Local delivery of cancer-cell glycolytic inhibitors in high-grade glioma

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Background. 3-bromopyruvate (3-BrPA) and dichloroacetate (DCA) are inhibitors of cancer-cell specific aerobic glycolysis. Their application in glioma is limited by 3-BrPA's inability to cross the blood-brain-barrier and DCA's dose-limiting toxicity. The safety and efficacy of intracranial delivery of these compounds were assessed.

Methods. Cytotoxicity of 3-BrPA and DCA were analyzed in U87, 9L, and F98 glioma cell lines. 3-BrPA and DCA were incorporated into biodegradable pCPP:SA wafers, and the maximally tolerated dose was determined in F344 rats. Efficacies of the intracranial 3-BrPA wafer and DCA wafer were assessed in a rodent allograft model of high-grade glioma, both as a monotherapy and in combination with temozolomide (TMZ) and radiation therapy (XRT).

Results. 3-BrPA and DCA were found to have similar IC50 values across the 3 glioma cell lines. 5% 3-BrPA wafer-treated animals had significantly increased survival compared with controls (P = .0027). The median survival of rats with the 50% DCA wafer increased significantly compared with both the oral DCA group (P = .050) and the controls (P = .02). Rats implanted on day 0 with a 5% 3-BrPA wafer in combination with TMZ had significantly increased survival over either therapy alone. No statistical difference in survival was noted when the wafers were added to the combination therapy of TMZ and XRT, but the 5% 3-BrPA wafer given on day 0 in combination with TMZ and XRT resulted in long-term survivorship of 30%.

Conclusion. Intracranial delivery of 3-BrPA and DCA polymer was safe and significantly increased survival in an animal model of glioma, a potential novel therapeutic approach. The combination of intracranial 3-BrPA and TMZ provided a synergistic effect.

Keywords: 3-bromopyruvate, dichloroacetate, glioma, glycolytic inhibitor, pCPP:SA.
only 2 ATP molecules for every molecule of glucose in contrast to the >30 ATPs produced through oxidative phosphorylation. Theories have been proposed regarding the evolutionary advantage to malignant cells of the additional role of glycolytic enzymes as regulators of transcription and apoptosis. The breakdown of glucose also provides many of the cellular building blocks required for production of the proteins, nucleic acids, and lipids required for replication. In addition, aerobic glycolysis facilitates extracellular acidosis, selecting for cancer cells with more resistant phenotypes that can withstand anaerobic conditions once the oxygen supply has been outgrown. Some glycolytic enzymes have also been noted to play a role in regulating apoptosis. With the upregulation of glycolysis, therefore, malignant cells would be less likely to undergo cell death.

Hexokinase (HK) is the first regulatory enzyme within glycolysis that catalyzes the conversion of glucose to glucose-6-phosphate, capturing glucose molecules within the cell. HK has 4 isoforms: I, II, III, and IV. HK II is found to be upregulated in many tumor cell lines and primarily promotes anabolic functions such as glycogen and lipid synthesis. Wolf et al. recently found HK II to be a key mediator of aerobic glycolysis in GBMs, as opposed to predominant HK I expression in normal brain tissue and low-grade gliomas. 3-Bromopyruvate (3-BrPA) is a potent ATP inhibitor and direct inhibitor of HK II. 3-BrPA has been shown to be well tolerated in vivo at doses required to significantly inhibit tumor ATP production, decrease lactic acid production, and promote apoptosis, with little noted toxicity to normal tissue at therapeutic doses. While shown to be effective in glioma cell lines, a limitation of the applicability of 3-BrPA to GBM therapy is its inability to cross the blood-brain barrier.

