Local Drug Delivery for Treatment of Brain Tumor Associated Edema

by

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Submitted to the Harvard-MIT Division of Health Sciences and Technology in partial fulfillment of the requirements for the degree of

Doctorate of Philosophy in Medical Engineering and Medical Physics at the MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Abstract

Brain tumor associated edema, a common feature of malignant brain neoplasms, is a
significant cause of morbidity from brain tumor. Systemic administration of corticosteroids, the
standard of care, is highly effective but can introduce serious systemic complications. Agents
that inhibit the vascular endothelial growth factor (VEGF) pathway, such as cediranib, are
promising alternatives, but are also associated with systemic toxicity as VEGF is essential for
normal physiological functions.

A miniature drug delivery device was developed for local drug delivery in rodents. It
comprises of a drug reservoir and a cap with orifice(s) through which drug is released. Drug
release kinetics is dependent on the payload, the drug solubility, and the surface area for
diffusion. Sustained releases of dexamethasone (DXM), dexamethasone sodium phosphate
(DSP), and solid dispersion of cediranib (AZD/PVP) were achieved. Employing the solid
dispersion technique to increase the solubility of cediranib was necessary to enhance its release.

Therapeutic efficacy and systemic toxicity of local drug administration via our devices
were examined in an intracranial 9L gliosarcoma rat model. Local delivery of DSP was effective
in reducing edema but led to DXM induced weight loss at high doses in a pilot study. DXM,
which is much less water-soluble than DSP, was used subsequently to reduce the dose delivered.
The use of DXM enabled long-term, sustained zero-order release and a higher payload than DSP.
Local deliveries of DXM and AZD/PVP were demonstrated to be as effective as systemic dosing
in alleviating edema. Edema reduction was associated with survival benefit, despite continuous
tumor progression. Animals treated with locally delivered DXM did not suffer from body weight
loss and corticosterone suppression, which are adverse effects induced by systemic DXM.

Local drug administration using our device is superior to traditional systemic
administration as it minimizes systemic toxicity and allows increased drug concentration in the
tumor by circumventing the blood brain barrier. A much lower dose can therefore be utilized to
achieve similar efficacy. Our drug delivery system can be used with other therapeutic agents
targeting brain tumor to achieve therapeutic efficacy without systemic toxicity.

Thesis Supervisor: Michael J. Cima, PhD
Title: David H. Koch Professor of Engineering
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CHAPTER 1

INTRODUCTION

1.1. SIGNIFICANCE

Brain tumor associated edema, a common feature of malignant brain neoplasms such as high-grade gliomas and metastases, is one of the major causes of morbidity from brain tumor [1]. It also correlates with poorer survival in patients with high-grade gliomas [2]. Malignant gliomas account for approximately 70% of the 22,500 new cases of malignant primary brain tumors diagnosed in the United States each year [3]. The median survival for patients with glioblastomas, the most common malignant glioma, is only 12 to 15 months despite optimal treatment [3]. Brain metastases develop in approximately 20% to 40% of cancer patients, and the subsequent median survival of these patients is generally less than 6 months [4]. Prognosis is grim in all these cases.

Corticosteroids are highly effective in treating brain tumor associated edema but can have adverse effects such as steroid myopathy, hyperglycemia, and neuropsychiatric syndromes that can impair quality of life [5]. Administration of corticosteroids has remained the mainstay of treatment due to no better alternative.

1.2. BRAIN TUMOR ASSOCIATED EDEMA

1.2.1. PATHOGENESIS

Brain tumor associated edema is vasogenic in nature, characterized by the breakdown of the blood-brain barrier (BBB). The BBB is composed of an intricate network of endothelial cells,
pericytes and astrocyte foot processes. It results from intact tight junctions, minimal endothelial pinocytosis, and lack of fenestration, restricting transport between the circulating blood and the central nervous system (CNS) [1].

The loss of integrity of the BBB in high-grade gliomas and brain metastases is attributed to increased endothelial pinocytosis, endothelial fenestrations and in particular, the opening of tight junctions [1]. Defective tight junctions are caused partly by the impaired ability of normal astrocytes to produce factors required to maintain a normal BBB [1], and compounded by the production of factors such as glutamate [6], leukotriene [7], and vascular endothelial growth factor (VEGF)-A [8] by the tumor cells. VEGF-A, commonly known as VEGF, has been shown to be upregulated in both malignant gliomas [9] and metastatic tumors [10], and its level in human gliomas correlates directly with the degree of malignancy [11]. There is a high degree of correlation between VEGF expression in high-grade gliomas and the occurrence of edema [12]. VEGF is secreted by both tumor and host stromal cells, and binds to its receptors, VEGF receptor (VEGFR)-1 and VEGFR-2, which are found mainly on the surface of endothelial cells. VEGFR-2 is the primary mediator of the downstream effects of VEGF in tumor angiogenesis. Binding of VEGF to its receptor stimulates the formation of gaps, fragmentations, and fenestrations in the brain endothelium, and leads to the degeneration of the basement membrane [13]. These structural changes lead to leakage of plasma fluid and proteins into the extracellular space of brain parenchyma, resulting in vasogenic edema (Figure 1.1). Edema is largely localized in the extracellular space of the white matter rather than the gray matter [14]. Brain metastases and primary brain tumor such as glioblastomas multiforme (GBM) produce up to 90 ml of edema fluid per day [1], overwhelming the sink effect provided by the ventricles and subarachnoid cerebrospinal fluid under normal physiological conditions [15].
Brain tumor associated edema exerts additional mass effect, often exceeding that induced by the tumor itself, and increases intracranial pressure within the rigid skull [15]. It also disrupts tissue homeostasis and reduces local blood flow, leading to debilitating neurological symptoms [15]. Severe brain edema can result in widespread ischemia, herniation, and ultimately, death.

1.2.2. STANDARD OF CARE - CORTICOSTEROIDS

Systemic corticosteroids are indicated in all patients with symptomatic brain tumor associated edema [14]. The efficacy of corticosteroids is well established. Significant decrease in tumor vascular permeability, reduction of intracranial pressure and improvement in neurological symptoms usually begins within 24 hours of treatment [17-19].

1.2.2.1. Mechanism of action

The mechanism of action of corticosteroids in controlling brain tumor associated edema is not fully understood, although corticosteroids have been used clinically since the 1950s based on empirical observations that they could significantly decrease mortality and morbidity in patients with brain tumors [20]. It has been suggested that corticosteroids reduce edema by
reducing the permeability of tumor capillaries [1, 21]. Corticosteroids diffuse through the plasma membrane and bind to the cytoplasmic receptor, allowing the steroid-receptor complex to move to the nucleus where it affects gene transcription and interacts with other transcription factors, mediating regulation of other signaling pathways [22]. Studies have shown that corticosteroids reduce edema by downregulating VEGF while upregulating angiopoietin-1, a strong BBB stabilizing factor [21, 23]. Corticosteroids can also cause dephosphorylation of the tight junction component proteins, occludin and ZO1, resulting in decrease in endothelial permeability [1].

1.2.2.2. Dose and schedule

Dexamethasone is the most widely used corticosteroid due to its long biological half-life (36 to 54 hours) and low mineralocorticoid (sodium retaining) activity [16]. A comparison of dexamethasone with other synthetic corticosteroids is shown in Table 1.1.

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Dose equivalent to 20 mg cortisol, mg</th>
<th>Relative anti-inflammatory activity (cortisol = 1)</th>
<th>Relative mineralocorticoid activity (cortisol =1)</th>
<th>Biological half-life, h</th>
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<tr>
<td>Hydrocortisone</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>8 – 12</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>5</td>
<td>4</td>
<td>0.8</td>
<td>12 – 36</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>4</td>
<td>5</td>
<td>0.5</td>
<td>12 – 36</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.75</td>
<td>25 – 30</td>
<td>&lt; 0.2</td>
<td>36 – 54</td>
</tr>
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</table>

A typical dexamethasone dosing regimen for patients with severe symptoms secondary to a brain tumor consists of a large 10 to 20 mg intravenous (IV) dose, followed by 4 mg four times per day or 8 mg twice per day [24-27]. Maintenance dosing comprising of oral or IV dexamethasone can range from 4 to 24 mg per day, in divided doses [28-30]. Patients with mild symptoms will benefit from a starting dose of 4 to 8 mg/day, considering the side effects of...
dexamethasone, while higher doses such as 16 mg/day are indicated in patients with more severe symptoms [24, 28, 31].

Tapering of dexamethasone after successful treatment of edema is necessary because of dexamethasone-induced suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis (Figure 1.2) [16]. The hypothalamus secretes corticotrophin-releasing hormone (CRH), which acts on the anterior pituitary. CRH stimulates the secretion of adrenocorticotropic hormone (ACTH), which in turn acts on the adrenal cortex. The adrenal cortex releases approximately 20 mg of cortisol per day in response to ACTH stimulation. Circulating cortisol will in turn inhibit the hypothalamus and the pituitary gland through a negative feedback cycle. Typical doses of corticosteroids used in treatment are supraphysiologic in nature, and will result in the inhibition of this cycle and suppression of its activity. Long term suppression of the axis can cause it to atrophy. Abrupt cessation or rapid tapering can lead to steroid withdrawal syndrome characterized by adrenal insufficiency, arthralgia, myalgia, and general symptoms such as headache and lethargy [16]. A regimen of twice daily dosing of dexamethasone, starting with 8 mg twice per day, tapering the dosage by 50% every 4 days and continuing with 2 mg twice per day until the last day of radiotherapy has provided good clinical results [14, 32]. Tapering should be slower, by 25% every 8 days in patients with extensive edema [16].

Chronic treatment with a low dose of 1 to 2 mg per day may be needed for some patients to have an acceptable quality of life [16]. One prospective cohort study found that 71% of malignant glioma patients were still on steroids at 3 months post radiotherapy [33]. A retrospective study of 138 patients showed that primary brain tumor patients required steroids for an average of 23 weeks post radiotherapy whereas secondary brain tumor patients required steroids for an average of 7 weeks [25]. There is much variability in corticosteroid prescribing.
practices. The dose and schedule of dexamethasone is often adjusted to the patient’s tolerance throughout the course of treatment in clinical practice.

![Diagram of corticosteroid-induced suppression of the HPA axis](image)

**Figure 1.2** Schematic representation of corticosteroid-induced suppression of the HPA axis.

### 1.2.2.3. Complications

Corticosteroids are associated with numerous adverse effects, which can contribute to overall treatment morbidity (Table 1.2). Gastrointestinal complications, steroid myopathy and opportunistic infections such as *Pneumocystis jirovecii* pneumonia (PJP) are of particular concern to brain tumor patients [34]. Patients on corticosteroid therapy are routinely treated with histamine H2 antagonists to lower the risk of gastrointestinal complications although no significant correlation between steroid usage and gastrointestinal complications was found [30]. Steroid myopathy is a common side effect with an estimated incidence between 2 and 20 percent, and causes significant morbidity in brain tumor patients [34]. PJP is a life-threatening opportunistic infection that occurs in immunocompromised hosts, such as patients receiving corticosteroids. The severity of complications usually correlates with the treatment duration (more than 3 weeks) and total dose [35]. Most complications resolve after cessation of therapy.
except for cataract and osteoporosis [5]. The frequency of these complications can be reduced by using the lowest possible dose [5].

<table>
<thead>
<tr>
<th>Table 1.2 Systemic side effects of corticosteroids (Adapted from Wen et al. and Kesari et al. [5, 36])</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
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<tr>
<td><strong>Neurologic</strong></td>
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<tr>
<td><strong>Dermatologic</strong></td>
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<tr>
<td><strong>Gastrointestinal</strong></td>
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<td></td>
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<tr>
<td><strong>Rheumatologic</strong></td>
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<tr>
<td><strong>Ophthalmologic</strong></td>
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<tr>
<td><strong>Endocrine/metabolic</strong></td>
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<tr>
<td><strong>Urogenital</strong></td>
</tr>
<tr>
<td><strong>Hematologic</strong></td>
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</tbody>
</table>

23
1.2.3. NOVEL DRUGS

The multitude of complications associated with corticosteroid therapy has sparked the interest in alternative treatments for brain tumor associated edema. Novel anti-edema agents that have been developed include corticotrophin-releasing factor, cyclooxygenase-2 (COX-2) inhibitors, and anti-VEGF agents.

1.2.3.1. Corticotrophin-releasing factor

Human corticotrophin-releasing factor (hCRF) is an endogenous neuropeptide that has been reported to reduce brain edema by acting directly on tumor vasculature, independent of the release of adrenal glucocorticoids [37]. It was effective in an animal model, but had limited efficacy in a phase I clinical trial [37, 38]. Corticorelin acetate, a synthetic analog of hCRF, was shown to significantly reduce steroid-related adverse events such as myopathy and Cushing syndrome, although the primary endpoint of at least 50% reduction in dexamethasone with stable or improved neurologic condition and Karnofsky performance was not achieved in a phase III trial [39]. The full clinical potential of this drug as a corticosteroid-sparing agent remains to be evaluated.

1.2.3.2. COX-2 inhibitors

Preclinical studies with COX-2 inhibitors suggest that they may be effective in treating brain edema. Rofecoxib, a selective COX-2 inhibitor, had a similar effect as dexamethasone in decreasing the diffusion of contrast agent into the brain parenchyma of glioma-bearing rats [40]. Another selective COX-2 inhibitor, SC-236, appeared to be as effective as dexamethasone in prolonging survival in a rat brain tumor model [41]. The effects of COX-2 inhibitors on brain tumor associated edema have yet to be examined in human clinical trials.
1.2.3.3. Anti-VEGF agents

Agents that target the VEGF signaling pathway are attractive therapeutic options for the treatment of brain tumor associated edema since VEGF plays an important role in the pathogenesis of brain tumor associated edema. They can normalize abnormal tumor vasculature, thus restoring the integrity of the BBB and reducing edema formation [42]. Two major classes of anti-VEGF agents are anti-VEGF ligands such as bevacizumab and aflibercept, and inhibitors of VEGFR such as cediranib (Table 1.3).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary Target</th>
<th>Other Targets</th>
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</thead>
<tbody>
<tr>
<td><strong>Anti-VEGF Ligands</strong></td>
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<tr>
<td>Bevacizumab</td>
<td>VEGF-A</td>
<td>None</td>
</tr>
<tr>
<td>Aflibercept</td>
<td>VEGF-A</td>
<td>VEGF-B, PIGF</td>
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<tr>
<td><strong>VEGFR inhibitors</strong></td>
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<td></td>
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<tr>
<td>Cediranib</td>
<td>VEGFR-2</td>
<td>All VEGFR subtypes, PDGFRβ, c-Kit</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>VEGFR-2</td>
<td>All VEGFR subtypes, PDGFRα and β, c-Kit</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR-2</td>
<td>VEGFR-3, B-Raf, PDGFRβ, c-Kit, Ras, p38α</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VEGFR-2</td>
<td>PDGFRβ, FLT3, c-Kit</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>VEGFR-2</td>
<td>EGFR</td>
</tr>
<tr>
<td>Vatalanib</td>
<td>VEGFR-2</td>
<td>All VEGFR subtypes, PDGFRβ, c-Kit</td>
</tr>
<tr>
<td>XL-184</td>
<td>VEGFR-2</td>
<td>c-Kit, c-Met, RET, FLT3, TIE2</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; FLT3, FL cytokine receptor; PDGFR, platelet-derived growth factor receptor; PIGF, placental growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Bevacizumab is a recombinant humanized monoclonal antibody against VEGF that works by binding VEGF with high affinity and inhibiting coupling of VEGF to its receptors, thus blocking the downstream effects of VEGF. It was granted approval by the Food and Drug
Administration (FDA) for treatment of recurrent GBM in May 2009 based on two phase II clinical trials [44]. The majority of the patients using systemic corticosteroids at baseline in the first trial experienced corticosteroid dose reduction while receiving bevacizumab, suggesting that bevacizumab may have corticosteroid-sparing effect [45, 46]. Edema was decreased in 50% of the patients, and 58% of the patients receiving corticosteroids at the start of therapy were able to reduce their corticosteroid doses by an average of 59% in the second trial [47]. The reduction in edema was apparent as early as 18 days after initiating treatment with bevacizumab in an early study [48].

Another anti-VEGF ligand is aflibercept, also known as VEGF Trap. It is a recombinant fusion protein of the extracellular domains of VEGF fused to the constant region of immunoglobulin G1. It inhibits tumor angiogenesis by binding with high affinity to VEGF and placental growth factor (PIGF). A phase II of aflibercept showed minimal activity in patients with recurrent malignant gliomas [49].

Inhibiting VEGFR by small molecule tyrosine kinase inhibitors (TKIs) is another approach to anti-VEGF therapy beside neutralization of the VEGF ligand by anti-VEGF ligands. Cediranib (AZD2171), an oral pan-VEGFR TKI, was demonstrated to induce vascular normalization within 24 hours and alleviates vasogenic brain edema in glioblastomas patients in a phase II clinical trial [50]. Corticosteroid usage was either reduced or discontinued in these patients as a result [50, 51]. Vatalanib, another pan-VEGFR inhibitor, decreased vascular permeability and cerebral blood volume in a phase I/II trial of patients with recurrent GBM [52]. Pazopanib, a TKI with multiple targets, reduced vasogenic edema in 32% of the patients in a phase II trial [53]. XL-184 also demonstrated its anti-edema effect and its corticosteroid-sparing
effect with at least 50% reduction in corticosteroid dose in 5 out of 14 patients, in a separate phase II study [54].

Treatment with anti-VEGF agents is associated with adverse effects, as with corticosteroids. This is expected since VEGF is essential for normal physiological functions. Common toxicities include hypertension, proteinuria, poor wound healing, and increased risk for venous and arterial thromboembolic events [55].

