Development of Experimental Models for Meningeal Neoplasia Using Intrathecal Injection of 9L Gliosarcoma and Walker 256 Carcinosarcoma in the Rat


ABSTRACT

Two models for meningeal neoplasia have been developed in rats using intrathecal injection of 9L gliosarcoma and Walker 256 carcinosarcoma cells. Tumor cells were injected in anesthetized animals through an indwelling catheter inserted at the cisterna magna to the level of the lumbar enlargement of the spinal cord. Survival of rats was dependent on the number of tumor cells injected. Spread of tumor was quantified by histology using a grading scale, and functional and behavioral changes were measured. Rats injected with 10^6 9L gliosarcoma cells showed progressive weight loss, flaccid paralysis, and neurogenic bladder dysfunction and had a median survival of 11 days. The tumor frequently grew as a mass compressing the spinal cord. The 9L gliosarcoma tumor cells markedly invaded the Virchow-Robin spaces but exhibited only minimal invasion of the central nervous system parenchyma. The tumor reached the brain by day 10. Rats injected with 2 x 10^6 Walker 256 carcinosarcoma cells showed progressive weight loss and weakness and had a median survival of 6 days. The tumor grew within the leptomeninges in a discontinuous multifocal fashion and reached the brain by day 4. There was extensive invasion of the central nervous system parenchyma by Walker 256 tumor cells along the Virchow-Robin spaces resulting in hemorrhage and necrosis of grey and white matter. Hot plate and tail flick response times were significantly delayed only in the days immediately preceding death of animals with either 9L or Walker 256 tumor and were not good indicators of tumor progression. Loss of motor coordination and failure of the stepping and placing reflex on the other hand showed good correlation with spread of tumor measured histologically. Control animals injected with 0.9% NaCl or with lethally irradiated tumor cells showed no significant weight loss or functional or behavioral changes. The intrathecal 9L gliosarcoma and Walker 256 carcinosarcoma models show different characteristics of human meningeal carcinomatosis and will be used for studies of experimental chemotherapy with intrathecally administered antitumor drugs.

INTRODUCTION

Tumor metastasis to the CNS occurs in 10 to 15% of cancer patients (1, 2). Tumor involvement of the meninges, the meninges surrounding the brain and spinal cord, occurs in 8% of cancer patients, most frequently originating from tumors of the lung, breast, and melanoma (3). Forty-four % of patients with small cell carcinoma of the lung develop meningeal metastases (4). One of the most devastating manifestations of solid tumors in the CNS is meningeal carcinomatosis, the diffuse or widespread multifocal invasion of the meninges with tumor. Survival of patients with meningeal carcinomatosis is usually no more than 3 mo after diagnosis (5–7). Although CNS radiation and administration of drugs such as methotrexate into the CSF have been successful in treating meningeal infiltration by leukemia (8), metastases from solid tumors to the meninges have been refractory to chemotherapy, either administered alone or as an adjuvant to radiation therapy (2).

Part of the problem in treating malignant tumors in the CNS is thought to be inadequate drug delivery across the blood-brain barrier into regions of the CNS harboring tumor cells (2, 10). Tumor cells within the CNS are protected by the blood-brain barrier from systemically administered hydrophilic drugs and even lipid soluble drugs may penetrate only short distances from the blood into CNS parenchyma (11). Administration of drugs directly into the CSF has been utilized in an effort to achieve and maintain high drug concentrations in the vicinity of tumors located on the meninges. A basic question that can be answered by animal models is whether a cytotoxic drug can be administered into the CSF at a dose that will inhibit the growth of sensitive tumors located within the meninges without damaging normal CNS tissue. In this paper we report the functional and histological characteristics of two models for meningeal neoplasia in rat using lumbar intrathecal injection of 9L gliosarcoma and Walker 256 carcinosarcoma. Later studies will deal with the assessment of toxicity and antitumor activity of a number of intrathecally administered cytotoxic drugs.

