

Intracranial microcapsule chemotherapy delivery for the localized treatment of rodent metastatic breast adenocarcinoma in the brain

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Metastases represent the most common brain tumors in adults. Surgical resection alone results in 45% recurrence and is usually accompanied by radiation and chemotherapy. Adequate chemotherapy delivery to the CNS is hindered by the blood–brain barrier. Efforts at delivering chemotherapy locally to gliomas have shown modest increases in survival, likely limited by the infiltrative nature of the tumor. Temozolomide (TMZ) is first-line treatment for gliomas and recurrent brain metastases. Doxorubicin (DOX) is used in treating many types of breast cancer, although its use is limited by severe cardiac toxicity. Intracranially implanted DOX and TMZ microcapsules are compared with systemic administration of the same treatments in a rodent model of breast adenocarcinoma brain metastases. Outcomes were animal survival, quantified drug exposure, and distribution of cleaved caspase 3. Intracranial delivery of TMZ and systemic DOX administration prolong survival more than intracranial DOX or systemic TMZ. Intracranial TMZ generates the more robust induction of apoptotic pathways. We postulate that these differences may be explained by distribution profiles of each drug when administered intracranially: TMZ displays a broader distribution profile than DOX. These microcapsule devices provide a safe, reliable vehicle for intracranial chemotherapy delivery and have the capacity to be efficacious and superior to systemic delivery of chemotherapy. Future work should include strategies to improve the distribution profile. These findings also have broader implications in localized drug delivery to all tissue, because the efficacy of a drug will always be limited by its ability to diffuse into surrounding tissue past its delivery source.

microcapsule | intracranial | chemotherapy | brain | metastases

The rationale behind localized drug delivery is that high concentrations of drug may be reached in target tissue while minimizing systemic exposure to the large quantities of drug that might otherwise be necessary to achieve the desired biologic effect. Localized drug delivery has been used, such as in drug-eluting stents for coronary artery disease, metered dose inhalers for the treatment of asthma, and intracranial chemotherapy wafers for brain tumors. This premise is particularly applicable to conditions affecting tissue that is sequestered from systemic circulation, such as conditions affecting the CNS.

Adequate and efficacious drug delivery to the CNS has proved challenging. Systemic delivery methods are hindered by low permeability at the blood–brain barrier (BBB) to nonlipophilic drugs, which requires high systemic doses to be administered, thereby increasing systemic toxicity (1, 2). One method of circumventing the BBB is to place polymers that can release drug in a time-controlled manner at the site of tumor resection (3–6). Localized drug delivery devices provide high drug concentrations to the tumor site while preventing drug degradation and clearance until its release (7, 8). The efficacy of current intracranial drug delivery, such as Carmustine wafers, has promise, with a doubling of median survival from 9 to 20 mo in the case of Carmustine

wafers (6, 9, 10). Phase III trial data indicated a 2-to 3-mo survival advantage in Carmustine wafers-treated patients (11), and Carmustine wafers continue to have a role combined with radiation and oral temozolomide (TMZ). However, nearly all patients still succumb to tumor progression.

The clinical focus of CNS drug delivery has been high-grade glioma (12). The modest increases in survival seen with such strategies have been attributed, in part, to the limited efficacy of the chemotherapies used and the disease target itself, because these tumors are among the most infiltrative and aggressive tumors studied (6, 8). It has also been postulated that the chemotherapy that has been used (Carmustine) does not diffuse well through brain tissue to produce tissue effects away from the implantation site. Such strategies have not been widely tested in more focal, noninfiltrative lesions of the brain, such as brain metastases. Several experiments have been conducted with polymer-based vehicles, which locally deliver chemotherapy, that suggest their efficacy in brain metastases (13). The microcapsules described here were studied in a rodent glioma model and showed tunable release kinetics and efficacy (12). These vehicles have, however, not been tested in brain metastases.