A second molecule for targeting aerobic glycolysis is dichloroacetate (DCA), which inhibits the mitochondrial enzyme pyruvate dehydrogenase kinase (PDK). PDK is a direct inhibitor of pyruvate dehydrogenase (PDH), which regulates the passage of pyruvate into mitochondria. Through the inhibition of an inhibitor, therefore, DCA promotes mitochondrial oxidation and decreases lactic acid formation. In essence, DCA promotes the aerobic metabolism of glucose through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, in place of the conversion of pyruvate into lactate. Due to the widespread distribution of oral DCA in the central nervous system, its efficacy at inhibiting growth in several GBM cell lines has been investigated. DCA led to rapid reversal of mitochondrial hyperpolarization in GBM cells (a marker of an apoptosis-resistant state), promoted p53 activation, and suppressed angiogenesis. In a trial of 3 participants with recurrent GBMs and 2 with primary GBMs, DCA was administered, in addition to standard chemotherapeutic and radiation protocols, and found to possibly be associated with prolonged radiological regression and stabilization. The dose of DCA, however, was limited by reversible peripheral neuropathy. Peripheral nerve toxicity had previously been noted during in vivo and human studies involving disorders of lactic acidosis.

Due to the reported inability of 3-BrPA to cross the blood-brain barrier and the dose-limiting toxicity of DCA, we set out to establish whether these molecules could be safely and effectively delivered locally at the tumor site. To maximize clinical relevancy, we selected a proven, FDA-approved method of local drug release using the biodegradable poly(anhydride, poly-(1,3 bis[p-carboxyphenoxy]propane-co-sebacic acid) (p[CPP:SA, 20:80]). PpCPP:SA, 20:80) has been shown to be biocompatible in the brain with no evidence of systemic or local toxicity. In multiple glioma studies no difference in survival has been noted between groups receiving empty p[CPP:SA] wafers and untreated animals.

We present the tolerability and efficacy of locally delivered 3-BrPA and DCA via p[CPP:SA, 20:80] polymer wafer. We then investigate the combination of 3-BrPA and DCA with temozolomide and radiation therapy.

Materials and Methods

In Vitro Cytotoxicity Analysis

The cytotoxicity of 3-BrPA and DCA were analyzed in the rodent 9L gliosarcoma (Brain Tumor Research Center, University of California at San Francisco) and F98 glioma (R. Barth, Ohio State University) cell lines as well as the human glioblastoma cell line U87 (ATCC). Cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) with 10% fetal bovine serum, penicillin/streptomycin (Invitrogen), and L-glutamine (Invitrogen) and kept humidified at 37°C with 5% CO₂.

Cells were plated in 96-well black-bottom plates (BD Falcon) at 2500 cells/well for the 9L and F98 cell lines and 1500 cells/well for the U87 cell line. After 24 hours, 3-BrPA and DCA in dimethyl sulfoxide were added at a concentration range of 0.0001 mM – 0.3 mM for 3-BrPA and 0.1 mM – 1 M for DCA. Cell counts were measured using the Cyquant cell proliferation assay (Invitrogen) at 450 nm with a microplate reader (Perkin Elmer Victor 3 Wallac) at 48 and 72 hours. Results were repeated no fewer than 3 times. Data were reported as mean inhibitory concentration 50% (IC₅₀) ± 95% confidence interval.

Incorporation of 3-BrPA and DCA into Biodegradable Polymer Wafers

pCPP:SA, 20:80 (Eisai) was synthesized by melt polycondensation, as previously described. To incorporate 3-BrPA into the polymer matrix, an appropriate amount of 3-BrPA was dissolved with pCPP:SA polymer into a 1:40 solution of methanol in methylene chloride. DCA was similarly incorporated using a 1:13 solution of methanol in methylene chloride. Resulting solutions were then placed in a vacuum desiccator until dried. The polymer mixtures were pressed into 10 mg cylindrical wafers and stored at −20°C.

In Vitro Release of 3-BrPA and DCA from Biodegradable Polymer Wafers

One 5% 3-BrPA wafer and one 50% DCA wafer were placed in separate vials containing 1 mL of phosphate-buffered saline (PBS) in triplicate at 37°C. Equal volumes of PBS were removed and replaced at set time points. PBS samples were frozen at −20°C until analyzed. The methods used for measurement of the released 3-BrPA and DCA are detailed in the Supplementary Material. Sample preparation involved a single liquid extraction
using acetonitrile. Phenylbutyric acid was used as an internal standard for both 3-BrPA and DCA.

