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Effects of lipid association on lomustine (CCNU) administered intracerebrally to syngeneic 36B-10 rat brain tumors

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Abstract

A syngeneic, intracerebral rat brain tumor model was developed and characterized and then used to evaluate the therapeutic enhancement of lipid-associated 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). The Fisher rat glioma cell 36B-10 (100,000–500,000 cells) was implanted intracranially to Fisher F-344 rats into the caudate nucleus. Animals treated with lipid-associated CCNU showed a 2- to 10-fold decrease in tumor size compared with animals treated with free CCNU, indicating that lipid association increases the therapeutic index of intracerebral CCNU treatment. Moreover, the syngeneic rat brain tumor model may be useful for evaluation of other therapeutic modalities.
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1. Introduction

Despite recent advances in neurological techniques of radiation treatment and chemotherapy, the prognosis of malignant gliomas remains unsatisfactory. Estimated median survival is less than 1 year, and less than 5% of brain tumor patients are expected to survive over 5 years [1]. Therefore, development of new treatment strategies and improved drug delivery techniques are urgently needed [2]. As radiation therapy could increase the risk of developmental delays in children, chemotherapy, after surgical intervention, is often recommended as a first-line therapy for brain tumors. However, the effectiveness of chemotherapy is limited by relatively short resident time and poor tumor penetration for most drugs used to treat malignant glioma. Specifically, it is well documented that systemic administration of the soluble forms of 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-nitrosourea (CCNU), routinely used for treating brain tumors, exhibits a dose-limiting toxicity of delayed myelosuppression and nephrotoxicity [3,4].

Recently, we developed a lipid-associated CCNU formulation that exhibits increased antitumor activity in vivo (i.e. by increasing circulation time and drug accumulation in tumor). After subcutaneous administration, we found therapeutic enhancement without exacerbating hematologic and neurologic side effects [5]. Lipid association has significantly reduced the decomposition ratio of CCNU, and treatment of primary CCNU in lipid association form has greatly reduced the growth of a brain tumor 36B-10 subcutaneously implanted in syngeneic Fisher F-344 rats [5]. The lipid formulation effect on CCNU is yet to be evaluated directly in brain tumor tissues.

A number of animal models in rats and mice used to develop and optimize therapeutic strategies to treat brain tumors have been described in the literature,

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and several of these reportedly allow monitoring of both pharmacokinetic and therapeutic assessments. Different glial cell lines, 9L, C6, T9, F98, RG2 (D74), RT-2, and CNS-1, are available for use in in vitro and in vivo rat studies [6]. Although these tumor models provide predictable and reproducible growth in rats, their usage to evaluate overall effectiveness of drug therapies is limited because of intense infiltration and early neurological deterioration and rejection, especially if survival is used as an endpoint. An additional syngeneic rat brain tumor model with high tumor take and consistent, localized, predictable tumor growth in the brain is needed to evaluate therapeutic effects. Therefore, we developed and characterized a syngeneic, intracerebral tumor model and then used it to evaluate the efficacy of lipid-associated CCNU.

2. Methods

2.1. Materials

The CCNU was kindly provided by the Drug Synthesis and Chemistry Branch of the Developmental Therapeutics Division of Cancer Treatment from the National Cancer Institute. The phospholipids, dimyristoylphosphatidyl glycerol (DMPG) and dimyristoylphosphatidyl choline (DMPC), were purchased from Syngena (Cambridge, MA). Other reagents were of analytical grade or higher.

2.2. Soluble and lipid-associated CCNU formulation

The preparation of CCNU encapsulation with lipid vesicles was performed by mixing 100 mg each of DMPC and DMPG (1:1 mol/mol) with CCNU to achieve a drug:lipid molar ratio of 1:3 [7]. Lipid and drug were mixed in 1 ml chloroform in a glass tube, and the solvent was subsequently evaporated under N_2 to produce a dry film, which was vacuum-desiccated for at least 30 min. The lipid with CCNU was resuspended in 1ml volume of sterile phosphate buffered saline, PH 7.4 composed of 8 g/l NaCl, 0.2 g/l KCl and KH_2PO_4, and 0.16 g/l Na_2HPO_4 to a final lipid concentration of 100 g/l. The mixture was then sonicated at room temperature for 20–30 min in a bath-type sonicator (Laboratory Supplies, Hicksville, NY) until translucent suspension of small unilamellar vesicles was obtained. The diameters of liposomes were determined by photon correlation spectroscopy (Malvern Zetasizer 5000, Southborough, MA) to be 75 ± 5 nm. Details of soluble and lipid-associated CCNU properties have been published by Bethune et al. [7]. Soluble CCNU dosage preparations were done right before drug inoculation and consisted of dissolving CCNU in a carrier solution of sterile 0.9% NaCl with 10% ethanol and 2% Tween 80.

2.3. Animals

Male and female Fisher F-344 rats, weighing 250–300 g, were obtained from Charles River Laboratories (Wilmington, MA). The rats were kept separately in a specific pathogen-free environment with free access to sterile laboratory pellets and water. All procedures were performed with an approved IACUC protocol.

2.4. Tumor 36B-10 cell inoculum

The ethylnitrosourea-induced Fisher F-344 rat malignant astrocytoma, 36B-10 cells originally harvested from rat brain, kindly provided by Alex Spence [8], were grown in tissue culture flasks in Waymouth's medium containing 10% fetal-bovine serum, 5% antibiotic/antimycotic, and 2 mM glutamine in a 5% CO_2 incubator at 37 °C immediately before inoculation. The 36B-10 cells in tissue culture flasks were collected by trypsin exposure for 2 min and then resuspended in Waymouth's medium and kept at 37 °C. Indicated numbers of cells were delivered stereotactically with a Hamilton syringe. The cells in injectae were suspended so that a consistent number of cells were injected into each animal.

2.5. Injection port and stereotaxic implantation of 36B-10 brain tumor cells

All rats were anesthetized with 80 mg/kg of ketamine HCl and 3 mg/kg of xylazine HCl intramuscularly. A sagital skin incision was made a few millimeters away from the midline over the anterior part of the brain to the left. The calvarium was exposed, and a burr hole was made. A plastic screw (2 mm in length, 2.15 and 0.71 mm in outer and center hole diameter, respectively) was placed at the following coordinates: 3 mm from the midline and 7 mm anterior to the vertical zero point [9]. After slow insertion of the needle through the hole to 6 mm depth, the indicated number of 36B-10 cells in 20 μl were injected into the caudate nucleus at a rate of 5 μl/min. A delay of 1 min was added before a gradual withdrawal of the syringe to minimize spread of the tumor cells to the injection canal or the surface of the brain. By injecting the cells directly into the caudate nucleus, contamination of the ventricles and CSF with cells was avoided, thereby greatly reducing early metastasis through this route to other parts of the brain. Finally, the skin was closed with a single nylon suture covering the injection port underneath, and the animals were allowed to regain consciousness under observation.

2.6. Drug treatment

A treatment regimen of soluble or lipid-associated CCNU in 20 μl volume was initiated to Fisher rats bearing brain glioma on day 4 post-tumor implantation. The single-dose study was done with treatment of 10 or 20 mg/kg free and lipid-associated CCNU ($n = 8$ rats for each treatment group)

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