Effect of trans sodium crocetinate on brain tumor oxygenation

Laboratory investigation

JASON SHEEHAN, M.D., PH.D., JONATHAN SHERMAN, M.D.,
CHRISTOPHER CIFARELLI, M.D., PH.D., JAY JAGANNATHAN, M.D., KASANDRA DASSOULAS, B.S.,
CLAIRE OLSON, B.A., JESSICA RAINNEY, AND SHAOJIE HAN, M.S.

Department of Neurological Surgery, University of Virginia Health System, Charlottesville, Virginia

Object. Glioblastoma multiforme tumors typically exhibit regions of hypoxia. Hypoxic regions within the tumor make cells less sensitive to radiosurgery and radiation therapy. Trans sodium crocetinate (TSC) has been shown to be a radiosensitizer. The goal of this research was to elucidate the underlying mechanism of TSC’s radiosensitizing effect.

Methods. A rat C6 glioma model was used. The C6 glioma cells were stereotactically injected into the rat brain to create a tumor. Two weeks later, MR imaging was used to confirm the presence of a glioma. Following demonstration on MR imaging of a brain tumor, animals were randomized into 1 of 2 groups: 1) TSC alone (100 µg/kg), or 2) saline control. Licox probes were inserted into the brain tumor and contralateral cerebral hemisphere. Tissue oxygenation measurements were recorded before and after intravenous infusion of either TSC or saline.

Results. Not surprisingly, tissue oxygenation measurements revealed that the brain tumor was hypoxic relative to the contralateral cerebral hemisphere brain tissue. Two to 8 minutes after TSC was infused, tissue oxygenation measurements in the brain tumor increased above baseline by as much as 60%. After this temporary elevation following TSC infusion, tumor oxygenation measurements returned to baseline. No significant elevations in tissue oxygenation were seen on the contralateral side. Similarly, the saline vehicle was not observed to increase tissue oxygenation in either the brain tumor or the contralateral brain tissue.

Conclusions. Administration of TSC transiently improves tissue oxygenation in hypoxic gliomas. Such an effect is one potential mechanism for the radiosensitization previously observed after addition of TSC.

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Key Words • trans sodium crocetinate • glioblastoma multiforme • radiosensitization • oxygen diffusion • rat

Glioblastoma multiforme is a very aggressive type of intracranial tumor. The mainstays of treatment are cytoreductive surgery followed by radiation therapy and chemotherapy.1,2 Areas within these tumors have been found to be hypoxic, and hypoxia may decrease the effectiveness of adjuvant therapy.13 Oxygen is known to be a powerful radiosensitizer of mammalian cells.4,15 It can increase the dose efficiency of ionizing radiation by a factor of 3. Considering the many intracranial tumors that have regions of relative hypoxia, the importance of raising partial brain O₂ concentrations to physiological levels during initiation of treatment cannot be overstated.

Trans sodium crocetinate, a carotenoid compound, has been shown to increase the diffusion of O₂ in plasma and, hence, to lead to improvements in O₂ availability to other tissues. The mechanism for this is believed to be due to interactions between the hydrophobic TSC molecules and water, and it should occur in any aqueous solution.17 In vitro measurements confirm that there is approximately a 30% increase in the diffusivity of O₂ through blood plasma.11,17 Because TSC also increases the diffusivity of O₂ through other aqueous-based systems, it may also have an effect on transport through extravascular fluid, although TSC appears to remain primarily in the intravascular space.

Previously, TSC was found to radiosensitize C6 glioma cells in vivo.14 In this study we examined the direct effects of TSC on the oxygenation of C6 gliomas in an in vivo model.

Methods

Study Design and Animals

A total of 30 Sprague-Dawley rats (Charles River Laboratories, Inc.) were included in the study. Each animal weighed between 200 and 250 g. They were allowed free access to food, and a 12:12 hour light/dark cycle was maintained. All aspects of this research were performed according to the National Institutes of Health animal experimentation guidelines and approved by the University of Virginia’s animal care and utilization committee.

This article contains some figures that are displayed in color online but in black and white in the print edition.

Abbreviations used in this paper: PBS = phosphate-buffered saline; TSC = trans sodium crocetinate.
After the tumor implantation, the animals were randomly assigned to 1 of 2 groups: 1) TSC alone; or 2) control (that is, saline infusion) alone.

Cell Line

The tumor cell line used was the C6 glioma line from American Type Culture Collection. Cells were cultured for 4–5 passages in F-12 Kaighn nutrient mixture medium (GIBCO) supplemented with 15% horse serum, 2.5% fetal bovine serum, and 1% antibiotic-antimycotic solution. The medium was changed twice per week, and the cells were grown in 75-cm² culture flasks. Cells were incubated at 37°C in 5% CO₂, passed weekly by washing with PBS, detached in 10 ml of 0.5 mM ethylenediaminetraacetic acid, and subcultured at a ratio of 1:10.

Cells for implantation were obtained by trypsinization from subconfluent cultures, washed twice with PBS, and resuspended in 10 mM PBS–glucose to a final concentration of $5 \times 10^5$ /μl. The cell density was determined using the microscope stage micrometer. The time between removal of the cells from culture to implantation in the rat host was < 90 minutes.