**Animals**

Female F344 rats, each weighing 160–200 grams, were purchased from Harlan Bioproducts. All rats were housed in standard facilities and provided free access to rodent chow and Baltimore city water. All animals were treated in accordance with the policies and guidelines of the Johns Hopkins University Animal Care and Use Committee.

**Safety of Intracranial Implanted 3-BrPA and DCA Polymer Wafers**

3-BrPA wafers were prepared in concentrations of 1%, 5%, 10%, 25%, or 50% 3-BrPA by weight. DCA wafers were prepared in concentrations of 10%, 25%, or 50% DCA by weight. Rats were anesthetized with an intraperitoneal injection of 3 mL/kg of a stock solution containing ketamine hydrochloride, 75 mg/mL (Ketesthesia, Butler Animal Health Supply), 7.5 mg/mL xylazine (Lloyd Laboratories); and 14.25% ethyl alcohol in 0.9% NaCl. The heads were shaved with clippers and prepared with Prepodyne solution (West Penetone). All surgical procedures were performed using standard sterile techniques. After a midline scalp incision, the galea overlying the left cranium was swept laterally. A 3 mm burr-hole was placed in the left parietal bone with its center 3 mm lateral and 5 mm posterior to bregma. A small dural incision was made, and the polymer was placed into the brain parenchyma. The animals were evaluated postoperatively every day for at least 120 days. Animals were closely monitored for signs of toxicity, including failure to thrive and neurological deficits. Survival was assessed, and necropsies were performed.

**Effect of Intracranial Delivery of 3-BrPA on L-lactate Production**

We measured the L-lactate level in brain extracts using the Lactate Assay Kit, EnzyChrom (BioAssay Systems) according to the manufacturer’s protocol. The brain lysates were obtained from 18 rats bearing 9L intracranial gliosarcoma tumors. Five days after tumor implantation, the rats were randomized as follows: 9 were intracranially implanted with a 5% Br-PA wafer, and 9 received no treatment (controls). Rats were euthanized on day 6, 24 hours after the polymer implantation, at which point most 3-BrPA would have been released (according to in vitro release kinetics). We defined the tumor as both visible and neurological deficits. Survival was assessed, and necropsies were performed.

**Combination 3-BrPA or DCA Polymer Wafers with Systemic Temozolomide and Radiation Therapy**

Study 4 investigated the combination of 5% 3-BrPA wafer and 50% DCA wafer with oral TMZ and radiation therapy (XRT) to more closely model clinical therapeutic regimens for high-grade glioma. Rats were implanted with 9L tumor and randomized to one of the following groups: group 1 (n = 8) received no treatment; group 2 (n = 10) received oral TMZ; group 3 (n = 8) received 5% 3-BrPA wafer; group 4 (n = 8) received 50% DCA wafer; and group 5 (n = 8) received oral TMZ and 5% 3-BrPA wafer. Study 2 assessed the combination of 3-BrPA and DCA polymer, and groups were designated as follows: group 1 (n = 8) received no treatment; group 2 (n = 8) received oral TMZ; group 3 (n = 8) received 5% 3-BrPA wafer; group 4 (n = 8) received 50% DCA wafer; group 5 (n = 8) received 5% 3-BrPA wafer and 50% DCA wafer; and group 6 (n = 8) received 5% 3-BrPA wafer + 50% DCA wafer + oral TMZ. To assess effects of 3-BrPA in combination with TMZ, Study 3 included the following groups: group 1 (n = 8) received no treatment; group 2 (n = 8) received oral TMZ; group 3 (n = 8) received 5% 3-BrPA wafer; and group 4 (n = 8) received 5% 3-BrPA wafer + oral TMZ.

