Cancer Research

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Cancer Res 2011;71:3753-3762. Published OnlineFirst April 20, 2011.

Published OnlineFirst April 20, 2011; DOI:10.1158/0008-5472.CAN-11-0451

Preclinical Validation of Electrochemotherapy as an Effective Treatment for Brain Tumors

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Abstract

Electrochemotherapy represents a strategy to enhance chemotherapeutic drug uptake by delivering electrical pulses which exceed the dielectric strength of the cell membrane, causing transient formation of structures that enhance permeabilization. Here we show that brain tumors in a rat model can be eliminated by electrochemotherapy with a novel electrode device developed for use in the brain. By using this method, the cytotoxicity of bleomycin can be augmented more than 300-fold because of increased permeabilization and more direct passage of drug to the cytosol, enabling highly efficient local tumor treatment. Bleomycin was injected intracranially into male rats inoculated with rat glia-derived tumor cells 2 weeks before the application of the electrical field (32 pulses, 100 V, 0.1 ms, and 1 Hz). In this model, where presence of tumor was confirmed by magnetic resonance imaging (MRI) before treatment, we found that 9 of 13 rats (69%) receiving electrochemotherapy displayed a complete elimination of tumor, in contrast to control rats treated with bleomycin only, pulses only, or untreated where tumor progression occurred in each case. Necrosis induced by electrochemotherapy was restricted to the treated area, which MRI and histology showed to contain a fluid-filled cavity. In a long-range survival study, treatment side effects seemed to be minimal, with normal rat behavior observed after electrochemotherapy. Our findings suggest that electrochemotherapy may offer a safe and effective new tool to treat primary brain tumors and brain metastases. *Cancer Res; 71(11); 3753–62. ©2011 AACR.*

Introduction

Primary and secondary malignant tumors of the brain constitute a significant challenge in cancer treatment. Surgery, radiotherapy, and medical treatment are advancing, but the prognosis is still grim and morbidity considerable (1, 2).

Electroporation-based treatments may be a new contributor in inhibiting tumor recurrence, while giving rise to limited side effects (3–7). Electroporation—permeabilization of membranes by electrical pulses—has become an expanding research field, both within nonthermal irreversible electroporation from which cell membranes do not recover and cell death ensues (3, 4, 7–9), and within reversible electroporation where cell membranes do reseal and only transiently allow drugs (10, 11) or genes (12–20) to enter the otherwise intact cell.

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doi: 10.1158/0008-5472.CAN-11-0451

Electrochemotherapy designates the use of electroporation to enhance local uptake of chemotherapeutic agents (21–24), enabling an increase in cytotoxicity of a staggering 300-fold in the case of bleomycin. What is well known is that electrochemotherapy with its high efficiency may be used as a once-only treatment, and that no treated cancer histology has yet been unresponsive to the treatment (25). Both these factors are an advantage for tumors in internal organs, including intracranial tumors. Electroporation-based treatments in internal organs are ongoing (www.clinicaltrials.gov; refs. 26, 27).

Bleomycin is well known in oncology and is used in standard treatment regimens; for example, its use in testicular cancer (28) and intracerebral use are well described (29). The choice of bleomycin as the cytotoxic agent for electrochemotherapy has been previously described (5, 6, 22, 25, 30–32) and has a solid scientific base; thus, bleomycin acts as an enzyme creating 10 to 15 DNA strand breaks per molecule (33), which is a far more efficient rate than any other chemotherapeutic agent. Bleomycin is large, charged, and hydrophilic, and the cell membrane will under normal circumstances keep it from the actual target of the drug, namely DNA. Electroporation offers direct access over the cell membrane in the area encompassed by the electrodes, allowing bleomycin to exert its cytotoxic potential much more efficiently. Bleomycin as a single drug has been tried in the treatment of brain tumors before, with acceptable toxicity but limited efficacy (29). Only one previous study, by Salford and colleagues (34), investigated the use of reversible electroporation in a rat brain tumor model facilitating the

Published OnlineFirst April 20, 2011; DOI:10.1158/0008-5472.CAN-11-0451

uptake of bleomycin (electrochemotherapy) by using 2 acupuncture needles inserted into rat brains inoculated with tumor cells. In this study, survival was improved by a few days; however, the electrical field between 2 single needles is of limited extension.

We have now developed an electrode device, which can be inserted through a single burr hole, with electrodes that may be subsequently deployed in a cone-shaped formation to treat a target area much larger than the burr hole. Electrochemotherapy, encompassing intracranial injection of bleomycin followed by pulses, was carried out in normal and tumor-bearing rats in short- and long-term studies with the use of this novel brain electroporation device to explore electrochemotherapy as a new treatment modality for brain tumors.

Materials and Methods

Study design

Three experimental designs, each conducted through 2 to 4 independent experiments, were carried out in rats.

Experimental setup 1 ($n = 60$): What are the appropriate brain electrodes and parameters for electrochemotherapy in the rat brain? Tumor cells were inoculated in the rat brain, and after 1 to 2 weeks electrochemotherapy, electroporation only, bleomycin only, or NaCl were injected followed by electroporation by using a 4- or 8-electrode device, respectively. After treatment, magnetic resonance imaging (MRI) was carried out repeatedly and animals were observed for 1.5 weeks.

Experimental setup 2 ($n = 25$): Could electrochemotherapy eliminate rat brain tumors? Tumor cells were inoculated and only after MRI verification of tumor, rats were treated by electrochemotherapy, electroporation alone, or bleomycin alone by using the 8-electrode device. After treatment, MRI was carried out repeatedly and the animals were observed for up to 3 weeks, with prior termination if symptoms or MRI indicated too large tumor size.