Metastases to the brain are the most commonly encountered tumors in the brain (14–17). It is estimated that 100,000–200,000 new cases are identified in the United States each year. Furthermore, 20% of patients with disseminated cancer have evidence of CNS involvement at autopsy (14). Certain tumors have

Significance

Brain metastases represent the most common intracranial tumors in adults. Adequate chemotherapy delivery to the CNS is hindered by the blood–brain barrier. Efforts in intracranial chemotherapy delivery aim to maximize CNS levels while minimizing systemic toxicity; however, the success has been limited thus far. In this work, intracranial implanted doxorubicin and temozolomide microcapsules are compared with systemic administration in a novel rodent model of breast adenocarcinoma brain metastases. These microcapsules are versatile and efficacious, but that efficacy may depend on the ability of the chemotherapy to diffuse in brain tissue. These insights apply to other non-CNS applications of local drug delivery, and drugs should be evaluated based on their ability to penetrate the targeted tissue as well as their inherent efficacy.

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a predilection to metastasize to the brain with nonsmall cell lung cancer, breast carcinoma, small cell lung carcinoma, malignant melanoma, renal cell carcinoma, gastrointestinal carcinoma, uterine carcinoma, and unknown primary carcinoma in decreasing frequency (14, 15). Cell surface markers seem to predict metastatic potential; for example, human epidermal growth factor receptor 2-positive breast carcinoma is more likely to present with CNS lesions (16). Metastases are focal in nature, with no true invasion of brain tissue in contrast to high-grade gliomas.

The treatment of brain metastases is often multimodal, with surgery, whole-brain radiotherapy (WBRT), stereotactic radiosurgery, and chemotherapy all playing a role (18, 19). The selection of specific modalities of treatment is dependent on patient-specific factors, such as number, size, and location of metastases, patient's overall health and prognosis, and degree of symptoms attributable to these metastases. Surgery remains a standard treatment for metastatic lesions, particularly those that are symptomatic for patients with good functional status and well-controlled extracranial disease (19). Metastases tend to be noninfiltrative histologically, suggesting that surgical treatment should reduce or eliminate tumor burden and be beneficial. Multiple retrospective analyses have shown survival benefit from resection of single brain metastases, particularly combined with WBRT (19–22). More recent data from 2005 suggest that patients with two or three metastases also show increases in overall survival when the dominant symptomatic lesion is surgically resected (23). The most promising trial of combination chemotherapy showed response rates of 40–59% in breast cancer metastases to the brain (24), although more recent studies show a wide range of response from 10% to 45%, with variability based on the primary tumor (19). Systemic chemotherapy delivery has played a limited role in the treatment of brain metastases, in part because of the poor penetration through the BBB (19). More recent studies suggest that chemotherapeutic agents that have better penetration of the BBB, such as TMZ, may have a role in newly diagnosed metastases, not just as salvage therapy (25, 26). The agents that have shown promise include cyclophosphamide, 5-fluorouracil, methotrexate and vincristine combined, and TMZ, although the use of TMZ has been restricted to recurrent breast metastases (19, 27, 28). The use of TMZ in recurrent breast cancer brain metastases has shown modest increases in survival, although this patient population represents a selection of the most aggressive and refractory cancers that have withstood surgery, WBRT, and multiple other systemic chemotherapies.

Doxorubicin (DOX) has been used to treat early-stage, node-positive breast cancer, particularly HER2-positive tumors, although it is more commonly used for treatment of non-Hodgkins lymphoma and certain leukemias (29). Its clinical use in breast metastases to the brain has largely been limited by the resistance of the tumor to the chemotherapy as well as its poor penetration of the BBB and its dose-related adverse effects. The risk of developing potentially lethal cardiotoxicity increased significantly when doses of DOX reach 500 mg/m² (30).

Microcapsules loaded with DOX or TMZ were administered intracranially in an experimental model of metastases to the brain in the following set of experiments. The CRL1666 cell line has been used in a metastatic spine model. This body of work represents the first time that it has been successfully used in a brain metastasis model to the best of our knowledge (31). Their efficacy was compared with systemic administration of DOX and TMZ. Tissue harvested from these animals was then processed for evidence of induction of apoptosis, and the distribution of drug released from the devices was also examined to test the relationship between drug distribution and biologic activity and efficacy.

Results

TMZ Liquid Crystal Polymer Devices. Liquid crystal polymer (LCP) is nonbiodegradable but biocompatible and inert, and its liquid crystal structure along with polymer chain rigidity do not allow drug partitioning through its membrane, which has been an issue with other polymer compounds used (such as polylactic acid).