**Efficacy of Locally Delivered 3-BrPA and DCA Polymer Wafers**

9L gliosarcoma was maintained as a subcutaneous mass, which was passaged every 3–4 weeks in the flanks of F344 rats. For intracranial implantation, the tumor was surgically excised from the carrier animal and sliced into 2 mm³ allografts. For intracranial implantation, 270 F344 rats (41 rats for Study 1, 48 rats for Study 2, 32 rats for Study 3, and 149 rats for Study 4) were anesthetized and prepared as described above. Under microscopic magnification, an opening was made through the dura and cortex, and a small area of cortex was resected. A 2 mm³ tumor allograft was placed in the resection cavity. In the polymer treatment groups, either one 5% 3-BrPA wafer or one 50% DCA wafer was placed at the time of tumor implantation. The skin was closed with surgical staples. In the oral DCA treatment group, rats received 80 mg/kg/day of DCA delivered via daily oral gavage for days 0–death (dose as previously published). Rats receiving oral temozolomide (TMZ) were dosed 50 mg/kg via daily gavage for days 5–9. All animals were evaluated daily postoperatively for up to 120 days. Brains were removed and preserved in 10% formalin for analysis. 

In Study 1, rats were randomized as follows: group 1 (n = 9) received no treatment; group 2 (n = 8) received oral DCA; group 3 (n = 8) received 5% 3-BrPA wafer; group 4 (n = 8) received 50% DCA wafer; and group 5 (n = 8) received oral DCA and 5% 3-BrPA wafer. Study 2 assessed the combination of 3-BrPA and DCA polymer, and groups were designated as follows: group 1 (n = 8) received no treatment; group 2 (n = 8) received oral TMZ; group 3 (n = 8) received 5% 3-BrPA wafer; group 4 (n = 8) received 50% DCA wafer; group 5 (n = 8) received 5% 3-BrPA wafer and 50% DCA wafer; and group 6 (n = 8) received 5% 3-BrPA wafer + 50% DCA wafer + oral TMZ. To assess effects of 3-BrPA in combination with TMZ, Study 3 included the following groups: group 1 (n = 8) received no treatment; group 2 (n = 8) received oral TMZ; group 3 (n = 8) received 5% 3-BrPA wafer; and group 4 (n = 8) received 5% 3-BrPA wafer + oral TMZ.
distance from the radiation source with a collimated beam (1 cm in diameter) centered at the allograft site. The remaining body was shielded with lead.

Statistical Analysis

In vitro cytotoxicity results are reported as the inhibitory concentration 50% (IC50) values for each cell line with the associated coefficient of determination ($R^2$). In vitro release of drug is reported as the mean and standard deviation plotted against each time point.

L-lactate levels per condition are expressed as mM/million cells. The values are reported as mean ± SEM for 9 different samples per condition, with each tested in duplicate. One-way ANOVA and Tukey post hoc test were used to analyze the data. Graphs were plotted and statistics calculated with GraphPad Prism software, version 6.1. Survival was the primary endpoint in all in vivo efficacy experiments. Kaplan-Meier analysis was used to analyze survival using GraphPad Prism software. Groups were compared using the Mantel-Cox test with 2-tailed $P$ value. Differences were considered statistically significant at $P < .05$.

Results

In Vitro Cytotoxicity Analysis

3-BrPA inhibited the growth and proliferation of 2 rodent glioma cell lines and the human glioma cell line (Fig. 1). The IC50 value for 3-BrPA was 15.8–25.5 mM at 48 hours, $R^2 = 0.941–0.971$. Similar IC50 values were found at 72 hours: IC50 = 22.3–25.0, $R^2 = 0.910–0.959$. The IC50 values for DCA at 48 hours were 24.2 mM for F98 and 33.7 mM for 9L with $R^2$ values of 0.994 and 0.948, respectively. Similar IC50 values were noted at 72 hours.

In Vitro Release of 3-BrPA and DCA from Biodegradable Wafers

In vitro release showed that the majority of 3-BrPA and DCA were released within the first 10 hours, followed by a steady,
slow release of the remainder over the course of the next 3 weeks (data shown in the Supplementary Section). This pCPP:SA polymer has previously been published as having a 2-phase degradation process.31 Water enters the polymer matrix and hydrolyzes the bonds (<10 h), and then the polymer breaks down and dissolves into solution (>3 weeks). In vivo, the polymer components have been shown to take 6–8 weeks to be entirely absorbed.31

**In Vivo Safety of Intracranial Implanted 3-BrPA and DCA Wafers**

Animals implanted with 1% or 5% 3-BrPA wafers displayed no systemic toxicity, however, toxicity was seen at concentrations of 10%, 25%, and 50% 3-BrPA, with median survival <15 days. The maximally tolerated dose for intracranial delivery of DCA was not reached. Wafers containing the maximal loading dose of 50% DCA revealed no neurological or systemic toxicity. Rats treated with 5% 3-BrPA or 50% DCA displayed normal weight gain with 100% survival to 120 days.