1.3. DRUG DELIVERY IN THE BRAIN

Progress has been made in drug discovery and development for treatment of malignant brain tumors, but clinical trials of new therapeutic agents have been disappointing, and prognosis for high grade gliomas has remained dismal. A significant part of the problem is the poor penetration of the systemically delivered drugs, which includes approximately 98% of all small molecule drugs and nearly 100% of large molecule therapeutics, across the BBB to achieve efficacious drug levels in the desired target [56]. Advancement in drug delivery may maximize efficacy of currently available drugs and improve treatment outcome. Drug delivery methods can be broadly classified into two categories – systemic delivery and local delivery. This section describes both traditional systemic delivery and local delivery strategies, with an emphasis on the latter.

1.3.1. TRADITIONAL SYSTEMIC DELIVERY

Drugs approved for treatment of brain tumor are usually administered systemically either by mouth or by intravenous injection. Lomustine and temozolomide are taken orally while bevacizumab is given intravenously. Systemic drug delivery to brain tumor is limited by multiple
factors, including the physical barrier posed by the BBB, active efflux transport mechanism, plasma protein binding and tumor interstitial pressure.

The BBB selectively restricts transport between the peripheral circulation and the cerebral parenchyma. The two major transport mechanisms across the BBB are passive diffusion and endogenous carrier- or receptor-mediated transport. Only lipid soluble small molecules with a molecular weight of less than 400 Da can readily penetrate the brain down their concentration gradient via passive diffusion [57]. Almost all other essential nutrients and metabolic substrates, such as glucose, amino acids, nucleosides, hormones, and growth factors, do not satisfy the criteria for passive diffusion, and have to rely on the carrier- or receptor-mediated transport systems to get into the brain [58]. After entering the BBB endothelium, molecules may be actively pumped back into the bloodstream by active efflux transporters, such as P-glycoprotein that is expressed in the luminal membrane of the endothelial cells [59]. These unique properties of the BBB serve to protect the brain from toxic substances, and to provide rigorous control of the brain microenvironment that is necessary for precise neural signaling, but also pose an insurmountable obstacle for most therapeutic drugs to reach efficacious levels in the brain parenchyma. Plasma protein binding and tumor interstitial pressure are other contributing factors to poor drug delivery to brain tumors. Many chemotherapeutics such as chlorambucil, vincristine, and paclitaxel bind more than 90% to plasma proteins, thus reducing the free fraction of drug in plasma that is available to cross the BBB [60]. Tumor interstitial fluid pressures can be more than 50 mmHg in peritumoral areas compared with 2 mmHg in a normal brain, generating a high pressure gradient which reduces drug diffusion into tumor and enhances drug diffusional loss into surrounding brain tissue [61].
The integrity of the BBB is often compromised in high-grade gliomas and brain metastases, as suggested by gadolinium enhancement of tumor during magnetic resonance imaging (MRI). However, vascular permeability in tumors has been shown to be heterogeneous. Vascular permeability is the greatest at or near the tumor core where the BBB is disrupted and sharply decreases at the tumor edge adjacent to normal brain parenchyma where the BBB remains relatively intact [62]. Clinical studies have confirmed that most antitumor agents accumulate to a greater extent and persist longer in the tumor core while drug levels are markedly lower or undetectable in the peritumoral region [63, 64].

Only a small fraction of a drug delivered systemically reaches the brain tumor while the bulk of it is lost to the rest of the body, which acts as a large sink [65]. Obtaining therapeutic drug levels will require administering a dosage much higher than what systemic organs can tolerate, resulting in systemic toxicity [66]. Maximum tolerated dose (MTD), rather than therapeutic dose, is often given due to the dose-limiting side effects of chemotherapeutic agents comprising of anemia, thrombocytopenia, leukopenia, stomatitis, nausea, and vomiting [67].

1.3.2. LOCAL DELIVERY

Local drug delivery in the brain can theoretically avoid the side effects of systemic exposure, and allow increased local drug concentration and prolonged drug exposure in the tumor by completely circumventing the BBB. High drug concentration in the tumor over a prolonged period is generally desirable for anti-tumor activity, thus local drug delivery may be more effective than traditional systemic approach. Some chemotherapeutic agents also only work in specific phases of the cell cycle. Maintaining substantial concentration of these agents for a prolonged period increases the chance of drug exposure during the sensitive phases of the cell
cycle [67]. Furthermore, local delivery may allow patients to maintain their quality of life by substantially reducing or eliminating side effects that are usually associated with systemic treatment.

Local drug delivery can also expand the drug arsenal against brain tumor. Agents that are intrinsically excluded from the brain by the BBB and/or unsuitable for systemic delivery (for instance, due to strong plasma protein binding) can realize their anti-tumor function through local drug delivery approaches. Although a wide range of drugs appears to be amenable to local drug delivery, a major limiting factor on drug choice and maximum drug dose is neurotoxicity.

The main disadvantages of local drug delivery are the need for invasive brain procedures and its inability to distribute drug to distant infiltrating tumors. However, local drug delivery approaches can be performed at the time of surgical tumor resection, which is the standard of care in cases of operable brain tumors, so no additional surgery is required. Local drug delivery remains a relevant method of drug delivery despite its limited drug distribution profile, since most brain tumors appear to remain localized to a single region of the brain and 90% of recurrences occur within 2 cm of the margin of the original tumor [68].

Table 1.4 Comparison of local delivery strategies

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Drug distribution</th>
<th>Refillability</th>
<th>Patient compliance</th>
<th>Ease of implement</th>
<th>Drug stability</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracavitary Infusion</td>
<td>Limited</td>
<td>Yes</td>
<td>Additional burden</td>
<td>Complex</td>
<td>Unstable liquid drug</td>
<td>Risk of infection/edema</td>
</tr>
<tr>
<td>CED</td>
<td>Enhanced but unpredictable</td>
<td>Yes</td>
<td>Additional burden</td>
<td>Complex</td>
<td>Unstable liquid drug</td>
<td>Risk of infection; Infusion-related edema</td>
</tr>
<tr>
<td>Implantable polymers</td>
<td>Limited</td>
<td>No</td>
<td>Easy</td>
<td>Simple</td>
<td>Stable solid drug</td>
<td>Risk of infection/edema</td>
</tr>
</tbody>
</table>
Some local drug delivery strategies used for treatment of brain tumor include intracavitary infusion, convection-enhanced delivery (CED), and implantable controlled-release polymer systems. Table 1.4 shows a comparison of these local delivery strategies.

### 1.3.2.1. Intracavitary infusion

The simplest approach to circumvent the BBB involves manual drug injection into the resected tumor cavity through a catheter.

![Image of Intracavitary delivery via an Ommaya reservoir into the tumor resection cavity. (Reproduced from Reulen [69])](image)

**Figure 1.3** Intracavitary delivery via an Ommaya reservoir into the tumor resection cavity. (Reproduced from Reulen [69])

The Ommaya reservoir is a device consisting of a fluid reservoir that is implanted under the scalp and connected to an outlet catheter, which is positioned in the tumor bed for intracavitary delivery (Figure 1.3). It offers the advantage of subsequent percutaneous access to the drug reservoir, enabling the delivery of intermittent bolus injections of drug. Drugs are injected percutaneously into the reservoir and delivered to the tumor by manual compression of the reservoir through the scalp [67]. Drug distribution within the tumor cavity relies on passive diffusion. The efficacy of a drug delivered by a diffusion based technique is dependent on its
diffusivity in the extracellular space of the brain, its uptake into tumor, and its rate of clearance [67]. Poor drug diffusion through tumor and brain parenchyma relative to drug clearance will result in treatment of only a small volume of tissue surrounding the drug source, leading to limited efficacy.

The Ommaya reservoir has been extensively used in clinical trials for intracavitary delivery of anti-tumor agents such as doxorubicin [70], mitoxantrone [71, 72], methotrexate [73], bleomycin [74, 75], interleukin-2 [76, 77], lymphokine-activated cells [77] and monoclonal antibodies labeled with cytotoxic radionuclides [72, 78]. Reservoir-related infection was rare. Local delivery using the Ommaya reservoir was generally well tolerated with minimal systemic and neurological toxicity, and no clinical deterioration; but its efficacy was controversial in the clinical studies, therefore hindering its use in clinical practice.

The Ommaya reservoir can be modified to achieve continuous drug release by covering the catheter with a semipermeable polyvinyl alcohol membrane [74]. Subcutaneously implantable pumps, such as the Infusaid pump and the Medtronic SynchroMed system, which are percutaneously refillable like the Ommaya reservoir, can also be used to deliver drug continuously over an extended period of time and at variable rates [67].

1.3.2.2. Convection-enhanced delivery

CED uses the pressure gradient generated from an infusion pump to drive bulk flow of infusate in the brain interstitium. A drug-containing fluid is delivered continuously to the targeted region in the brain via a catheter that is connected to an infusion pump. Using convection to supplement simple diffusion, CED can significantly enhance the spatial distribution of drugs beyond what is possible with diffusion alone, while producing drug
concentration in the brain that is orders of magnitude higher than that can be achieved by systemic administration [79, 80].

Convection relies mainly on the pressure gradient generated and is independent of the molecular weight of the delivered agent. Delivery of both small and large molecules, such as paclitaxel [81, 82] and targeted toxins [83-89], is therefore possible with convection. The drug distribution profile is constant during infusion but drops abruptly when infusion ends [90]. Drug diffusion, loss within capillaries, and drug metabolism determine the drug distribution over longer time periods after infusion [80].

Several factors, such as brain regions, catheter placement, catheter size, catheter design, rate of infusion and infusion volume, can affect the distribution of drug delivered by CED [90]. Regional differences in drug distribution exist between gray and white matter. Drug distribution tends to be spherical in the isotopic gray matter, while distribution in the anisotropic white matter preferentially occurs along the parallel fiber tracts [61]. Infusing into the white matter produces edema that appears similar to brain tumor associated edema [80]. Infusion-related edema, which is often present at a greater extent than brain tumor associated edema, can significantly affects the flow of the infused agent, and exacerbate morbidity [80]. Another major concern during infusion is backflow as it can lead to diminished drug delivery to the target tissues and spreading of the infused agent into unintended regions of the brain. Backflow along the insertion track into the subarachnoid space, in the case of cortical infusions, can cause subsequent widespread distribution of the agent via the circulating CSF, increasing the possibility of widespread neurotoxicity [91]. Drug leakage into the CSF, due to bad catheter placement, was reported to be responsible for chemical meningitis [81].
Additional challenges arise in the application of CED in the setting of brain tumors. The complex anatomy and heterogeneous physical properties of brain tumor-infiltrated tissue, coupled with the effect of tumor resection surgery and the presence of edema causes uneven and unpredictable drug distribution [91]. Dramatically higher interstitial fluid pressure in brain tumor compared to that in normal brain tissues creates a pressure gradient, which drives the infusate out of the tumor toward the normal surrounding tissues [61]. Rapid efflux of drug from brain tumor shortens drug residence time within tumor, leading to decreased drug penetration and drug activity in tumors [61]. Inadequate drug delivery due to CED has resulted in limited efficacy in clinical trials. Adverse effects, including surgical complications and significant neurological deterioration, further hinder the progress of CED in the clinical setting.

Osmotic micro-pumps have been utilized extensively for CED of therapeutic agents, such as angiogenesis inhibitors [92, 93], heat shock protein 90 (Hsp 90) inhibitor [94] and dexamethasone [95], in preclinical studies of treatment of brain tumor.

1.3.2.3. Implantable polymer systems

Surgically implantable controlled-release polymer systems can be placed in the tumor resection cavity for continuous local drug delivery. These systems can be matrix-based or reservoir based. Drugs are dissolved or dispersed throughout a solid polymer phase in a matrix system while drug is enclosed within a polymer in a reservoir system [96].

An example of a matrix-based polymer system is the Gliadel® wafer, the first and only FDA approved local drug delivery therapy for malignant glioma [97]. It is a biodegradable polyanhydride poly[bis(p-carboxyphenoxy-propane)-sebacic acid] (PCPP-SA) polymer impregnated with carmustine (1,3-bis(2-chloroethyl)-1-nitrosoourea or BCNU). Treatment with
Gliadel® wafers improved survival of newly diagnosed and recurrent malignant glioma patients by 2 months on average, which is considered significant survival benefit for this disease [98, 99]. Drug is released by means of both drug diffusion and polymer degradation at the surface [100]. Drug diffusion is dependent on the permeability of the matrix and the diffusional properties of the drug. Polymer degradation can be controlled to occur over days or years by altering the ratio of the monomers in the polymer matrix. Constant rate of drug release is achieved since breakdown of the polymer is limited to the surface in a process known as surface erosion [100]. No foreign body is left behind due to the biodegradable nature of the polymer, and the breakdown products are not mutagenic, cytotoxic or teratogenic [101]. Any drug can theoretically be incorporated into the polymer matrix as long as it does not react chemically with the matrix backbone [67]. The PCPP-SA polymer matrix has been used to deliver other chemotherapeutics, such as doxorubicin [102], taxol [103], and temozolomide [104], in preclinical models.

Non-biodegradable, inert, ethylene vinyl acetate copolymer (EVAc) is another polymer matrix that has been used for diffusion-controlled drug delivery. It has been used experimentally to deliver carmustine [105] and dexamethasone [106] locally within brain tumor, resulting in prolonged survival and reduction in brain tumor associated edema respectively.

Reservoir-based polymer systems offer the advantages of high drug payload and long-term, nearly zero-order drug release kinetics over matrix-based systems [96]. Local delivery of temozolomide via a reservoir-based, polymeric microcapsule device has led to improvement in survival in a rodent model [107]. Drug is released through the orifices in the device and any drug can theoretically be used with this device without the need for further drug modification. Silicone rubber capsules have also been used to deliver anti-tumor drugs for treatment of brain
tumors [108, 109]. The rate of drug release is governed by drug diffusivity within silicone rubber, thus only drugs with certain diffusivity can be delivered satisfactorily with this approach.

The effect of drug delivered by polymer systems is restricted to residual tumor cells at the resection margin due to limited drug distribution, which occurs mainly by diffusion. Drug concentrations generally fall exponentially away from the source and drug penetration into brain tissues is limited to a few millimeters [110]. The brain concentration of carmustine was found to decrease 90% at a distance of around 1 mm removed from the polymer [111]. An important consideration when translating polymer systems from preclinical to clinical studies is that diffusion distance in the human brain is more prohibitive than that in the rat brain since the weight of the human brain is a 1000-fold greater than that of the rat brain [112]. Other factors like drug elimination rate, brain peculiar extracellular matrix and tumor local environment can further hinder diffusion in brain parenchyma [113]. One approach to improve spatial drug distribution is to implant multiple polymers at the tumor site. Up to 8 Gliadel® wafers may be implanted in the tumor cavity depending on the size of the cavity [114].

Unreleased drug in solid form is usually protected from degradation in polymer systems while drug is usually stored in a liquid reservoir at body temperature in catheter systems and many drugs are not stable under these conditions [96]. Polymers are also not subject to clogging and blockage by tissue debris, which are major issues with catheter technology [67]. Another advantage of polymer systems is that they do not pose additional burden to the patient as no further intervention is usually required after implantation. A disadvantage of polymer systems delivery over catheter systems is that it is not refillable. Surgery is required if any changes in dose is necessary in polymer-based delivery.
1.4. THESIS OBJECTIVES

The overall goal of this work is to develop a local drug delivery device that delivers anti-edema agents in the brain for treatment of brain tumor associated edema with minimal systemic complications. Systemic administration of corticosteroids, the current standard of care, is highly effective but can cause serious adverse effects. Treatment outcome can generally be improved with novel therapeutic agents and better drug delivery to the brain. VEGF inhibitors, such as bevacizumab and cediranib, are novel anti-edema agents that have demonstrated their potential as alternatives to corticosteroids in clinical trials [47, 50, 51]. They can have systemic side effects like corticosteroids, though it is not clear how their side effects compared to those of corticosteroids. Local drug delivery in the brain is a promising approach that can avoid the side effects of systemic exposure and increase local drug concentration and exposure in the tumor by completely circumventing the BBB. Local administration of dexamethasone via an implantable polymer matrix system [106] and an osmotic pump [95] have shown efficacy in reducing edema. However, these local delivery technologies have limitations and systemic effects have not been thoroughly examined in these experimental studies. Systemic toxicity has been reported in both preclinical and clinical studies when very high doses of carmustine are locally delivered in the brain [115, 116]. Based on these findings, we hypothesize that optimizing local drug delivery can minimize systemic side effects while being as effective as systemic dosing in treating brain tumor associated edema. A reservoir-based, diffusion-controlled drug delivery device that can be easily used with any drug without further chemical modification and be easily altered to give varying drug release rates has been developed to test this hypothesis. Our specific objectives are to:
1) Identify valid animal model(s) for study of brain tumor associated edema (Chapter 2)

2) Develop a dexamethasone-loaded intracranial drug delivery device whose drug release rate can be easily optimized, characterize its release kinetics, and evaluate its efficacy and toxicity in valid animal model(s) (Chapters 3 and 4)

3) Develop an intracranial drug delivery device that delivers a VEGF inhibitor, characterize its release kinetics, evaluate its efficacy and toxicity in valid animal model(s) (Chapter 5)
CHAPTER 2

CHARACTERIZATION OF BRAIN EDEMA ANIMAL MODELS

2.1. INTRODUCTION

Accurate disease modeling is important in evaluating therapy efficacy. While no animal model can perfectly emulate all aspects of the human glioma, valid models should fulfill the following criteria: 1) clinically relevant glioma-like characteristics; 2) sufficient host survival time following tumor formation to permit therapy evaluation; and 3) similar response to conventional therapy as in human glioma [117].

