

A novel polymer gel for the delivery of local therapies to intracranial tumors: *In vivo* safety evaluation

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Abstract: The treatment of intracranial malignancies is limited by the ability of systemically administered therapies to cross the blood–brain barrier. Royer resorbable matrix, or R-Gel, is a dextran polymer administered in liquid form via needle injection. Within minutes of preparation, the polymer forms a gel and subsequently solidifies, thereby conforming to the dimensions of the injection cavity. R-Gel can accommodate a wide variety of therapeutic agents that may provide new options for local treatment delivery. This preclinical study evaluates the neurotoxicity of R-Gel implanted in the rat brain. Fifteen rats underwent intracranial administration of R-Gel ($N = 9$) or saline ($N = 6$) were monitored for sys-

temic and neurotoxicity, and sacrificed at pre-determined time points. Animals that received the R-Gel injection demonstrated no behavioral changes or weight loss. Histopathologic analysis revealed an inflammatory response in both groups on day 3 and day 7 after implantation, which resolved by day 42. These results suggest that intracranial R-Gel is well tolerated. Therapeutic studies of chemotherapy-complexed R-Gel are underway. © 2011 Wiley Periodicals, Inc. *J Biomed Mater Res Part A*: 99A: 479–484, 2011.

Key Words: glioma, polymer, local therapy, interstitial chemotherapy, brain tumor

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INTRODUCTION

Despite recent therapeutic advances, high-grade gliomas continue to have poor clinical outcomes. Due to the infiltrative nature of these tumors, complete resection is rarely achieved; over 80% of glioblastomas recur within two centimeters of the resection cavity.^{1,2} To address this clinical course, the primary management of glioblastoma has focused on local therapies. Surgical resection, followed by external beam radiation therapy to the surgical cavity and surrounding tissues, served as standard therapy for years. Although the addition of temozolomide, an orally available alkylating agent, has improved clinical outcomes,³ the blood–brain barrier hinders the delivery of many medical therapies. The blood–brain barrier may become an even greater issue in the era of molecular targeted therapies, many of which are substantially larger and less lipophilic than conventional chemotherapeutics.

Accordingly, a number of local delivery methods have arisen. The most widely used local approach is the biode-

gradable BCNU (carmustine) polymer wafer (Gliadel).⁴ At the time of surgery, up to eight 1.5-cm Gliadel disks are placed along the walls of the resection cavity. Gliadel has been shown to be safe and effective in patients with newly diagnosed and recurrent glioblastoma.^{5–7} In a randomized, double-blind, placebo-controlled clinical trial of adults with newly diagnosed glioblastoma, median overall survival was 13.8 months among patients receiving Gliadel, compared with 11.6 months for a placebo polymer wafer (HR = 0.73; 95% CI = 0.56–0.95), providing proof of principle for interstitial chemotherapy.^{7,8}

Nevertheless, as currently formulated, chemotherapy-impregnated wafers have certain shortcomings. Some of the Gliadel surface area may never come in contact with surrounding tissue; conversely, some of the adjacent brain may never come in contact with the Gliadel wafer. *In vivo* pharmacokinetic studies have shown that Gliadel wafers release their carmustine content over a relatively brief period of approximately 2–3 weeks.⁹

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(a)



(b)



(c)

FIGURE 1. R-Gel polymer preparation. (a) Via a luer lock system, syringes containing adipic dihydrazide (the cross-linking agent) (left) and oxidized dextran (right) are mixed reciprocally. Active therapeutic ingredients, such as anti-neoplastic agents, would otherwise be added to the cross-linking agent syringe but were not used in the current experiments; (b) Immediately after mixing, the polymer remains a liquid; (c) Within 90 s of mixing, the polymer forms a gel and conforms to the configuration of the injection cavity. After approximately 120 s, the polymer solidifies. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The Royer resorbable matrix, or R-Gel (Royer Biomedical, Frederick, MD), may provide a number of advantages over a preformed rigid design. R-Gel, a dextran polymer administered in liquid form, solidifies *in situ* to conform to the contours of the injection cavity, thereby optimizing contact with surrounding tissue. In addition, R-Gel release kinetics may be potentially modified to deliver therapeutics

over an extended period of time. Given the unique environment of the central nervous system, we performed preclinical toxicity studies of intracranially administered R-Gel. In these studies, unloaded R-Gel polymer was evaluated, with plans to move forward with future trials of R-Gel loaded with anti-neoplastic therapeutic agents in the absence of neurotoxicity.