Experimental setup 3 ($n = 15$): What is the long-term effect of electrochemotherapy in the rat brain? The long-term effects of electrochemotherapy by the 8-electrode device in healthy brain tissue were studied with repeated MRI, and observation for 8 weeks.

Animals

Male rats [Fischer (F344/SCA), Scanbur AB (#1), and Sprague Dawley, Taconic (#2 and #3)], were 7 to 11 months old and were kept at Glostrup Research Institute. Protocol was approved by The Danish Animal Experiments Inspectorate.

Anesthesia for surgery or MR scanning was by s.c. injection of Hypnorm (VetaPharma Ltd.) with Midazolam (Hameln Pharmaceuticals GmbH) or Dormicum (Roche, F. Hoffmann-La Roche AG,). Analgesia was administered twice over 48 hours with Rimadyl (50 mg/mL, Pfizer Aps.).

Inoculation of N32 glioma-derived cells

N32 cells were kindly provided by Leif Salford (Lund University, Sweden; ref. 30) in 2008. They were maintained and prepared as previously described (36) and tested by rapid MAP-27 panel (Taconic) last in November 2010 without signs of infection. The N32 rat glioma cells have been shown to grow readily in *in vitro* systems, as well as intracerebrally to develop tumors, and are only weakly immunogenic (35). A cell culture sample from stock solution was prepared 2 to 3 weeks before inoculation, and 3,000 N32 cells were inoculated (5 μL). The skull area around bregma was accessed with scalpel and a burr hole (5 mm diameter) made by trepan drill. Injection was at 4-mm depth at the stereotaxic coordinates $X = 2$, $Y = 1$, $Z = -4$ (Kopf 962 Dual Ultra Precise Small Animal Stereotaxic Instrument). Micrometer positioning ensured gentle needle passage (Neoflon 24GA or Spinal Needle 27GA; Becton Dickinson and company) into the brain tissue, and a pump (Univentor U-802 Syringe Pump; AgnTho's AB) was used to inject cells. The burr hole was covered by bone wax (B|Braun) and the skin sutured.

Electrochemotherapy

In experimental setup 1, an initial prototype with only 4 electrodes as well as an improved 8-electrode device were developed (Sonion A/S; Fig. 1); after this only the 8-electrode device was used. Electrochemotherapy was carried out by initial intracranial injection of 42 IU of bleomycin contained in 14 μL (freeze-dried 15,000 IU Bleomycin, Baxter A/S, dissolved in 5 mL isotonic NaCl; $X = 2$, $Y = 1$, $Z = -4$), by using a pump, and leaving the needle in the tissue for 5 minutes before retraction. The 8-electrode device was deployed through the burr hole to treatment depth at 5 mm ($X = 2$, $Y = 1$, $Z = -5$), and 32 pulses (4 × 8 pulses; Fig. 1), each of 100 V, 0.1 ms duration, 1 Hz were applied. This electrode gave rise to an electrical field of at least 280 V/cm in an area of 100 mm^3. The 4-electrode device was used for 8 pulses (Fig. 1). Pulses were delivered by a square pulse generator (Cliniporator; IGEA), and polarity of the electrodes was switched after each pulse by using a switch box (Sonion A/S). All treatments were carried out as "once only" in each rat, and no rats received more than one treatment modality.

MRI

A human 3 Tesla MRI system was used (Achieva, Philips). The head of the sedated rat was placed in a 50-mm 4-channel phased array animal coil (Animal Coil; Shanghai Chenguang Medical Technologies). Both T2-weighted turbo spin echo sequences (TR/TE: 4847/100; FOV/matrix: 50/248; 1-mm slices and scan time: 5:58 minutes), and T1-weighted sagittal gradient echo sequences (TR/TE/FA: 31/10/30; FOV/matrix: 50/500; 1 mm slices and scan time: 7:21 minutes) before and after i.v. contrast agent administration by tail vein (0.3 mmol/kg Dotarem; Guerbet) were carried out, supplemented by a postcontrast transverse T1-weighted gradient echo (TR/TE/FA: 31:10:30, FOV/matrix: 50:625, 1-mm slices, scan time 13:37 minutes). Blankets and heating pads were used.

Tumor volume

From the magnetic resonance scans, volume of tumor alone (before treatment), or the combination of tumor and tissue in vicinity reactive to treatment were approximated by in 1 plane measuring the longest diameter (a) and the longest diameter (b) perpendicular to a by Achieva scan software 2.6.3.3 (Philips

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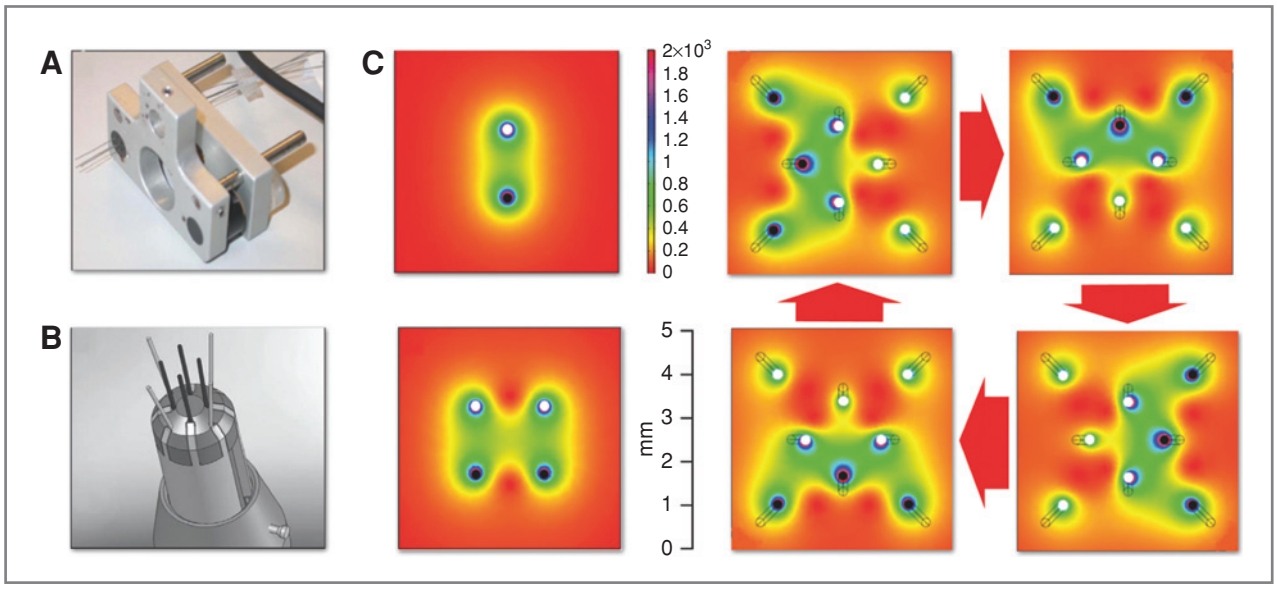