Fick's Law of Diffusion was used to estimate the rate of drug delivery from microcapsule devices for the device prototype. Release kinetics were controlled by the diameter of device orifices, the number of orifices, and the solubility of the drug. The inner volume of the reservoir measured 1.5 mL, which allowed for higher drug loading per device volume compared with previous drug delivery devices (10, 32). One important innovation in this work was the use of the injection molding process for high-precision device production, which gave reliable and consistent results, including very small tolerances in volume. These devices can be engineered to achieve desired release kinetics. These devices are not biodegradable and were intact on retrieval from brain tissue at the time of animal euthanasia without evidence of biofouling. Multiple-orifice microcapsules were fabricated to increase the release rate of TMZ. These devices were manufactured with a total of five orifices for drug release: one 889- μ m-diameter orifice in the cap and four 403- μ m-diameter orifices around the circumference of the microcapsule reservoir. The theoretical release rate of TMZ from a single 889- μ m-diameter orifice microcapsule was calculated by calculation of the flux (J) and the area (A) over which drug release occurs. The release rate of TMZ from these devices was found to be 2.5×10^{-5} mg/s or 2.16 mg/d (Fig. 1).

In Vitro TMZ Drug Release Kinetics. The in vitro release of TMZ from the LCP microcapsules into HPLC-grade water was assayed by HPLC. Control devices with sealed orifices were fabricated to test the hermeticity of the UV-curable epoxy and polymer housing. A theoretical release curve based on Fick's Law was constructed for comparison with experimental drug release data. The calculated mass flow rate was 90 mg/h. The experiment was completed in triplicate. Results were reported as average values with SDs. The experimental release curves were in agreement with the theoretical release curve. The experimental TMZ release rate during the first 100 h of release was $88 \text{ mg/h} \pm 6.75 \text{ }\mu\text{g}$. At this time, 67% of the total 12-mg payload was released. The release rate decreased after 100 h, with 71% after 190 h. The devices fabricated for drug leak tests showed that the epoxy sealed the devices, preventing leakage. No drug partitioning through the polymer housing was detected, suggesting that LCP is capable of storing TMZ throughout the length of therapy.

In Vitro DOX Drug Release Kinetics. The in vitro release of DOX from LCP microcapsules was assayed using spectroscopy. A theoretical release curve was again constructed for comparison with experimental release data. The calculated mass flow rate for single-orifice devices was 1.21 mg/d. The calculated rate for multiple-hole devices was 2.16 mg/d (Fig. 2). The experiment was completed in triplicate. Results were reported as average values with SDs. At 300 h, nearly 70% of the 1-mg payload was released from single-orifice devices. Multiple-hole devices released 70% of the payload from multiple-hole devices over a shorter time period (Fig. 2). The experimental and theoretical release curves were in agreement.

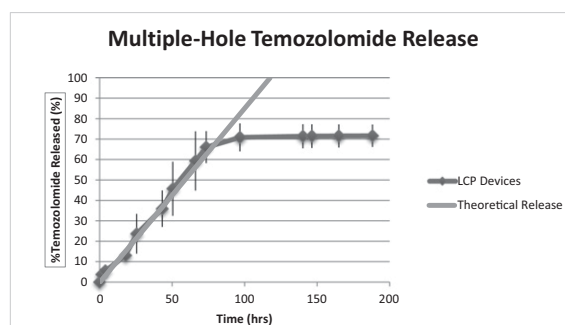


Fig. 1. TMZ LCP device in vitro release.

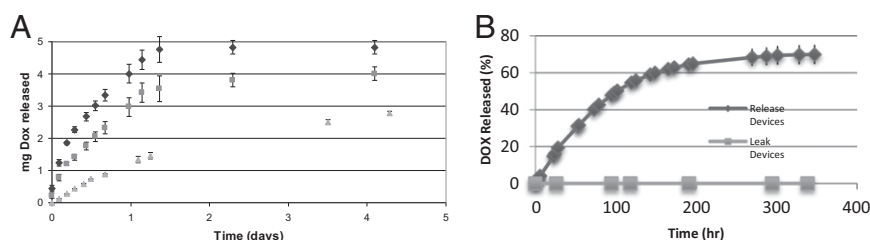


Fig. 2. (A) In vitro release of DOX from one- (green), three- (red), and five-hole (blue) microcapsule devices loaded with 5 mg DOX. (B) DOX LCP single-orifice device in vitro release compared with leak devices.