MATERIALS AND METHODS

Intrathecal Injection of Tumor Cells. Rats were prepared for receiving intrathecal injection using a technique previously described by Yaksh and Rudy (12). Briefly rats were anesthetized with 2.5 to 4% halothane in air and placed in a stereotaxic holder. The head was immobilized, shaved, and washed with Thimerosal tincture, United States Pharmacopeia. The cisterna magna was exposed by dissection through a midline incision at the base of the skull and a small transverse incision was made in the cisternal dura mater. A 10-μl PE-10 polyethylene catheter (Intramedic; Clay Adams, Parsippany, PA) filled with 0.9% NaCl was inserted 8.5 cm to the level of the lumbar cord. The catheter was exteriorized by passing it through a trochar inserted s.c. at the top of
the head of the animal and secured by a small loose knot in the tubing coated with cranioplast cement that remained below the forehead skin. The open end of the catheter was occluded with a short length of stainless steel wire. Animals were observed for 3 days and only animals showing normal motor and sensory function were used for the study (>97% of animals given implants). Three days after implanting the catheter tumor cells were injected through the catheter in a volume of 20 μl 0.9% NaCl using a microsyringe followed by a 10-μl 0.9% NaCl wash. Previous studies have shown that intrathecal injection volumes of 20 to 30 μl are well tolerated in the rat (12).

**Tumor Cells and Animals.** 9L/5F gliosarcoma cells were obtained from M. Barker, Brain Tumor Research Center, University of California, San Francisco, CA. 9L gliosarcoma cells were maintained in cell culture in Dulbecco's minimal essential medium containing 10% fetal calf serum with 1% glutamine and gentamicin, 0.05 mg/ml. Confluent tumor cell monolayers were passed weekly for no more than 30 passages using 0.25% trypsin in Ca²⁺ and Mg²⁺ free phosphate buffered 0.9% NaCl media, pH 7.4, to liberate the cells. Cells in log-phase growth were used for intrathecal injection. Cells were counted with a hemocytometer using trypan blue exclusion to ensure that viability was greater than 95%. Male Fischer 344 rats (Harlan Sprague-Dawley, Madison, WI) weighing 180 to 205 g were given injections intrathecally with 10⁶ 9L gliosarcoma cells or 2 x 10⁵ Walker 256 carcinosarcoma cells. Female colony 205 Sprague-Dawley rats weighing 200 to 250 g were given injections intrathecally with 4 x 10⁴ to 10⁶ tumor cells. Studies were also conducted to establish on the blunt instrument. An abnormal response in one or both feet was considered a failure of the test. Coordinated motor function was measured by the ability of the animal to negotiate a 60° inclined ramp (13). Thermally evoked tail flick response time, which reflects C fiber induced activation of a segmentally organized ventral root reflex, was measured as the time taken to remove the tail from over a slit through which showed a 300-W projection lamp. If the animals did not respond in 6 s it was considered to have failed the test (13). Hot plate response time, which identifies the integrity of ascending systems through which Aδ/C nerve fiber evoked information travels, was measured as the time taken for the animal to respond by lifting the hind paw and licking the plantar surface when the animal was placed on a surface heated to 52.5°C (13). If no response was observed by 60 s the animal was considered to have failed the test and removed from the hot plate.

**RESULTS**

**Tumor Cell Dose Response**

The effect of different numbers of tumor cells injected intrathecally upon survival of rats is shown in Table 1. The maximum number of tumor cells that could be injected in a volume of 20 μl was 10⁶. Median survival times of rats with tumors decreased with increasing numbers of tumor cells injected. A dose of 1 x 10⁴ 9L gliosarcoma cells and 2 x 10⁵ Walker 256 carcinosarcoma cells was chosen for further studies of tumor progression. In these studies the median day of death of rats with 9L gliosarcoma tumors was 11 days and of those with Walker 256 carcinosarcoma tumors was 6 days.
INTRATHecal TUMor MODELS

Table 1

Survival of rats given intrathecal injections of tumor cells

<table>
<thead>
<tr>
<th>Tumor cells injected into the lumbar spinal CSF space as described in the text.</th>
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<tbody>
<tr>
<td>No. of tumor cells/rat</td>
</tr>
<tr>
<td>9L gliosarcoma</td>
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<tr>
<td>4 x 10⁴</td>
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<td>Walker 256 carcinosarcoma</td>
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Functional and Behavioral Changes

All rats given injections intrathecally of 9L gliosarcoma showed progressive flaccid paralysis beginning several days before death of the animal, with paresis involving first the hind limbs and then the upper body. A characteristic feature of animals with 9L gliosarcoma tumors was incontinence and hematuria consistent with neurogenic bladder from loss of autonomic control due to spinal cord damage. All rats given injections intrathecally of Walker 256 carcinosarcoma showed progressive, generalized debilitation characterized by marked weakness. Control animals receiving lethally irradiated tumor cells or 0.9% NaCl exhibited no obvious signs of functional or behavioral impairment.