METHODS

Materials

R-Gel was provided by Royer Biomedical (Frederick, Maryland). Briefly, the R-Gel formulation is produced starting with USP dextran (M_w 70,000). The polymer is oxidized with sodium metaperiodate. Purification is accomplished with ultrafiltration, yielding a solution of oxidized dextran that contains 150 mg/mL of polymer with dialdehyde groups on 10% of residues. The cross-linking agent, adipic dihydrazide, is weighed and loaded into syringes as a solid.

The formulation is supplied in a two-syringe system. Syringe A, containing oxidized dextran solution, is attached to Syringe B, containing the adipic dihydrazide cross-linker plus excipients [Fig. 1(a)]. The contents are mixed by reciprocation (20 times in about 30 s). This action initiates a gelation reaction, shown in Figure 2. A hydrazide nitrogen is added to an aldehyde carbon atom, and the product subsequently dehydrates. Because the reaction proceeds at pH 6 or below, reaction of aldehyde groups with amines, which are charged at pH 6, is precluded. Under these conditions,

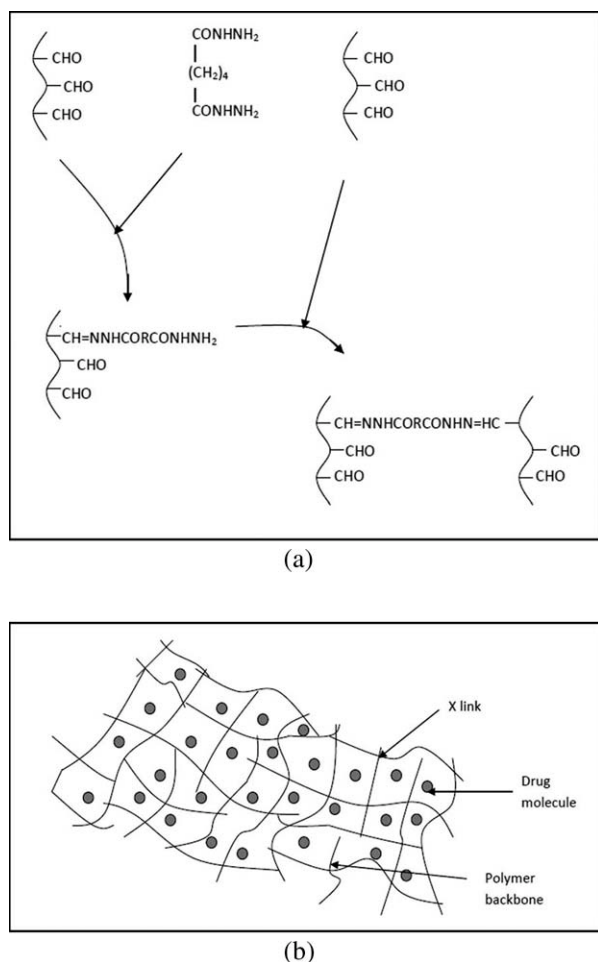


FIGURE 2. R-Gel polymer reaction. (a) On mixing of the dextran polymer and dihydrazide cross-linking agent, a hydrazide nitrogen is transferred to an aldehyde carbon atom. The product subsequently dehydrates; (b) Schematic of the resulting cross-linked polymer.