Figure 1. A 4-electrode (A) and 8-electrode device (B), respectively, for use in rat brain tissue. The 4 electrodes in the 4-electrode device will go straight forward when deployed in the brain tissue. When the 8-electrode device is deployed in the brain, the outer 4 electrodes will create a cone shape, whereas the inner 4 electrodes will go straight forward. Electrical field distributions [V/cm; (C)] shown as central slice plots perpendicular to the electrodes; white and black circles correspond to different potentials. Each window is 5 × 5 mm. Electrical field distribution is shown for a 2-, 4-, and an 8-electrode device at a particular polarity pattern (100 V applied). For the 8-electrode device, each pulse consists of 4 polarity patterns, such that each is rotated 90 degrees compared with the previous, to complete a full revolution.

Healthcare). A score for tumor volume was calculated as $V = ab^2\ \pi/6$ (31, 35, 37).

Termination

Rats were terminated by anesthesia followed by lethal pentobarbital injection. Animals were perfused with isotonic NaCl followed by 4% paraformaldehyde and kept at 5°C for at least 24 hours before further procedures.

Histology

Hematoxylin and eosin (H&E), PAS, and immunohistochemical staining were done according to standard protocols. Immunohistochemical staining of neurofilaments (NF) was done with monoclonal mouse anti-human, 2F11 (Dako), and for glial filament (GFAP) polyclonal rabbit anti-GFAP (Dako) was used. The following characteristics were listed and graded by an experienced pathologist, blinded with respect to treatment status: presence of (i) tumor or tumor cells, (ii) necrotic tissue, (iii) macrophages, (iv) erythrocyte traces, and (v) condensed or lacking NF or glial filaments shown by immunohistochemical staining, all graded 0 to 3. The score 0 was given for the normal situation, whereas a score of 3 indicated a highly abnormal situation in the area of interest. We used an Olympus BX50 microscope (Olympus lenses, plan objective ×2), Olympus color view 1 camera (Tubus, UTV, 0.5xG3), and Olympus soft imaging system (analySIS getIT; Olympus).

Statistical analysis

Log-rank (Mantel–Cox) test was used for survival curve comparison, *t*-test for the tumor volume measurement, and Mann–Whitney *U*-test for statistical handling of the pathologic scores. *P*-values less than 0.05 were considered statistically significant on a 2-sided scale.

Results

Brain tumor response to treatment

Electrochemotherapy induced regression and elimination of tumor in the targeted brain areas in 9 of 13 rats over 2 to 3 weeks, whereas 8 control rats showed tumor progression as shown in Fig. 2 (experimental setup 2, 8-electrode device), where tumor volume measured on MRI is shown (Fig. 2A) along with a survival curve (Fig. 2B) indicating when rats were euthanized due to large tumor size. Tumors were verified before treatment by MRI and had a mean size of 7 mm^3 (range, 1–34).

Immediately after electrochemotherapy or electroporation and lasting for up to 1 week, treatment effects appeared as similar sized areas (low SEM) with a diffused transition zone between contrast and noncontrast-enhanced brain tissue. Images from 6 consecutive MRI scans of a rat that had received electrochemotherapy are shown in Fig. 3. In general, we observed that this short-term treatment effect on contrast enhancement is not clearly distinguishable from contrast enhancement reflecting a tumor, and the measurements listed in Fig. 2A include the entire contrast enhancement observed.

Among the 13 rats treated with electrochemotherapy, 9 rats (69%) showed regression of tumor and then no tumor, and 4 rats (31%) showed tumor progression. Of the 4 rats showing progression, 1 had tumor cell remains outside the treatment target area, and 3 rats were characterized by having the 3