**In Vivo Efficacy of Locally Delivered 3-BrPA and DCA Wafers**

In Study 1, intracranial 5% 3-BrPA and 50% DCA wafers both significantly improved survival in animals with 9L gliosarcoma (Table 1) (5% 3-BrPA median survival, 18 days; \( P = .0027 \) and 50% DCA median survival, 17 days; \( P = .02 \)) relative to the control group (median survival, 13 days). Animals treated with oral DCA (median survival, 11 days) did not demonstrate an improvement compared with the control group. When the 5% 3-BrPA wafer was combined with oral DCA, the combination therapy (median survival, 16 days) was not found to increase survival in comparison with the 5% 3-BrPA wafer (Fig. 3).

Study 2 confirmed that the group receiving the 5% 3-BrPA wafer and the group receiving the 50% DCA wafer had median survivals of 19 days, which significantly prolonged survival compared with the untreated control group (median survival, 14 days; \( P < .01 \)). Combining 5% 3-BrPA and 50% DCA wafers did not provide a survival benefit over either therapy alone. In addition, the efficacy of 5% 3-BrPA and 50% DCA wafer implantation combined with oral TMZ therapy was assessed. This triple combination was well tolerated and resulted in significantly prolonged survival as compared with the controls (\( P = .01 \)). However, it was not found to provide any survival benefit as compared with animals that received oral TMZ only (Fig. 4A).

Study 3 was conducted to assess combined therapy of intracranial 5% 3-BrPA and oral TMZ. Both 5% 3-BrPA wafer and oral TMZ significantly prolonged survival as compared with the untreated controls (\( P < .001 \)). The combination therapy of 5% 3-BrPA wafers and 50% DCA wafers significantly prolonged survival in comparison with the controls (\( P < .01 \)).

**In Vivo Effect of Intracranial 3-BrPA Delivery on L-lactate Production**

The mechanism of 3-BrPA cytotoxicity is not fully understood, but its ultimate effect, blocking glycolysis, is undisputed. The end product of glycolysis is lactate, and the level of lactate reflects glycolytic activity. We performed an in vivo study to measure the lactate level in brain extracts and, specifically, to compare the lactate level in the tumor side with the corresponding contralateral side (Fig. 2). Eighteen brains obtained from rats bearing 9L gliosarcoma were used, 9 of which received no treatment and 9 that received a 5% 3-BrPA wafer on day 5. The lactate level in the tumor of the control groups was significantly higher than the contralateral side (0.89 ± 0.1 vs 0.60 ± 0.05 mM, \( P < .05 \)). In rats with 5% 3-BrPA, the lactate level in the tumor was significantly lower compared with the contralateral side (0.43 ± 0.02 mM vs 0.60 ± 0.05 mM, \( P < .005 \)) as well as when compared with the tumor location in untreated rats (0.43 ± 0.02 mM vs 0.89 ± 0.1 mM, \( P < .005 \)). These data confirm results that were previously shown only in vitro.32,33 These data also provide direct evidence of the antiglycolytic action of 3-BrPA, as well as indirect evidence of its cytotoxicity and effective intracranial release in an in vivo glioma model.