Magnetic Resonance Imaging

Each rat's brain was imaged 13 days after tumor implantation by using a Varian MR imaging system (4.7-T, 40-cm-bore magnet; accessible diameter of 32.4 cm) (Varian Medical System) in the University of Virginia's Small Animal Multimodality Imaging Core. A Gd-enhanced, T1-weighted pulse sequence was used for imaging (TR/TE 400/20, number of excitations 2, field of view 8 cm, matrix size 256 × 192, slice thickness 3 mm). The pulse sequences allowed 30 slices per scan, which covered the entire brain. The average scan time was 3 minutes. All imaging was performed 1 minute after an intraperitoneal injection of 1 ml (70 mg) of gadoteridol (Prohance; Bracco Diagnostics, Inc.). The images obtained in the sessions were analyzed to verify tumor implantation in each animal.

Animals were excluded from the tissue oxygenation measurements if there was no discernible evidence of tumor, as indicated by the presence of a circumscribed lesion with Gd enhancement on the postoperative MR images. In those animals with tumor visible on MR images, the lesion’s depth and cross-sectional diameters were measured to determine the appropriate insertion depth for the Licox monitor probe’s tip. Probes were inserted into the tumor side through the previously placed bur hole, which had been used for stereotactic insertion of the glioma cells.

Administration of TSC

The TSC was provided as a gift by Diffusion Pharmaceuticals. The TSC was administered to the animals by tail vein injection starting on the 14th day after tumor implantation. Following 20 minutes of baseline Licox tissue measurements in each animal, the TSC was delivered intravenously in bolus form. This 20-minute interval permitted equilibration of the probe in the brain or tumor tissues. Rats in the TSC group were treated with 100 μg/kg of the drug. Control animals received a comparable volume of saline intravenously.

Licox Measurements

A total of 9 animals were excluded from the tissue oxygenation measurements due to either poor tumor grafting as noted on MR imaging or a substantial clinical deterioration within the first 2 weeks following surgery. During tissue oxygenation measurements, animals were anesthetized with ketamine/xylazine for ~60 minutes. The previous cranial incision was opened and the bur hole overlying the tumor was localized. A contralateral bur hole was placed overlying the opposite cerebral hemisphere.

Small-animal Licox probes (model #CC1, Integra) were used. The probe is 0.5 mm in diameter and has a PO₂ sensitive area of 7 mm². Probe error is typically 3.8 ± 3.5%. There is a tendency for the probe to underestimate PO₂ tension; there is typically little in the way of drift in the probe readings during the time span of these experiments (that is, 1–2 hours). Twenty minutes of baseline measurements were taken with each probe, and stabilization of the PO₂ measurements was confirmed prior to injection of the agent or vehicle. Two Licox probes were inserted, one through each bur hole, thereby allowing for simultaneous measurements of PO₂ on the ipsilateral (that is, with tumor) and contralateral (without tumor) cerebral hemispheres. On the tumor side, the Licox probe was inserted at a depth derived from the MR imaging that would put the probe tip within the tumor itself (Fig. 1). Animals spontaneously breathed room air during tissue oxygenation measurements.

Ultimately, tissue oxygenation measurements were made in 12 TSC-treated animals and in 9 saline-treated controls. Tissue oxygenation changes were expressed as a ratio of the tissue oxygenation at a single time point following TSC or saline infusion, divided by the mean tissue oxygenation over a 20-minute time period prior to the infusion. Measurements were taken at 2-minute intervals.

Statistical Analysis

To evaluate for differences in tissue oxygenation between treatment groups, a commercial statistical package (SPSS version 15.0; SPSS, Inc.) was used. A probability value of ≤ 0.05 was considered to be statistically significant.
and brain (B).

ing the location of tissue oxygenation measurements of the tumor (T) and chemotherapy.16 It may also stimulate angiogenesis and radiosurgery, radiation therapy, and hypoxia-induced gene expression.10

The ability to alter hypoxia in tissue has many potential roles for CNS lesions. In the setting of a brain tumor, shifting hypoxic tissue to normoxia may permit more beneficial effects from radiation or chemotherapy. Similarly, increased oxygenation may facilitate improvements in the rate and extent of recovery from hypoxic injury associated with a stroke or neurotrauma.

Normoxia rather than hyperoxia appears to be more appropriate in the clinical arena for pathological entities in the CNS. Normal brain tissue is very well perfused and therefore well oxygenated. Quantitative MR imaging of human brain perfusion reveals that gray matter is perfused at a rate of 93 ± 16 ml/100 g/min, white matter at a rate of 38 ± 10 ml/100 g/min, and whole brain at a rate of 52 ± 8 ml/100 g/min.2 This translates to a typical tissue oxygenation in the brain of > 25 mm Hg. In the setting of normoxic brain tissue, TSC did not appreciably alter partial tissue oxygenation. However, in the context of hypoxia associated with a high-grade glioma, a single intravenous bolus of TSC temporarily elevated tissue oxygenation above its baseline hypoxic state.

