Therapeutic Efficacy of Aldoxorubicin in an Intracranial Xenograft Mouse Model of Human Glioblastoma[1,2]

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Abstract

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor with a median survival of 12 to 15 months after diagnosis. Acquired chemoresistance, high systemic toxicity, and low penetration of the blood brain barrier by many anticancer drugs contribute to the failure of anti-GBM therapies. To circumvent some of these obstacles, we tested a novel prodrug approach to evaluate anti-GBM efficacy by utilizing serum albumin-binding doxorubicin (Doxo), aldoxorubicin (Aldoxo), which is less toxic, is released from albumin in an acidic environment and accumulates in tumor tissues. A human GBM cell line that expresses a luciferase reporter (U87-luc) was stereotactically injected into the left striatum of the brain of immunodeficient mice. Following initial tumor growth for 12 days, mice were injected once a week in the tail-vein with Aldoxo [24 mg/kg or 18 mg/kg of doxorubicin equivalents—3/4 maximum tolerated dose (MTD)], Doxo [6 mg/kg (3/4 MTD)], or vehicle. Aldoxo-treated mice demonstrated significantly slower growth of the tumor when compared to vehicle-treated or Doxo-treated mice. Five out of eight Aldoxo-treated mice remained alive more than 60 days with a median survival of 62 days, while the median survival of vehicle- and Doxo-treated mice was only 26 days. Importantly, Aldoxo-treated mice exhibited high levels of Doxo within the tumor tissue, accompanied by low tumor cell proliferation (Ki67) and abundant intratumoral programmed cell death (cleaved caspase-3). Effective accumulation of Aldoxo in brain tumor tissues but not normal brain, its anti-tumor efficacy, and low toxicity, provide a strong rationale for evaluating this novel drug conjugate as a treatment for patients afflicted with GBM.

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Abbreviations: GBM, Glioblastoma multiforme; Doxo, Doxorubicin; Aldoxo, Aldoxorubicin; HPLC, High-performance liquid chromatography; MTD, Maximum tolerated dose; TMZ, Temozolomide; BBB, Blood-brain barrier

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Introduction

Glioblastoma multiforme (GBM) is the most aggressive primary neoplasm of the central nervous system (CNS) accounting for approximately 60% of all primary brain tumors with 12,500 new cases diagnosed in the US annually [1,2]. Standard of care for newly diagnosed GBM remains a multimodal regimen consisting of surgical resection with concomitant daily temozolomide (TMZ) and radiation therapy, followed by adjuvant TMZ [3]. Nevertheless, the increase in median survival is only 2.5 months compared with individuals treated with radiotherapy alone [3,4]. Additionally, acquired chemoresistance is a major problem with this therapy. Essentially all patients develop recurrent or progressive disease after the initial therapy showing no response to repeated challenges with TMZ [5]. Currently, bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor, remains the only Food and Drug Administration approved drug as a single-agent for the treatment of patients with recurrent or progressive GBMs. Despite the encouraging beneficial effects seen in a number of clinical trials, this anti-angiogenic therapy has not produced the therapeutic responses initially envisioned [6,7]. Emerging evidence from both the clinical and laboratory studies suggests that GBM rapidly adapts to anti-vascular endothelial growth factor therapy leading to rapid tumor progression, and the patients who progress following bevacizumab treatment, poorly respond to bevacizumab-based combinations [7,8]. Thus, considering the overall failure of these approaches in the treatment of GBM, there is an urgent need for more effective therapies to achieve improved outcomes in newly diagnosed and recurrent GBM patients.

Chemotherapy is of limited use as treatment for GBM either because of acute systemic toxicities, or poor penetration of the blood-brain barrier (BBB) [9,10]. Doxorubicin (Doxo), an anthracycline antibiotic, is a highly effective therapeutic agent for the treatment of many malignant tumors, however, its dose-related systemic toxicity and lack of penetration through the BBB limit its use in the treatment of intracranial tumors [11,12]. Several different formulations of doxorubicin have been developed, including pegylated liposomal doxorubicin (Doxil) [13–15]. However, none of these agents showed activity in preclinical studies or in phase I/II clinical trials as a treatment option for brain tumors.