**Fig. 2.** (A) Schematic representation of the effect of 3-BrPA on the metabolism of glioma cells. 3-BrPA has been shown to inhibit both hexokinase (HK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).32,33 The 3-BrPA inhibits aerobic glycolysis and leads to a decrease of ATP and lactate production. (B) In vivo intracranial measurement of L-lactate. Figure depicts the contralateral and ipsilateral (tumor) areas and their respective lactate levels measured at 565 nm on day 6 and normalized to cell number. Values are shown as mean ± SEM of 9 brains per each condition, each tested in duplicate.
The combination of 3-bromopyruvate (3-BrPA) or dichloroacetate (DCA) wafers with systemic temozolomide and radiation therapy significantly increased survival compared to either therapy alone.

### Discussion

This study presents the successful integration of 2 cancer cell glycolytic inhibitors into biodegradable wafers for local, intracranial chemotherapeutic delivery. These results demonstrate that intracranial 3-BrPA and DCA wafers are well tolerated and significantly increase survival relative to the control group and systemic delivery of these compounds in a rodent model of glioma. In combination with oral TMZ, the intracranial 3-BrPA wafer was found to significantly improve survival over both therapies alone. When both 3-BrPA and DCA wafers were combined with the current clinical regimen for high-grade glioma, oral TMZ and XRT, both were well tolerated. The addition of either glycolytic inhibitor with TMZ and XRT treatment did not provide any statistical survival advantage as compared with the combined 3-BrPA or DCA wafer therapy alone.

**Table 1.** Treatment of 9L rat gliosarcoma with 3-bromopyruvate, dichloroacetate, temozolomide, and radiation therapy

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Survival, Days (Range)</th>
<th>P Value</th>
<th>Long-term Survivors (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>13 (10–17)</td>
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<tr>
<td>80 mg/kg oral DCA (n = 8)</td>
<td>11 (10–15)</td>
<td>NS vs control</td>
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</tr>
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<td>5% 3-BrPA wafer (n = 8)</td>
<td>18 (11–24)</td>
<td>.003 vs control</td>
<td>0</td>
</tr>
<tr>
<td>50% DCA wafer (n = 8)</td>
<td>17 (11–24)</td>
<td>.02 vs control</td>
<td>0</td>
</tr>
<tr>
<td>Oral DCA + 5% 3-BrPA wafer (n = 8)</td>
<td>16 (11–24)</td>
<td>NS vs 5% 3-BrPA</td>
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</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td>5% 3-BrPA wafer (n = 8)</td>
<td>19 (14–26)</td>
<td>&lt;.01 vs control</td>
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</tr>
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<td>50% DCA wafer (n = 8)</td>
<td>10 (19-LT)</td>
<td>&lt;.001 vs control</td>
<td>1</td>
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<td>Oral TMZ (n = 8)</td>
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<td>&lt;.01 vs control</td>
<td>0</td>
</tr>
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<td>5% 3-BrPA wafer + 50% DCA wafer (n = 8)</td>
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<tr>
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<td>NS vs oral TMZ</td>
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<tr>
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<tr>
<td>Oral TMZ (n = 8)</td>
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<td>&lt;.05 vs 5% 3-BrPA wafer; &lt;.01 vs oral TMZ</td>
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</tr>
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<td>XRT (n = 8)</td>
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<td>&lt;.0001 vs control; &lt;.0001 vs oral DCA</td>
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<td>NS vs control</td>
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<td>5% 3-BrPA wafer day 0 + TMZ + XRT (n = 10)</td>
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<td>&lt;.05 vs 5% 3-BrPA wafer day 0; NS vs TMZ + XRT</td>
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<tr>
<td>5% 3-BrPA wafer day 5 + TMZ + XRT (n = 10)</td>
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<td>50% DCA wafer day 5 + TMZ + XRT (n = 10)</td>
<td>40 (19-LT)</td>
<td>&lt;.0001 vs 50% DCA wafer day 5; NS vs TMZ + XRT</td>
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</table>

Abbreviations: * 3-BrPA, 3-bromopyruvate; DCA, dichloroacetate; LT, long-term survivor(s); NS, no statistical significance; TMZ, temozolomide; XRT, radiation therapy.

3-BrPA wafer with oral TMZ significantly improved survival over either therapy alone (P < .05) (Fig. 4B).
those receiving TMZ and XRT, although the triple combination therapy was associated with a few long-term survivors. The treatment strategy assessed through this series of experiments is based on the following factors: (i) the route of administration (local release of 3-BrPA and DCA) and (ii) the potential for additional therapeutic benefit when glycolytic inhibitors are given in combination with systemic TMZ and XRT.