Animals given injections of 9L gliosarcoma and Walker 256 carcinosarcoma exhibited progressive weight loss (Charts 1 and 2). The weight loss became significantly different on day 3 for rats given 9L gliosarcoma injections and on day 5 for those given Walker 256 carcinosarcoma injections from control animals given 0.9% NaCl injections. Both tumors caused a progressive increase in the number of animals failing to negotiate a 60° inclined plane and in the number of animals failing the placing and stepping reflex test. The changes became significant for both tests beginning on day 5 for animals given injections of Walker 256 carcinosarcoma tumor and on day 8 for animals given injections of 9L gliosarcoma tumor compared to control animals given 0.9% NaCl injections. Rats given injections of 9L gliosarcoma cells showed a significant change in the tail flick response times only on day 10, the last day of testing. The single animal with Walker 256 carcinosarcoma tumor surviving to day 6 showed an increase in the tail flick response time but all animals that died before day 6 had normal tail flick response times. On day 9 animals given injections of 9L gliosarcoma showed an increase in the hot plate response time that became significantly different compared to control animals given 0.9% NaCl injections (Chart 1). Animals with Walker 256 carcinosarcoma tumor exhibited a gradual increase in the hot plate response time that became significantly different on day 4 compared to control animals receiving 0.9% NaCl (Chart 2). Animals receiving lethally irradiated 9L gliosarcoma and Walker 256 carcinosarcoma cells showed no significant change in body weight or in functional or behavioral parameters.

Histology

9L Gliosarcoma. The 9L gliosarcoma cells appeared to be anaplastic and hyperchromatic. Two discrete cell types were seen growing in the CNS; one was rounded and the other was more elongated and spindle shaped. Tumor cells exhibited different patterns consisting of streaming, whorling, and palisading within the tumor mass. The graded results of 9L gliosarcoma infiltration of the CNS and neuraxial spread are shown in Table 2. The tumor margin of the 9L gliosarcoma in the spinal cord was well demarcated (Fig. 1A) and there was minimal infiltration of tumor cells beyond the perivascular (Virchow-Robin) spaces into the parenchyma. There was frequent compression of the spinal cord by a tumor mass surrounding the intrathecal catheter close to the site of injection (Fig. 2A). The 9L gliosarcoma showed only moderate spread to the brain parenchyma with a propensity for the cerebellar folia (Fig. 3A). In sections from animals receiving lethally irradiated 9L gliosarcoma a minimal number of dead cells were seen surrounding the area of the catheter in the lumbar region.

Walker 256 Carcinosarcoma. Walker 256 carcinosarcoma...
cells exhibited hyperchromatic and eccentric nuclei, pleomorphism, and many abnormal mitoses. The graded results for Walker 256 carcinosarcoma progression in the CNS are shown in Table 3. Walker 256 carcinosarcoma cells spread along the neuraxis from the lumbar site of administration to reach the cortex of the brain by 4 days after injection. The tumor spread to the base of the brain and infiltrated the hypothalamus but did not directly invade the cranial nerves or the pituitary gland. Occasional tumor cells were seen in the choroid plexi of the lateral and frontal ventricles. Simultaneous to the rostral tumor spread, the cells were seen invading the interstices of the CNS parenchyma along the Virchow-Robin spaces. This produced perivascular cuffing...
and then infiltration beyond these spaces, first into the white and then into the gray matter of the spinal cord. Perivascular cuffing and parenchymal infiltration also occurred in the brain. The degree of infiltration by Walker 256 carcinosarcoma cells was greatest in the spinal cord and the tumor margins were ill defined (Fig. 1, C and D). Neuronophagia was evident in the areas of infiltrated gray matter and centers of the tumors were necrotic and occasionally hemorrhagic.

DISCUSSION

We have studied two rat tumor cell lines which when injected into the lumbar intrathecal space of rat produce different patterns of tumor growth within the meninges. The 9L gliosarcoma frequently formed a large focal mass that compressed the spinal cord. It invaded the Virchow-Robin spaces but only minimally infiltrated the parenchyma of the CNS. In contrast, Walker 256 carcinosarcoma grew within the leptomeninges in a discontinuous multifocal fashion and extensively invaded the CNS parenchyma.