Brain tumor animal models that have been used for the study of brain tumor associated edema are either xenografts or allografts. These tumors are easy to develop, highly reproducible, highly penetrant and develop rapidly but they lack their native stroma and may be genetically different from the original tumor due to selective pressure during cell culture [118, 119]. Human glioma xenografts implanted in immune-deficient mice may retain many of the genetic mutations but there is a lack of immunological interaction between tumor and host [118]. Allografts, on the other hand, have an intact immune system but they do not fully recapitulate the genetics and pathobiology of human glioma [118]. The 9L gliosarcoma, an allogeneic tumor model in syngeneic Fischer rats, has been the most widely used experimental model for brain tumor associated edema [21, 41, 106, 120, 121]. Other models that have been used include C6 glioma in rats [122], RG2 rat glioma in cats [123], VX2 carcinoma in rabbits [95], Walker 256 carcinosarcoma in rats [124, 125], CNS-1 rat glioma and U87 human glioma in mice [126]. The
use of these models remains controversial as they have been criticized for not recapitulating the features of human brain tumor.

Genetically engineered mouse (GEM) models with spontaneous tumor formation arising from mutations in the genes characteristic of human malignancy are attractive as they can recapitulate the complex tumor genetics, angiogenesis, tumor-host interactions and pathophysiology of human GBM [119]. However, most GEM models are not practical for therapy studies due to their low tumor penetrance, poor reproducibility, prolonged tumor formation latency, and inherent difficulty in diagnosing the tumor in a timely fashion [127]. No GEM model has been used for investigating brain tumor associated edema to date.

Zhu et al. has developed a GEM model which has a fully penetrant, rapid-onset high-grade malignant glioma phenotype with prominent pathological and molecular resemblance to human GBM, when the mutant (vIII) epidermal growth factor receptor (EGFR) is activated along with the loss of function of the tumor suppressor genes p16Ink4a/p19ARF and PTEN [94, 128]. More than 25% of all GBM patients are found with the combination of activated EGFR, loss of p16Ink4a/p19ARF and PTEN [94]. An important characteristic of this model is the ability to have spatiotemporal control over EGFRvIII expression using the Cre/loxP system. An adenovirus transducing Cre recombinase (Ad-Cre), which is injected in the basal ganglia of adult mice, mediates the removal of the floxed stop cassette and leads to localized somatic expression of EGFRvIII. Another appealing feature of this model is the presence of a Cre/loxP conditional luciferase reporter transgene that allows longitudinal, noninvasive tumor monitoring via bioluminescence imaging (BLI). Only cells exposed to Ad-Cre give rise to EGFR-dependent gliomas and express the bioluminescent luciferase reporter [94]. BLI output has been demonstrated to correlate linearly with glioma volume in this model [129].
The brain tumor, its associated edema and survival of this GEM model were characterized to determine the validity of this mouse model for the study of brain tumor associated edema. Tumor growth was longitudinally monitored using BLI and edema measurement was attempted using MRI. Edema was also quantified at the end of the study using wet/dry weight ratio. Survival curves were generated to evaluate if host survival time following tumor formation was sufficient for therapy evaluation. The well-established intracranial 9L gliosarcoma model was also characterized and compared with this GEM model.

2.2. MATERIALS AND METHODS

2.2.1. ANIMAL CARE

All the animal procedures were conducted in accordance with the Massachusetts Institute of Technology (MIT) Committee for Animal Care guidelines.

2.2.2. TUMOR INDUCTION IN TRANSGENIC MICE

Tumor induction procedures were adapted from Zhu et al. [94]. Adult compound Coll al\textsuperscript{tm2(CAG-EGFR*)Char} /tm2(CAG-EGFR*)Char; Cdkn2a\textsuperscript{tm1Rdp/tm1Rdp}; Pten\textsuperscript{tm1Hwub/m1Hwub}; Tg(CAG-luc)C6Char conditional transgenic mice, at least 3 months old, were anesthetized with an intraperitoneal (IP) injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). The scalp was shaved and the surgical site was prepped in a sterile fashion with betadine/ethanol scrub. The animals were mounted on a Stoelting stereotaxic frame with non-puncturing ear bars (Stoelting, Wood Dale, IL, USA). A midline incision was made and a 1 mm burr hole was drilled at the stereotactically defined location of the striatum (0.5 mm rostral to the bregma, 1.5 mm lateral to the midline, 2 mm depth to the pia surface). $1 \times 10^8$ adeno-CMV-Cre viral particles (Gene
Transfer Vector Core, University of Iowa, Iowa City, IA, USA) in approximately 1 μL was injected at a rate of 0.1 μL/min using a NanoFil 10 μL syringe (World Precision Instruments, Sarasota, FL, USA) mounted onto a Quintessential stereotaxic injector (Stoelting). After careful retraction of the syringe, the skin was sutured and the animal was allowed to recover.

### 2.2.3. 9L TUMOR IMPLANTATION IN RATS

Fischer 344 (F344) rats were obtained from Charles River Laboratories (Wilmington, MA, USA). 9L gliosarcoma was provided by Dr. Betty Tyler from Johns Hopkins University and propagated in the flanks of carrier F344 rats. Tumor was harvested from a carrier rat and cut into 1 mm³ pieces for intracranial tumor implantation. Animals were anesthetized with IP injection of ketamine (75mg/kg) and xylazine (7mg/kg). The scalp was shaved and the surgical site was prepped in a sterile fashion with betadine/ethanol scrub. A midline incision was made and the pericranium was carefully removed. A 3 mm burr hole was drilled over the left hemisphere with its center 3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture. The underlying cortex was gently aspirated to expose the sulcus, where the tumor piece was placed. The skin was sutured and the animal was allowed to recover.

### 2.2.4. BIOLUMINESCENCE IMAGING

Bioluminescence, a reporter of tumor size, was monitored noninvasively using the IVIS Spectrum imaging system (PerkinElmer, Waltham, MA). The mice were anesthetized with 2% isoflurane and their heads were shaved. D-Luciferin (PerkinElmer) was administered via IP injection at a dose of 165 mg/kg approximately 10 min prior to imaging. Signal intensity was quantified as photon count rate per unit body area per unit solid angle subtended by the detector.
Image analysis was performed using Living Image software.

2.2.5. MAGNETIC RESONANCE IMAGING

MRI was performed on 7T Varian MRI scanner (Agilent Technologies). All the animals were anesthetized with 2% isoflurane and placed prone in the cradle. The field of view used for mice and rats were 25 mm x 25 mm and 32 mm x 32 mm respectively. T2 weighted images (TR = 4,000 ms, TE = 40 ms, 1 mm slice thickness, 128x128 matrix size) were acquired to assess the development of tumor and its associated edema. Edema and tumor volumes were measured using OsiriX. T2 relaxation maps was also generated in Osirix from multi-echo spin-echo images (TR = 2,000 ms, TE = 10 ms, 16 echoes, 2 mm slice thickness, 128x128 matrix size) acquired. T2 maps were used to confirm the regions of tumor and its associated edema.

2.2.6. BRAIN WATER CONTENT

Animals were euthanized by carbon dioxide asphyxiation and their brains were collected. The brains were divided into ipsilateral and contralateral cerebrums. The cerebrums were weighed immediately to obtain the wet weight, and then dried at 100°C for up to a week, until a final dry weight was achieved. The tissue water content was calculated as water content = (wet weight − dry weight)/ wet weight, and expressed as a percentage.

2.2.7. HISTOLOGY

Brains were excised after the animals were euthanized, rinsed in cold phosphate buffered saline (PBS), and fixed in 10% neutral buffered formalin solution (Sigma-Aldrich, St Louis, MO, USA) at 4°C overnight. Fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained
with hematoxylin and eosin (H&E) by the Hope Babette Tang Histology Facility at MIT for histopathological analysis.

2.2.8. STATISTICAL ANALYSIS

All the data were expressed as mean ± standard error of the mean (S.E.M.). Paired Student’s t test was performed for brain water content comparison. \( P < 0.05 \) was considered statistically significant.

2.3. RESULTS AND DISCUSSION

2.3.1. CHARACTERIZATION OF TRANSGENIC MOUSE MODEL

\[\text{Figure 2.1} \ (A) \text{ Schematic illustration of the activation of the mutated (vIII) human EGFR in transgenic mice. A strong ubiquitous promoter (CAGGS) is positioned upstream of a transcriptional stop cassette (STOP) which is flanked by 2 loxP sites (blue triangles), followed by EGFR_{vIII} cDNA and a polyA signal sequence (purple rectangle). Localized expression of EGFR_{vIII} is conditional to the removal of the stop cassette, which is mediated by Ad-Cre.} \]

\[\text{Figure 2.1} \ (B) \text{ Experimental design. Tumor growth was monitored longitudinally by BLI post tumor induction by injection of Ad-Cre. When mice were determined to have bioluminescent signal output of more than } 10^6 \text{ p/s/cm}^2/\text{sr, MRI was performed to further assess tumor and its associated edema.}\]
Glioma was induced in the transgenic mouse model by injecting Ad-Cre in the basal ganglia. Ad-Cre mediates the removal of the floxed stop cassette, which is positioned between a strong ubiquitous promoter (CAGGS) and the mutated (vIII) human EGFR cDNA, leading to localized somatic expression of EGFR\textsuperscript{vIII} (Figure 2.1A). Tumor growth was monitored periodically by BLI. MRI was carried out in animals with bioluminescent signal output of more than $10^6 \text{p}/\text{s}/\text{cm}^2/\text{sr}$ to further assess tumor and its associated edema (Figure 2.1B).

![Image](image.png)

**Figure 2.2** Longitudinal monitoring of tumor growth by BLI. (A) Representative bioluminescent images for a mouse imaged 7, 14, 21, and 28 days after Ad-Cre injection. (B) Individual measurements of bioluminescent outputs from mice. The solid lines represent outputs from mice which had tumors, while the dotted lines represent outputs from mice which did not form tumor.
Our results from BLI suggest that this transgenic mouse model of glioblastoma is highly penetrant. 20 out of 23 mice (87%) injected with Ad-Cre showed continuous increase in bioluminescent output with time, suggesting that tumors were growing in these mice (Figure 2.2). Histology was used to confirm the presence or absence of tumors. Bioluminescent output generally increased exponentially and varied a lot among the mice. The time taken for the exponential growth of tumor to occur varied from 20 to 60 days post tumor induction. Most tumor-bearing mice suffered from poor body conditions and died or had to be euthanized after the tumors grew exponentially. The survival of these mice ranged from approximately 3 to 10 weeks (Figure 2.3), which was similar to that published by Zhu et al. [128]. A major concern with survival analysis was the development of lymphoma which led to decrease in survival. Homozygous null mice for the Ink4a/ARF locus reportedly were reported to develop lymphoma at a median age of 30 weeks [128]. Mice which were not manipulated at all had died from lymphoma, which was characterized by splenomegaly (Figure 2.4). 19 out of 23 mice (83%) used in this study were found to have enlarged spleens at the time of sacrifice. It might be difficult to distinguish if the mice had succumbed to glioma or lymphoma.

The huge disparity in tumor growth and survival among tumor-bearing mice made experiment planning, i.e. scheduling of MRI and treatment, very challenging. Not many mice were evaluated by MRI as a result. T2 weighted images and multi-echo spin-echo images were acquired (Figure 2.5). Hydrocephalus was common and tumor cores appeared to be necrotic in some mice. Edematous peritumoral regions were not detected on the T2 weighted images. We attempted to evaluate edema using T2 maps generated from and multi-echo spin-echo images, since T2 signal correlates with water content. It was hard to demarcate tumor regions on T2 maps, although T2 signal of tumor was found to be slightly higher than that of normal cerebral
cortex. Low T2 signal from tumor necrotic core might affect the accuracy of using T2 signal to evaluate edema. Some mice also did not survive the imaging sessions due to the long duration. MRI might not be an appropriate tool to assess edema in this transgenic mouse model.

Figure 2.3 Kaplan-Meier survival curves of mice with tumor (n = 14) and mice with no tumor despite Ad-Cre injection (n = 3).

Figure 2.4 Enlarged spleen, a sign of lymphoma, in a typical mouse.
Figure 2.5 Tumor and edema assessment using MRI. (A) Representative consecutive T2-weighted coronal MR images of a mouse. Hydrocephalus and tumor with a necrotic core were noted. (B) A T2-weighted image and its corresponding T2 map. Tumor region could be distinguished from normal brain tissue on the T2-weighted image, but not with certainty on the T2 map. The green highlighted regions of interest, normal cerebral cortex on the left and tumor on the right, were used to make T2 measurements on the T2 map. T2 signal of tumor was higher than that of cerebral cortex.

Figure 2.6 Water content of the ipsilateral cerebrum and contralateral cerebrum. The water content of the ipsilateral cerebrum was significantly higher than that of the contralateral cerebrum in tumor-bearing mice (*P = 0.016), while the water content of the ipsilateral cerebrum and the contralateral cerebrum were lower and not significantly different from each other in control animals. Data represent mean values ± S.E.M. (n = 3 for control, n = 14 for tumor).
Brain water content was used to assess edema at the time of sacrifice (Figure 2.6). Water content of both ipsilateral and contralateral cerebriums of tumor-bearing mice was higher than that of control mice. The water content of the ipsilateral cerebrum was significantly higher than that of the contralateral cerebrum in tumor-bearing mice ($P = 0.016$), while the water content of the ipsilateral cerebrum and the contralateral cerebrum was not significantly different from each other in control animals that were not manipulated. Presence of hydrocephalus and invasiveness of tumor might explain the increase in water content of contralateral cerebrum.

**2.3.2. CHARACTERIZATION OF 9L RAT MODEL**

![Figure 2.7](image_url)

Figure 2.7 Characteristics of 9L gliosarcoma rat model. (A) Representative $T_2$-weighted coronal MR images of a rat imaged 5, 7 and 9 days after intracranial tumor implantation. Tumor (outlined by a white dotted line) and its associated edema (outlined by a black dotted line) can be seen on day 9. (B) Water content of the ipsilateral cerebrum of tumor-bearing rats was significantly higher than that of the control rats (****$P < 0.0001$). (C) Kaplan-Meier survival curve of rats with intracranial 9L gliosarcoma.
The intracranial 9L gliosarcoma rat model is more predictable and reproducible than the transgenic mouse model. Longitudinal monitoring using MRI showed that edema usually started to develop 9 days after tumor implantation (Figure 2.7A). Tumors were hard to distinguish on MR images before day 9 and were shown to grow exponentially after day 9. Water content of the ipsilateral cerebrum of tumor-bearing rats was significantly higher than that of control rats that were not manipulated (Figure 2.7B). Rats with brain tumors survived for 13 to 16 days post tumor implantation, with a median survival of 14 days (Figure 2.7C). Survival time after edema development was about 4 to 7 days, thus this model is appropriate for testing effects of short-term treatment but not long-term treatment of brain tumor associated edema.

2.4. CONCLUSIONS

Edema evaluation in the transgenic mouse model was challenging. We failed to measure edema with MRI, and brain water content measurement might be obscured by hydrocephalus and invasion of tumor across the midline. There was a huge variation in the time taken for the tumor to grow exponentially, making it hard to plan treatment and MRI sessions. MRI techniques were also limiting factors. Survival analysis of this mouse model could be complicated by the development of lymphoma, in addition. We therefore concluded that this transgenic mouse model was not appropriate for the preclinical study of treatment of brain tumor associated edema, although it bears resemblance to human glioma in many aspects. 9L gliosarcoma rat model is relatively more predictable and reproducible, thus subsequent in vivo therapeutic efficacy studies were carried out using the 9L rat model.
CHAPTER 3

LOCAL DELIVERY OF DEXAMETHASONE SODIUM PHOSPHATE

3.1. INTRODUCTION

Local drug delivery is a promising approach that can theoretically avoid the side effects of systemic exposure, and allow increased local drug concentration and prolonged drug exposure in the tumor by completely circumventing the blood brain barrier. High drug doses are generally desirable at the tumor site to achieve sufficient anti-tumor activity but systemic toxicity has been noted above a certain threshold. A dose escalation study was carried out in patients with recurrent malignant glioma to determine the maximum tolerated dose (MTD) of carmustine incorporated into the implantable polymers, in an attempt to improve the efficacy of the commercially available Gliadel®, which contains 3.85% of carmustine by weight [116]. Adverse events, such as brain edema, seizures and infection, were more prominent in patients given polymers with 20% carmustine by weight than in patients given lower doses; severe brain edema and seizures were reported when the dose was escalated to 28% [116].

Although systemic toxicity was not identified in a preclinical study where local delivery via polymer wafer incorporated with dexamethasone (DXM) was demonstrated to be effective in reducing edema [106], intracranial implantation of similar wafer in normal rats resulted in progressive weight loss over time, which was postulated to be a sign of systemic toxicity [130].

We hypothesize that optimizing DXM dosage in local delivery is important in achieving treatment efficacy while minimizing systemic side effects. A reservoir-based, diffusion-
controlled drug delivery device that allowed for any drug to be used without further chemical
modification and for drug release rate to be easily altered, was developed based on the
microcapsule device design of Scott et al [107]. We loaded this device with different payloads of
dexamethasone sodium phosphate (DSP), a highly water soluble, inorganic ester of DXM, to
obtain varying drug release rates. The in vitro and in vivo release profiles of devices with
different payloads were characterized. An in vivo study in an intracranial 9L gliosarcoma rat
model was conducted to assess the effects of local delivery via these devices on reducing edema
and systemic toxicity. Brain water content, measured by the wet/dry weight method, was utilized
to evaluate edema while body weight and DXM level in plasma were used as indicators of
systemic toxicity.