Effect of 3-BrPA on Cancer Cell Metabolism and the Necessity for Local Delivery

Several oncoproteins have been recognized to induce glycolytic enzymes, which in turn have been shown to play a regulatory role in cancer cell apoptosis.8 One oncogene in particular, AKT, has been found to promote both aerobic glycolysis and inhibit apoptosis by both transcriptional and nontranscriptional mechanisms.8,34 AKT inhibits apoptosis in part by negatively regulating the enzyme, glycogen synthase kinase 3 beta (GSK3beta). Inhibition of GSK3beta promotes the binding of HK II to the outer mitochondrial membrane.35 HK II, in contrast to other forms of hexokinase, contains a N-terminal hydrophobic domain that allows binding to the outer mitochondrial membrane protein, voltage-dependent anion channel (VDAC). The binding of HK II to VDAC yields a 5-fold increase in its affinity to ATP and provides feedback inhibition from G-6-P. VDAC has also been found to be a key protein in mitochondria-mediated apoptosis with HKII acting in an apoptosis-suppressive capacity.16,17 3-BrPA has been found to cause dissociation of HK II from VDAC and significantly inhibit the phosphorylated, active form of AKT.38 Therefore, 3-BrPA is able to decrease cancer cell aerobic glycolysis and promote apoptosis.39 3-BrPA has also been noted to interact with other intracellular proteins as an alkylating agent.40

Effective 3-BrPA therapy for glioma requires determination of sufficient drug delivery to the brain for maximal cytotoxic effect against the tumor cells. Vali et al assessed the biodistribution using radio-labeled (14C)3-BrPA and found a consistently low uptake in the highly metabolic tissue of the brain, suggesting poor penetration of the blood-brain barrier.18 Dose escalation studies of 3-BrPA in rodents have revealed systemic toxicity.
at doses >20 mg/kg with death at doses >30 mg/kg. In our study, 3-BrPA at a systemic dose of 12 mg/kg/day, a dose found to be efficacious for in vivo tumor models outside of the CNS, was not found to be effective in the 9L intracranial gliosarcoma model. The potential solution is local drug delivery. Local administration of 3-BrPA was initially described as transarterial delivery in a rabbit model of liver cancer. Intracranial local delivery of 3-BrPA has not yet been described. Our study
demonstrates that intracranial controlled-release of 3-BrPA at a concentration required to achieve a survival benefit is safe. The 5% 3-BrPA wafer consistently provided a >30% survival benefit in the rodent 9L gliosarcoma model. As previously noted, the rapid release of 3-BrPA within the first 24 hours is consistent with the release of other compounds from p(CPP:SA 20:80) such as 1,3-bis(2-chloroethyl)-1-nitrosourea.21

Role of DCA in Promoting Oxidative Phosphorylation and Benefit of Local Delivery

Aerobic glycolysis relies on increased lactic acid production in place of the mitochondrial-based oxidative phosphorylation. The gate-keeping enzyme PDH regulates whether pyruvate, the product of glycolysis, is broken down to lactic acid or enters the mitochondria to undergo the TCA cycle and electron transport chain.21 PDH functions to decarboxylate pyruvate to acetyl-coenzyme A in order for it to enter the TCA cycle. The flow of electrons down the electron transport chain is associated with production of reactive oxygen species (ROS). PDH is inactivated by its phosphorylation via PDK. DCA, an inhibitor of PDK, has been found to inhibit lactic acid production and promote oxidative phosphorylation.19 Oxidative phosphorylation has been associated with increased production of ROS and efflux of proapoptotic mediators from mitochondria with induction of mitochondria-dependent apoptosis in cancer cells but not in normal cells.21

