was usually perceptible as loss of appetite, decreased level of consciousness, ataxic gait, or weakness of the extremities between Days 11 to 14. Eight animals died between Days 11 and 15, with a mean ($\pm$ standard deviation) survival period of 12.6 $\pm$ 1.4 days. The animals receiving the smaller dose of inoculum lived longer and were sacrificed 30 to 60 days postinjection because of neurological symptoms. The rabbit that did not develop tumor was sacrificed and examined postmortem 68 days after infusion of tumor cells.

Ocular involvement with VX2 tumor was observed as early as the 3rd day postinfusion, with a range of 3 to 11 days and a mean time to visualized metastasis in the anterior chamber of 6.3 $\pm$ 2.2 days. These findings usually progressed, with the involved eye becoming proptotic and large tumor nodules appearing in the anterior chamber.

On pathological examination, the cerebral metastases were most prevalent at the junction of the gray and white matter in the cerebral hemisphere ipsilateral to the infusion (Fig. 1). They varied in size from microscopic to approximately 6 mm in diameter. Multiple metastases were observed in the cerebrum and cerebellum in 24 and in the eye in 22 of the 25 animals. However, some contralateral metastases developed as a result of spread via the posterior circulation. Ocular metastases were found in the iris, sclera, and chordoid, with growth of tumor into the vitreous humor. Ophthalmic nerve metastasis and lacrimal gland involvement were also noted. All 22 rabbits found to have ocular involvement at necropsy also had CNS metas-

† L-glutamine solution obtained from Whitaker MA Bioproducts, Walkersville, Maryland; bovine serum obtained from Flow Laboratories, McLean Virginia.

‡ Magnetic resonance imaging system manufactured by Picker International, Highland Heights, Ohio.
FIG. 1. Gross section of the brain showing multiple VX2 tumor metastases (darker areas). H & E, × 3.5.

Fig. 2. Photomicrographs of rabbit brain inoculated with VX2 tumor. Left: Tumor is seen within and growing through a capillary wall. Arrows denote capillary endothelial cells. H & E, × 200. Right: Section of an early VX2 tumor metastasis (T) to the choroid. The retina (R) and sclera (S) are identified. H & E, × 60.

MRI studies detected 21 of the 22 ocular metastases (both in the anterior chamber and choroid) and all of the cerebral metastases. In the four rabbits that received the lower dose of tumor cell inoculum (10⁵ cells), serial MRI demonstrated progressive growth and a number of metastatic foci.

Discussion

A major goal in developing a brain-tumor model is duplication of natural pathogenesis. Previous techniques are severely flawed due to the trauma caused by the method of tumor cell implantation. Infusion of tumor cells directly into the CNS circulation directly mimics hematogenous spread. It should be noted that this method for producing hematogenous spread of VX2 tumor was originally reported as unsuccessful in producing brain metastasis. In addition, several animals died acutely of unknown causes 1 to 3 days after infusion of VX2 tumor cells. The use of heparin to decrease the incidence of thrombosis is probably critical, allowing for microscopic tumor emboli to lodge in the capillaries of the brain with minimal complications. Moreover, the 96% reproducibility rate reported here is greater than the 85% rate found in the literature describing implantation of the VX2 tumor. Survival times after tumor implantation were also slightly prolonged.

This technique resulted in ocular metastases in approximately 92% of the animals, occurring as early as 3 days after inoculation of the tumor in 19 of 24 cases. Other ocular metastatic models have success rates of...
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50% to 60%.\(^9\) As all animals with ocular metastases had CNS metastases, ocular metastases were a reliable sign of successful tumor implantation within the brain. This provides for the first time a simple visual means of confirming tumor implantation within the CNS, without the use of radiographic techniques. Moreover, since ocular metastasis (mean time of onset Day 6.3) preceded the neurological symptoms (mean time of onset Day 12.6), new diagnostic techniques and treatments for CNS metastases can be tested early in the animals' clinical course with the knowledge that tumor is in fact present.

These results demonstrate that MRI is a sensitive technique for the early detection of VX2 tumor metastases to the brain. This was expected, since in humans MRI has been as much as 30% more sensitive than computerized tomography (CT) in detecting CNS lesions.\(^2\) In addition, MRI was able to delineate clearly choroidal and anterior-chamber metastases within the eye, although these were not appreciated on CT scans.

Conclusions

A brain-tumor model that mimics hematogenous spread of malignancy to the CNS and eye has been developed in rabbits. Infusing tumor cells into the ICA produced hematogenous spread of tumor to the brain without the trauma or inflammation associated with direct implantation of tumor within the parenchyma. Ocular metastases developed in 92% of animals and served as a visual confirmation of tumor implantation in the brain and a precursor of neurological deterioration. It was shown that MRI was useful in documenting metastases in both the CNS and eye. This model could be used for evaluating different CNS and eye tumors.

References


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