Intrathecal chemotherapy with ACNU in a meningeal gliomatosis rat model

T. Ken Yoshida, M.D., Ph.D., Keiji Shimizu, M.D., Athanasios Koulousakis, M.D., and Volker Sturm, M.D.

Departments of Neurosurgery, Köln University Medical School, Köln, Germany, and Osaka University Medical School, Osaka, Japan

Intrathecal administration of ACNU (1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride) had a remarkable chemotherapeutic effect in a rat model of meningeal gliomatosis. This effect was evaluated in rats with meningeal gliomatosis induced by an intracisternal inoculation of rat C6 glioma cells. The median survival time of the rats treated with a single dose of intrathecal ACNU (1 mg/kg) on Day 1 or Day 3 after tumor inoculation was significantly prolonged by 35.7% to 42.9% or 25.0% to 28.6%, respectively, as compared with that of the control animals. Meningeal gliomatosis rat models treated intrathecally with ACNU (1 mg/kg) 5 days after tumor inoculation or intravenously with ACNU (15 mg/kg) both failed to prolong the survival time of the animals. These findings suggest that intrathecal chemotherapy with a low dose of ACNU is effective in the early stages of meningeal gliomatosis, whereas intravenous chemotherapy with a high dose of ACNU is always ineffective.

KEY WORDS • meningeal gliomatosis • ACNU • intrathecal drug infusion • drug delivery • glioma • rat

Meningeal gliomatosis is characterized pathologically either by its multifocal nature or its diffuse infiltration of glioma cells into the subarachnoid space. Although meningeal gliomatosis has been believed to occur comparatively rarely, Arita, et al., reported that the incidence of meningeal gliomatosis is relatively high and that the prognosis of these patients has not been significantly modified to date. Systemic chemotherapy cannot achieve a drug concentration in the cerebrospinal fluid (CSF) sufficient to attain the cell kill that can bring about clinical response. Thus, the treatment of meningeal gliomatosis is limited to radiotherapy of the brain and spinal cord and/or the use of a limited number of antitumor drugs that can be administered directly into the subarachnoid space (intrathecally) or cerebral ventricles. In spite of the considerable efficacy of radiotherapy, it is known to have toxic effects on the brain that result in intellectual impairment even at low doses. Intrathecal chemotherapy for leptomeningeal neoplasms with drugs such as methotrexate, cytosine arabinoside, thiopetidine phosphoramide, bleomycin, or neocarzinostatin has proved effective. However, glioma cells are not as sensitive to these drugs as they are to the chloroethylnitrosoureas. It is, therefore, essential to design an alternative method for the treatment of meningeal gliomatosis.

Treatment with the chloroethylnitrosourea ACNU (1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea) has demonstrated a strong cytotoxic effect against brain tumors. Nevertheless, systemic administration of this drug has often proved ineffective in treating meningeal gliomatosis. This compound has the following pharmacological characteristics: 1) it crosses the blood-brain barrier (BBB); 2) its half-life is short in blood or CSF; and 3) its capillary transfer constant is high. Accordingly, from the viewpoint of local toxicity to the normal brain, these characteristics are considered to be advantageous for intrathecal chemotherapy. In order to study the feasibility of intrathecal chemotherapy with ACNU in the treatment of meningeal gliomatosis, experimental meningeal gliomatosis was treated intrathecally and intravenously with ACNU according to its pathophysiological stages. In this report, a remarkable efficacy of intrathecal treatment with a low dose of ACNU is de-
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described in a rat model of meningeal gliomatosis, and several pathophysiological problems associated with the treatment of meningeal gliomatosis are discussed.

Materials and Methods

Tumor Tissue and Animals

Male Wistar rats, each weighing approximately 100 gm, and the well-established rat C6 glioma cell line were used in these experiments. The C6 glioma cells were cultured in Eagle’s minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (50 U/ml), and streptomycin (50 μg/ml) at 37°C in a humidified atmosphere supplied with 5% CO₂.

Measurement of Intrathecal ACNU Toxicity

In order to assess the systemic and local toxicity of ACNU, the rats (in groups of 10 each) received an intrathecal injection of ACNU, 0.5, 1.0, or 2.0 mg/kg, at a constant rate of 0.1 ml/100 gm body weight or of equal volumes of drug-free diluent as a control. The survival time, changes in body weight, behavioral changes, and neurological signs of the treated and control groups of rats were recorded daily. In another group of rats, local toxicity of ACNU in the brain was pathologically studied. The rats treated intrathecally with ACNU at each dose (three rats in each group) were sacrificed 30 minutes after an intravenous injection of Evans blue dye (1 ml of 0.5% solution) on Day 1, 3, 5, 10, 20, or 30 after intrathecal ACNU administration. A leakage of Evans blue dye was examined macroscopically and microscopically. Histopathological examination was also performed on the specimens.

Meningeal Gliomatosis Model

Details regarding the present models have been published previously.21-24 Briefly, 0.1 ml of 1 × 10⁷ viable C6 glioma cells was transplanted percutaneously via a 27 gauge needle into the cisterna magna of the rats under ether anesthesia. These rats are referred to in the present study as “meningeal gliomatosis rats.” The viable cells were counted by the trypan blue dye exclusion method.