3.2. MATERIALS AND METHODS

3.2.1. MATERIALS

Dexamethasone sodium phosphate injection solution was acquired from Baxter
(Deerfield, IL, USA). Dexamethasone, USP and dexamethasone sodium phosphate powder, USP
was obtained from Spectrum Chemicals & Laboratory Products (New Brunswick, NJ, USA).
High-performance liquid chromatography (HPLC) grade acetonitrile, water, sodium acetate,
methylprednisolone and dichloromethane were purchased from Sigma Aldrich (St. Louis, MO,
USA). HPLC grade glacial acetic acid was procured from Fisher Scientific (Fair Lawn, NJ,
USA). Phosphate buffered saline (PBS), pH 7.4, was bought from Corning (Manassas, VA,
USA).
3.2.2. DEVICE FABRICATION AND ASSEMBLY

Drug delivery devices, made of biocompatible Vectra MT1300 liquid crystal polymer, were injection molded by microPEP (East Providence, RI, USA). Each device is comprised of a reservoir and a cap. A single hole, 100 μm in diameter, was laser drilled in each cap at the Massachusetts Institute of Technology (MIT)’s microsystems technology laboratories using a laser ablation system. Profile of every laser-drilled hole was inspected under a VK laser scanning microscope (Keyence Corporation, Elmwood Park, NJ, USA).

All the device parts were cleaned with 70% isopropanol before drug loading. DSP powder was packed into a reservoir, which was then covered with a cap at the top. Biomedical-grade epoxy was applied around the circumference of the cap to seal the cap to the reservoir. The epoxy was allowed to dry for at least 24 h before drug release was initiated by removing residual air trapped within the device using a vacuum system, while the device was submerged in sterile PBS.

3.2.3. IN VITRO AND IN VIVO DRUG RELEASE

Activated devices (n = 3 for each payload) were incubated at 37°C in PBS for in vitro drug release. PBS was replaced every 24 h to maintain approximate sink conditions. The amount of drug released in PBS was determined by HPLC.

Drug release rates in vivo were determined indirectly by subtracting the amount of drug left in the explanted devices from the original payload. Explanted devices were retrieved from rat brains at the end of the in vivo experiment on day 12 post tumor implantation, and their drug contents were dissolved in sufficient PBS for measurement using HPLC.
3.2.4. HPLC ANALYSIS

HPLC analysis was performed using an Agilent 1200 series system (Agilent Technologies, Wilmington, DE, USA) with the column temperature set at 30°C.

The HPLC method for determination of both DXM and DSP in \textit{in vitro} samples was adapted from Lamiable \textit{et al.} [131]. The assay was conducted using an Agilent Zorbax Eclipse Plus C18 column (75 x 4.6 mm, 3.5 μm) with a mobile phase of 58% 2 mM sodium acetate buffer (pH 4.8) and 42% acetonitrile ran at a flow rate at 1 mL/min, a detection wavelength at 246 nm, and an injection volume of 20 μL. Good linearity was achieved for both DXM ($R^2 = 0.9997$) and DSP ($R^2 = 0.9997$) in the concentration ranges of 1 μg/mL to 10 μg/mL and 1 μg/mL to 1,000 μg/mL respectively.

Blood samples were collected in heparinized tubes immediately after the animals were euthanized by carbon dioxide asphyxiation on day 12 post tumor implantation. Plasma was obtained by centrifuging heparinized blood at 4°C, 1,300 g for 10 min, and stored at -80°C until analysis. The internal standard (1 μg of methylprednisolone) and 1 mL of dichloromethane were added to 200 μL of the sample. The mixture was stirred for 15 min and centrifuged for 5 min at 2,600 g. The aqueous layer and creamy interface were discarded. The organic layer was transferred to a reaction vial, evaporated to dryness at 45°C under a stream of nitrogen and reconstituted with 120 μL of mobile phase. The HPLC method used for the plasma samples was similar to that used for the \textit{in vitro} samples except that the mobile phase was delivered in a gradient with the concentration of acetonitrile varying from 42% to 100% and then back to 42% in a 15 min run. A linear calibration curve with $R^2 = 0.9999$ was obtained for DXM in the concentration range of 0.1 μg/mL to 10 μg/mL.
3.2.5. **IN VIVO EXPERIMENT**

Male Fischer 344 (F344) rats, 200 – 300 g, were obtained from Charles River Laboratories (Wilmington, MA, USA). All the animal experiments were conducted in accordance with the MIT's Committee for Animal Care guidelines.

9L gliosarcoma was provided by Dr. Betty Tyler from Johns Hopkins University and propagated in the flanks of carrier F344 rats. Tumor was harvested from a carrier rat and cut into 1 mm³ pieces for intracranial tumor implantation. Animals were anesthetized with intraperitoneal (IP) injection of ketamine (75mg/kg) and xylazine (7mg/kg). The scalp was shaved and the surgical site was prepped in a sterile fashion with betadine/ethanol scrub. A midline incision was made followed by careful removal of the pericranium. A 3 mm burr hole was drilled over the left hemisphere with its center 3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture. The underlying cortex was gently aspirated to expose the sulcus, where the tumor piece was placed. The skin was sutured and the animal was allowed to recover.

5 days after tumor implantation, rats were randomly assigned to the following groups (n = 5 per group): 1) no treatment (control), 2) intracranial blank devices (PBS Device), 3) intracranial devices with an initial payload of 100 µg of DSP and a mean release rate of 7 µg/day (DSP Device 7 µg/day), 4) intracranial devices with an initial payload of 800 µg of DSP and a mean release rate of 36 µg/day (DSP Device 36 µg/day), 5) intracranial devices with an initial payload of 1,600 µg of DSP and a mean release rate of 50 µg/day (DSP Device 50 µg/day), 6) intracranial devices with initial payload of 2,400 µg of DSP and a mean release rate of 88 µg/day (DSP Device 88 µg/day), 7) daily IP injection of DSP at 1.644 mg/kg (DSP IP 411 µg/day*).

*411 µg/day is the dose for a 250g rat. Rats assigned to receive intracranial devices underwent a second surgery for device implantation. The incision was reopened, and the connective tissue
overlying the burr hole was displaced. The device was implanted within the tumor, with its orifices facing downwards and towards the tumor. Animals were euthanized 12 days after tumor implantation. Rats in the DSP IP group were euthanized 2 h after the last IP dose. Their body weights were recorded daily to monitor potential systemic toxicity.

3.2.6. BRAIN WATER CONTENT

Animals were euthanized by carbon dioxide asphyxiation on day 12 and their brains were collected. Tumors were excised by blunt dissection before dividing the brains into ipsilateral and contralateral cerebrums. Tumor, ipsilateral and contralateral cerebrums were weighed immediately to obtain the wet weight, and then dried at 100°C for up to a week, until a final dry weight was achieved. Tissue water content was calculated as water content = (wet weight – dry weight)/ wet weight, and expressed as a percentage.

3.2.7. STATISTICAL ANALYSIS

All the data were expressed as mean ± standard error of the mean (S.E.M.). Brain water content, change in body weight and plasma DXM level were analyzed using ordinary one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparisons tests for pair comparisons among the different groups. $P < 0.05$ was considered statistically significant. All the statistical analyses were performed using Graphpad Prism.
3.3. RESULTS AND DISCUSSION

3.3.1. DRUG RELEASE CHARACTERISTICS

Figure 3.1 Drug delivery device. (A) Illustration of the device. (B) Schematics of how the device works. Some drug inside the reservoir dissolved in PBS with the influx of PBS after vacuum. Drug then diffuses across the orifice down a concentration gradient into the external solution. (C) Image of a laser-drilled orifice, 100 μm in diameter, taken under a laser scanning microscope.

The drug delivery device, as shown in Figure 3.1, is a diffusion-controlled device comprising of a reservoir of drug in its solid form and a cap with a 100 μm orifice through which the drug is released. Influx of sterile PBS into the reservoir through the orifice, after removal of residual gases within the device under vacuum, causes some drug to dissolve. Drug then diffuses across the orifice in the cap down a concentration gradient into the external solution. Drug release kinetics is determined by the drug payload, the solubility of the drug, the surface area of diffusion (i.e. surface area of the orifice and number of orifices) and the diffusion distance (i.e. distance between the solid drug and the exterior of the device).

DSP was chosen for its high solubility in water, estimated to be around 500 mg/mL. High drug solubility was thought to be desirable since it would theoretically lead to a high drug release rate, ensuring that sufficient dose was delivered locally for effective treatment. Our preliminary study showed that implantation of a device that had a 100 μm orifice and a maximum payload of
2,400 μg of DSP led to weight loss in rats (data now shown). As the smallest orifice that can be laser-drilled reliably is 100 μm in diameter, the only way to decrease the release rate of DSP from our device is to reduce the drug payload.

Figure 3.2A shows the release profiles of DSP from devices with different drug payloads in PBS, plotted as cumulative amount of drug released against time, over the course of 7 days. The amount of drug released decreased gradually with time for all the payloads. *In vitro* releases were fairly reproducible as the coefficient of variation was < 10% for all the release rates. A linear relationship was observed when plotting the cumulative release versus the square root of time for every device (Figure 3.2B). The slope and correlation of the linear regression line of best fit, in addition to the average release rate and coefficient of variation, for each payload are shown in Table 3.1. The slopes of the lines of best fit varied linearly with the drug payloads (Figure 3.2C). This linear relationship shows that the release kinetics of DSP from this drug delivery device can be predicted based on the drug payload.

The Higuchi model [132] could be used to describe drug release from devices containing payload above ~880 μg. The basic equation of the Higuchi model is:

\[
\frac{M_t}{A} = \sqrt{DC_o(2C_o - C_s)t} , \quad C_o > C_s \tag{3.1}
\]

where \(M_t\) is the cumulative amount of drug released at time \(t\), \(A\) is the surface area of diffusion, \(D\) is the drug diffusivity in the polymer carrier, \(C_o\) and \(C_s\) are the initial drug concentration and the solubility of the drug in the polymer respectively [132, 133]. Equation 3.1 can be expressed as:

\[
\frac{M_t}{M_\infty} = K\sqrt{t} \tag{3.2}
\]
where $M_\infty$ is the initial amount of drug loaded in the system, and $K$ is a constant reflecting the design variables of the system. An important condition is $C_o > C_s$ for the justification of the pseudo-steady state assumption. Our drug delivery system may behave according to the Higuchi model if drug diffusion in the reservoir is not fast, resulting in a depletion layer adjacent to the device cap (Figure 3.3) [134]. The device cap which has holes through which the drug is released, acts as a rate-limiting membrane. $C_s$ can be assumed to be the saturated concentration of DSP. A saturated solution is expected near the solid drug reservoir for drug payloads above $\sim 880 \mu g$ in the early phase of drug release.

A proportionality between the cumulative amount of drug released and the square root of time can also be derived from an exact solution of Fick's second law of diffusion for thin films of thickness $\delta$ under perfect sink conditions for $C_o < C_s$. The amount of drug released is given by:

$$\frac{M_t}{M_\infty} = 4 \left( \frac{Dt}{\delta^2} \right)^{1/2} \left\{ \pi^{-1/2} + 2 \sum_{n=1}^{\infty} (-1)^n \text{erfc} \left( \frac{n\delta}{2\sqrt{Dt}} \right) \right\}$$

(3.3)

An approximation of equation 3.3 for short times and $M_t/M_\infty < 0.60$ can be written as:

$$\frac{M_t}{M_\infty} = 4 \left( \frac{Dt}{\pi\delta^2} \right)^{1/2} = k'\sqrt{t}$$

(3.4)

where $k'$ is a constant. This equation might describe drug release from the device loaded with 100 $\mu g$ of DSP.
Figure 3.2 DSP release characteristics. (A) *In vitro* and *in vivo* release profiles of DSP from devices with different drug payloads. *In vitro* release was performed in PBS while *in vivo* release at day 7 was obtained indirectly by subtracting the amount of drug left in explanted device from the original drug payload. Data represent mean values ± S.E.M. (n = 3 for each payload). (B) Same *in vitro* release data from (A) plotted against the square root of time. Cumulative release was demonstrated to vary linearly with square root of time for each payload. The lines represent linear regression lines of best fit. (C) A linear relationship ($R^2 = 0.9$) was observed between the slopes of the lines of best fit and the drug payloads.
Table 3.1 *In vitro* release data

<table>
<thead>
<tr>
<th>Payload (µg)</th>
<th>Average release rate (µg/day)</th>
<th>Coefficient of variation (%)</th>
<th>Slope of line of best fit (µg day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>7</td>
<td>≤ 8.54</td>
<td>23 (R² = 0.995)</td>
</tr>
<tr>
<td>800</td>
<td>36</td>
<td>≤ 2.76</td>
<td>130 (R² = 0.993)</td>
</tr>
<tr>
<td>1,600</td>
<td>50</td>
<td>≤ 9.86</td>
<td>178 (R² = 0.999)</td>
</tr>
<tr>
<td>2,400</td>
<td>88</td>
<td>≤ 6.87</td>
<td>319 (R² = 0.995)</td>
</tr>
</tbody>
</table>

Figure 3.3 (A) Schematic illustration of the Higuchi model. (B) Theoretical concentration profile within the device reservoir where the drug transport is not fast. The device cap which has holes through which the drug is released, acts as a rate-limiting membrane. The solid line in each diagram represents the concentration gradient after time, t. The shift in concentration profile after interval of Δt is shown as a dotted line.

*In vivo* drug release rates were determined indirectly by measuring the amount of drug left in the explanted devices at the end of the *in vivo* experiment. The *in vivo* cumulative drug release on day 7 was calculated by subtracting the amount of drug left in the explanted device
from the original payload. The \textit{in vivo} cumulative drug release at the end of 7 days was found to be close to the \textit{in vitro} drug release for all the four payloads (Figure 3.2A), suggesting that \textit{in vitro} drug release could be a good approximate of actual \textit{in vivo} release.

### 3.3.2. BRAIN WATER CONTENT

![Graph showing water content of the tumor, ipsilateral cerebrum, and contralateral cerebrum.](image)

**Figure 3.4** Water content of the tumor, ipsilateral cerebrum, and contralateral cerebrum. Animals treated with systemic or local administration of DSP had significantly lower water content in the tumor and ipsilateral cerebrum than the untreated animals ($P < 0.001$). Animals that received PBS device are not significantly different from the untreated animals. *411 \(\mu\)g/day is the DSP IP dose for a 250g rat. Data represent the mean ± S.E.M. (\(n = 5\) per group).

Water content of tumor, ipsilateral cerebrum, and contralateral cerebrum were measured by the wet/dry weight method and compared across all the groups relative to the control group (Figure 3.4 and Table 3.2). Rats that received systemic and local DSP exhibited significantly lower ($P < 0.001$) water content in the tumor and ipsilateral cerebrum than the rats in the control and PBS device group at the end of the 7-day treatment. Edema reduction appeared to be independent of the route of administration and the dosage of DSP. There was no significant
difference among the groups treated with systemic and local DSP. Rats that received intracranial blank devices were not significantly different from the untreated rats, suggesting that the device alone did not worsen edema. The water content of the contralateral cerebrum was not significantly different among all the groups. Based on these results, DSP seemed to exert its effect on brain tumor associated edema only and not on normal brain tissues.

Table 3.2 Comparison of water content of tumor, ipsilateral cerebrum, contralateral cerebrum across all the groups. *** $P < 0.001$, **** $P < 0.0001$, relative to the control group. Data represent the mean ± S.E.M. (n = 5 per group). + 411 µg/day is the dose for a 250g rat.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Tumor (Water Content (%))</th>
<th>Ipsilateral Cerebrum (Water Content (%))</th>
<th>Contralateral Cerebrum (Water Content (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.4 ± 0.2</td>
<td>80.0 ± 0.1</td>
<td>78.9 ± 0.0</td>
</tr>
<tr>
<td>PBS Device</td>
<td>83.5 ± 0.2</td>
<td>79.9 ± 0.1</td>
<td>79.0 ± 0.2</td>
</tr>
<tr>
<td>DSP Device 7 µg/day</td>
<td>81.1 ± 0.4 ***</td>
<td>79.3 ± 0.1 ****</td>
<td>78.9 ± 0.1</td>
</tr>
<tr>
<td>DSP Device 36 µg/day</td>
<td>80.8 ± 0.4 ***</td>
<td>79.3 ± 0.1 ****</td>
<td>79.0 ± 0.0</td>
</tr>
<tr>
<td>DSP Device 50 µg/day</td>
<td>80.8 ± 0.4 ***</td>
<td>79.0 ± 0.1 ****</td>
<td>78.8 ± 0.1</td>
</tr>
<tr>
<td>DSP Device 88 µg/day</td>
<td>80.7 ± 0.3 ****</td>
<td>79.0 ± 0.1 ****</td>
<td>79.0 ± 0.1</td>
</tr>
<tr>
<td>DSP IP 411 µg/day</td>
<td>80.7 ± 0.4 ****</td>
<td>78.9 ± 0.1 ****</td>
<td>78.9 ± 0.1</td>
</tr>
</tbody>
</table>

3.3.3. SYSTEMIC DRUG EXPOSURE

DSP is converted to DXM by plasma enzymes. DXM levels in plasma samples, which were analyzed by HPLC, are shown in Table 3.3. Dose-dependent systemic drug exposure was observed with local delivery using our device. No DXM was detected in the plasma samples collected from animals treated with DSP-loaded devices that had average release rates of 7 µg/day and 36 µg/day. The amount of DXM present could be too low to be detected by the HPLC method used, which has a detection limit of 10 ng/mL. Only 2 out of 5 plasma samples
from animals that were given DSP-loaded devices with a mean release rate of 50 µg/day had detectable amounts of DXM while all the plasma samples from animals that received DSP-loaded devices with a mean release rate of 50 µg/day had measurable amounts of DXM. Our results demonstrated that DSP released from our intracranial devices leaked into systemic circulation at higher release rates. Average DSP release rate of not more than 36 µg/day appeared to be ideal for minimizing systemic drug exposure, which can lead to a cascade of systemic side effects. Peak DXM levels in plasma of rats in the DSP IP group, measured 2 h after the last IP dose, were significantly higher than the DXM levels measured in all the other groups (**** \( P < 0.0001 \)). Sustained, continuous, local drug delivery can prevent peak drug level in plasma which is commonly seen in traditional routes of drug administration.

