Convection-Enhanced Drug Delivery: Increased Efficacy and Magnetic Resonance Image Monitoring

Yael Mardor,1,3 Ofer Rahav,4 Yacov Zauberman,2 Zvi Lidar,4 Aharon Ocherashvili,1 Dianne Daniels,1 Yiftach Roth,1,7 Stephan E. Maier,1 Arie Orenstein,1,8 and Zvi Ram1

1Advanced Technology Center, 2Neurosurgery Department, Sheba Cancer Research Center, Sheba Medical Center, Tel Hashomer, Ramat Gan; 3Neurosurgery Department, Sourasky Medical Center, Tel Aviv; Israel; 4School of Physics and Astronomy, Sackler School of Medicine, Tel-Aviv University; and 5Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Abstract
Convection-enhanced drug delivery (CED) is a novel approach to directly deliver drugs into brain tissue and brain tumors. It is based on delivering a continuous infusion of drugs via intracranial catheters, enabling convective distribution of high drug concentrations over large volumes of the target tissue while avoiding systemic toxicity. Efficient formation of convection depends on various physical and physiologic variables. Previous convection-based clinical trials showed significant diversity in the extent of convection among patients and drugs. Monitoring convection has proven to be an essential, yet difficult task. The current study describes the application of magnetic resonance imaging for immediate assessment of convection efficiency and early assessment of cytotoxic tissue response in a rat brain model. Immediate assessment of infusate distribution was obtained by mixing Gd-diethylenetriaminepentaacetic acid in the infusate prior to infusion. Early assessment of cytotoxic tissue response was obtained by subsequent diffusion-weighted magnetic resonance imaging. In addition, the latter imaging methodologies were used to establish the correlation between CED extent and infusate’s viscosity. It was found that low-viscosity infusates tend to tend to backflow along the catheter track, whereas high-viscosity infusates tend to form efficient convection. These results suggest that CED formation and extent may be significantly improved by increasing the infusate’s viscosities, thus increasing treatment effects. (Cancer Res 2005; 65(15): 6858-63)

Introduction
Fluid convection (bulk flow) which occurs in the brain interstitial fluid under normal conditions (1) with vasogenic edema (2) and after infusion of solutions directly into brain parenchyma (3), is a promising technique for distribution of solutions into brain tissue. It has been shown that fluid convection, established by maintaining a pressure gradient during interstitial infusion, could be used to greatly enhance the distribution of various molecules in the brain (4, 5). These studies showed the ability of convection to obtain in situ drug concentrations several orders of magnitude greater than those achieved by systemic administration over large volumes of brain in reasonably obtainable time intervals. The concentration profile obtained using convection-enhanced drug delivery (CED) is relatively flat up to the flow front, providing control over undesired toxicity (6). The application of CED to brain pathology is an emerging field. Most of the studies carried out thus far have focused on the treatment of brain tumors.

We have recently conducted a phase I/II clinical trial where 15 patients with recurrent glioblastoma multiforme received a total of 20 cycles of intratumoral CED of Taxol with a significant antitumor response rate of 73% (7). In a phase II clinical trial in which patients were treated with CED of TF-CRM107 (a conjugate protein of diphtheria toxin with a point mutation linked by a thioester bond to human transferrin), the response rate was 35% (8). Other drugs have also been tested in clinical trials, such as TP-38 (9) and IL4(38-37)-PE38KDEL (10) with evidence of some clinical activity and IL13-PE38QQR (11) with no definite conclusive statements regarding efficacy. The expansion of the application of this technique for other brain pathologies such as neurodegenerative diseases, seizure disorders, head trauma, etc., is currently under investigation.

Convection is known to depend on variables such as catheter size, flow rate, tissue consistency, catheter localization, infusate concentration, and molecular weight (12, 13). After accounting for these variables, initial clinical experience shows that there is significant variability in the extent of convection among patients, as well as in differential tumor response to the therapeutic drugs. Therefore, increasing tumor response to convection-based therapies in a reliable/reproducible manner is essential, as well as real-time, noninvasive monitoring of the extent of convection and its early effects on the tissue.