Administration of Intrathecal or Intravenous ACNU

Each group of 10 meningeal gliomatosis rats was treated intrathecally or intravenously with ACNU (0.1 ml, dissolved in 0.9% NaCl solution) at a dose of 1 or 15 mg/kg, respectively, on Day 1, 3, or 5 after tumor inoculation. The control group received injections of equal volumes of drug-free diluent. The percentage increase in life span (%ILS) was calculated from the median survival time (MST) as follows:

\[
\text{%ILS} = \frac{\text{MST (treated)} - \text{MST (control)}}{\text{MST (control)}} \times 100.
\]

All experiments were performed in duplicate. Dosages of intrathecal and intravenous ACNU were determined by both the toxicity test described herein and the results of our previous studies.24-26

Results

Toxicity of Intrathecal ACNU

Body weight changes in each group of rats and the number of rats that died due to acute toxicity of ACNU after intrathecal administration are illustrated in Fig. 1. The body weight of the rats in the control group and in the groups that received intrathecal ACNU at doses of up to 1.0 mg/kg displayed a constant increase. No loss of appetite, behavioral disorders, or neurological signs were observed in these groups. However, the group of rats treated with intrathecal ACNU at 2.0 mg/kg exhibited a rapid decrease in body weight to approximately 70% of baseline, and 60% of the animals died due to acute toxicity of ACNU. These animals showed various physiological and neurological signs, such as loss of appetite, agitation, a decrease in activity, a tendency toward violence, paresis, and paralysis.

In the group of rats treated with intrathecal ACNU at doses of up to 1.0 mg/kg, no extravasation of Evans blue dye, gliosis, or neuronal loss was observed for at least 30 days after drug administration. In the group treated with ACNU at a dose of 2.0 mg/kg, the subpial region of the brain was stained with Evans blue dye 3 days after drug injection into the ambient cistern, the hippocampal fissure, and the base of the brain. Neu-
Table 1: Efficacy of intrathecal ACNU (1 mg/kg) treatment in a meningeal gliomatosis rat model

<table>
<thead>
<tr>
<th>Day of Treatment*</th>
<th>Experiment No.</th>
<th>Median Survival Time (days)</th>
<th>Increased Life Span (% control)</th>
<th>p Value†</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Treated</td>
<td>Control</td>
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<td></td>
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<td>20.0</td>
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</tr>
<tr>
<td>2</td>
<td>14.5</td>
<td>14.0</td>
<td>10.7</td>
<td>NS</td>
</tr>
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</table>

* Day of Treatment = number of days after tumor inoculation.† Significance computed by Student’s t-test. NS = not significant.

Table 2: Efficacy of intravenous ACNU (15 mg/kg) treatment in a meningeal gliomatosis rat model

<table>
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<tr>
<th>Day of Treatment*</th>
<th>Experiment No.</th>
<th>Median Survival Time (days)</th>
<th>Increased Life Span (% control)</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

* Day of Treatment = number of days after tumor inoculation.† Significance computed by Student’s t-test. NS = not significant.

Discussion

Treatment of malignant brain tumors has advanced along with the development of new techniques of surgery, radiotherapy, chemotherapy, and immunotherapy. However, an effective countermeasure for the treatment of meningeal gliomatosis has not been established to date. It is, therefore, vital to develop a new therapy for this disease.

Treatment of Meningeal Gliomatosis

Treatment of meningeal gliomatosis is limited to conventional radiotherapy and chemotherapy. Although radiotherapy has proved to be effective, the problem of its neurotoxic side effects remains to be solved. The chloroethylnitrosourea ACNU has proved to be considerably effective on malignant brain tumors during the last decade because its pharmacological property permits it to cross the BBB. However, the parenteral administration of chloroethylnitrosoureas in the treatment of meningeal gliomatosis has failed to show any remarkable efficacy. The basis of this failure has been postulat-

![Fig. 2: Graphs showing Kaplan-Meier survival curves of meningeal gliomatosis rats treated with intrathecal ACNU on Day 1 (left), Day 3 (center), and Day 5 (right) after tumor inoculation. Each group of 10 meningeal gliomatosis rats was intrathecal (it) or intravenously (iv) treated with 0.1 ml ACNU at a dose of 1 mg/kg or 15 mg/kg, respectively, after an intracisternal inoculation of $1 \times 10^7$ C6 glioma cells. The control group received injections of equal volumes of drug-free diluent. N = the number of rats in each group.](image-url)

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ed as follows: 1) the differential sensitivity of clonogenic tumor cells against the cytotoxic agents; 2) the heterogeneity of the tumor cells; 10 and 3) the low drug concentration attained in the subarachnoid space. 11