### Table 3.3
Concentration of DXM in plasma samples. Data represent the mean ± S.E.M. (n = 5 per group). *411 µg/day is the dose for a 250g rat.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>DXM Concentration in Plasma (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSP Device 7 µg/day</td>
<td>undetectable</td>
</tr>
<tr>
<td>DSP Device 36 µg/day</td>
<td>undetectable</td>
</tr>
<tr>
<td>DSP Device 50 µg/day</td>
<td>46.9 ± 29.1</td>
</tr>
<tr>
<td>DSP Device 88 µg/day</td>
<td>194.8 ± 29.5</td>
</tr>
<tr>
<td>DSP IP 411 µg/day (^+)</td>
<td>2661.0 ± 124.5</td>
</tr>
</tbody>
</table>

### 3.3.4. DEXAMETHASONE-INDUCED WEIGHT LOSS

Systemically administered DXM has been shown to cause body weight loss in rodents [135-138]. The percentage body weight change between day 5 and day 12 post tumor implantation is plotted in Figure 3.5 to evaluate the body weight change induced by treatment.
Figure 3.5 Scatter plot of the percentage weight gain from day 5 to day 12 post tumor implantation, after 7 days of treatment. Dose-dependent weight loss was observed in animals treated with local delivery of DSP. Systemic DSP dosing also led to significant weight loss. The lines represent the mean ± S.E.M. (n = 5 per group). *** P < 0.001, **** P < 0.0001, relative to the control group. *411 μg/day is the dose for a 250g rat.

The mean weight change of the rats that received blank devices (1.2 ± 0.7%) was not significantly different from that of the untreated rats, suggesting that the device did not induce toxicity. Dose-dependent weight loss was observed in the animals that received intracranial DSP-loaded devices. Increase in DSP release rate was associated with greater weight loss. Rats that were given DSP devices with 1,600 μg of DSP, DSP devices with 2,400 μg of DSP and IP DSP injection experienced weight changes of -9.2 ± 1.9 % (P < 0.001), -19.4 ± 1.1 % (P < 0.0001), and -17.1 ± 0.9 % (P < 0.0001) respectively, which were significantly lower compared with that of the untreated controls (-0.5 ± 1.1 %). Rats that were given DSP devices loaded with 100 μg
and 800 μg of DSP, had weight changes of 0.2 ± 1.1% and -1.5 ± 0.9% respectively, which were not significantly different from each other and from that of controls. It, therefore, appeared that the mean DSP release rate should not exceed 36 μg/day in order to reduce edema without systemic toxicity. This result is similar to that obtained from measuring DXM concentration in plasma samples. Furthermore, the mean weight change of animals that were given the highest local dose of 88 μg/day was not significantly different from that of animals dosed systemically at 411 μg/day, implying that continuous local drug delivery can be as toxic as once-a-day systemic dosing even though a lower total dose was administered in local delivery.

3.4. CONCLUSIONS

We have developed a reservoir-based, diffusion-controlled intracranial drug delivery device that was capable of delivering DSP in a reproducible and predictable manner for treatment of brain tumor associated edema. Drug release was proportional to the initial payload of DSP. Good \textit{in vitro-in vivo} correlation was also observed. Local delivery of varying doses of DSP was demonstrated to be as effective as systemic dosing in reducing edema. However, local delivery of DSP can lead to dose-dependent systemic toxicity, as reflected by the concentration of DXM in plasma and the change in body weight. Local administration of DSP via our device, at an average rate of not exceeding 36 μg/day, was found to be effective in treating edema while avoiding systemic side effects.
CHAPTER 4

LOCAL DELIVERY OF DEXAMETHASONE

4.1. INTRODUCTION

Optimizing dosage in local drug delivery can potentially improve treatment outcomes by achieving efficacy with minimal systemic toxicity. We have shown in Chapter 3 that only a very little amount of dexamethasone sodium phosphate (DSP) needs to be locally delivered to treat brain tumor associated edema without systemic complications. A low drug release rate of DSP was previously achieved by using a low drug payload. Another approach to obtain low drug release rate was to use drug of low solubility in water. Dexamethasone (DXM) is an ideal drug candidate. The solubility of DXM in water (~50 µg/mL) is approximately 10,000 times less than the solubility of DSP (~500 mg/mL).

The release rate of DXM will primarily depend on the surface area of diffusion, i.e. the size and number of orifices in the device cap, instead of the initial payload. Varying drug release rates were obtained by changing the number of orifices, each 300 µm in diameter. The drug delivery device was micro-machined, instead of injection molded as in Chapter 3, to facilitate the fabrication of the orifices. We investigated the in vitro stability and release kinetics of DXM from this micro-machined intracranial drug delivery device. In vivo distribution of DXM in rat brain was also examined. A more comprehensive in vivo study than the one in Chapter 3 was carried out to evaluate the effects of local delivery, using the same intracranial 9L gliosarcoma rat model. 2 local doses were examined and compared in this in vivo study. Therapeutic efficacy was assessed by analyzing survival, measuring tumor and its associated edema using magnetic
resonance imaging (MRI), in addition to measuring brain water content by the wet/dry weight method. Body weight, DXM and corticosterone levels in plasma were used to measure systemic toxicity. Plasma DXM level was used as an indicator of systemic drug exposure while the concentration of corticosterone, the equivalent of cortisol in human, in plasma was used to assess hypothalamic-pituitary-adrenocortical (HPA) activity, which could be suppressed by DXM administration.

4.2. MATERIALS AND METHODS

4.2.1. MATERIALS

Dexamethasone sodium phosphate injection solution was acquired from Baxter (Deerfield, IL, USA). Dexamethasone, USP and dexamethasone sodium phosphate powder, USP was obtained from Spectrum Chemicals & Laboratory Products (New Brunswick, NJ, USA). High-performance liquid chromatography (HPLC) grade acetonitrile, water, sodium acetate, methylprednisolone and dichloromethane were purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade glacial acetic acid was procured from Fisher Scientific (Fair Lawn, NJ, USA). Phosphate buffered saline (PBS), pH 7.4, was bought from Corning (Manassas, VA, USA). Biocompatible polysulfone blocks and sheets were acquired from AmazonSupply (Seattle, WA, USA).

4.2.2. DEVICE FABRICATION AND ASSEMBLY

Drug delivery devices, each comprising of a reservoir and a cap, were micro-machined from biocompatible polysulfone blocks using the computer numerical control (CNC) micro
machining center (Cameron Micro Drill Presses, Sonora, CA, USA). The reservoirs and caps were micro-machined in bulk separately (Figure 4.1).

The reservoirs were fabricated in a polysulfone block by first pocket milling to create the insides of the reservoirs where the drug will be contained, and then profile milling to make the walls of the reservoirs with attached bridges to the surrounding block. The bridges held the machined reservoirs in place so that the block could be flipped and face milled to remove excess material. The bridges were then manually cut away to obtain the reservoirs.

The caps were manufactured from a polysulfone sheet taped to a sacrificial block. The polysulfone sheet was faced to the thickness of the cap before holes, 300 μm in diameter, were drilled. Individual caps were produced by profile milling and then carefully removed from the adhesive tape. Profile of every drilled hole was inspected under a VK laser scanning microscope (Keyence Corporation, Elmwood Park, NJ, USA) for quality control.

All the device parts were cleaned with 70% isopropanol before drug loading. DXM powder was packed into a reservoir, which was then covered with a cap at the top. Biomedical-grade epoxy was applied around the circumference of the cap to seal the cap to the reservoir. The epoxy was allowed to dry for at least 24 h before drug release was initiated.

4.2.3. IN VITRO DRUG RELEASE

Drug release was initiated by removing residual air trapped within the device using a vacuum system, while the device was submerged in PBS. Activated devices (n = 5 for each type of device) were incubated at 37°C in PBS for in vitro drug release. PBS was replaced every 24 h to maintain approximate sink conditions. The amount of drug in PBS was determined by HPLC.
A. Device reservoir fabrication

B. Device cap fabrication

C. Device assembly

Figure 4.1 Device fabrication and assembly. Fabrication of (A) device reservoir and (B) device cap were performed separately. Schematics of the micro-machining processes were shown. (C) Device assembly involved loading the drug into the reservoir, covering the reservoir with a cap, and then sealing the cap to the reservoir with biomedical grade epoxy.
4.2.4. DRUG STABILITY

The stabilities of solid DXM and DXM in PBS at 37°C were evaluated over the course of 10 days. A 1 mg/mL stock solution of DXM in ethanol was divided into aliquots such that each aliquot contained 10 µg of DXM. Ethanol was then evaporated to dryness at room temperature overnight, and the resultant solid was incubated at 37°C to assess the stability of solid DXM. A 10 µg/mL stock solution of DXM in PBS was divided into aliquots and incubated at 37°C to examine the stability of DXM in PBS. The aliquots (n = 3) were collected at different time points and stored at -20°C until analysis by HPLC. Aliquots containing DXM in PBS were thawed and analyzed directly with HPLC while solid DXM was dissolved in PBS before HPLC analysis.

4.2.5. HPLC ANALYSIS

HPLC analysis was performed on an Agilent 1200 series system (Agilent Technologies, Wilmington, DE, USA) with the column temperature set at 30°C. The method for measuring DXM in *in vitro* samples was adapted from Lamiable *et al.* [131]. The assay was conducted using an Agilent Zorbax Eclipse Plus C18 column (75 x 4.6 mm, 3.5 µm) with a mobile phase of 58% 2 mM sodium acetate buffer (pH 4.8) and 42% acetonitrile ran at a flow rate at 1 mL/min, a detection wavelength at 246 nm, and an injection volume of 20 µL. Good linearity was achieved for DXM (*R*² = 0.9997) in the concentration range of 1 µg/mL to 10 µg/mL.

4.2.6. ANIMAL CARE

Female Fischer 344 (F344) rats, 150 – 175 g, were bought from Charles River Laboratories (Wilmington, MA, USA). All the animal experiments were done in accordance with the Massachusetts Institute of Technology’s Committee for Animal Care guidelines.
4.2.7. DRUG DISTRIBUTION IN RAT BRAIN

Rats were randomly assigned to one of the following groups (n = 3 per group): 1) intracranial DXM-loaded device with 1 hole (DXM 1H Device), 2) intracranial DXM-loaded device with 9 holes (DXM 9H Device), 3) daily intraperitoneal (IP) injection of DSP at 1.64 mg/kg (DXM IP). Rats assigned to receive intracranial devices underwent a surgical procedure for device implantation. They were anesthetized with IP injection of ketamine (75mg/kg) and xylazine (7mg/kg). The scalp was shaved and the surgical site was prepped in a sterile fashion with betadine/ethanol scrub. A midline incision was made followed by careful removal of the pericranium. A 3 mm burr hole was drilled over the left hemisphere with its center 3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture. The dura was gently removed to expose the underlying cortex, where the device was implanted with its orifice(s) facing downwards and against the cortex. The skin was sutured and the animal was allowed to recover.

Animals were euthanized by carbon dioxide asphyxiation 14 days after start of treatment, and their brains were harvested immediately. Rats in the DXM IP group were euthanized 2 h after the last IP dose. Each brain was sectioned into 1 mm thick coronal slices using an Alto™ stainless stain brain matrix (Roboz Surgical Instrument Co. Inc., Gaithersburg, MD, USA). Each brain slice (~100 mg) was weighed, placed in ~0.2 mL of ice-cold PBS with ~100 mg of 0.5 mm zirconium oxide beads (Next Advance, Averill Park, NY, USA) in a micro-centrifuge tube, and homogenized for 3 min at the maximum speed setting using the Bullet Blender® (Next Advance). The brain homogenate samples were centrifuged at 4°C, 15,000 g for 15 min, and stored at -80°C until analysis. The concentration of DXM in plasma samples was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Neogen, Lexington, KY, USA) according to the vendor’s protocol. Samples were sufficiently diluted so that their DXM concentrations can be
read from the standard curve, which was plotted utilizing a 4-parameter logistic curve fit algorithm for the concentration range of 0.5 ng/mL to 10 ng/mL.

4.2.8. Efficacy Study

9L gliosarcoma was provided by Dr. Betty Tyler from Johns Hopkins University and propagated in the flanks of carrier F344 rats. Tumor was harvested from a carrier rat and cut into 1 mm³ pieces for intracranial tumor implantation. Animals were anesthetized with intraperitoneal (IP) injection of ketamine (75mg/kg) and xylazine (7mg/kg). The scalp was shaved and the surgical site was prepped in a sterile fashion with betadine/ethanol scrub. A midline incision was made followed by careful removal of the pericranium. A 3 mm burr hole was drilled over the left hemisphere with its center 3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture. The underlying cortex was gently aspirated to expose the sulcus, where the tumor piece was placed. The skin was sutured and the animal was allowed to recover.

9 days after tumor implantation, rats were randomly assigned to one of the following groups (n = 6 per group): 1) no treatment (control), 2) intracranial blank device (PBS Device), 3) daily IP injection of DSP at 1.64 mg/kg (DXM IP), 4) intracranial DXM-loaded devices with 1 orifice (DXM 1H Device), 5) intracranial DXM-loaded device with 9 orifices (DXM 9H Device). Rats assigned to receive intracranial devices underwent a second surgery for device implantation. The incision was reopened, and the connective tissue overlying the burr hole was displaced. The device was implanted within the tumor, with its orifices facing downwards and towards the tumor. Animals were checked twice daily and euthanized when found in poor body condition. Rats in the DXM IP group were euthanized 2 h after the last IP dose. The body weights of all the animals were recorded daily from day 9 onwards to monitor their health.
4.2.9. MAGNETIC RESONANCE IMAGING

MRI was performed on 7T Varian MRI scanner (Agilent Technologies). Rats were anesthetized with 2% isoflurane and placed prone in the cradle. T2 weighted images (TR = 4,000ms, TE = 40ms, 1mm slice thickness, 12 image slices, 128x128 matrix size, 32mm x 32mm field of view) were acquired to assess tumor and its associated edema on day 9 and on day 12 post tumor implantation. Tumor and edema volumes were measured using OsiriX imaging software.

4.2.10. BRAIN WATER CONTENT

Animals were euthanized by carbon dioxide asphyxiation when they were found in poor body condition. Their brains were collected immediately after sacrifice. Tumors were excised by blunt dissection before dividing the brains into ipsilateral and contralateral cerebrums. Tumor, ipsilateral cerebrum and contralateral cerebrum were weighed immediately to obtain the wet weight, and then dried at 100°C for up to a week until a final dry weight was achieved. The tissue water content was calculated as water content = (wet weight – dry weight)/ wet weight, and expressed as a percentage.

4.2.11. PLASMA DEXAMETHASONE AND CORTICOSTERONE

Blood samples were collected in heparinized tubes immediately after the animals were euthanized. All the animals were euthanized around the same time of the day, between 5pm and 7pm, to minimize the influence of daily fluctuations of corticosterone levels. Plasma was obtained by centrifuging heparinized blood at 4°C, 1,300 g for 10 min, and stored at -80°C until analysis. The concentration of DXM in plasma samples was determined by an enzyme-linked
immunosorbent assay (ELISA) kit (Neogen, Lexington, KY, USA) according to the vendor’s protocol. Samples were sufficiently diluted so that their DXM concentrations can be read from the standard curve, which was plotted utilizing a 4-parameter logistic curve fit algorithm for the concentration range of 0.5 ng/mL to 10 ng/mL. Plasma corticosterone level was also quantified using an ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) following the manufacturer’s protocol. Samples were sufficiently diluted so that their corticosterone concentrations can be determined from the standard curve, which was plotted utilizing a 4-parameter logistic curve fit algorithm for the concentration range of 32 pg/mL to 20,000 pg/mL.

4.2.12. STATISTICAL ANALYSIS

All the data were expressed as mean ± standard error of the mean (S.E.M.). Tumor and edema volumes were analyzed using repeated measures two-way analysis of variance (ANOVA) in combination with Tukey’s and Holm-Sidak’s multiple comparisons tests for pair comparisons among the groups and for comparison between day 9 and day 12 within each group respectively. Log-rank (Mantel-Cox) test was used to compare the survival curves. One-way ANOVA followed by Tukey’s multiple comparisons test for pair comparisons was performed for all the other experiments. \( P < 0.05 \) was considered statistically significant. All the statistical analyses were performed using Graphpad Prism.
4.3. RESULTS

4.3.1. IN VITRO RELEASE

Our device is comprised of a reservoir of solid drug and a cap with hole(s) through which drug is released. Influx of PBS into the device through the hole(s), after removal of residual trapped air within the device via vacuum, caused some drug to dissolve. Drug then diffuses across the hole(s) down a concentration gradient into the external solution (Figure 4.2).