In Vivo Efficacy Experiments.

Efficacy of locally delivered DOX and TMZ. The primary efficacy outcome was animal survival (Fig. 3). The median survival of untreated control animals was 11 d, with mean of 10 d. Animals with unfilled (blank) LCP-implanted devices had mean and median survival of 12 d. Animals with DOX-loaded LCP devices (payload = 5 mg) had mean and median survival of 14 d. Rats from the systemic DOX and intracranial TMZ microcapsule groups exhibited longer survival rates. TMZ LCP device-treated animals had median and mean survival of 19 and 18 d, respectively, with all dying at 21 d. DOX-treated animals had mean and median survival of 19 d, with all animals dying by 25 d. Statistical analyses of the survival data were performed, and significant differences were observed between the control/DOX ($P < 0.05$), control/TMZ ($P < 0.05$), blank/DOX ($P < 0.05$), and blank/TMZ ($P < 0.05$) groups. There was no statistical significance shown between the control and DOX microcapsule groups.

The second in vivo experiment tested the efficacy of DOX microcapsules loaded with lower payload (3.5 and 5 mg) against other groups. Animal survival was the primary outcome measure (Fig. 4). Untreated control animals experienced a median survival of 13 d. Those animals treated with DOX LCP microcapsules (payload = 5 mg) had median survival of 13 d and mean survival of 15 d. Animals treated with DOX LCP devices (payload = 3.5 mg) had median and mean survival of 12 and 13 d, respectively. DOX animals had median survival of 18 d, with mean survival of 13 d. Animals treated with oral TMZ had mean and median survival of 19 d, with all animals dying by 21 d. Animals with TMZ microcapsules had median and mean survival of 21 and 25 d, respectively, with all dying by 56 d. Significant differences were observed between control/oral TMZ ($P < 0.05$), control/TMZ LCP ($P = 0.01$), DOX LCP (5 mg)/TMZ LCP ($P = 0.02$), DOX LCP (3.5 mg)/TMZ LCP ($P = 0.02$), and DOX/TMZ LCP ($P = 0.01$). Increased survival in TMZ LCP microcapsules was not statistically significant compared with oral TMZ administration ($P = 0.09$).

Cleaved caspase 3 expression on immunohistochemistry. Brain tissue harvested from the in vivo efficacy studies was formalin-fixed and paraffin-embedded for immunohistochemistry. Coronal sections were obtained from each treatment group. Cells expressing the cleaved caspase 3 protein were counted per high-power field (hpf) in all sections and compared between treatment groups (Fig. 5). Untreated control animals had an average of 12 cells/hpf. DOX animal tissue had, on average, 10 cells/hpf. DOX LCP (5 mg) animal tissue had 81 cells/hpf. Blank LCP tissue had an average of 2 cells/hpf. TMZ LCP animal tissue had 103 cells/hpf. Using the unpaired t test, significant differences were observed between control/DOX microcapsule ($P < 0.05$), control/TMZ microcapsule ($P < 0.05$), control/oral TMZ ($P = 0.001$), DOX/DOX microcapsule ($P < 0.05$), and oral TMZ/TMZ microcapsule ($P = 0.003$).

Drug distribution after release from microcapsules. Healthy rodent brain tissues exposed to microcapsules filled with DOX or TMZ were examined for the spatial distribution of drug released over time (Fig. 6). Microcapsules loaded with DOX achieved narrow distribution, with a large peak seen at the sight of implantation and a steep drop out of drug level within 0.25 cm. Rodent tissue exposed systemically to DOX showed low but uniform levels of DOX. Microcapsules loaded with TMZ achieved broader

distribution, with more uniform levels of drug seen within 0.5 cm, and a dropout was seen around 0.7 cm. Systemic TMZ achieved a similar distribution profile but with lower exposure levels than TMZ delivered through microcapsule.