TABLE I. Summary of Histopathologic Findings

Animal	Hemosiderin	Necrosis	Macrophages
Control			
Day 3	0	2+	3+
Day 3	0	1+	3+
Day 7	0	1+	3+
Day 7	0	0	3+
Day 42	2+	2+	0
Day 42	1+	1+	0
R-Gel			
Day 3	0	1+	3+
Day 3	0	2+	3+
Day 3	0	2+	3+
Day 7	0	1+	3+
Day 7	0	1+	3+
Day 7	0	1+	3+
Day 42	3+	2+	0
Day 42	1+	2+	0
Day 42	2+	1+	0

Cumulative hemorrhage (hemosiderin), necrosis, and inflammation are semi-quantitatively scored 0 (absent), 1+ (mild), 2+ (moderate), or 3+ (severe).

the dihydrazides are not protonated and retain their nucleophilicity, thereby serving as effective cross-linking reagents. Within 90 s of mixing, a gel conforming to the injection cavity is formed [Fig. 1(c)]. Within 120 s, the R-Gel solidifies.

Animal experiments

Fifteen adult female Fisher 344 rats, 150–175 g, were used for these experiments. Six animals received control (saline) injections and nine animals received unloaded R-gel polymer matrix injections. Animals were maintained in standard animal facilities with free access to food and water. Following anesthesia induction, the animals' heads were shaved and prepped in sterile fashion with betadine. A midline incision was made and a burr hole drilled over the parietal region. Once the dura was exposed, animals were transferred to a stereotactic frame. A small amount of parietal brain tissue was removed with gentle suction. Subsequently, a needle was placed into the cavity at a depth of 3.5 mm and either 100 μ L unloaded R-Gel polymer matrix (treatment) or 100 μ L saline (control) was injected. The R-Gel polymer matrix was prepared at the time of injection by mixing the contents of the dextran solution and cross-linking agent syringes, then transferring their contents to the stereotactic needle for administration. After stereotactic injection, the needle was removed and the surgical area was washed with sterile saline. The wound was closed with sterile clips.

Animals were then monitored on a daily basis for signs of toxicity (e.g., ataxia, lack of grooming, delayed response to stimulus), and weights were obtained twice weekly. All aspects of this study were approved by the Johns Hopkins University Animal Care and Use Committee. On days 3, 7, and 42, animals were sacrificed (two each of the saline injected controls and three each of the R-Gel polymer matrix