The release of DXM from our drug delivery system can be described by Fick’s first law,

\[ J = -D \frac{\partial C}{\partial x} \]

(4.1)

\[ J = -D \frac{dc}{dx} = \frac{dM_t}{A \ dt} \]

(4.2)
\[
\frac{dM_t}{dt} = \frac{DA}{\delta} (c_2 - c_1)
\]  
(4.3)

\[
M_t = \frac{DA}{\delta} (c_2 - c_1)t
\]  
(4.4)

where \(J\) is the diffusion flux, \(D\) is the drug diffusivity, \(dc/dx\) is the concentration gradient (spatial derivative), \(M_t\) is the cumulative amount of drug released up to time \(t\), \(A\) is the surface area for diffusion, \(c_2\) is the concentration of drug inside the device, \(c_1\) is the concentration of drug outside the device and \(\delta\) is the distance over which the concentration gradient exists. Steady-state, unidirectional diffusion is assumed in equations 4.2, 4.3, and 4.4. \(c_1\) can be assumed to be constantly zero in perfect sink conditions while \(c_2\) can be assumed to be the saturated concentration of DXM in the device reservoir during the early phase of drug release. \(\delta\) can be assumed to be the thickness of the cap. As the term \(\frac{DA}{\delta}(c_2 - c_1)\) in equation 4.4 is a constant, \(M_t\) varies linearly with time as in zero-order release kinetics.

Releases of DXM from our drug delivery devices with different number of orifices in PBS were observed to follow zero-order kinetics over the course of 10 days (Figure 4.3A). The empirical release curves, plotted as cumulative amount of drug released against time, were fitted using a linear regression model, and the mean release rates were determined from the slopes of the linear regression lines of best fit. The mean release rates of DXM-loaded devices with 1 hole (DXM 1H device), 5 holes (DXM 5H device), and 9 holes (DXM 9H device) were 0.58 ± 0.00 \(\mu\)g/day (\(R^2 = 0.998\)), 3.37 ± 0.03 \(\mu\)g/day (\(R^2 = 0.982\)), and 5.91 ± 0.06 \(\mu\)g/day (\(R^2 = 0.984\)) respectively. The coefficients of variation were \(\leq 4.2\%\), \(\leq 15.0\%\), and \(\leq 8.4\%\) for the release
rates of DXM 1H device, DXM 5H device, and DXM 9H device, suggesting that the releases were fairly reproducible.

![Graph A](image1.png)

**Figure 4.3** *In vitro* releases of DXM from drug delivery devices. (A) Cumulative release curves of DXM from devices with 1 hole (DXM 1H Device), 5 holes (DXM 5H Device) and 9 holes (DXM 9H Device). Releases of DXM from these devices were shown to obey zero-order kinetics. The lines represent linear regression lines of best fit. (B) Plot of the mean release rate against the number of orifices in the device. A linear relationship ($R^2 = 0.998$) was observed between the mean release rates, determined from the slopes in (A), and the number of orifices. Data represent mean values ± S.E.M. ($n = 5$).

Equation 4.4 shows that drug release ($M_t$) is directly proportional to the surface area for diffusion ($A$), which is defined by the number of orifices in our drug delivery system. This is consistent with our plot of the mean release rate versus the number of orifices (Figure 4.3B). A linear relationship ($R^2 = 0.998$) was revealed between the mean release rate and the number of orifice, implying that the desirable DXM release rate can be obtained by manipulating the number of holes in the device.
4.3.2. DRUG STABILITY

![Graph showing drug stability over time]

**Figure 4.4** Stabilities of DXM in solid form and in PBS over the course of 10 days. Data were expressed as the mean percentage of the initial amount of drug on day 0 ± S.E.M. (n = 3 for each time point).

DXM was stable in solid form and in PBS over the course of 10 days at 37°C (Figure 4.4). DXM was dissolved in PBS and its concentration was analyzed using HPLC for the stability test of solid DXM, while the concentration of DXM in PBS was measured directly with HPLC for the stability test of DXM in PBS. There was no evidence of drug degradation in both tests. Stability of DXM in both solid and solution forms makes it a good drug candidate for reservoir-based controlled drug delivery.

4.3.3. DRUG DISTRIBUTION IN RAT BRAIN

DXM penetration was limited in brains of rats that received intracranial devices (Figure 4.5). Systemic DXM IP dosing, on the contrary, resulted in a relatively uniform distribution of DXM throughout the brain. DXM-loaded devices with 9 holes were found to give very high DXM concentration, which was more than 3 times that found in systemically dosed rats, at the
site of device implantation. The concentration of DXM dropped exponentially away from the device and no drug was measured more than 4 mm away from the device. When devices with a single hole were implanted in the rat brains, the concentration of DXM at the site of implantation was similar to that in systemically dosed rats, 2 h after the last dose. DXM levels dipped sharply away from the device and were not detectable beyond 3 mm from the 1-hole device.

Figure 4.5 Distribution of DXM in rat brain resulting from systemic DXM dosing (DXM IP), intracranial implantation of DXM-loaded devices with 1 hole (DXM 1H Device) and 9 holes (DXM 9H Device). Data represent mean values ± S.E.M. (n = 3).

4.3.4. TUMOR AND EDEMA QUANTIFICATION FROM MRI

Representative T<sub>2</sub>-weighted coronal MR images of all the treatment groups before (day 9) and 3 days after treatment (day 12) are shown in Figure 4.6A. Intracranial 9L gliosarcoma was evident in the left hemisphere as an isointense or hyperintense region with a distinct boundary. Peritumoral regions with hyperintense signal were identified as brain tumor associated edema. When the tumor reached a minimum volume of 8 mm<sup>3</sup> on day 9, as measured on MR images, tumor-bearing rats were randomized to one of the following treatment groups: 1) no treatment
(control), 2) intracranial implantation of blank device (PBS device), 3) daily IP injection of DSP at 1.64 mg/kg (DXM IP), 4) intracranial implantation of DXM-loaded device with 1 hole (DXM 1H device), 5) intracranial implantation of DXM-loaded device with 9 holes (DXM 9H device).

Tumor volumes on day 12 were significantly higher \((P < 0.05)\) than those on day 9 in all the groups (Figure 4.6B). No significant difference was observed among all the groups on either day 9 or day 12. The average relative increase in tumor size ranged from 4.6 to 6.1 fold, with no statistical difference among all the groups (Figure 4.6C).

Treatment with DXM via either local or systemic administration was demonstrated to impede the development of edema. The volume of edema increased significantly \((P < 0.01)\) from day 9 to day 12 in animals that were not treated with DXM, i.e. the control and PBS device groups; but no significant increase was exhibited in the DXM IP, DXM 1H device, and DXM 9H device groups – groups that were treated with DXM (Figure 4.6D). The volume of edema was not significantly different among all the groups on day 9, before the start of treatment. After 3 days of treatment on day 12, rats that were treated with DXM had significantly less \((P < 0.001)\) edema than the untreated rats, while rats that received blank devices showed no significant difference compared with the controls. The device alone did not appear to improve or worsen edema. The effect of DXM on edema was not dose-dependent as the volume of edema was not significantly different among the groups that received some form of DXM treatment. These observations were consistent with the results obtained from plotting the relative change in edema volume from day 9 to day 12 (Figure 4.6E). The relative change in edema volume was \(-0.48 \pm 0.08\), \(-0.45 \pm 0.07\), and \(-0.58 \pm 0.07\) fold in the DXM IP, DXM 1H device, and DXM 9H device groups respectively. These changes were significantly lower \((P < 0.0001)\) than the relative changes of \(1.86 \pm 0.23\) and \(2.18 \pm 0.38\) fold in the control and PBS device groups respectively.

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Figure 4.6 Tumor and edema assessment using MRI. (A) Representative T2-weighted coronal MR images before (day 9) and after (day 12) treatment. (B) Tumor volumes measured on MR images. There was no significant difference among all the groups on both day 9 and day 12. (C) Relative change in tumor volume calculated from volumes in (B) confirmed that increase in tumor was not significantly different. (D) Edema volumes measured on MR images. Edema on day 12 was significantly reduced in groups treated with systemic or local DXM compared with controls (***P < 0.001). (E) Relative change in edema volume calculated from volumes in (D) confirmed that the decrease in edema was significant relative to control (****P < 0.0001). Data are expressed as the mean values ± S.E.M. (n = 6 per group).
4.3.5. BRAIN WATER CONTENT

Figure 4.7 Water content of the tumor, ipsilateral cerebrum and contralateral cerebrum. Animals treated with systemic or local administration of DXM had significantly lower water content in the tumor and in the ipsilateral cerebrum than the control animals ($^*P < 0.05$, $^{**}P < 0.01$). Data represent mean values ± S.E.M. (n = 3 per group).

Water content of the tumor, ipsilateral cerebrum and contralateral cerebrum were measured by the wet/dry weight method and compared across all the groups relative to the control group (Figure 4.7). Rats that were administered systemic or local DXM in the DXM IP, DXM 1H device and DXM 9H device groups had significantly lower ($P < 0.05$) water content in the tumor and in the ipsilateral cerebrum than the rats in the control and PBS device groups. Edema reduction appeared to be independent of the route of administration and the dosage of DXM since there was no significant difference among the 3 groups that were treated with DXM. The device alone did not worsen edema as the brain water content of the rats that received intracranial blank devices was not significantly different from that of the untreated rats. The water content of the contralateral cerebrum was not significantly different among all the groups,
suggesting that DXM exerted its effect on brain tumor associated edema without affecting the water content of normal brain tissues.

4.3.6. SURVIVAL

![Kaplan-Meier survival curves](image)

**Figure 4.8** Kaplan-Meier survival curves. Systemic and local delivery of DXM led to significant survival benefit ($P < 0.05$, $n = 6$ per group).

The Kaplan-Meier survival curves of all the treatment groups are depicted in Figure 4.8. Untreated control animals and animals that were given blank intracranial devices had median survival of 14 and 14.5 days respectively, with all the animals succumbing to the tumor within 15 days of tumor implantation. Survival of the control group was not significantly different from that of the PBS device group, implying that the blank device had no impact on survival. When compared with the control group, survival was significantly prolonged ($P < 0.05$) in the DXM IP, DXM 1H device, and DXM 9H device groups, which had median survival of 18.5, 17.5 and 17.5 days respectively. Rats in these 3 groups survived for at least 16 days and at most 21 days. There was no significant difference in survival among these groups. The improvement in survival in rats treated with DXM was independent of dose and route of DXM administration.
4.3.7. DEXAMETHASONE-INDUCED WEIGHT LOSS

Figure 4.9 Relative body weight. (A) Plots of relative body weight versus time since the start of treatment on day 9. All the groups had decreasing weight with time, with the DXM IP group experiencing the most weight loss. (B) Comparison of the mean relative weights on day 12 and day 16. The DXM IP group had significantly lower relative body weight than all other groups on both days (*P < 0.05). Data represent mean values ± S.E.M. (n = 6 per group).

Body weight loss was used as an indicator of systemic toxicity. The body weight of all the groups, which was monitored daily from day 9 (the start of treatment) onwards, is plotted as a percentage of the weight on day 9 in Figure 4.9A. All the groups displayed downward trends in body weight with time, with the group that received systemic DXM losing the most weight.

Comparisons of the mean relative weights were made among all the groups on day 12 and day 16 to study the effect of short-term (3-days duration) and long-term (7-days duration)
treatment (Figure 4.9B). Both short-term and long-term systemic administration of DXM led to significantly lower \((P < 0.05)\) relative body weight than any other treatment. The DXM IP group had a mean relative weight of 93.1 ± 0.7 %, compared with 97.9 ± 1.3 % for the control group, 97.9 ± 0.8 % for the PBS device group, 97.9 ± 1.3 % for the DXM 1H device group, and 97.9 ± 0.4 % for the DXM 9H device group on day 12, after 3 days of treatment. There was no significant difference among the control, PBS device, DXM 1H device, and DXM 9H device groups, suggesting that local delivery of DXM could avoid DXM induced weight loss. 7 days after treatment on day 16, rats in the DXM IP group, again, had significantly lower \((P < 0.05)\) relative body weight (88.1 ± 1.2 %) than rats in the DXM 1H device (92.9 ± 1.2 %) and the DXM 9H device (92.1 ± 0.3 %) groups. No dose-dependent effect on body weight was observed in the rats treated with locally administered DXM. The mean relative body weight of the DXM 1H device group was not significantly different from that of the DXM 9H device group on both day 12 and day 16 although a higher local dose was delivered in the DXM 9H device group.

### 4.3.8. PLASMA DEXAMETHASONE AND CORTICOSTERONE

An important goal of local drug delivery is to minimize systemic drug exposure. The concentration of DXM in plasma at the time of sacrifice was measured and compared among the different groups to study the effect of treatment on systemic drug exposure (Figure 4.10A). Local delivery of DXM in the brain failed to prevent DXM from leaking into systemic circulation, but managed to reduce systemic exposure to DXM. DXM level in plasma of rats in the DXM IP group, 2 h after the last IP dose, was found to be 3265 ± 146 ng/mL, which was approximately 3 orders of magnitude higher than the DXM levels measured in plasma of rats treated with locally delivered DXM. The concentration of DXM in plasma of rats that received local sustained
delivery of DXM via our 1-hole device and 9-holes device were 1.2 ± 0.1 ng/mL and 8.1 ± 0.8 ng/mL respectively, showing that the systemic level of DXM increased with rise in locally administered DXM dose. Both route of administration and dosage, even in the case of local delivery, were found to be important factors of systemic exposure to DXM.

Figure 4.10 Plasma levels of (A) DXM and (B) corticosterone at the time of sacrifice. Plasma DXM level in the DXM IP group was 3 orders of magnitude higher than the plasma DXM levels in the DXM 1H device and DXM 9H device groups. Plasma corticosterone level in the DXM IP group was significantly lower than that in any other group (*P < 0.05). Data represent the mean values ± S.E.M. (n = 6 per group).

An adverse effect due to systemic exposure to DXM is corticosterone suppression.

Plasma corticosterone levels at the time of sacrifice, depicted in Figure 4.10B, were measured to assess systemic toxicity. The plasma concentration of corticosterone in rats that were treated with systemic DXM, 6.4 ± 0.9 ng/mL, was significantly lower (P < 0.05) than that in all other rats. Plasma corticosterone levels of the control, PBS device, DXM 1H device and DXM 9H device groups were 362.3 ± 73.6 ng/mL, 399.0 ± 92.3 ng/mL, 361.0 ± 95.4 ng/mL and 349.0 ± 93.4 ng/mL respectively, and there was no significant difference among these groups. Local delivery of DXM in the brain, independent of the dose, was shown to avoid corticosterone suppression which was associated with systemic dosing of DXM.
4.4. DISCUSSION

We have developed a reservoir-based, highly tunable drug delivery system for the local delivery of DXM in the rat brain. A key feature of this system is the ability to modify drug release rates by controlling the number of holes in the device. Sustained, zero-order releases of DXM from devices with different number of orifices were achieved and accurately predicted by Fick's first law. Drug degradation did not need to be accounted for during release studies since DXM was found to be extremely stable at body temperature in both solid form and when dissolved in PBS. The high stability of DXM also implies that DXM is suitable for our drug delivery system. It is important that the solid DXM stored in the device reservoir will not degrade when the device is implanted in the brain.

A similar type of drug delivery system was reported in an earlier study, but the parts were manufactured by injection molding [107]. A novelty of this work is the use of micro-machining for device fabrication. Micro-machining is an appealing method for making prototypes in pilot studies as it enables fast turnaround time and flexibility in design alteration without compromising on quality. The devices that we micro-machined have tight tolerances and can be reliably reproduced, resulting in reproducible drug releases with these devices. The number of orifices in our devices can be easily changed to obtain different release rates by simply changing the number of times the devices are drilled during the micro-machining process. If the devices were to be injection molded, different molds would be required for making devices with different number of orifices. Micro-machining generally requires no tooling, therefore it is more cost efficient than injection molding for production in small quantities. Although injection molding can give a much lower cost per part than micro-machining in large-scale production, tooling cost,
which makes up the bulk of the upfront production cost, can make injection molding prohibitive for small-scale prototype production.

Biocompatibility of the device is evident from the *in vivo* results. Blank devices did not induce body weight loss, which is a reflection of systemic toxicity. No abnormalities were observed. Blank devices also had no adverse or beneficial impact on tumor growth, edema formation, and overall survival. Outcomes of rats that were given blank devices were essentially indistinguishable from those of control rats.

*In vivo* evaluations were carried out with the 1-hole and 9-holes devices to investigate whether different DXM release rates had an impact on drug distribution, therapeutic efficacy and systemic side effects. The DXM release rate of the 9-holes device was an order of magnitude higher than that of the 1-hole device, and less than twice that of the 5-holes device. Devices with 5 holes were not tested in *in vivo* experiments as it was thought that its performance would be somewhere between that of the 1-hole and 9-holes devices.

Both 1-hole and 9-holes devices showed limited drug distribution profiles, which are expected, since drug distribution occurs mainly by diffusion. The concentration of DXM was high at the site of device implantation but dropped exponentially away from the device. The concentration gradient and the distance over which DXM was distributed were greater for the 9-holes device than that for the 1-hole device due to higher drug release. The concentration of DXM at the site of device implantation obtained with 9-holes devices was about 4 times more than that with 1-hole devices, although the drug release rate of the 9-holes device was about 10 times higher than that of the 1-hole device. Absence of perfect sink conditions at the site of device implantation may explain the discrepancy. Furthermore, the concentration of DXM at the site of implantation of the 1-hole device was found to coincide with the concentration of DXM in
brains of rats 2 h after the last systemic dose. Peak DXM levels in rat brain should be observed 2 h after systemic dosing, based on a previous pharmacokinetic study [130]. Our finding suggests that the 1-hole device is capable of maintaining peak DXM level seen in systemic dosing, in the tumor region throughout the duration of treatment. The cumulative amount of DXM that the tumor was exposed to could be similar or greater in rats that were treated with the 1-hole devices intracranially than in rats that were dosed with IP injection of DXM. This cumulative amount of DXM delivered in the tumor region is projected to be much higher in rats that were treated with the 9-holes devices that in rats that received the 1-hole devices or daily systemic dosing.