Discussion

A growing trend in pharmacology is designing targeted therapies that minimize indiscriminate delivery and maximize delivery to the desired end organ. Multiple strategies have been used, including nanoparticle-driven delivery, liposomal formulation, and local delivery. Each strategy aims to minimize systemic toxicity. In these experiments, intracranial drug delivery was explored in metastatic breast tumors to the brain. The findings suggest that local chemotherapy delivery may be more efficacious than its systemic delivery, and this efficacy may hinge on drug diffusion. The broader implication for delivery outside the CNS is that successful local delivery depends on the efficacy and distribution of the drug. For those drugs that show strong biologic efficacy but with narrow distribution, strategies to improve distribution, such as chemical modification, should be deployed to improve their tissue penetration.

In Vitro Drug Release. The rates of microcapsule devices exhibited close agreement with theoretical rates for the first 100 h of release. The release from single- and multiple-hole microcapsules plateaus after this time as chemical driving force decreases for diffusion. This plateau results in slower drug diffusion than theoretically predicted. The stability of TMZ also has a profound impact on the in vitro release kinetics. TMZ is a prodrug that quickly degrades into the bioactive MTIC (3-methyl-(triazene-1-yl)imidazole-4-carboxamide) molecule under basic conditions and in high-ionic strength solvents (32). This instability does not seem to be the case for DOX, because it is not a prodrug. In vitro release into water differs from in vivo conditions. The half-life of TMZ in water is considerably greater than its half-life in PBS, in FBS, and in vivo (32). This difference accounts for the close agreement with theory that is seen during the first 100 h of the TMZ water release profiles. Degradation of TMZ because of its instability in solution may account for the observed plateau and may explain why 100% drug is not released experimentally.

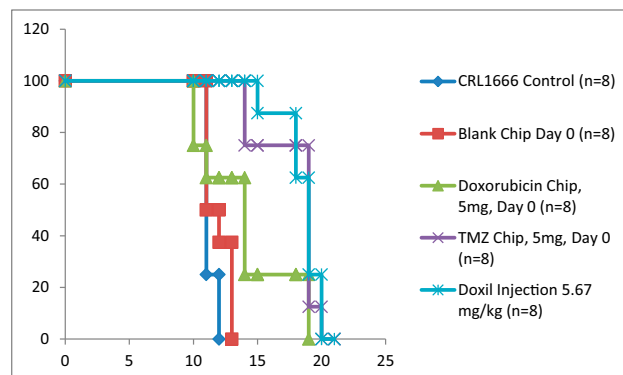


Fig. 3. Efficacy of locally delivered DOX and TMZ in breast metastasis model.