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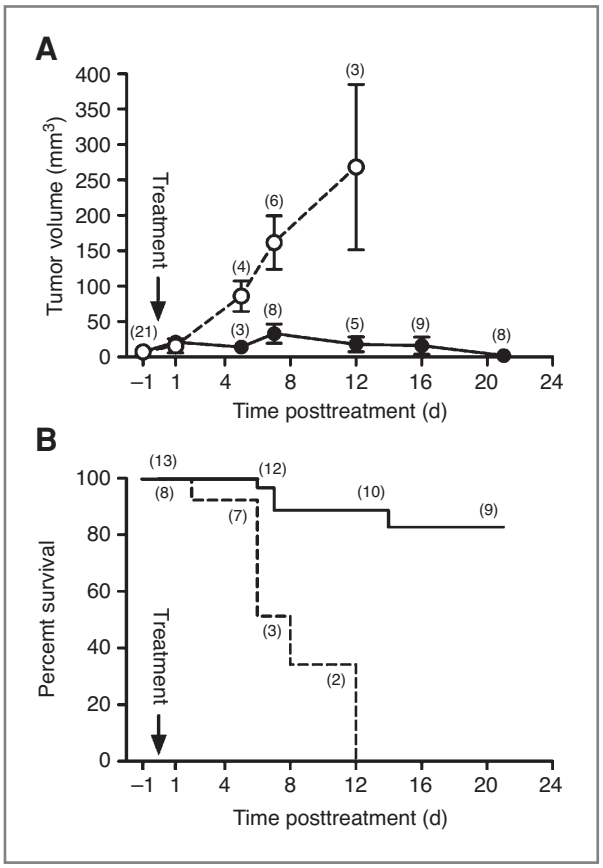

Figure 2. Tumor volume estimated as contrast-enhanced area of tumor and brain tissue reaction to treatment if present, mean ± SEM (A), and Kaplan–Meier plot showing survival in percentage (B), both as a function of days; day -1, MRI confirmed rat brain tumor. Closed circle (●) and solid line (—) designate electrochemotherapy (n = 13 animals). Open circle (○) and dotted line (---) designate electroporation, bleomycin, or no treatment (n = 8 animals). Numbers in parentheses indicate animals investigated at that particular time point; discrepancies between numbers in (A) and (B) reflect availability of MRI. Electrochemotherapy refers to electrical pulses in the presence of bleomycin, with the aim of accomplishing enhanced bleomycin uptake.

largest initial tumors before treatment. There were 8 rats in the control treatment group, represented by untreated rats ($n = 4$), rats treated with electroporation only ($n = 2$), or with bleomycin only ($n = 2$). All control rats had statistically significantly increased tumor size (contrast enhancement) already within 1 week after tumor appeared on MRI ($P < 0.01$), as well as after 2 weeks ($P < 0.01$), compared with rats given electrochemotherapy; all control rats needed to be terminated prior to the end of experiment because of tumor progression, and none showed any sign of treatment effect. The statistical tests are based on results including treatment effect seen within the first week. The Kaplan–Meier survival curve (Fig. 2B) reflects termination because of tumor progression, and the difference between the 2 groups was significant ($P < 0.001$).

Rats were only included if they had a verified tumor visible on MRI. Later exclusion criteria were (i) odd growing tumor located near surface (1 rat), histologic confirmed fibrotic tissue showing contrast enhancement similar to tumor (1 rat), and small tumors on MRI not later confirmed (2 rats).

Histologic analyses showed consistency with the end-stage MRI confirming presence or absence of tumors (Fig. 4). The initial sizes of the tumors are similar for the 2 rats in Fig. 4, but the rat receiving electrochemotherapy had no tumor or tumor cell remains at termination (30 days). The rat receiving electroporation only had a tumor with characteristics also seen in human glioblastoma multiforme (intratumoral necrosis, pseudo-palisading necrosis) at termination (24 days).

In total, 9 of 13 rats (69%), with MRI-confirmed tumor that were given electrochemotherapy had no tumor on termination, and all of 8 rats receiving no treatment ($n = 4$), bleomycin ($n = 2$), or pulses only ($n = 2$) had tumor progression and early termination was necessary.

Rat brain tissue reactions to treatment

In experimental setup 1, the effect of electrochemotherapy for the initial 4-electrode device prototype and the improved 8-electrode device showed dose–response, with the 8-electrode device having the highest impact on brain tissue both visually (Fig. 5) and quantitatively (Fig. 6). Electrochemotherapy is characterized by impacting rat brain tissue in the targeted area only (Fig. 5) corresponding to and restricted by the electrical field distribution (Fig. 1). The 8-electrode device was significantly superior to the 4-electrode device, showing more severe morphologic changes with extensive necrosis, macrophage invasion, bleeding spots, loss of neurons and astrocytes (represented by their filaments), and no presence of tumors or tumor cell remains shown by H&E, NF, and GFAP staining (Fig. 6). These effects of electrochemotherapy are easily distinguishable from brain tissue reaction to plain electroporation, bleomycin, or NaCl, where only macrophage invasion could match the effect found with electrochemotherapy, except for bleomycin, which also showed moderate impact on astrocytes (Fig. 5 and Fig. 6).

Long-term effect of electrochemotherapy in healthy rat brain tissue

Experimental setup 3 pursued the long-term effect of electrochemotherapy ($n = 8$), bleomycin, or electroporation ($n = 7$) in healthy rat brain tissue, with no prior inoculation of tumor cells. We found no long-term effect of either bleomycin or electroporation. For the rats treated with electrochemotherapy, repeated MRI (data not shown) suggests an ongoing process toward increased tissue degradation and increased presence of tissue fluid other than blood (e.g., cerebrospinal fluid) in the treated area at *in vivo* follow-up by MRI for 8 weeks. The targeted area gradually lost contrast enhancement and gradually increased appearance of dark areas (on T1-weighted scans), suggesting an emerging fluid-filled space, as shown in Fig. 7A1 and B1. At termination, all these brains showed substantial loss of tissue in targeted areas. Histologic analyses showed healthy brain tissue in proximity to clearly distinguishable cavities at the location of the targeted area of the brain for all 8 rats after electrochemotherapy (Fig. 7A2 and B2). We found no histologic