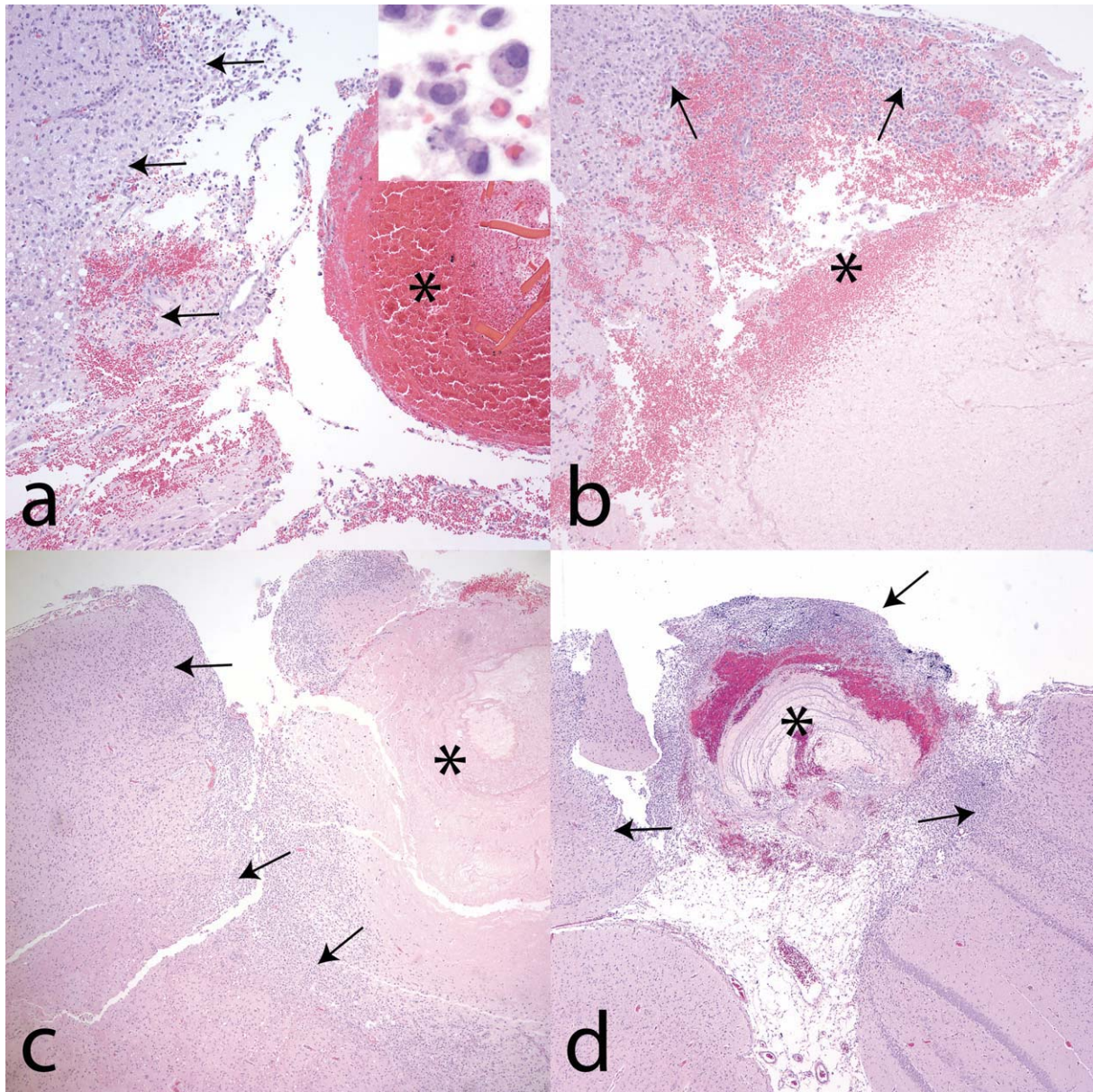


FIGURE 3. Operative site changes. Acute hemorrhage (asterisks) was present at the operative site of both saline (a) and R-Gel (b) injected animals sacrificed after 3 days (original magnification 40 \times). Surrounding inflammatory cells (arrows) included rare neutrophils and more prominent lymphocytes and macrophages (a, inset). In animals sacrificed 7 days after injection with saline (c) or R-Gel (d), central organizing hemorrhage was present (asterisk) along with surrounding chronic inflammation (arrows) now predominantly comprised of macrophages (original magnification 100 \times). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

injected animals), and their brains were harvested. Specimens were prepared by slicing the brain in a coronal plane at the level of R-Gel polymer matrix or saline injection. Brain slices were then formalin fixed and paraffin embedded. Representative sections adjacent to and more distant from the injection site were stained with hematoxylin and eosin.

Histologic analysis was performed by a neuropathologist (C.G.E.) blinded to animal treatment assignment. Changes around the surgical site and at distant sites in the brain, including the contralateral hemisphere, were evaluated. Cumulative hemorrhage (determined by hemosiderin deposits),

necrosis, and inflammation (predominantly macrophages) were semi-quantitatively scored 0 (absent), 1+ (mild), 2+ (moderate), or 3+ (severe). Note was made whether or not the injection site was visualized.

RESULTS

All 15 animals tolerated the intracranial injection procedure well. Neither the R-Gel- nor control-treated rats demonstrated clinical behavior suggestive of neurotoxicity. Animal weights remained similar throughout the course of the experiment. On day 0, control animals had a mean weight of

139.0 ± 3.5 g; R-Gel animals had a mean weight of 141.4 ± 4.4 g ($p = 0.28$). On day 7, control animals had a mean weight of 146.0 ± 4.5 g; R-Gel animals had a mean weight of 147.7 ± 6.4 g ($p = 0.67$). On day 42, control animals had a mean weight of 175.0 ± 0.0 g; R-Gel animals had a mean weight of 171.7 ± 7.6 g ($p = 0.60$).