Recent publications have shown the use of surrogate markers, such as liposomes or macromolecules (albumin) traced with contrast agents that are visible on images obtained using noninvasive techniques, such as iopanoic acid for computed tomography and Gd-diethylenetriaminepentaacetic acid (Gd-DTPA) for magnetic resonance imaging (MRI; refs. 14–17). These markers are confused with the therapeutic agent to enable real-time monitoring of the distributed drugs.

Diffusion-weighted MRI (DWMRI) enables noninvasive characterization of biological tissues based on their water diffusion characteristics. We have recently shown that DWMRI may serve as a surrogate marker for assessing the propagation of the convective wave of Paclitaxel in brain tumors and predict tumor response in tumor volumes covered with convection (7, 18).

In the current study, we show the use of the readily available MRI contrast agent, Gd-DTPA, as a surrogate marker for immediate assessment of convection efficiency and infusate distribution, by simply mixing it with the infusate prior to infusion. Early assessment of brain tissue cytotoxic response to therapeutic agents infused by CED is obtained using subsequent T2-weighted and DWMRI. In addition, our data presents a significant correlation between infusate viscosity and CED extent, suggesting that drug distribution may be significantly improved by increasing the infusate viscosity.
Materials and Methods

Experimental design. Solutions containing combinations of Crema-phore, Taxol, Carboplatin, Ethanol, sucrose and human serum albumin in different concentrations were mixed with Gd-DTPA (1:70) and infused into the striatum of normal Sprague-Dawley rats (males, 250-300 g). T1-weighted MRI were acquired immediately post-treatment to assess the extent of convection, and T2-weighted and DWMRI were acquired 24 hours later to assess tissue response and its correlation to the extent of convection. Some rats were monitored by MRI for an additional period of 4 days in order to show the subsequent formation of necrosis following the earlier changes observed on T2-weighted and DWMRI.

Convection-enhanced drug delivery procedure. Under full anesthesia, a midline scalp incision was made to identify the bregma. A 1 mm burr hole was made in the right region of the skull, 3 mm anterior and 2 mm lateral to the bregma. A 33-gauge needle attached to a 1,000 μL syringe (Gastight, Hamilton, Reno, NV) was placed stereotactically 5.5 mm deep into the striatum. The infusion was done using a BASI syringe pump at a rate of 1 L/min for a duration ranging from 17 to 90 minutes.

Diffusion-weighted magnetic resonance method. Diffusion-weighted images are usually obtained by acquiring conventional T2-weighted images with the addition of diffusion-weighting gradients that filter out the signal from high-mobility water molecules and sensitize the MRI to molecular diffusion/mobility (19). Hence, regions of accumulated liquids or severe necrosis appear dark in DWMRI, whereas regions of slow water accumulation, such as in the case of intracellular water accumulation or inflammation, appear bright. In this method, the normalized intensity of the water signal is given by:

$$I/I_0 = \exp[-bADC]$$  

(A)

$I$ and $I_0$ denote the signal intensities in the presence and absence of diffusion-weighting gradients, ADC is the molecular apparent diffusion coefficient and $b$ is the diffusion weighting factor, which is expressed in units of s/mm². By acquiring DWMRI at $b = 5$ and $1,000$ s/mm² and using Eq. (A), it was possible to calculate ADC maps.

Magnetic resonance image monitoring. Immediate assessment of CED formation and extent was done using a General Electric 0.5 T interventional MRI machine [Signa SP/i (special proceeding/interventional)] with the 1.0 T MRI machine with the 10.4 mol/L lx operating system, gradients intensity of up to 4.3 G/cm and the line scan diffusion-weighted imaging (20) acquisition software package. Specially designed animal volume coils of 5 cm diameter were used for data acquisition.

T2-weighted fast spin echo MRI were acquired with: $256 \times 128$ matrix, $12 \times 9$ cm² field of view, repetition time of 3,000 ms, echo time of 90 ms and 2 mm slices with 0.5 mm gap. Line-scan DWMRI were acquired with: $256 \times 128$ matrix, $12 \times 9$ cm² field of view, repetition time of 5,440 ms, echo time of 142 ms and 2 mm slices. T1-weighted images were acquired with: $256 \times 128$ matrix, $12 \times 9$ cm² field of view, repetition time of 400 ms, echo time of 12 ms and 3 mm slices, no gap.