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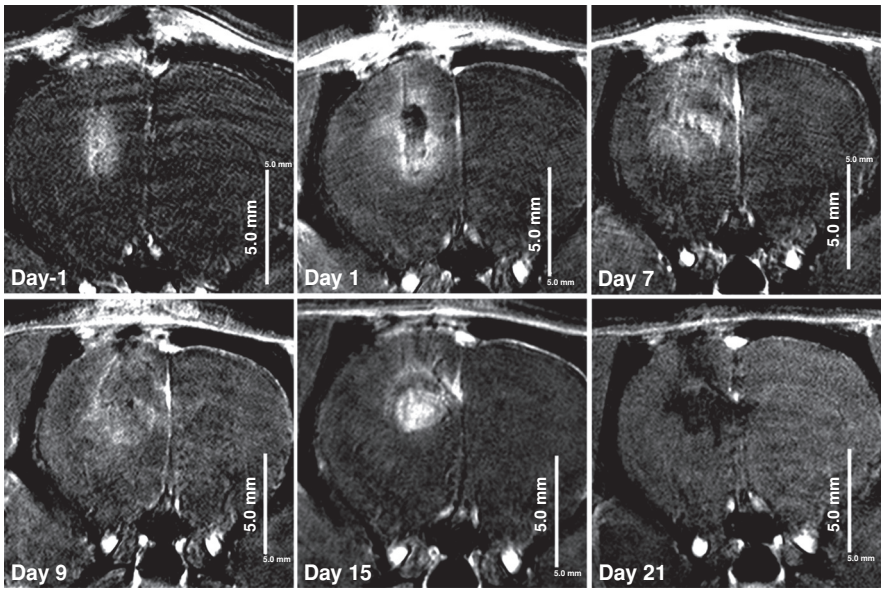

Figure 3. MRI of a rat brain (T1-weighted after injection of contrast). Craniocaudal scans show a dorsoventral orientation. Day −1, appearance of a rat brain tumor (contrast enhanced) 12 days after inoculation of N32 rat glial derived tumor cells; Day 1, day after electrochemotherapy, contrast enhanced of tumor overlaid with tissue reaction to electrochemotherapy. Days 7, 9, 15, and 21 are shown in following panels as indicated.

evidence of remains of necrotic cells or bleeding but occasional presence of very few macrophages in bordering healthy tissue. Histologic analyses of control rats showed no sign of cavity formations, but minor traces after intrusion with intracranially bleomycin injection were observed.

Tolerability

On the basis of our records from the pilot project as well as from the reported experiments, the anesthesia and analgesia were in general well tolerated by the rats. Incidence of rupture of superior sagittal sinus during the surgical procedure (drilling) was less than 5%. All rats recovering from analgesia regained normal rat behavior within 48 hours with no observed characteristic or adverse behavior related to treatment status. Posttreatment mortality was observed in less than 1% of the treated rats. Long-term effect of electrochemotherapy revealed loss of tissue corresponding to the targeted area, but basic normal rat behaviors were maintained until termination date. We did not observe any obvious difference in basic behavior between rats receiving different treatments in the short-term studies. Specifically, no abnormalities in terms of motor function or behavior were observed, and the treated and control groups responded similarly to sedation and experimental procedures.

Discussion

The use of electrical pulses to create transient permeabilization in the cell membrane (electroporation) is well characterized (21, 23, 24), and so is the combination with a cytotoxic agent to enhance the efficacy of this agent by creating direct access to the cell cytosol (5, 6, 12, 22, 25, 30–32). This study is the first report of electrochemotherapy of experimental brain tumors in rats with a novel expandable electrode, and we have shown that this treatment method is highly effective with complete resolution of 69% of treated tumors after once-only treatment. Furthermore, treatment effects are localized to the target area, and the toxicity profile is favorable, with no apparent influence on rat behavior or morbidity.

A grim prognosis and substantial morbidity continue to be associated with both primary and secondary (metastatic) brain tumors (1, 2). From previous experience with electrochemotherapy of superficial tumors (5, 6, 25, 30, 32), it is known that this treatment modality has proven highly efficient regardless of tumor histology and as a once-only treatment also after previous radio- or chemotherapy (5, 6, 25, 38). Furthermore, electrical pulses may be delivered in a few seconds, enabling intraoperative treatment. We therefore decided to test a novel electrode device for electroporation of brain tumors in the hope that the encouraging preclinical results in this study could be translated to treatment of primary and secondary brain tumors in the clinic.

Development of electrodes

Only one previous study reports the use of electrochemotherapy in an animal model of brain cancer, dating back to 1993 (34). Here, the investigators used 2 opposing acupuncture needles to apply the electrical field, giving rise to a rather small treatment area. In this study, we have tested an electrode which may enter through a burr hole and expand to cover an area larger than the insertion (39, 40). Furthermore, pulse sequencing has been developed, to ensure sufficient coverage of the treatment area (Fig. 1).

Electrochemotherapy of brain tumors

Having the electrode device it was possible to move on to the first and fundamental question: Does the electrode device