Histopathologic findings are summarized in Table I, and representative images are shown in Figure 3. The operative site was clearly visualized in all but one case. Microscopic examination revealed acute and subacute changes in and around the operative site characteristic of any surgical resection, including hemorrhage, infiltration of macrophages, local reactive astrocytosis, and some cell death. Fresh hemorrhage (red blood cells) was seen in all specimens in and around the operative site at the day 3 and day 7 time points, and did not vary significantly between the control and R-Gel injected groups. Rare necrotic foci were present directly adjacent to the operative site, or in nearby structures. These foci were relatively small, and were sometimes associated with dystrophic calcification. No necrotic foci were observed in the contralateral hemisphere, or in sites distant from the implantation.

Macrophage infiltrates were also seen in and around the operative site in animals sacrificed in the first week after surgery. In addition to these local infiltrates, in some animals sacrificed on day 7, macrophages and lymphocytes could be found at a greater distance from the implantation site. In these cases, inflammatory infiltrates were often seen in white matter tracts. This was most prominent in one R-Gel implanted animal, in which inflammatory cells were present in the white matter tracts crossing the midline of the brain, but less pronounced contralateral inflammation was also noted in control animals. Fragments, possibly representing R-Gel polymer, were visualized in specimens from some R-Gel-treated animals sacrificed on days 3 and 7.

DISCUSSION

The Royer resorbable matrix (R-Gel) may have a number of properties favorable for use as a chemotherapeutic delivery mechanism. As a dextran-based polymer matrix, it is unlikely to be susceptible to degradation by proteolytic enzymes. In addition, by modifying polymer concentration, it may be possible to modulate drug release kinetics. In this study, we have demonstrated that intracranial administration of unloaded R-Gel is well tolerated in rat brains. No significant histopathologic differences were seen between animals administered R-Gel or saline, with the possible exception of more prominent inflammation in distant white matter tracts in the former group. In both the R-Gel and saline groups, the inflammatory response had subsided by day 42. No differences in animal behavior or weight were noted, and all animals lived to the scheduled dates of sacrifice.

Drug delivery to the central nervous system has challenged clinicians and researchers for decades. The blood-brain barrier limits the bioavailability of systemically administered therapeutics. Preferentially, drugs that are small, highly lipid soluble, and exhibit little binding to plasma

proteins are able to pass through the endothelial tight junctions of the blood-brain barrier. Unfortunately, many recently developed cancer therapeutics do not fit this profile. Accordingly, local treatment delivery remains an important consideration in neuro-oncology.

The polymer evaluated in this study offers a number of advantages. The liquid formulation permits conformation to the contours of a surgical cavity and, therefore, a high level of contact with surrounding tissue. As a liquid, R-Gel could potentially be readministered without the need for repeat craniotomy, but instead via stereotactic needle guidance. Indeed, these preclinical experiments demonstrate the feasibility of polymer preparation and delivery within the 2-min window before the R-Gel polymer, as currently formulated, solidifies. Nevertheless, many aspects of intracranial R-Gel administration remain to be determined, including *in vivo* safety, pharmacokinetic, and efficacy data of R-Gel complexed to chemotherapy or to other cancer treatments.

Principal limitations of this study include the inability to visualize directly the implantation, solidification, and retention of R-Gel in the brain. Hypothetically, injected R-Gel may have been lost into the subarachnoid space or through the burr hole. However, these events seem unlikely. First, the R-Gel was injected as a slow push, and no extracranial extravasation was observed. Second, the inherent viscosity of R-Gel may prevent migration (see Fig. 1). In terms of R-Gel degradation, little can be concluded from this initial safety study, as only unconfirmed pieces of gel were noted in the brain at early time points. Future studies are required to answer this question.

In conclusion, the Royer resorbable matrix (R-Gel), a dextran polymer administered in liquid form, appears biocompatible with the rat brain. In these experiments, R-Gel was not associated with clinical changes such as weight loss or behavioral changes, or with histopathologic evidence of neurotoxicity. Future research will focus on the therapeutic potential of this novel drug delivery system for the treatment of central nervous system malignancies.

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