With regard to delivery of drug to the tumor cells spread in the subarachnoid space, an intrathecal administration of the chemotherapeutic agents is generally considered to be a useful method.3,7,10,11 In order for the drugs to be applicable, the following pharmacological conditions must be met: 1) a low capillary transfer constant; 2) a rapid diffusion into the CSF; and 3) a slow rate of metabolism and degradation.3 The pharmacological profiles of ACNU are not always consistent with these characteristics because of its high capillary transfer constant and short biological half-life. Therefore, from a pharmacological standpoint, ACNU would not appear to be useful intrathecally. Nevertheless, the pharmacological dispositions of ACNU are important and of great advantage to the deterrence of the neurotoxicity of intrathecal chemotherapy. Considering drug delivery to the subarachnoid space and neurotoxicity, intrathecal chemotherapy with ACNU has been acclaimed as a new therapy for meningeal neoplasms.9,10

Toxicity and Pharmacokinetics of Intrathecal ACNU

Neurotoxicity, one of the major problems of intrathecal chemotherapy, eliminates most of the anticancer drugs from consideration for intrathecal application. In the current study, it was demonstrated that the animals could tolerate an intrathecal administration of ACNU at doses of up to 1 mg/kg and that neither pathological changes nor systemic toxicity were observed at these doses. It has also been reported that doses of up to 1.5 mg/kg were well tolerated in rats.12 Intrathecal administration of ACNU at the low dose (1 mg/kg) selected in the present study demonstrated remarkable efficacy in the early stages of meningeal gliomatosis, whereas intravenous chemotherapy with a high dose (15 mg/kg) of ACNU failed to show any efficacy. It is likely that an effective drug concentration was not obtained in the CSF4 with intravenous therapy or that the drug was inactivated before reaching the subarachnoid space,9,11 although the intravenous dose was 15 times greater than the intrathecal dose.

Blasberg, et al.4 reported that drug delivery into the brain tissue through the vertebral wall is remarkably low after administration of BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) or ara-C (beta-D-arabinosyl-cytosine) into the cisterna magna of a monkey. Accordingly, it seems to be difficult to attain a high concentration of the drugs in the parenchyma via intrathecal therapy in order to treat malignant brain tumors that have already deeply involved the parenchyma. However, as shown in this study, intrathecal administration of ACNU can prevent tumor growth in a meningeal gliomatosis model in its early stages. This is because an effective drug concentration was obtained by intrathecal administration, similar to that claimed by other investigators in pharmacokinetic studies of intrathecal ACNU administration in dogs9 and humans.10

Pathophysiology of Meningeal Gliomatosis

In contrast to the results in the early stages of meningeal gliomatosis (1 and 3 days after tumor inoculation), intrathecal chemotherapy with ACNU failed to show any efficacy in its late stage (5 days after tumor inoculation). In order to clarify this phenomenon, the pathophysiology of meningeal gliomatosis should be understood. We have previously studied the pathophysiology of meningeal gliomatosis in an animal model.25 Briefly, tumor cells inoculated into the subarachnoid space remained floating in the CSF, forming spheroids 2 days after tumor inoculation. On the 3rd day, the tumor cells began to invade the parenchyma through the perivascular space of the penetrating vessels, followed by a complete invasion into the parenchyma by the 5th day after tumor inoculation. Tumor growth is unexpectedly rapid in the subarachnoid space and, consequently, the tumor occupies the subarachnoid space, involving the parenchyma by Day 5 after tumor inoculation. Furthermore, no remarkable physical symptoms are observed in the early stages. This might explain why the diagnosis is rarely made early enough to treat clinical meningeal spread. For our preliminary study, we selected the treatment regimen based on these pathophysiological changes observed in an animal model, and the results of intrathecal chemotherapy described here demonstrate that a radical pathophysiological alteration takes place between Days 3 and 5 after tumor implantation.

Treatment Problems of Meningeal Gliomatosis

It appears possible to categorize the phases of meningeal gliomatosis into four stages in animal models, based on its symptomatology and pathophysiology as observed in our previous communication.24 According to this categorization, intrathecal chemotherapy with ACNU proved to be effective and superior to systemic chemotherapy in the early stages (1 and 2) of meningeal gliomatosis in rats, but not in the late stages (3 and 4). Considering these facts, we stress that the diagnosis should be made in the early stages and that the treatment should be designed according to the clinicopathological stage. Moreover, additional therapies, including a combination of chemotherapy, radiotherapy, and immunotherapy, are necessary in the treatment of the advanced stages of meningeal gliomatosis.

It should be kept in mind that tumor cells are apt to acquire resistance during chemotherapy.6,22,23,25-27 Although glioma cells are comparatively sensitive to chloroethyl nitrosoureas, the drug concentration required to attain an effective cell kill becomes considerably higher as the tumor cells acquire resistance.26 It is, therefore, difficult to reach an effective drug concentration in the tumor cells with systemic chemotherapy.10 Further studies using animal models are under way to determine the
most effective regimen and protocols of intrathecal chemotherapy.

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Address reprint requests to: T. Ken Yoshida, M.D., Ph.D., Department of Neurosurgery, Köln University Medical School, Joseph-Stelzmann-Strasse 9, 5000 Köln 41, Germany.