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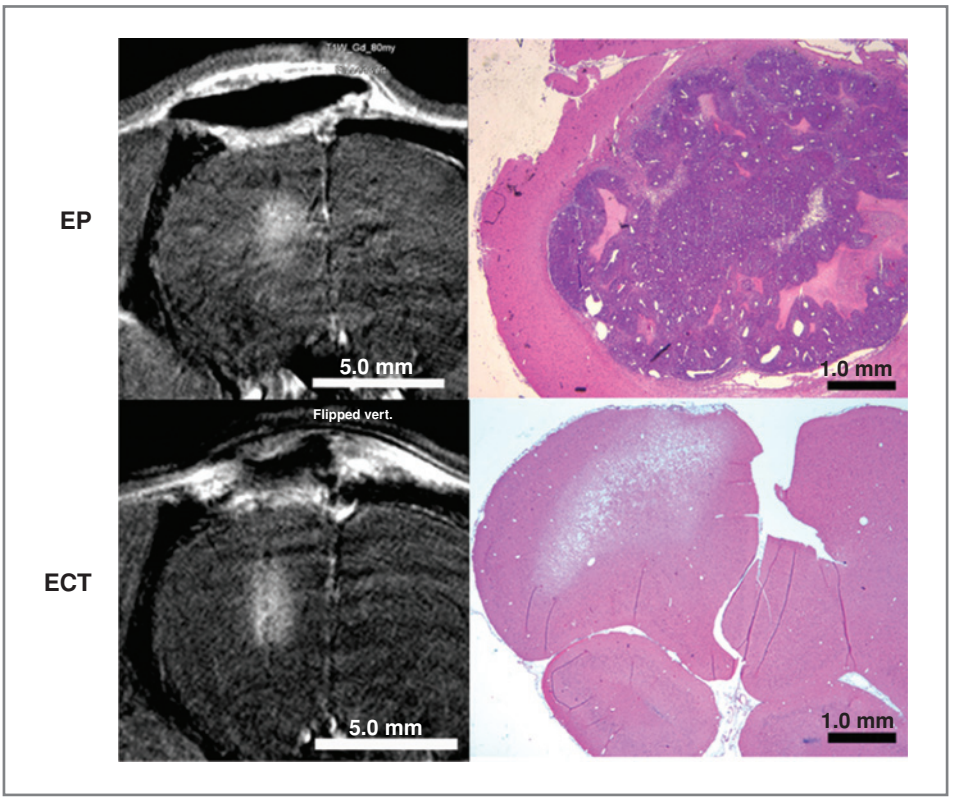

Figure 4. Electrochemotherapy versus electroporation alone. Left, MRI of 2 rats at the time point for confirmation of presence of similar sized tumors prior to treatment. Right, pathologic H&E staining showing the development resolution of the tumor at the end of the experiment for a rat given electroporation (EP; top) and electrochemotherapy (ECT; bottom), respectively. MRI show craniocaudal scans in dorsoventral orientation.

work? In experimental setup 1, 4- and 8-electrode devices were tested, and it was found that areas of necrosis after electrochemotherapy corresponded to the configuration of the 2 electrode devices. Furthermore, the combination of drug and pulses gave rise to necrosis in the treatment area whereas only pulses or only drug did not, as previously described (38, 41).

The second very important pair of questions was as follows: Can we determine presence of established tumors on MRI before treatment, and what is the success rate of electrochemotherapy when macroscopic tumors are present? We found that tumors could be seen on MRI, but also that we could see the overlay of the treatment area as an extended zone of contrast enhancement around the tumor itself. The initial increase in volume, also for tumors that later disappeared, could be caused by a treatment-related effect around the tumor. We could not distinguish the boundary between the actual tumor and treatment effects on MRI; however, the latter subsided within a week. Recent research indicates that in the future diffusion-weighted MRI may give additional information on the treatment volume (42).

The complete disappearance of 69% of treated tumors was highly encouraging, but not necessarily unexpected. Thus, in the treatment of cutaneous tumors complete remission rates have been reported to be between 60% and 90% (25, 41, 43). In our animal model, we treated an area measuring approximately 100 mm^3 and observed a 69% remission. It has been shown that in larger tumors the success rate is lower (43), in concordance with the fact that every part of the tumor must be covered by the electrical field to achieve success (42, 44–46). It is noteworthy that 3 of the 4 rats that were not successfully treated in the study had the largest tumors when treated, indicating that it is likely that the success rate is very high when the tumor is encompassed by the electrode. The electrode used in this study is a prototype for use in rats, and it was only placed once. We are anticipating use of an electrode device for treatment in humans, but this will be with a larger electrode device (39), that can be sequentially deployed to cover a larger tumor volume.

To respond to the third very important question—is brain electrochemotherapy safe?—the next step of the study was to subject healthy rats to electrochemotherapy. By not inoculating the rats with brain tumor cells, possible interference from tumor growth could be avoided. MRI scans and behavioral assessments were carried out, as well as histology at the end of the observation period.

Again, histologic sections revealed far more pronounced effects after electrochemotherapy than after pulses or drug alone. This was also evident from MRI scans, and as shown in Fig. 7, the treatment area was subjected to necrosis and subsequent resolution, revealing a fluid-filled cavity not unlike radiological findings after stroke.

The behavioral observations included basic motor ability and orientation behavior (i.e., neurologic dysfunction and hemiparesis), and no obvious effects on these were found, despite the marked findings on MRI. Although treatment-related effects were present at MRI (Fig. 3), this did not seem