Therapeutic efficacies of local DXM delivery via 1-hole and 9-holes devices and systemic administration of DXM were not significantly different one another, despite the differences in drug dosage and distribution in the brain. Local delivery of DXM using the devices was shown to be as effective as systemic dosing of DXM in alleviating edema, as measured by MRI and by brain water content. Edema reduction was associated with modest but significant survival benefit, despite continuous tumor progression.

Systemically administered DXM is known to induce body weight loss and corticosterone suppression in rodents [135-138]. Local administration of DXM using 1-hole and 9-holes devices were shown to be equally effective in eliminating these systemic side effects, although systemic level of DXM was higher in rats treated with 9-holes devices than in rats with 1-hole devices. Leakage of locally delivered DXM into systemic circulation was minimal but inevitable, despite the limited drug distribution profile of the devices. It was possible that systemic exposure to DXM due to both 1-hole and 9-holes devices was below the threshold that would trigger corticosterone suppression. Another possibility was that plasma corticosterone changes might be detectable if the plasma samples were obtained at peak times in the circadian cycle. The samples
were drawn from euthanized rats within 2 hours before the end of the light cycle in our study so corticosterone level would not be at its peak in a Fischer rat [139].

DXM has been shown to be a better drug candidate for our drug delivery system than DSP, the highly water soluble, inorganic ester of DXM, when a low release rate is required. The differences in release rates and release profiles between these 2 drugs arise from the huge disparity in solubility. The release rate of DSP is higher and decreases gradually with time, while the release rate of DXM is lower and characterized by zero-order kinetics. A device with maximum load of DSP and a single 100 μm hole has a mean release rate of 88 μg/day; a device with maximum load of DXM and a single 300 μm hole delivers DXM at a constant rate of 0.58 μg/day, which is 2 orders of magnitude lower than the DSP device, even though the DSP device has a smaller hole. Delivery of DXM via our device therefore offers the advantage of long-term, zero-order release, which is not possible with DSP. Another advantage of using DXM is that maximum payload can be used to achieve the release rate(s) at which brain tumor associated edema is reduced without systemic side effects. All the DXM-loaded devices used in this study had maximum payloads. Drug delivery via our device, that had the smallest orifice possible and the maximum payload of DSP, was shown to result in systemic toxicity in Chapter 3. The only way to reduce release of DSP was to utilize lower initial payload. Lowering the payload helped to eliminate systemic toxicity but the space within the device was not maximized. Maximizing payload is essential for a device designed for implantation in the brain, since an intracranial device should ideally be as small as possible to minimize additional mass effect, which can lead to increased intracranial pressure within the rigid skull.

Local DXM delivered via the 1-hole and 9-holes devices were merely 2.36% and 0.23% of the systemic dose (Table 4.1). The systemic dose used in our study was translated from typical
human dose (16 mg/day) using the body surface area normalization method [140]. Local drug delivery with our device offers the benefit of using a small fraction of the dose used in systemic delivery to achieve similar efficacy while minimizing the risk of toxicity associated with systemic delivery.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose and Schedule</th>
<th>Total dose in 7 days/ μg</th>
<th>Comparison with systemic dose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXM IP (systemic)</td>
<td>250 μg once a day</td>
<td>1750+</td>
<td>-</td>
</tr>
<tr>
<td>DXM 1H Device (local)</td>
<td>sustained release, 5.9 μg/day</td>
<td>41.4</td>
<td>2.36</td>
</tr>
<tr>
<td>DXM 9H Device (local)</td>
<td>sustained release, 0.58 μg/day</td>
<td>4.1</td>
<td>0.23</td>
</tr>
</tbody>
</table>

4.5. CONCLUSIONS

Reproducible, sustained release of DXM from our novel, micro-machined, drug delivery device was achieved. Drug release was characterized by zero-order kinetics and could be easily altered by changing the number of orifices in the device. Local delivery of DXM in rat brains via our devices was demonstrated to be as effective as systemic delivery in alleviating edema. Edema reduction was associated with survival benefit, despite continuous tumor progression. Local DXM delivery also eliminated side effects, such as weight loss and corticosterone suppression, caused by systemic dosing of DXM. Edema alleviation and systemic complications were not dose-dependent with local delivery of DXM. Local drug administration using our drug delivery system is superior to systemic administration as it allows the use of a much lower dose to achieve similar efficacy with minimal risk of systemic toxicity. Its use can be extended to other therapeutic agents targeting brain tumor to achieve similar outcome.
CHAPTER 5
LOCAL DELIVERY OF CEDIRANIB

5.1. INTRODUCTION

Brain tumor associated edema is vasogenic in nature, characterized by the disruption of blood brain barrier (BBB). The exact mechanism behind the breakdown of BBB is unclear. One school of thought is that the tumor production of vascular endothelial growth factor (VEGF) stimulates the formation of gaps in brain endothelium and leads to plasma leakage into the extracellular space of brain parenchyma, resulting in edema [13]. VEGF has been shown to be upregulated in both malignant gliomas [9] and metastatic tumors [10], and its level in high-grade gliomas correlates with the occurrence of edema [12]. Therefore, agents that target the VEGF signaling pathway are thought to be promising alternatives to corticosteroids, the standard of care for brain tumor associated edema [42]. Cediranib (AZD2171), an oral pan-VEGF receptor tyrosine kinase inhibitor, induced vascular normalization within 24 hours and alleviated brain edema in glioblastoma patients in a phase II trial [50]. Corticosteroid usage was reduced or discontinued as a result [50, 51]. Bevacizumab, a monoclonal antibody against VEGF, also demonstrated anti-edema activity and corticosteroid-sparing effect in a phase II study [47].

Treatment with systemically administered VEGF inhibitors, as with corticosteroids, is associated with systemic side effects [55]. This is not surprising since VEGF is essential for normal physiological functions. Another challenge with the systemic delivery of VEGF inhibitors is the BBB. The brain distribution of cediranib was shown to be limited by P-glycoprotein, an active efflux transporter at the BBB [141].
Local drug delivery strategies can avoid the side effects of systemic exposure, and allow increased drug concentration in the tumor by circumventing the BBB. One approach that has much success is the implantation of diffusion-controlled, sustained-release polymer systems at the tumor site. Treatment with Gliadel®, a carmustine impregnated biodegradable polymer wafer, improved median survival by 2 months without systemic toxicity in patients with malignant glioma [98, 99]. The matrix-based polymer system used in Gliadel® has also been used to deliver other chemotherapeutics, such as doxorubicin [102], taxol [103], and temozolomide [104], in preclinical models. Another type of polymer system is the reservoir-based polymer device. Local delivery of temozolomide using such a system has led to prolonged survival in a 9L rat model [107]. A reservoir-based system offers the advantages of high drug payload and long-term, nearly zero-order drug release kinetics over a matrix-based system [96].

A reservoir-based drug delivery device, same as the one used in Chapter 4, was used to test the hypothesis that local delivery of cediranib can reduce brain tumor associated edema effectively with minimal systemic toxicity. Cediranib is practically insoluble in aqueous media. Increasing its solubility, by employing the solid dispersion technique, was necessary to enhance its delivery via diffusion. We investigated the in vitro stability, release kinetics and biological activity of this solid dispersion of cediranib (AZD/PVP). Therapeutic efficacy was assessed by analyzing survival, measuring tumor and its associated edema using magnetic resonance imaging (MRI), in addition to measuring brain water content by the wet/dry weight method. Body weight was used to measure systemic toxicity. The therapeutic efficacy and systemic toxicity associated with local delivery of AZD/PVP were examined in an intracranial 9L gliosarcoma rat model, and compared with other treatments, including local delivery of dexamethasone (DXM), systemic delivery of DXM, and systemic delivery of cediranib.
5.2. MATERIALS AND METHODS

5.2.1. MATERIALS

Cediranib was obtained from Selleck Chemicals (Houston, TX, USA). Dexamethasone sodium phosphate (DSP) injection solution was acquired from Baxter (Deerfield, IL, USA). Povidone K-30, polysorbate 80, and micronized dexamethasone, USP were bought from Spectrum Chemicals & Laboratory Products (New Brunswick, NJ, USA). Dimethyl sulfoxide (DMSO), high-performance liquid chromatography (HPLC) grade acetonitrile, water, methanol, sodium acetate, ammonium formate and formic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade glacial acetic acid was procured from Fisher Scientific (Fair Lawn, NJ, USA). Polysulfone blocks and sheets were obtained from AmazonSupply (Seattle, WA, USA).

5.2.2. PREPARATION OF SOLID DISPERSION OF CEDIRANIB

Solid dispersion of cediranib in povidone K-30 (PVP) was prepared by the conventional solvent evaporation method, adapted from Sethia and Squillante [142]. Briefly, cediranib and PVP, in the weight ratio of 1 to 2, were dissolved in minimum volume of methanol. Methanol was removed under vacuum in a rotavapor at room temperature for 15 minutes, followed by evaporation under vacuum for at least 12 hours. The resultant solid dispersion was scraped out with a spatula and refrigerated for 2 days at 4°C to harden. It was pulverized using a mortar and pestle before use.
5.2.3. DEVICE FABRICATION AND ASSEMBLY

The drug delivery devices, each comprising of a reservoir and a cap, were micro-
machined as previously described in Chapter 4.2.2. All the caps used in this study had 9 holes, each 300 μm in diameter. Profile of every drilled hole was inspected under a VK laser scanning microscope (Keyence Corporation, Elmwood Park, NJ, USA) for quality control. All the device parts were cleaned with 70% isopropanol before drug loading. Each reservoir was packed with either AZD/PVP or DXM, and then covered with a cap at the top. Biomedical-grade epoxy was applied around the circumference of the cap to seal the cap to the reservoir. The epoxy was allowed to dry for at least 24 h before drug release was initiated.

5.2.4. IN VITRO DRUG RELEASE

Drug release was initiated by removing residual air trapped within the device using a vacuum system while the device was submerged in sterile PBS. Activated devices (n=5 for each drug) were incubated at 37°C in PBS for in vitro drug release. PBS was replaced every 24 h to maintain approximate sink conditions. The amount of drug in PBS was determined by HPLC.

5.2.5. DRUG STABILITY

The stabilities of solid AZD/PVP and AZD/PVP in phosphate buffered saline (PBS) at 37°C were evaluated over the course of 28 days. A 1 mg/mL stock solution of AZD/PVP in methanol was divided into aliquots such that each aliquot contained 10 μg of AZD/PVP. Methanol was then evaporated to dryness at room temperature overnight, and the resultant solid AZD/PVP was incubated at 37°C to assess the stability of solid AZD/PVP. A 10 μg/mL stock solution of AZD/PVP in PBS was divided into aliquots and incubated at 37°C to examine the
stability of AZD/PVP in PBS. The aliquots (n = 3) were collected at different time points and stored at -20°C until analysis by HPLC. Aliquots containing AZD/PVP in PBS were thawed and analyzed directly with HPLC while solid AZD/PVP was dissolved in PBS before analysis using HPLC.

5.2.6. HPLC ANALYSIS

HPLC analysis was performed on an Agilent 1200 series system (Agilent Technologies, Wilmington, DE, USA) with the column temperature set at 30°C. The HPLC method for determination of DXM was adapted from Lamiable et al. [131]. 20 μL of sample was quantified using an Agilent Zorbax Eclipse Plus C18 column (75 x 4.6 mm, 3.5 μm) and a mobile phase, consisted of 58% 2 mM sodium acetate buffer (pH 4.8) and 42% acetonitrile, delivered at 1 mL/min. The effluent was monitored at 246 nm. Concentration of cediranib was quantified using a HPLC method modified from Wang et al. [143]. The assay was conducted using a Phenomenex (Torrance, CA, USA) Kinetex C18 column (50 x 4.6 mm, 2.6 μm), with a mobile phase of 10 mM ammonium formate containing 0.1% formic acid: acetonitrile (62: 38) ran at a flow rate of 1 mL/min, a detection wavelength of 235 nm, and an injection volume of 20 μL.

5.2.7. IN VITRO HUVEC PROLIFERATION ASSAY

Cell cultures were maintained at 37°C with 5% carbon dioxide. Human umbilical vein endothelial cells (HUVEC), purchased from Life Technologies (Carlsbad, CA, USA), were grown in Medium 200 supplemented with low serum growth supplement (Life Technologies), at a density of 1,000 cells/ well in 96 well plates. Stock solutions of cediranib and AZD/PVP were prepared in DMSO at a concentration of 10 mM, and diluted to varying concentrations in an
assay medium composed of phenol red-free Medium 200, 2% v/v fetal bovine serum, 1 μg/mL hydrocortisone (Life Technologies), 3 μg/mL heparin (Sigma Aldrich), and 3 ng/mL VEGF<sub>165</sub> (R&D Systems, Minneapolis, MN, USA). After an attachment period of 24 h, the cells were dosed with varying concentrations of cediranib or AZD/PVP. Cells dosed with vehicle (DMSO) were used as control. The cell cultures were then incubated for 4 days and cellular proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma Aldrich). Briefly, MTT was added and incubated for 4 h. The resultant formazan crystals were dissolved in acidified isopropanol and the absorbance was measured by a microplate reader at 570 nm with a reference wavelength of 690 nm. The experiments were performed in triplicate and the relative cell viability was expressed as a percentage relative to the control.

5.2.8. IN VIVO EXPERIMENT

5.2.8.1. Animals

Female Fischer 344 (F344) rats, 150 – 175 g, were obtained from Charles River Laboratories (Wilmington, MA, USA). All animal experiments were conducted in accordance with the Massachusetts Institute of Technology’s Committee for Animal Care guidelines.

5.2.8.2. Intracranial 9L gliosarcoma rat model

9L gliosarcoma was provided by Dr. Betty Tyler from Johns Hopkins University and propagated in the flanks of carrier F344 rats. Tumor was harvested from a carrier rat and cut into 1 mm<sup>3</sup> pieces for intracranial tumor implantation. Animals were anesthetized with intraperitoneal (IP) injection of ketamine (75mg/kg) and xylazine (7mg/kg). The scalp was shaved and the surgical site was prepped in a sterile fashion with betadine/ethanol scrub. Midline incision was made, followed by careful removal of the pericranium. A 3 mm burr hole was drilled over the
left hemisphere with its center 3 mm lateral to the sagittal suture and 5 mm posterior to the
coronal suture. The underlying cortex was gently aspirated to expose the sulcus, where the tumor
piece was placed. The skin was sutured and the animal was allowed to recover.

5.2.8.3. Study design

9 days after tumor implantation, rats were randomly assigned to one of the following
groups (n = 7 per group): 1) no treatment (control), 2) intracranial blank device (PBS device), 3)
daily IP injection of DSP at 1.64 mg/kg (DXM IP), 4) intracranial device delivering DXM
(DXM device), 5) daily oral gavage of cediranib suspended in 1% (w/v) aqueous polysorbate 80
at 4.63 mg/kg (AZD oral), 6) intracranial device delivering AZD/PVP (AZD device). Rats
receiving intracranial devices underwent a second surgery for device implantation. The incision
was reopened, and the connective tissue overlying the burr hole was displaced. The device was
implanted within the tumor, with its orifices facing downwards and towards the tumor. Animals
were checked twice daily and euthanized when found in poor body condition. Their body
weights were recorded daily from day 9 onwards to monitor their health.

5.2.8.4. Magnetic resonance imaging

MRI was performed on 7T Varian MRI scanner (Agilent Technologies). Rats were
anesthetized with 2% isoflurane and placed prone in the cradle. T2 weighted images (TR = 4,000
ms, TE = 40 ms, 1 mm slice thickness, 12 image slices, 128 x 128 matrix size, 32 mm x 32 mm
field of view) were acquired to assess tumor and its associated edema on day 9 and on day 12
post tumor implantation. Edema and tumor volumes were measured using Osirix imaging
software.
5.2.9. BRAIN WATER CONTENT

Animals were euthanized by carbon dioxide asphyxiation and their brains were collected. Tumors were excised by blunt dissection before dividing the brains into ipsilateral and contralateral cerebrums. Tumor, ipsilateral cerebrum and contralateral cerebrum were weighed immediately to obtain the wet weight, and then dried at 100°C for up to a week until a final dry weight was achieved. The tissue water content was calculated as water content = (wet weight – dry weight)/ wet weight, and expressed as a percentage.

5.2.10. STATISTICAL ANALYSIS

All the data were expressed as mean ± standard error of the mean (S.E.M.). Dose-response curves were fitted with a nonlinear regression model to determine the drug concentration where the response was reduced by half (IC$_{50}$). Logarithms of IC$_{50}$ values were compared using extra sum-of-squares F test. Tumor and edema volumes were analyzed using repeated measures two-way analysis of variance (ANOVA) in combination with Tukey’s and Holm-Sidak’s multiple comparisons tests for pair comparisons among the groups and for comparison between day 9 and day 12 within each group respectively. Log-rank (Mantel-Cox) test was used to compare the survival curves. One-way ANOVA followed by Tukey’s multiple comparisons test for pair comparisons was performed for all other experiments. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using Graphpad Prism.
5.3. RESULTS

5.3.1. IN VITRO DRUG RELEASE

The drug delivery device, as shown in Figure 5.1, is comprised of a reservoir of solid drug and a cap with orifices through which drug is released. Influx of PBS into the reservoir, after removal of residual gases within the device via vacuum, caused some drug to dissolve. Drug then diffuses across the orifices down a concentration gradient into the external solution.

