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Injectable Hydrogels for Localized Chemotherapy and Radiotherapy in Brain Tumors

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ABSTRACT

Overall survival of patients with newly diagnosed glioblastoma (GBM) remains dismal at 16 months with state-of-the-art treatment that includes surgical resection, radiation, and chemotherapy. GBM tumors are highly heterogeneous, and mechanisms for overcoming tumor resistance have not yet fully been elucidated. An injectable chitosan hydrogel capable of releasing chemotherapy (temozolomide [TMZ]) while retaining radioactive isotopes agents (iodine, [^{131}I]) was used as a vehicle for localized radiation and chemotherapy, within the surgical cavity. Release from hydrogels loaded with TMZ or ^{131}I was characterized *in vitro* and *in vivo* and their efficacy on tumor progression and survival on GBM tumors was also measured. The *in vitro* release of ^{131}I was negligible over 42 days, whereas the TMZ was completely released over the first 48 h. ^{131}I was completely retained in the tumor bed with negligible distribution in other tissues and that when delivered locally, the chemotherapy accumulated in the tumor at 10-fold higher concentrations than when delivered systemically. We found that the tumors were significantly decreased, and survival was improved in both treatment groups compared to the control group. Novel injectable chemo-radio-hydrogel implants may potentially improve the local control and overall outcome of aggressive, poor prognosis brain tumors.

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Introduction

Overall survival of patients with newly diagnosed glioblastoma (GBM) remains dismal at 16 months with state-of-the-art treatment that includes surgical resection, external beam radiation, and chemotherapy. The majority of GBM tumors recur locally despite targeting the tumor and its surrounding margin of the normal brain without distant failure. Necessary radiotherapy (RT) doses required for improved local tumor control exceeds normal brain tissue tolerance and increased RT necrosis. In addition, the blood-brain barrier has stymied the development of effective radiosensitizers and systemic chemotherapies. GBM tumors are highly heterogeneous, and mechanisms for overcoming tumor resistance have not yet fully been elucidated. Alternative therapeutic strategies for the treatment of GBM are therefore clearly warranted.

Localized RT offers a potential strategy for improved tumor-targeting precision while sparing normal tissues.[1,2] Traditional placement of radioactive brachytherapy seeds in the surgical cavity has proved difficult in achieving the desired homogeneity of RT dose distributed to margins due to irregularities in cavity shape. More recent efforts have used balloons and a catheter to deliver a radioactive source (Gliasite® Radiation Therapy System).[3]

Conflicts of interest: Dr. Azab receives research support from Verastem, Selexys, Karyopharm, Cell Works, Cleave Bioscience, Glycomimetics, Abbvie, and Vasculox and is the founder and owner of Targeted Therapeutics LLC and Cellatrix LLC; however, there has been no contribution of the aforementioned entities to the present study. Dr. de la Puente is a co-founder of Cellatrix LLC; however, there has been no contribution to the present study. Dr. de la Puente and Dr. Azab have a provisional patent application on the technology described in this manuscript. Other authors have no conflicts of interest to disclose.

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Implantation of iridium-192 and iodine-125 seeds and the balloon-based brachytherapy have shown activity in primary malignant gliomas.[4-8] However, randomized studies using interstitial radiation boosts have not demonstrated an improved outcome compared to standard therapy.[9] The use of an injectable biodegradable hydrogel, loaded with chemotherapy and RT, with the ability to fill the postresection cavity entirely regardless of its shape and degree of deformity should facilitate greater homogeneity of doses and may lead to improved outcomes.

In lieu of intravenous chemotherapy, treatment plans are increasingly turning to localized methods of delivery. Biodegradable carmustine wafers (Gliadel) placed in the surgical cavity after the removal of GBM have gained increased acceptance. Gliadel wafers loaded with carmustine were shown to significantly increase median survival versus placebo wafers in the treatment of GBM, with a diffusion capacity of about 2 mm.[10,11] The wafers are solid and thus do not entirely fill the postresection cavity. This can contribute to nonhomogeneous distribution of chemotherapy.

The goal of the present study was to test the feasibility of a novel delivery system for local chemoradiotherapy treatment of GBM. A biodegradable injectable chitosan hydrogel capable of releasing chemotherapy while retaining radioactive isotopes agents was used as a vehicle for localized radiation and chemotherapy within the surgical cavity. The study involved exploring new approaches of previous efforts of using brachytherapy techniques to treat central nervous system lesions and development of an efficient and highly controllable means of delivering localized chemotherapy to address and enhance the chemotherapeutic effectiveness across the blood-brain barrier.