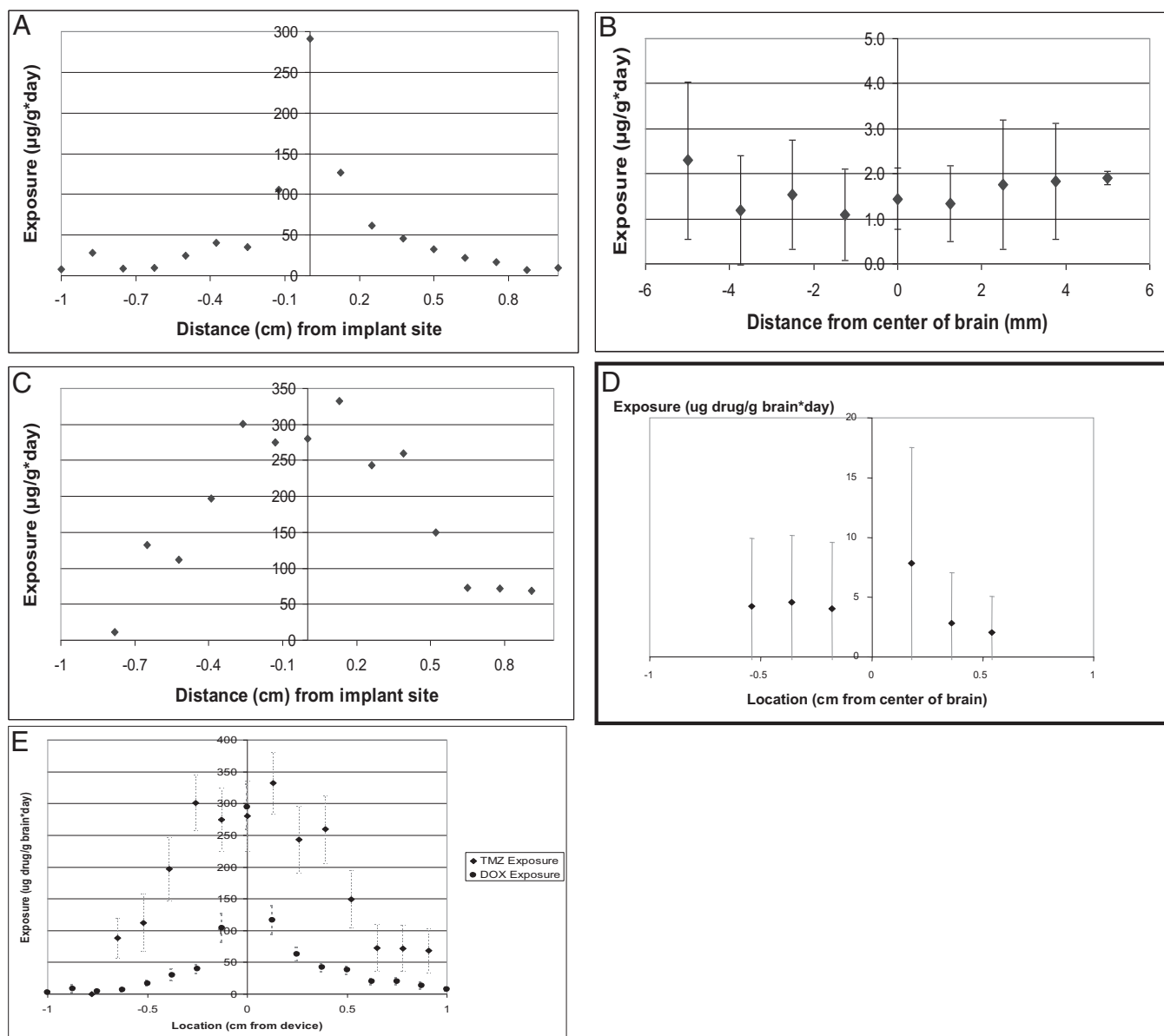


Fig. 6. (A) DOX distribution in rodent tissue when released from the microcapsule. (B) DOX distribution in rodent tissue when delivered systemically. (C) TMZ distribution in rodent tissue when released from the microcapsule. (D) TMZ distribution in rodent tissue when delivered systemically. (E) Comparison of drug distribution profiles when TMZ and DOX were delivered through the microcapsule.

whole-brain radiation therapy may not be attractive given its long-term effects on cognition. This body of work is not meant to suggest that intracranial TMZ therapy alone may cure metastatic breast cancer; however, these studies represent a proof of concept that a chemotherapy-like TMZ, which until now, has been used with limited success, may be more beneficial when delivered intracranially in a device with tunable release kinetics.

Intracranial chemotherapy delivery has largely focused on high-grade gliomas, which are infiltrative by nature and quite different from metastatic lesions (13, 35). The limited efficacy of current intracranial delivery schema, including Carmustine wafers, may be attributed to the inability of chemotherapy to penetrate brain tissue well. Our experiments support the notion that differences in chemotherapeutic drug distribution seem to correlate to tissue response to chemotherapy and length of survival. Therefore, efforts in exploring intracranial drug delivery should focus, in part, on strategies to improve and broaden the distribution of the drug through tissue.

Conclusion

This work has shown that polymer microcapsules are capable of achieving tunable rates of release of various chemotherapies. TMZ microcapsules and systemic DOX seem to show promising efficacy against breast metastases to the brain. We have also shown that the tissues harvested from these experiments indicate that TMZ microcapsules induce apoptosis at higher levels than DOX microcapsules or systemic DOX. Finally, we have shown that the drug distribution profiles for intracranial and systemic DOX are quite different from that of intracranial TMZ and may explain the differences observed in survival. Microcapsules offer versatile targeted delivery without the requirement for intensive drug formulation necessary for liposomal, nanoparticle, or solvent drug-loading methods. These devices also have the unique ability to simultaneously deliver multiple therapies and may be used to deliver a variety of treatments beyond chemotherapy, including nanoparticles and small molecules. The ability of a drug to penetrate tissue requires additional investigation; drugs that show