Calculation of convection-enhanced drug delivery extent. The volume (in cm³) of infusate distribution was calculated from the T1-weighted MRI acquired immediately post-CED treatment. Regions of interest were defined over the entire enhancing region in each slice (excluding the ventricles). The number of pixels in the regions of interest were counted and multiplied by the volume of a single pixel.

Establishment of the application of immediate T1-weighted magnetic resonance imaging for assessment of convection-enhanced drug delivery extent. In order to verify that T1-weighted MRI acquired immediately post-treatment with a Gd-DTPA containing infusate, represent the true distribution of the agent in the tissue, 11 rats were treated by CED with an infusate containing 0.1% Evans blue and Gd-DTPA (1:70) for 30 minutes. Seven out of these 11 rats were treated with a higher viscosity infusate obtained by adding sucrose at a concentration of 17%. T1-weighted MRI were acquired immediately post-treatment, after which the brains were extracted and fixated in formalin.

Figure 1. A and B, T1- and T2-weighted axial MRI of normal rat brain. C, D, and E, T1-weighted MRI acquired immediately post-CED treatment with infusates containing Gd-DTPA and Evans blue, showing examples of different convective efficiency as depicted by MRI: poor (C), moderate (D), and efficient (E) convection. F, G, and H, fixated brain samples of the same rats demonstrating similar distributions of the dye in the tissue.
Establishment of the application of diffusion-weighted magnetic resonance imaging for assessment of cytotoxic tissue response. In order to study the correlation between the extent of CED as depicted in the immediate T1-weighted images and the cytotoxic tissue response as depicted in the later DWMRs, nine Sprague-Dawley rats were treated by CED of Taxol (0.54 mg/mL). In order to obtain a wide range in volumes of distribution, the infusion times were varied between 17 and 60 minutes.

The effect of infusate viscosity on convection-enhanced drug delivery efficacy. In order to study the effect of infusate viscosity on the efficient formation and extent of convection, eight groups of rats (four to seven rats in each group) were treated by CED of eight solutions with different viscosity values: human serum albumin at different concentrations (0.2%, 1%, 2.5%), sucrose at different concentrations (5%, 20%), Ethanol (5.5%) and Taxol (0.66 mg/mL). Infusion time was 90 minutes. The relative (to saline) viscosities of the solutions were measured at room temperature using a W. Oswald viscosity meter. CED efficacy was determined from the immediate T1-weighted MRI as described above. The final convection volume for each infusate was defined as the average convection volume over the group of rats treated with it.

Later treatment effects of increased infusate viscosity. In order to test whether increasing the infusate viscosity changes later treatment effects, 13 rats were treated with Carboplatin (4 mg/mL, 17 minutes infusion). In six of those, the infusate viscosity was increased by adding sucrose to the solution prior to infusion (Carboplatin, 4 mg/mL; sucrose concentration, 12%). Rats were followed by MRI up to 4 days post-treatment.

Results

Examples of using T1-weighted magnetic resonance imaging for immediate assessment of convection-enhanced drug delivery extent. The extent of convection formation was reflected by T1-weighted MRI acquired immediately post-treatment with infusates mixed with Gd-DTPA. Poor convection was characterized by significant backflow along the catheter and into the ventricles, depicted in the images as significant enhancement in the ventricles and little/no enhancement in the striatum. Efficient convection presented significant spread into the striatum with minimal backflow into the ventricles. Examples of poor, moderate, and good convections are shown in Fig. 1.

Validation of the application of immediate T1-weighted magnetic resonance imaging for assessment of convection-enhanced drug delivery extent. In order to study whether the extent of CED, as depicted by the T1-weighted images, represents the volume in which the agent is distributed, the relation between the volume of the immediate Gd enhancement and the volume of the Evans blue distribution in the fixated rat brain was studied for 11 rats treated with infusates containing Evans blue and Gd-DTPA. The correlation between the two volumes of distribution was found to be highly significant ($r^2 = 0.95$, $P < 0.0001$, Pearson correlation). Examples showing the infusate distribution as depicted by MRI and in the fixated samples are shown in Fig. 1.