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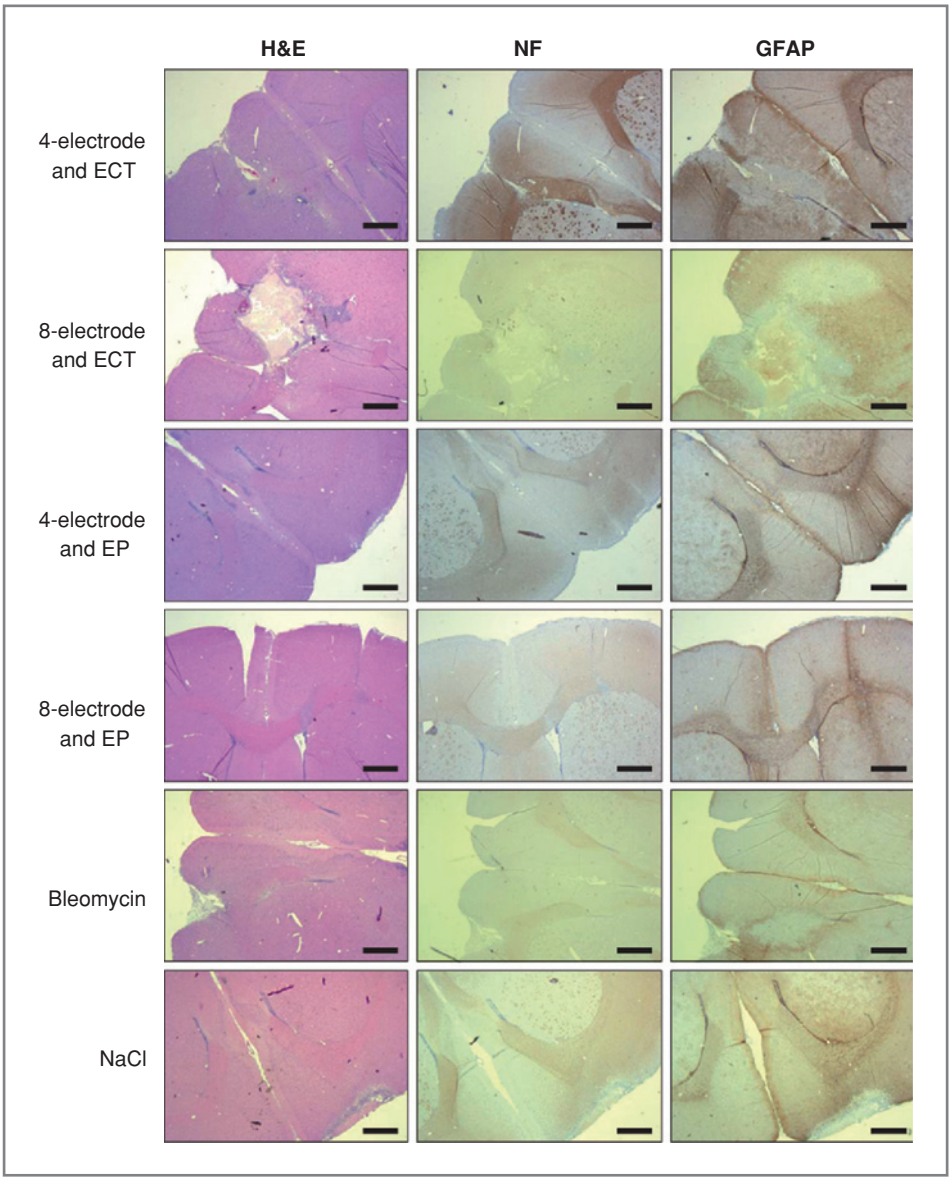

Figure 5. Paraformaldehyde-perfused rat brains stained with H&E (left column), NF (middle column), and GFAP (right column). Treated groups underwent electrochemotherapy (ECT) using either the 4- or 8-electrode device. Controls received EP (just pulses), bleomycin injection only, and saline injection. Original magnification, ×20; bars, 1 mm.

to affect rat behavior, probably because it was limited to part of the brain.

What is known from previous studies of electrochemotherapy with bleomycin is that the number of molecules internalized may influence the type of cell death that ensues (33). A window of opportunity exists in which normal tissue may be spared whereas tumor tissue will be severely affected (25). As described in this article, intratumoral injections of bleomycin were carried out in our study. This regimen tends to give a high number of molecules internalized, leading to necrosis of both tumor tissue and normal tissue (47). From clinical studies, we know that normal tissue may be spared whereas efficient tumor cell kill is obtained with i.v. administration (25), because i.v. administration gives a lower and more evenly distributed tissue dose, and this may allow treatment within a therapeutic window. Further experiments on lowering bleomycin tissue dose, including i.v. administration may further elucidate the question of how to obtain complete tumor regression while preserving normal tissue in the brain.

Several studies have documented an antivascular effect of both electrical pulses and the combination of electrical pulses and bleomycin (electrochemotherapy; refs. 3, 48). Thus, in addition to electrical pulses affecting perfusion and endothelial cells, bleomycin also has known activity against endothelial cells and has been used in treatment of vascular malformations (49). This antivascular effect may in fact be very important in the tumor response seen with both irreversible electroporation and electrochemotherapy (3, 4, 8, 48, 50).

Recently published studies on irreversible electroporation (3, 4, 7–9) have shown encouraging results in tumor treatment

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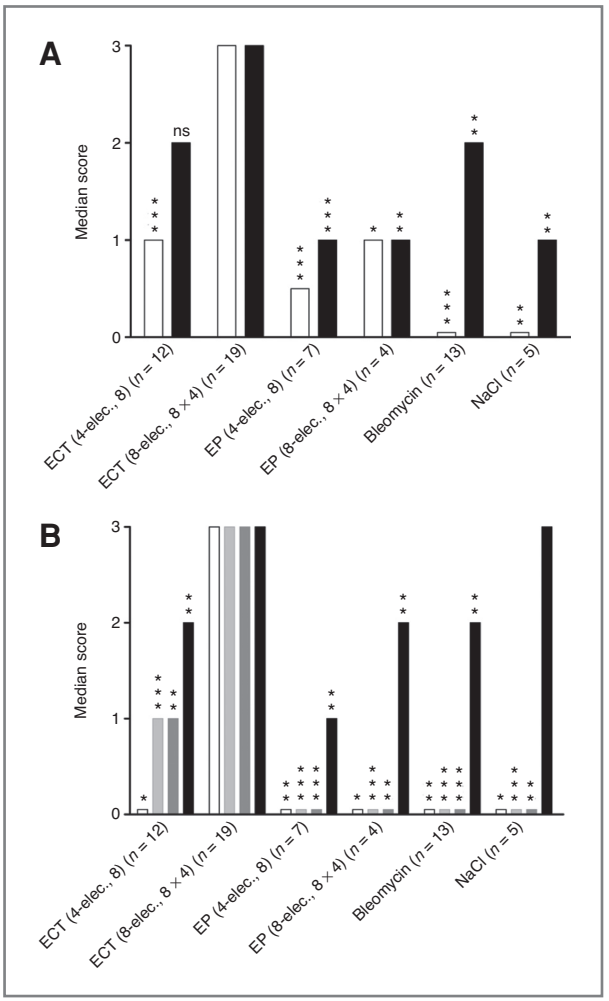