DCA at a dose of 50 mg/kg/day was found to possibly prolong radiographic regression and stabilization in human patients with GBM. Unfortunately, the dose was associated with reversible peripheral neuropathy.6 DCA administered orally at a dose of 80 mg/kg/day was found to decrease the tumor size in a rodent model of subcutaneous adenocarcinoma, although animal survival was not analyzed.21 We did not find that 80 mg/kg/day of oral DCA provided a survival benefit in the intracranial 9L rodent gliosarcoma model, which is perhaps evidence for a higher systemic dose requirement for glioma therapy. Higher systemic doses, however, may entail even greater toxicity. DCA in a 3-month chronic administration study was found to have a lethal dose of 2000 mg/kg in rats, while a lethal dose of 75 mg/kg was noted in dogs.62 The success seen with the local delivery of DCA via a 50% DCA wafer suggests that local delivery of DCA may be required to achieve an efficacious dose at the tumor site without the toxicity associated with systemic DCA delivery.

Combination of Glycolytic Inhibitors with Temozolomide and Radiation Therapy

The combination of 3-BrPA wafer and DCA, both in oral and polymer wafer form, with TMZ and XRT may warrant further investigation because 3-BrPA wafer in combination with TMZ was found to provide a synergistic effect when compared with either TMZ or 5% 3-BrPA wafer alone. Glioma stem cells are thought to be a major contributor to the resistance of gliomas to standard treatments.2 The chemoresistant state may be due to the upregulation of multidrug resistant genes, which leads to reduced intracellular drug accumulation through increased expression of membrane efflux transporters, as well as the inhibition of apoptosis.43,44 Nakano et al recently elucidated that glycolysis may be related to cancer cell chemoresistance.45 Since the function of membrane efflux transporters is dependent on ATP and ATP production in tumor cells is largely based on aerobic glycolysis, Nakano et al hypothesized that inhibition of glycolysis will preferentially decrease the efflux of anticancer agents, allowing for maximal therapeutic effect. They found that 3-BrPA restored the cytotoxic effect of daunorubicin and doxorubicin in resistant tumor cell lines expressing membrane efflux transporters. Indirect evidence from numerous studies has shown that enzymes targeted by 3-BrPA are implicated in TMZ chemoresistance, which suggests that TMZ could be potentially more effective when combined with 3-BrPA.46,47 Our study shows that 3-BrPA enhances TMZ efficacy in vivo. This synergistic and chemosensitizing effect is either due to HKII inhibition,54 which has been shown to sensitize glioma cells to TMZ, or to the downregulation of p-Akt, which plays a key role in the overexpression of a GLUT 3 transporter46 and Mcl-1,47,48 an anti-apoptotic Bcl-2 family related to TMZ resistance.

Radiosensitivity is thought to be a common property of cancer stem cells, possibly due to their ability to more efficiently repair DNA relative to noncancer stem cells.49 It has been noted that cancer stem cells also contain lower levels of ROS due to enhanced expression of free radical scavenger systems. ROS are the predominant mediators of cell death induced by ionizing radiation; therefore, enhanced scavenging of free radicals may result in increased radiosensitivity.50 El Sayed et al recently showed that 3-BrPA increases H2O2 production, and thus increases ROS.16 Similarly, DCA has been shown to increase whole cell H2O2 within GBM cell lines.

Conclusion

Both 3-BrPA and DCA were found to be cytotoxic in vitro in a human glioma cell line as well as rodent glioma and gliosarcoma cell lines. These glycolytic inhibitors were successfully integrated into pCPP:SA polymer, which allowed for their intracranial delivery. In vivo experiments showed a significant increase in survival with 5% 3-BrPA and 50% DCA wafers in the intracranial 9L rodent gliosarcoma model. 5% 3-BrPA wafer in combination with TMZ significantly increased survival as compared with either therapy alone and in combination with TMZ and XRT, while no statistical difference was found, and an increase in the number of long-term survivors was noted. Oral DCA and 50% DCA wafer, given in combination with TMZ and XRT, also yielded a few long-term survivors. Further studies should be conducted to investigate the local delivery of 3-BrPA and DCA in additional cell lines and to assess potential mechanisms for increased efficacy of combined local delivery of 3-BrPA and DCA with current standard clinical regimens.

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