Materials and Methods

Materials and Reagents

Unless stated otherwise, all materials were purchased from Sigma (St. Louis, MO). Chitosan (low molecular weight, 50,000-190,000 Da based on viscosity, Sigma Product #448869), acetic acid 99.7%, and glutaraldehyde (GA) solution 25% were used for the preparation of the chitosan hydrogels. Alginic acid sodium salt from brown algae (low viscosity 4-12 cP), sodium bicarbonate, and calcium chloride were used for the elaboration of microparticles. Fluorescein isothiocyanate (FITC) and bovine serum albumin (BSA)-FITC (BSA-FITC) were purchased from MP Biomedicals (Solon, OH) and used for evaluation of *in vitro* release. Volumex (iodine-131 [^{131}I] human serum albumin [HSA]) was purchased from Daxor Corporation (New York, NY). Doxorubicin and temozolomide (TMZ) were purchased from Selleckchem (Houston, TX). Corning Matrigel Basement Membrane Matrix Growth Factor Reduced (Corning, New York, NY) was prepared following manufacturer instructions for *in vivo* implantation.

Cells

Glioma (D54 and D54-GFP-luc) cell lines were a kind gift from Dr. Dinesh Thotala (Department of Radiation Oncology, Cancer Biology Division, Washington University in Saint Louis School of Medicine). All cell lines were cultured in Dulbecco's Modified Eagle's medium (DMEM) with F-12 Nutrient Mixture in a 1:1 ratio supplemented with 10% fetal bovine serum (FBS; Gibco, Life Technologies, Grand Island, NY), 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (CellGro; Mediatech, Manassas, VA). Before plating, cells were washed with phosphate-buffered saline (PBS, Corning CellGro; Mediatech, Manassas, VA), trypsinized with 0.05% Trypsin-EDTA 1× (Gibco, Life Technologies), spun for 5 min (1000 rpm), and resuspended in fresh DMEM:F12 media.

Animals and Tumor Development

Nude (Hsd: Athymic Nude-Foxn1nu) 5- to 6-week-old female mice (Harlan) were used for animal studies. Animal procedures were approved by the Institutional Animal Care and Use Committee at Washington University School of Medicine in St. Louis. Mice were anesthetized with ketamine/xylazine and injected subcutaneously with 150 μL matrigel at a final concentration of 6 mg/mL containing 1 million D54-GFP-luc cells into the right flank, as previously described.[12] After 10-14 days, tumors were palpable, and mice were stratified for treatments.

Preparation of Chitosan Hydrogels

Chitosan hydrogels were produced based on previously described methods[13] with the following modifications. Briefly, chitosan (1 g) was dissolved in 100 mL of 0.1 M acetic acid. While chitosan solution was being stirred, different GA solution concentrations (0.1%-5% w/v in water) were added. A hydrogel was immediately formed, and stirring was stopped thereafter. Chitosan hydrogels were allowed to stabilize for 4 h before characterization.

Cross-Linking and Shear-Stress Characterization of Chitosan Hydrogels

Cross-linking was measured by quantification of absorbance uptake of hydrogels and FTIR spectroscopy. Representative pictures of the chitosan hydrogels were taken 4 h after cross-linking to show their appearance. Then, cross-linked chitosan hydrogels' absorbance at 360 nm (SpectraMax i3; Molecular Devices, Sunnyvale, CA) was determined. FTIR was used to characterize the presence of specific chemical groups in the materials. Chitosan hydrogels cross-linked with GA were analyzed by FTIR spectroscopy using transmittance mode. FTIR spectra were obtained in the range of wave number from 4000 to 500 cm^{-1} during 32 scans (NEXUS 470 FT-IR, Smart Performer, Thermo Nicolet). The FTIR spectra were normalized by reducing the background noise of non−cross-linked chitosan hydrogels, and major vibration bands were associated with chemical groups.

In addition, chitosan hydrogels from varying GA concentrations were characterized by one of 3 appearance levels: liquid, semisolid, or solid. The injectability of chitosan hydrogels was analyzed by using shear-stress characterization. The shear-stress technique analyzed 4 mL of chitosan hydrogel contained in a 5 mL syringe. The syringe was loaded with 4 mL of pre−cross-linked hydrogels using a pipette (liquid hydrogels), a spatula (semisolid hydrogels), or small pieces by hand (solid hydrogels). A constant force was applied to the top of the syringe, and dispersing time of various amounts of hydrogel (1/4, 2/4, 3/4, and full volume) was measured. The results (amount of hydrogel dispersed vs. time × force) showed the shear-stress properties of the cross-linked chitosan hydrogels. A qualitative determination of injectability was performed by 10 properly trained participants following the previously described method.[14] The participants were asked to evaluate the injectability of 4-mL aliquots of each formulation in a syringe by rating injection difficulty and the formulation flow through the syringe, using an arbitrary score from 1 to 4. In particular, the arbitrary score for both parameters was defined as following:

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