![Figure 5.1](image)

**Figure 5.1** (A) Illustration and (B) photograph of an assembled drug delivery device with (C) 9 holes, each 300 μm in diameter.

![Figure 5.2](image)

**Figure 5.2** Cumulative release profiles of DXM and AZD/PVP. Data represent mean values ± S.E.M. (n = 5 for each drug)
Figure 5.2 shows the release profiles of DXM and AZD/PVP from the drug delivery device in PBS, plotted as cumulative amount of drug released against time. The release of DXM was observed to follow zero-order kinetics ($R^2 = 1.00$) with a mean rate of $5.91 \pm 0.06 \mu g/day$ for the duration of the 10-day release experiment. The release profile of AZD/PVP, on the other hand, was characterized by an initial burst effect ($13.22 \pm 0.43 \mu g$) on the first day, followed by a decreased, steady release at an average rate of $2.23 \pm 0.08 \mu g/day$ ($R^2 = 0.95$). In vitro releases of both drugs were fairly reproducible. The coefficient of variation was $\leq 8.41\%$ and $\leq 7.24\%$ for mean release rates of DXM and AZD/PVP respectively.

### 5.3.2. DRUG STABILITY

![Graph showing drug stability over time](image)

**Figure 5.3** Stabilities of AZD/PVP in solid form and in PBS for 28 days. Data were expressed as the mean percentage of the initial amount of drug on day 0 ± S.E.M. (n = 3 for each time point).

The stabilities of AZD/PVP in solid form and in PBS, over the course of 28 days at 37°C, are depicted in Figure 5.3. AZD/PVP was extremely stable in solid form with no evidence of degradation throughout the course of 28 days. The concentration of AZD/PVP in PBS, in contrast, decreased steadily with time, and approximately 10% of the drug was lost at the end of
28 days. AZD/PVP is expected to be protected from degradation when stored in solid state in a device that is implanted intracranially. Stability of solid AZD/PVP at 37°C makes it a good drug candidate for reservoir-based drug delivery.

5.3.3. BIOACTIVITY

VEGF-stimulated proliferation of HUVEC was inhibited by cediranib and AZD/PVP in a similar dose-dependent manner, with IC\textsubscript{50} values determined to be 21.92 nM and 48.33 nM respectively (Figure 5.4). Logarithms of their IC\textsubscript{50} values were not significantly different, suggesting that AZD/PVP had similar biological activity as cediranib.

![Figure 5.4 Dose-response curves of AZD/PVP and cediranib (AZD). Both drugs inhibited VEGF-induced HUVEC proliferation in similar dose-dependent fashion. Data represent mean values ± S.E.M., performed on triplicate experiments.](image)

5.3.4. IN VIVO EFFICACY STUDY

The effects of different treatments on brain tumor, its associated edema, and survival were evaluated in an intracranial 9L gliosarcoma rat model.
5.3.4.1. Tumor and edema quantification from MRI

Representative T2-weighted coronal MR images before (day 9) and 3 days after treatment (day 12) are shown in Figure 5.5A. Intracranial tumor was evident in the left hemisphere as an isointense or hyperintense region with a distinct boundary. Peritumoral regions with hyperintense signal were identified as brain tumor associated edema. Rats with tumors that were \( \geq 8 \text{ mm}^3 \) on day 9 were randomly assigned to one of the following groups: 1) no treatment (control), 2) intracranial implantation of blank device (PBS device), 3) daily IP injection of DSP (DXM IP), 4) intracranial implantation of device delivering DXM (DXM device), 5) daily oral gavage of cediranib (AZD oral), 6) intracranial implantation of device delivering AZD/PVP (AZD device).

Tumor volumes on day 12 were significantly higher \((P < 0.0001)\) than those on day 9 in all the groups (Figure 5.5B). No significant difference was observed among all the groups on both day 9 and day 12. The average relative increase in tumor size ranged from 4.1 to 5.5 fold, with no statistical difference among all the groups (Figure 5.5C).

Treatment with DXM or cediranib via either local or systemic administration was demonstrated to impede the development of edema. The volume of edema increased significantly \((P < 0.0001)\) from day 9 to day 12 in the control and PBS device groups, but no significant increase was detected in the DXM IP, DXM device, AZD oral and AZD device groups – groups that were treated with systemic or local DXM or cediranib (Figure 5.5D). The volume of edema was not significantly different among all the groups on day 9, before the start of treatment. After 3 days of treatment on day 12, rats in the DXM IP, DXM device, AZD oral and AZD device groups had significantly less \((P < 0.001)\) edema than the controls, while rats in the PBS device group showed no significant difference compared with the controls. The device alone did not appear to improve or worsen edema.
Figure 5.5 Tumor and edema assessment using MRI. (A) Representative T$_2$-weighted coronal MR images before (day 9) and after (day 12) treatment. (B) Tumor volumes measured on MR images. (C) Relative change in tumor volume calculated from the volumes in (B). Both (B) and (C) show that there was no significant difference in tumor growth among all the groups. (D) Edema volumes measured on MR images. Edema on day 12 was significantly reduced in groups treated with DXM or cediranib compared with the control group (**P < 0.001). (E) Relative change in edema volume calculated from the volumes in (D) confirmed that the decrease in edema was significant relative to control (****P < 0.0001). Data represent mean values ± S.E.M. (n = 7 per group).
These observations were consistent with the results obtained from plotting the relative change in edema volume from day 9 to day 12 (Figure 5.5E). The relative change in edema volume was -0.53 ± 0.07, -0.45 ± 0.04, -0.45 ± 0.09 and -0.63 ± 0.08 fold in the DXM IP, DXM device, AZD oral and AZD device groups respectively. These changes were significantly lower ($P < 0.0001$) than the relative change of 1.75 ± 0.30 and 1.90 ± 0.29 fold seen in the control and PBS device groups respectively. No significant difference was observed among the groups treated with DXM or cediranib.

### 5.3.4.2. Brain water content

![Graph showing water content of tumor, ipsilateral cerebrum, and contralateral cerebrum](image)

**Figure 5.6** Water content of the tumor, ipsilateral cerebrum and contralateral cerebrum. Animals treated with systemic or local administration of DXM and cediranib had significantly lower water content in the tumor and ipsilateral cerebrum than the control animals ("$P < 0.05$). Data represent the mean ± S.E.M. ($n = 4$ per group).

Water content of the tumor, ipsilateral cerebrum and contralateral cerebrum were measured by the wet/dry weight method and compared across all the groups relative to the control group (Figure 5.6). DXM and cediranib were equally effective in reducing brain tumor
associated edema. Rats that were systemically or locally administered DXM or cediranib – in the DXM IP, DXM device, AZD oral and AZD device groups – exhibited significantly lower ($P < 0.05$) water content in the tumor and ipsilateral cerebrum than the control group (Figure 5.6).

There was no significant difference among these 4 groups. The water content of the ipsilateral cerebrum and the contralateral cerebrum were not significantly different in these 4 groups, whereas the water content of the ipsilateral cerebrum was significantly higher ($P < 0.01$) than that of the contralateral cerebrum in both the control and PBS device groups. The water content of the contralateral cerebrum was not significantly different among all the groups, suggesting that DXM and cediranib exerted their effects on brain tumor associated edema without affecting the water content of normal brain tissues.

5.3.4.3. Survival

![Figure 5.7 Kaplan-Meier survival curves. Systemic and local delivery of both dexamethasone and cediranib led to significant survival benefit ($P < 0.05$, $n = 7$ per group).](image)

Figure 5.7 Kaplan-Meier survival curves. Systemic and local delivery of both dexamethasone and cediranib led to significant survival benefit ($P < 0.05$, $n = 7$ per group).
The Kaplan-Meier survival curves of all the treatment groups are depicted in Figure 5.7. Both the control and PBS device groups had the same median survival of 14 days, with all rats succumbing to the tumor within 15 days of tumor implantation. No significant difference was found between them. When compared with control, survival was significantly prolonged \((P < 0.05)\) in the DXM IP, DXM device, AZD oral and AZD device groups, which shared the same median survival of 16 days, minimum survival of 15 days and maximum survival of 19 days. There was no significant difference in survival among these 4 groups. DXM and cediranib, independent of the route of drug delivery, were equally effective in improving survival.

5.3.5. SYSTEMIC TOXICITY

Body weight was used as an indicator of systemic toxicity. The body weight of all the groups, which was monitored daily from day 9 onwards, is plotted as a percentage of the weight on day 9 in Figure 5.8A. All groups displayed decreasing body weight with time, with the DXM IP group losing the most weight.

Comparisons of the mean relative weights were made among all the groups on day 12 and day 16 to study the effect of short-term (3-days duration) and long-term (7-days duration) treatment (Figure 5.8B). Both short-term and long-term systemic administration of DXM led to more severe weight loss than any other treatment. The DXM IP group had a significantly lower relative weight of 92.3 \(\pm\) 0.6 \%, compared with 97.0 \(\pm\) 1.4 \% for the control group \((P < 0.05)\), 97.0 \(\pm\) 0.7 \% for the PBS device group \((P < 0.05)\), 99.7 \(\pm\) 1.0 \% for the DXM device group \((P < 0.001)\), 100.7 \(\pm\) 0.7 \% for the AZD oral group \((P < 0.0001)\), and 101.0 \(\pm\) 1.6 \% for the AZD device group \((P < 0.0001)\) on day 12. There was no significant difference among the control, PBS device, DXM device, AZD oral and AZD device groups. Similar trend continued on day 16,
with the DXM IP group (83.2 ± 1.2 %) having significantly lower relative body weight than the DXM device (92.6 ± 1.5 %, P < 0.01), AZD oral (94.8 ± 1.7 %, P < 0.001) and AZD device (93.1 ± 2.1%, P < 0.01) groups. No significant difference was found among the DXM device, AZD oral and AZD device groups. Local delivery of DXM in the brain prevented DXM induced weight loss seen with systemic dosing of DXM. Cediranib, on the other hand, did not seem to have an effect on body weight.

Figure 5.8 Relative body weight as a measure of systemic toxicity. (A) Plots of body weight versus time since start of treatment on day 9. All groups had decreasing weight with time, with the DXM IP group experiencing the most weight loss. (B) Relative body weights of the groups on day 12 and day 16. Mean relative weight of the DXM IP group is significantly lower than that of any other group on day 12 and day 16 (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). Data represent mean ± S.E.M. (n = 7 per group).
5.4. DISCUSSION

The efficacy of corticosteroids in treating brain tumor associated edema is well established [30]. Systemic corticosteroids have remained the standard of care as there is no better option [5]. Corticosteroids are not only associated with numerous detrimental systemic complications, but they may also antagonize the cytotoxic and anti-proliferative effects of concurrently administered chemotherapy. The most commonly used corticosteroid, DXM, has been shown to protect glioblastoma cell lines from apoptosis induced by temozolomide [144, 145] and cisplatinium [146].

Efforts in improving treatment outcome can be broadly classified into two categories: drug development and development of drug delivery method. Cediranib and bevacizumab are two VEGF inhibitors – a new class of anti-edema agents – that have demonstrated their potential as alternatives to corticosteroids in clinical trials. Cediranib has been proven to be active in rats [147]; while bevacizumab, a recombinant humanized antibody to human VEGF, does not bind to rodent VEGF [148]. Cediranib was therefore chosen for testing in the rat intracranial 9L gliosarcoma model, which is widely used for the study of brain tumor associated edema [21, 41, 106, 120, 121]. Furthermore, cediranib has been shown to synergistically sensitize glioma cells to temozolomide [149, 150], unlike DXM, which has been shown to mitigate the efficacy of temozolomide [144, 145]. Cediranib may therefore be a better drug candidate for the treatment of brain tumor associated edema than DXM.

Cediranib has always been given orally in studies of glioblastoma [50, 51, 126] but never locally in the brain. Local delivery of cediranib in the brain was investigated in this study, with the goal of alleviating brain edema while minimizing systemic toxicity. Common toxicities
associated with systemic cediranib include hypertension, fatigue and diarrhea [51]. Comparisons between cediranib and DXM and between local and systemic delivery were made to determine if local delivery of cediranib gave the best treatment outcomes.

Reproducible, sustained release of DXM and AZD/PVP from our device was achieved. Release profile is dependent on drug solubility, surface area of diffusion (i.e. surface area of the orifices) and diffusion distance (i.e. distance between the solid drug and the exterior of the device). When a saturated or near saturated solution is maintained inside the device reservoir, zero-order release kinetics is expected. This was demonstrated with the release of DXM. Difference in the release profile of AZD/PVP from that of DXM might be explained by the higher solubility of AZD/PVP which led to a greater initial release. Drug loss with this initial burst decreased the chemical driving force. A concentration gradient formed within the device reservoir and diffusion distance increased. Consequently, diffusion decreased and a near-steady state was reached, giving rise to a diminished, steady release of AZD/PVP.

Our data validated that the bioactivity of AZD/PVP was comparable to that of cediranib. It is important that AZD/PVP retains the biological activity of cediranib for it to be efficacious when delivered locally in the brain. Cediranib is a highly potent inhibitor of the kinase activity of VEGF receptor-2 (KDR), which plays a key role in mediating VEGF-induced responses [147]. It has been reported to inhibit VEGF-stimulated HUVEC proliferation with an IC$_{50}$ value of 0.4 nM [147], which is lower than what we determined. This discrepancy is probably due to different culture conditions and the use of different assays to evaluate proliferation.

The device alone was established to have no adverse or beneficial effects on tumor growth, edema formation, overall survival and body weight. Outcomes of the rats that were given blank devices were essentially indistinguishable from those of the untreated control rats.

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Local delivery using our device was demonstrated to be as effective as systemic delivery in reducing edema for both DXM and cediranib. Both drugs were equally effective in alleviating edema. Animals treated with either drug via either local or systemic administration experienced significant decrease in edema compared with the control animals. There was no significant difference among rats treated with DXM and cediranib. Importantly, edema reduction was associated with survival benefit. Median survival showed a modest but significant improvement from 14 to 16 days despite continuous tumor progression. The increase in tumor volume from day 9 to day 12 observed in rats given DXM or cediranib was not significantly different from that in controls, suggesting that DXM and cediranib had no effect on tumor growth. These effects of DXM and cediranib on brain tumor and its associated edema mirror the observations in other preclinical studies [106, 126].

Toxicity, together with efficacy, determines the success of a therapy. Treatment-related adverse effects can worsen a patient’s condition and lead to termination of therapy. Systemically administered DXM has been shown to cause body weight loss in rodents [135-138]. It was found to aggravate tumor-induced weight loss in our study. Local delivery appeared to eliminate the toxicity that was observed with systemic delivery of DXM as weight loss in rats treated with locally delivered DXM was significantly less than in rats given systemic DXM, but was not statistically different from controls. The method of administration of cediranib, in contrast, had no significant consequence on body weight in our study. This result is not surprising. No changes in body weight or other signs of toxicity related to the systemic use of cediranib have been reported in preclinical studies [126, 151, 152], although adverse effects of cediranib have been observed in clinical trials [51, 153]. Systemic toxicity of cediranib might manifest in other ways. Soft stools were present in all the rats that received cediranib by oral gavage but were absent in
all the other rats. It is uncertain if the soft stools were due to cediranib or polysorbate 80, the vehicle used for oral gavage of cediranib.

Local DXM and cediranib doses delivered via our device were merely 2.36% and 0.21% of their respective systemic doses (Table 5.1). The systemic doses used in our study were translated from typical human doses (16 mg/day for DXM and 45 mg/day for cediranib) using the body surface area normalization method [140]. Local drug delivery with our device offers the benefit of using a small fraction of the dose used in systemic delivery to achieve similar efficacy while minimizing the risk of toxicity associated with systemic delivery. Lower dose also makes local delivery more cost-effective than traditional systemic routes of administration, especially when it comes to delivering expensive therapeutic agents. Our device can be easily used with other drugs without the need for intensive drug formulation. Local delivery of drugs targeting brain tumor using our device is a promising approach to improve overall treatment outcome by attaining efficacy without systemic toxicity.

Table 5.1 DXM and cediranib dose comparison. *Dose converted to dexamethasone equivalent. **Dose converted to cediranib equivalent.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose and Schedule</th>
<th>Total dose in 7 days/ µg</th>
<th>Comparison with systemic dose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic (DXM IP)</td>
<td>250 µg once a day*</td>
<td>1750*</td>
<td>-</td>
</tr>
<tr>
<td>Local (DXM Device)</td>
<td>sustained release, 5.9 µg/day</td>
<td>41.4</td>
<td>2.36</td>
</tr>
<tr>
<td>Cediranib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic (AZD Oral)</td>
<td>690 µg once a day</td>
<td>4830</td>
<td>-</td>
</tr>
<tr>
<td>Local (AZD Device)</td>
<td>sustained release, 4.4 µg on first day, subsequent 0.74 µg/day**</td>
<td>10.2**</td>
<td>0.21</td>
</tr>
</tbody>
</table>
5.5. CONCLUSIONS

A water-soluble solid dispersion of cediranib (AZD/PVP), which has similar bioactivity as cediranib, was developed for diffusion-based drug delivery. Reproducible, sustained release of both DXM and AZD/PVP from our novel, intracranial drug delivery device was achieved. Local delivery of DXM and cediranib with our device was demonstrated to be as effective as systemic delivery in alleviating edema. Edema reduction was associated with survival benefit, despite continuous tumor progression. Local delivery of dexamethasone was shown to avoid dexamethasone-related weight loss, a systemic adverse effect seen in animals treated with systemic dexamethasone. Local drug administration using our device is superior to systemic administration as it allows the use of a much lower dose to achieve similar efficacy with minimal risk of systemic toxicity. Its use can be extended to other therapeutic agents targeting brain tumor to achieve similar outcome.
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