Early assessment of cytotoxic tissue response, depicted by T2-weighted and diffusion-weighted magnetic resonance imaging. Efficient convection with toxic infusates was followed by significant enhancement in T2-weighted and DWMRI acquired 24 hours post-treatment. Figure 2 shows examples of cytotoxic tissue response, as depicted in images acquired following efficient CED with Taxol, Cremaphore, and Carboplatin. The radiological cytotoxicity of these three drugs seemed similar and all lead to necrosis as depicted by later images acquired with various MR sequences (T1, T2, and DWMRI).

Pathological samples taken from rats treated with Taxol and sacrificed immediately after the DWMRI scan, show severe damage in regions appearing bright in the T2-weighted and DWMRI. An example is shown in Fig. 2.

Efficient prolonged CED (90 minutes) of nontoxic infusates, such as sucrose and human serum albumin solutions, was followed by minor enhancement on T2-weighted and DWMRI, at the site of the catheter tip. When convection was not achieved, including with
Enhanced treatment effects obtained by increased infusate viscosity. In order to test whether the enhanced drug distribution, obtained by increased infusate viscosity, is followed by enhanced treatment effects, 13 rats were treated with Carboplatin at a cytotoxic drug dose (4 mg/mL). Six out of those were treated with high-viscosity infusates, obtained by adding sucrose to the Carboplatin solution. The rats treated with the higher viscosity infusates presented larger volumes of distribution, as depicted by the immediate T1-weighted MRI ($P < 0.02$, one-tailed nonparametric Mann-Whitney test), as well as larger volumes of cytotoxic tissue response, as depicted by the later DWMRI images ($P < 0.002$, one-tailed nonparametric Mann-Whitney test). Figure 5 shows examples of two rats, one treated with the original Carboplatin solution and the other with high-viscosity Carboplatin. The immediate MRI show increased backflow along the catheter path and into the ventricles, whereas high-viscosity infusates tend to form efficient convection, resulting in a significant distribution of the infusate in the striatum.

Discussion
In this paper, we present the application of Gd-DTPA and MRI for immediate assessment of CED formation and extent as well as of T2-weighted and DWMRI for early assessment of cytotoxic tissue response to the distributed drug. As mentioned in the introduction, Gd-DTPA has been previously used as a surrogate marker for CED distribution, by chemically binding or entrapping it to macromolecules or other particles such as liposomes and confining the...
labeled particles with the original infusate. Gd-DTPA has also been used to monitor the extent of CED with saline (21, 22). In the current study, we show that Gd-DTPA could simply be mixed in the original infusate prior to infusion, thus enabling real-time MRI of the efficient formation and extent of CED. The significant correlation between the extent of CED, as depicted by the immediate T1-weighted images, and the distribution of the Evans blue dye (similar molecular weight to Taxol) in the fixated rat brain samples, as well as the significant correlation with the later cytotoxic tissue responses, as depicted by the DWMRI in the cases of Taxol and Carboplatin, confirms the validity of this methodology for these agents. It is reasonable to assume that this methodology would produce similar results for other small molecular drugs. The fact that the T1-weighted images consistently depict a larger distribution for higher concentrations of human serum albumin, shows the feasibility of using this methodology for larger molecules as well. Nevertheless, this methodology should be tested for CED of large particles over longer infusion times. Because Gd-DTPA is a small particle, it is reasonable to assume that it will be carried along with the convective wave of the original infusate. 

Nevertheless, using this imaging methodology may enable immediate assessment of CED formation and efficiency of distribution even in such cases. Early assessment of tissue cytotoxic response to treatment is essential, especially in the case of brain tumors, where both efficient treatments of pathologic regions as well as sparing of normal tissue are critical. Accurate noninvasive treatment monitoring may enable treatment adjustment in real-time, thus optimizing treatment outcome on a patient by patient basis.

The nonspecific cytotoxic tissue response, depicted as enhancing regions on T2-weighted and DWMRI, is consistent with our previous clinical findings (7, 18). We have previously presented data of 15 patients with recurrent glioblastoma multiforme which were treated by CED of Taxol. The clinical data showed that early changes in the DWMRI were followed by later tumor necrosis. Patients who did not present early changes on the DWMRI acquired during treatment had no later radiological responses to treatment. The current study clarifies that the changes observed in the DWMRI of the responding patients were tissue cytotoxic responses to Taxol. Therefore, the most probable explanation for the absence of responses in some of the patients in the clinical Taxol trial is that convection was not formed, thus Taxol was not distributed in the tissue.