Aldoxorubicin (Aldoxo; CytRx Corporation), formerly known as INNO-206, is a (6-maleimidocaproyl) hydrazone conjugate of doxorubicin (Doxo), which binds rapidly and selectively to the Cysteine-34 position of circulating serum albumin after intravenous administration, and releases Doxo selectively at tumor sites because of the low pH of the tumor environment [16]. Once released intracellularly, the Doxo intercalates DNA, inhibits DNA synthesis, and induces apoptosis. Preclinically, Aldoxo has demonstrated superior anti-tumor activity relative to Doxo in tumor xenograft mouse models for breast, ovarian, pancreatic, and lung cancers [17,18]. More recently, Aldoxo has demonstrated activity against multiple myeloma cells *in vitro* and *in vivo*, as well as enhancement of the anti-tumor effect of bortezomib (velcade), a drug approved for the treatment of relapsed multiple myeloma [19]. A phase 1 study of Aldoxo demonstrated its safety and favorable clinical responses in a variety of tumor types [20]. Because of these encouraging results we examined the preclinical efficacy of Aldoxo against GBM using a mouse intracranial GBM model in which progression of the tumor growth, CNS invasion, as well as penetration of the drug into tumor and normal brain could be studied in a quantitative fashion.

Materials and Methods

Intracranial Implantation of U87-luc Glioma Cells in Mice

A human GBM U87MG subline, U87-luc stably integrated with a luciferase reporter gene [21], kindly provided by Dr. Daniel A. Vallera [22], was used for establishing intracranial xenograft GBM tumors. Female athymic nude mice, 6 to 8 weeks of age (Harlan Laboratories), were anesthetized with a ketamine/xylazine cocktail solution. Animals were secured in a Harvard Apparatus stereotaxic head frame, a 1-cm midline scalp incision was made, and 5×10^5 cells in 5 μl serum-free DMEM were injected into the left striatum (coordinates: 2.5 mm lateral and 0.5 mm posterior to the bregma) through a burr hole in the skull using a 10-μl Hamilton syringe to deliver tumor cells to a 3.5-mm intraparenchymal depth. The burr hole in the skull was sealed with bone wax and the incision closed using Dermabond. Tumor growth was monitored and measured via bioluminescence imaging *in vivo*. All experiments were performed in accordance with the Institutional Animal Care and Use Committee guidelines.

Drug Treatment of Mice

Aldoxorubicin (Aldoxo; CytRx Corporation, Los Angeles, CA) and Doxo (Sigma-Aldrich, cat # 44583) were prepared on each day of injection in sterile vehicle (10 mM of sodium phosphate, 5% of D-(+)-glucose, pH 6.4) at a concentration of 10 mg/3 ml and 3 mg/3 ml, respectively. The dosing formulations were stored at 4°C before injection and were injected within 1 hour of formulation. Both drugs were administered intravenously in a volume of 0.15 ml to achieve 75% of the maximum tolerated Aldoxo dose of 32 mg/kg per injection and Doxo dose of 8 mg/kg per injection for a 20-g mouse. The study consisted of 8 vehicle-treated control mice (group C), 8 Doxo-treated mice (group D), and 8 Aldoxo-treated mice (group A). Treatment was initiated 12 days after intracranial implantation of GBM cells. Vehicle or Aldoxo was administered for a total of six injections (i.e., 12, 19, 26, 42, 50, and 56 days after cell implantation). Doxo was administered intravenously for a total of only two injections (i.e., 12 and 19 days after cell implantation), because seven of the eight mice in this group died before the third injection.

In Vivo Imaging of Intracranial Tumors

Intracranial tumor growth was quantified by biophotonic imaging using a Xenogen IVIS 200 system (Xenogen, Palo Alto, CA). Mice were administered a 100-μl intraperitoneal injection of 30-mg/ml D-luciferin (PerkinElmer) suspended in DPBS (Gibco) 10 minutes before imaging as a substrate for the luciferase enzyme. Prior to imaging, anesthesia was induced with isoflurane gas by placing mice in the chamber of an XGI-8 vaporizer, and sustained by inhalation via nose cones inside the imaging chamber. Images were captured and quantified with Living Image 4.1 software based on equivalent regions of interest over the head. Image intensities were expressed as photons per sec/cm^2 per steradian.

Neurotoxicity and Morbidity Assessments

Neurotoxicity was assessed by signs of motor disturbances and/or imbalance, and morbidity by rapid weight loss, impaired mobility, decreased food intake, and signs of lethargy.

High-Performance Liquid Chromatography (HPLC) System and Conditions

The HPLC system used was an Agilent 1100 Series (Wilmington, DE) equipped with a scanning fluorescent detector with excitation

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