Both tumors produced progressive weight loss and anorexia in the animals. The reason for the weight loss is unknown and may relate to progressive paralysis of the animal. It has been suggested that cancer-induced cachexia and anorexia are due to a modification of hypothalamic function (15). Of particular interest therefore was infiltration of the hypothalamus by Walker 256 carcinosarcoma cells. However, animals with 9L gliosarcoma tumor also lost weight although hypothalamic infiltration was not seen. In both groups of animals with tumors the hot plate response time increased as the tumor progressed but for apparently different reasons. The hot plate response time measures a species-specific stereotypic behavior dependent on the integrity of descending systems through which A5/C fiber evoked information travels. The rats with 9L gliosarcoma tumor became paraplegic because of focal spinal cord compression and were unable to move to the stimulus. In contrast, rats with Walker 256 carcinosarcoma tumor were quite weak yet often still ambulatory as the tumor grew. It appeared that animals with Walker 256 carcinosarcoma tumor were unable to sense the heat and thus did not respond to the hot plate. Neither tumor produced a marked change in tail flick response time which reflects C fiber evoked activation of segmentally organized ventral root reflexes and remains intact in animals with spinal cord transection (13). Both groups of animals with tumors began to show failure of the placing and stepping and inclined plane tests in a time frame which correlated with progression of the tumor. The placing and stepping response is mediated by Aβ afferents while the inclined plane test gives an indication of coordinated motor function. The placing and stepping response, the inclined plane test, and weight loss appear to be useful early noninvasive tests for evaluating tumor progression. The hot plate response time, which showed a significant change only on the days immediately preceding death of the animal, is a less useful test for tumor progression.

The tumor models exhibited different characteristics of human meningeal carcinomatosis. In human meningeal cancer the tumor infiltrates the subarachnoid space, maximally over the base of the brain, and in the posterior aspect of the spinal cord (16). The more apparent infiltration is often found in the cauda equina and invasion into the CNS parenchyma commonly takes place via the perivascular Virchow-Robin spaces (7). The 9L gliosarcoma is a model for cases in which localized growth of tumor causes...

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spinal cord compression. The Walker 256 carcinosarcoma model with disseminated meningeal spread of tumor and little mass effect is quite similar to human meningeal carcinomatosis. In addition, the functional abnormalities exhibited by the Walker 256 carcinosarcoma rats are similar to the neurological deficits seen in patients with meningeal cancer.

There have been previous attempts to develop animal models for meningeal tumor involvement. Peterson et al. (17) and Varakis et al. (18) reported murine T-cell lymphoma lines that when injected i.v. produced visceral metastases and in some cases CNS disease by infiltration of the cerebral leptomeninges. However, because of extensive systemic disease the model cannot be used for studies of chemotherapy of meningeal tumors. Ushio et al. (19, 20) has reported a rat model for meningeal carcinomatosis using Walker 256 carcinosarcoma cells injected percutaneously into the cisterna magna. Difficulties with models using percutaneous implantation for brain tumor models are the possibility of trauma to the CNS (21) and erratic location of the tumor (22). Tumor cells can seed along the track of the needle used to inject the cells forming a tumor located partly outside the CNS. This could cause problems in studies of intrathecal chemotherapy for meningeal tumors.

In the models we have developed, tumor cells are deposited in the lumbar intrathecal space and grow within the meninges with infiltration of the CNS parenchyma as seen in human meningeal carcinomatosis. The models will be useful for investigating the antitumor effects of intrathecally administered cytotoxic drugs. The catheter used to inject tumor cells is left permanently in place and can be used to introduce drugs into the intrathecal space and to study the toxicity and antitumor activity of intrathecally administered agents. These studies will form the basis of a later report.

REFERENCES

Fig. 1. A, 9L gliosarcoma forming a solid mass and compressing the spinal cord. The tumor grows in a circumferential manner around the catheter (ca) used for tumor inoculation. Note the clear demarcation between the tumor and spinal cord white matter, without tumor infiltration; B, 9L gliosarcoma cells disseminated over the surface of the cerebellar folia surrounding a blood vessel. Note tumor infiltration along the Virchow-Robin spaces (arrows); C, infiltration of the meninges of a rat spinal cord by Walker 256 carcinosarcoma cells. Note tumor surrounding but not invading multiple roots of the cauda equina. Tumor cells can be seen invading the white matter of the spinal cord; D, invasion of the spinal cord white matter by Walker 256 carcinosarcoma cells. Tumor cells are hyperchromatic with eccentric nuclei. Arrowheads, edge of the spinal cord. Note the ill-defined tumor margin.
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