The selection of the 100 µg/kg dose of TSC is based on a dose-response curve generated in an ischemic rat model and the effective dose for radiosensitization seen by this group (unpublished data).14 It is also within the effective TSC range of 60–180 µg/kg reported by other groups.7–9 The short duration of the elevation in tumor oxygenation following TSC administration may have been a function of the method of administration (bolus) and the pharmacokinetics of the drug. Refinements in delivery, dose, and half-life of the agent may improve the therapeutic window of improved tissue oxygenation.

The increased order within plasma as facilitated by TSC would not seem to increase the oxygenation of already well-perfused brain tissue. The TSC appears to alter the hydrogen bonding patterns in serum and thereby cause an increase in the rate of O2 diffusion across capillary membranes.7 In the setting of hypoxia, the effect of increased O2 diffusion seems to be accentuated. In a previous study,17 TSC has been shown to increase the diffusion of glucose and O2 through water by 25–30%. This effect is probably achieved through induction of order in the surrounding water structure through increased hydrogen bonding of water molecules.17,18 In the setting of hemorrhagic shock, TSC has been shown to reduce lactate levels, presumably through increased O2 diffusivity.15 Also, in animals receiving supplemental O2, TSC was found to increase O2 delivery to baseline normoxic brain tissue.11

In previous work by our group, TSC was seen to increase the sensitivity of the C6 glioma to radiation.14 The current results would serve as a reasonable explanation for this effect. During irradiation, tumor cell death results from direct ionizations within the pathological tissue. Increased oxygenation can enhance free-radical generation within the treated volume.3 Thus, temporary improvement in oxygenation of a hypoxic tumor appears to afford a therapeutic advantage during delivery of certain forms of adjuvant treatment. Further study of this compound in

Discussion

High-grade gliomas are well known to have regions of tissue hypoxia, which is likely to contribute to the resistance of gliomas to radiosurgery, radiation therapy, and chemotherapy.16 It may also stimulate angiogenesis through vascular endothelial growth factor upregulation and lead to glioma cell invasion.5,20 The development of antiangiogenic therapies for CNS malignancies will probably require a better understanding of tumor hypoxia and hypoxia-induced gene expression.10

Results

Licox measurements were taken in the cerebral hemispheres ipsilateral and contralateral to the C6 glioma. Absolute tissue oxygenation measurements of C6 glioma tissue were consistent with hypoxic tissue (< 25 mm Hg), whereas the contralateral cerebral hemisphere typically showed normal partial tissue oxygenation. During the ~1 hour of tissue oxygenation monitoring, there were no statistically significant differences noted in the normalized oxygenation values among the following groups: 1) brain parenchyma in saline-treated rats; 2) tumor in saline-treated rats; and 3) brain parenchyma in TSC-treated animals.

Nevertheless, a marked increase in tissue oxygenation measurements was observed within the brain tumors of those animals in which TSC was administered. A statistically significant elevation in tissue oxygenation was seen from 22 to 28 minutes into the experiments (p < 0.05; t-test). Because the agent (or saline) was administered after 20 minutes of baseline oxygenation monitoring, this amounted to elevations in tissue oxygenation ~ 2-8 minutes after administration of TSC. The TSC increased brain tumor oxygenation measurements by factors of 1.66, 1.48, 1.38, and 1.33 at 2, 4, 6, and 8 minutes, respectively, following TSC bolus (p < 0.05). Tissue oxygenation in the brain tumors of the TSC-treated animals was increased by as much as 66% before returning to baseline (Fig. 2).

Fig. 1. Coronal T1-weighted contrast-enhanced MR image illustrating the location of tissue oxygenation measurements of the tumor (T) and brain (B).
Tissue oxygenation in a glioblastoma multiforme

Fig. 2. Graph showing normalized partial tissue oxygenation following administration of TSC in a rat model of C6 glioma. The TSC was administered 20 minutes after the start of Licox monitoring. Statistically significant differences (denoted by asterisks) were noted at 22-, 24-, 26-, and 28-minute time points in the tumor tissue of the animals receiving TSC.

the setting of hypoxic conditions within the CNS seems warranted.

Conclusions

Intratumoral hypoxia is a common finding in the setting of high-grade gliomas, and has been shown to diminish the effectiveness of radiosurgery. Treatment with TSC appears to increase the tissue oxygenation temporarily in hypoxic C6 gliomas. Under a normal fraction of inspired oxygen, TSC did not elevate the oxygenation concentration of normal brain tissue. Additional testing of TSC appears warranted for evaluation of therapeutic advantages afforded by a compound that reduces hypoxia in pathological tissue.

Disclosure

The TSC was provided by Diffusion Pharmaceuticals (Charlottesville, VA), and Licox probes were provided by Integra Lifesciences Corp. (Plainsboro, NJ). Also, Diffusion provided a grant to fund a portion of this research. Diffusion Pharmaceuticals did not devise the scientific methodology and had no role in the data analysis or preparation of the manuscript. The investigators have not received any financial payment from these companies.

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