improved distribution profiles are likely to show more promise in treating lesions of the brain. Finally, the limitations of prior efforts at intracranial chemotherapy delivery may be overcome with focus on new therapeutic targets and strategies to improve the ability of a given chemotherapy to diffuse through CNS tissue. These insights apply to non-CNS applications of local drug delivery, where drug diffusion and inherent efficacy should be analyzed in target tissue. In cases where tissue penetration is poor, drug formulation strategies, although cumbersome, should improve the drug's overall effect. When this two-pronged strategy is used, many new applications of localized drug delivery may be identified and further studied.

Materials and Methods

In Vitro Drug Release Kinetics of TMZ. Twelve milligrams TMZ was loaded into LCP single- and multiple-hole devices. TMZ quantification was done using HPLC with acetonitrile (92:8) mobile phase and UV absorption at 316 nm. Interpolation of the readings with a standard curve was used to quantify the amount of drug released.

In Vitro Drug Release Kinetics of DOX. DOX (3.5 and 5 mg) was loaded into LCP single- and multiple-hole polymer microcapsules for in vitro drug release characterization. DOX was quantified using the UV spectrophotometer at 481 nm. Interpolation of readings with a standard curve was used to quantify the amount of drug released.

In Vitro Cytotoxicity. CRL1666 mammary adenocarcinoma cells were exposed to TMZ and DOX at varying concentrations to determine cytotoxicity. Cell proliferation was quantified using the MTT assay (Invitrogen).

In Vivo Experiments

Tumor and device implantation. Briefly, animals were anesthetized, and the dura was exposed. Stereotactic injection consisted of 75,000 tumor cells in 2.5 μ L that were placed into the brain at a depth of 3.5 mm and delivered over 2 min. Devices were implanted at the time of tumor injection.

Efficacy of locally delivered TMZ and DOX. Two experiments were conducted to determine the efficacy of locally delivered TMZ and DOX in tumor-bearing rats. All TMZ microcapsules used in this experiment had a total TMZ payload of 12 mg with a single 889- μ m-diameter orifice cap. All DOX microcapsule devices used in this experiment had a total payload of 5 mg with a single 180- μ m-diameter orifice cap and one 430- μ m-diameter orifice on the reservoir. The first experiment included five experimental groups and 40 F344 rats: (i) no treatment ($n = 8$), (ii) i.p. DOX (Doxil; Johnson & Johnson) on days 0, 6, and 13 after tumor implantation ($n = 8$), (iii) empty devices ($n = 8$), (iv) TMZ-filled devices ($n = 8$), and (v) DOX-filled devices ($n = 8$). The second study included 80 F344 rats divided into seven experimental groups: (i) no treatment control ($n = 16$), (ii) oral TMZ by gavage on days 5–9 after tumor implantation ($n = 8$), (iii) TMZ-filled LCP microcapsule ($n = 8$), (iv) DOX-filled LCP microcapsule with 5 mg DOX payload ($n = 8$), (v) DOX-filled LCP microcapsule with 3.5 mg DOX payload ($n = 16$), (vi) i.p. DOX on days 0, 6, and 13 ($n = 16$), and (vii) empty device controls ($n = 8$). Overall, animal survival was compared with that of untreated controls. Death was the primary end point for efficacy studies. Kaplan–Meier survival curves were constructed.

Immunohistochemical staining. Cleaved caspase 3 staining was developed by Cell Signaling Technology. The whole brain was sectioned and counted in aggregate, including tumor as well as peritumoral tissue.

Spatial drug distribution. Nondiseased F344 rats underwent implantation of LCP devices with DOX or C^{13} -radiolabeled TMZ. DOX LCP device animals were killed on days 2, 4, 6, 8, and 10 ($n = 4$). Radiolabeled TMZ animals were killed on days 2, 4, 6, 8, and 10 ($n = 5$). Rodent brain tissue was homogenized and processed for quantification of radiolabeled TMZ or DOX.

Western blotting. Anti-cleaved caspase 3 Western blotting was done on rat brain tissue homogenates obtained from efficacy trials. Cleaved caspase 3 rabbit mAb was obtained from Cell Signaling Technology.

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