Figure 6. Median scores of observed changes in brain tissue on histologic sections as a function of treatment with various solutions, devices, and electroporation parameters. A, top, neuro-filaments (white) and glial filaments (black). B, bottom, necrotic cells with (light-gray) and without cavity (gray), trace of erythrocytes (middle-gray) and macrophages (dark-gray). 0, not present; 1, just present or only a few; 2, clearly present; and 3, extensively present. All animals were inoculated with N32 tumor cells except for those treated with NaCl. Bleomycin was injected intracranially. Mann–Whitney *U*-test (2-sided) with electrochemotherapy (ECT). (8-elec, 8 × 4) as reference, *, $P < 0.05$, **, $P < 0.01$, and ***, $P < 0.001$. EP, electroporation; NaCl, sodium chloride; 4-elec, 8, 4-electrode device, 8 pulses; 8-elec, 8 × 4, 8-electrode device, 8 × 4 pulses.

in canine models, as well as evidence of normal tissue preservation (9). When combining electrical pulses with bleomycin, a lower field strength can be used, as shown in this study. The overall, and highly encouraging conclusion, is that a novel treatment paradigm with the use of electrical pulses in cancer treatment may lead to efficient ablation with limited side effects, and that further research may expand the use of this technology even further.

An electroporation device has been developed for the clinic, namely a 13-electrode device which will be able to encompass a larger treatment area (www.clinicaltrials.gov ref. 42).

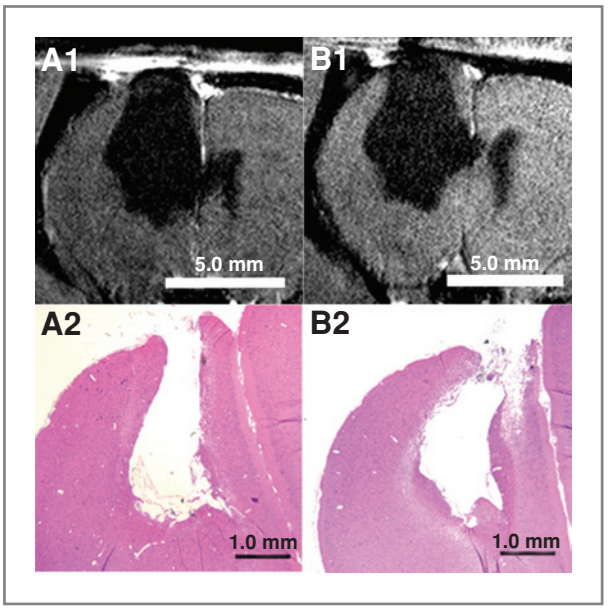

Figure 7. Long-term effect of electrochemotherapy by using intratumoral bleomycin injection, in healthy rat brain tissue. Images of 2 rats (A and B), both receiving electrochemotherapy. T1-weighted contrast enhanced MRI (top, craniocaudal scans in a dorsoventral orientation) and H&E staining (bottom). Eight weeks after electrochemotherapy, loss of contrast enhancement was observed in the targeted areas in exchange for evidence of fluid-filled cavities (A1 and B1, dark areas). Histologic analyses revealed cavities in the targeted areas (A2 and B2).

Potential side effects to electrochemotherapy in the human brain encompass risk of anesthetic complication, hemorrhage, and infection. Relaxation to prevent peripheral motor response during the electrochemotherapy procedure and general anesthesia will be necessary. However, the electrochemotherapy procedure can be short because the electrical pulses are quickly delivered. Edema will ensue, but focally where the electrochemotherapy has been carried out, as has indeed been seen at the MRI studies in the present study. Steroids may ameliorate edema.

Conclusions

The data presented here represent the first study on brain electrochemotherapy with the use of an expandable electrode device, enabling treatment of larger brain tumors through a burr hole. Results are highly encouraging, showing a high level of efficacy with a 69% complete response rate and limited toxicity. The results are similar to what has been shown in clinical trials of electrochemotherapy for skin metastases, and this would lead to optimism regarding the possibilities for electrochemotherapy to be of benefit to patients suffering from primary or secondary cancers of the brain.

Disclosure of Potential Conflicts of Interest

J. Gehl, F. Mahmood, and B. Agerholm-Larsen have patents granted or pending related to this work.

Acknowledgments

Leif Salford, Lund University Hospital, Sweden, is thanked for providing the N32 tumor cells. We also acknowledge skilful technical assistance from Birgit Hertz, Lone Bojesen, and the Technical Team at Sonion.

Grant Support

The Danish National Advanced Technology Foundation (J. Gehl, H.K. Iversen), Copenhagen County Foundation (J. Gehl, H.K. Iversen), and Einar Willumsens Foundation (B. Agerholm-Larsen, J. Gehl). J. Gehl is a Royal Swedish Academy of Sciences Research Fellow supported by a grant from the Acta Oncologica Foundation.

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Received February 8, 2011; revised April 7, 2011; accepted April 11, 2011; published OnlineFirst April 20, 2011.

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Published OnlineFirst April 20, 2011; DOI:10.1158/0008-5472.CAN-11-0451

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