Efficient CED with toxic drugs resulted in significant enhancement in T2-weighted images and DWMRI (Fig. 2). Efficient CED of

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8 S. Kunwar, personal communication.
nontoxic infusates, such as solutions containing sucrose or human serum albumin, showed no cytotoxic tissue response or (in the case of prolonged efficient CED) was followed by minor enhancement on T2-weighted and DWMRI (Fig. 3), at the site of the catheter tip. This minor damage to the tissue may be explained by the large pressure gradient caused by the continuous infusion. This finding is consistent with the calculations by Chen et al. (13), which showed that at long infusion times, the pore fraction increases at short radial distances from the catheter tip. These results suggest that Gd-DTPA, at the concentration used in our study (1:70), does not cause cytotoxicity. Nevertheless, in-depth investigation of the dose-toxicity relationship of Gd with potential therapeutic infusates will be crucial for clinical application of specific drugs.

The current animal study shows that the cytotoxic tissue response could be detected in T2-weighted images as well as in diffusion-weighted images. Nevertheless, DWMRI is advantageous because it provides additional information over T2-weighted images, as shown in Fig. 3. Moreover, DWMRI is especially advantageous in the clinical setting, because most brain pathologies are accompanied by vasogenic edema which appears bright in T2-weighted MRI. The heterogeneous appearance of pretreatment pathology, in addition to vasogenic brain edema, may screen the T2 changes resulting from the treatment. It is our experience that DWMRI, in which most of the signal from vasogenic edema is filtered out, is far more effective than T2-weighted MRI in clearly depicting CED treatment response effects.

As mentioned in the Introduction, the efficient formation of convection depends on many variables, including patient-related variables, such as tissue consistency and catheter positioning. The response rate of the few convection-based trials published thus far is promising, although some drugs seem to be more efficient than others. The significant correlation between infusate viscosity and convection efficacy category suggests that some drugs may be less efficient than others due to their low viscosity, hindering efficient formation of convection, resulting in poor treatment effects. This significant correlation suggests that by increasing the infusates’ viscosity (which may be simply obtained by increasing the solvent concentration, such as more human serum albumin or more sugar), it is possible to significantly increase the efficiency of CED formation and extent. Moreover, the example in which Carboplatin distribution was increased by adding sucrose to the infusate establishes the increased treatment efficacy obtained by using this approach.

The use of high-viscosity infusates for obtaining efficient CED may be advantageous in several aspects. It may enable high-efficacy distribution of large particles, extending the use of CED to therapy regimens which have thus far been limited due to their size. These include liposomes, gene therapy and related products, nanoparticles containing/not containing iron oxide, etc. Current CED therapy is usually prolonged by several days. Efficient formation and extent of convection, obtained by using high-viscosity infusates, enables coverage of larger volumes of distribution in less time. This may enable shorter CED treatments. In most cases, small particles distributed by CED tend to clear out of the tissue in relatively short periods. Large particles tend to remain in the tissue for long periods of time, thus enabling long exposure of the tissue to the drug and slow release of drugs. Therefore, in addition to efficient and quick distribution of the particles, high-viscosity infusates of large particles may enable shorter CED treatment periods with long-lasting treatment effects. CED of large particles may enable targeted delivery, because the large particles may be targeted to specific sites, tissue types, cells, etc., thus enabling the use of high-efficacy drugs, such as Taxol, whereas avoiding nonspecific cytotoxic effects.

Acknowledgments
Received 1/18/2005; revised 3/14/2005; accepted 5/17/2005.
Grant support: Israel Science Foundation (to Y. Mardor), NIH grant # R01 NS39335 (to S. Maier), Adams Super Center for Brain Studies (to Z. Ram), Herman Showder Fund (to Z. Ram), and Israel Cancer Association (to Z. Ram).

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We thank Dr. Ouzzi Nissim for many helpful discussions and Dr. Jacob Baram for his assistance in providing some of the infusate materials.

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www.aacrjournals.org 6863 Cancer Res 2005; 65: (15). August 1, 2005
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