Convection-Enhanced Drug Delivery of Interleukin-4 *Pseudomonas* Exotoxin (PRX321): Increased Distribution and Magnetic Resonance Monitoring

Y. Mardor, D. Last, D. Daniels, R. Shneor, S. E. Maier, D. Nass, and Z. Ram

The Advanced Technology Center, Sheba Medical Center, Ramat-Gan, Israel (Y.M., D.L., D.D., R.S.); Department of Radiology, Brigham and Woman's Hospital, Boston, Massachusetts (S.E.M.); Pathology Institute, Sheba Medical Center, Ramat-Gan, Israel (D.N.); Department of Neurosurgery, Tel-Aviv Medical Center, Tel-Aviv, Israel (Z.R.); and Medical School, Tel-Aviv University, Tel-Aviv, Israel (Y.M., Z.R.)

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ABSTRACT

Convection-enhanced drug delivery (CED) enables achieving a drug concentration within brain tissue and brain tumors that is orders of magnitude higher than by systemic administration. Previous phase I/II clinical trials using intratumoral convection of interleukin-4 *Pseudomonas* exotoxin (PRX321) have demonstrated an acceptable safety and toxicity profile with promising signs of therapeutic activity. The present study was designed to assess the distribution efficiency and toxicity of this PRX321 using magnetic resonance imaging (MRI) and to test whether reformulation with increased viscosity could enhance drug distribution. Convection of low- [0.02% human serum albumin (HSA)] and high-viscosity (3% HSA) infusates mixed with gadolinium-diethylenetriamine pentaacetic acid and PRX321 were compared with low- and high-viscosity infusates without the drug, in normal rat brains. MRI was used for assessment of drug distribution and detection of early and late toxicity. Rep-

resentative brain samples were subjected to histological examination. Distribution volumes calculated from the magnetic resonance images showed that the average distribution of 0.02% HSA was larger than that of 0.02% HSA with PRX321 by a factor of 1.98 ($p < 0.02$). CED of 3.0% HSA, with or without PRX321, tripled the volume of distribution compared with 0.02% HSA with PRX321 ($p < 0.015$). No drug-related toxicity was detected. These results suggest that the impeded convection of the PRX321 infusate used in previous clinical trials can be reversed by increasing infusate viscosity and lead to tripling of the volume of distribution. This effect was not associated with any detectable toxicity. A similar capability to reverse impeded convection was also demonstrated in a CED model using acetic acid. These results will be implemented in an upcoming phase IIb PRX321 CED trial with a high-viscosity infusate.

Targeted fusion toxins (FT) represent a new antineoplastic treatment modality with significant therapeutic potential. The toxin moiety of FT is catalytically active, making these

compounds more potent than chemotherapeutic agents. In addition, such toxins may be active against chemotherapy-resistant hypoxic tumor cells and chemotherapy-refractory tumors (Hall and Fodstad, 1992). Furthermore, the selective targeting of the toxin to tumor cells can limit the side effects associated with antineoplastic treatment. Most targeted FTs consist of a toxin moiety fused to a tumor-specific ligand, which specifically targets it to a receptor that is overexpressed on certain tumor cells (Puri et al., 1994; Rahaman et al., 2002; Rainov and Söling, 2006; Shimamura et al., 2006).

Selectivity can be further enhanced by localized administration, thus maximizing exposure in focal and diffuse portions of the tumor while limiting systemic exposure and toxicity. Intratumoral administration can be achieved by surgical implantation of biodegradable toxin-containing polymers (e.g., Gliadel carmustine-containing wafers) (Olivi and

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ABBREVIATIONS: FT, fusion toxin; CED, convection-enhanced drug delivery; Gd-DTPA, gadolinium-diethylenetriamine pentaacetic acid; MRI, magnetic resonance imaging; PRX321, interleukin-4 *Pseudomonas* exotoxin; GBM, glioblastoma multiforme; MR, magnetic resonance; HSA, human serum albumin; T, time.

Brem, 1994; Westphal et al., 2003) or by infusion (Seiki et al., 1991; Wersäll et al., 1997), although diffusion into surrounding tissue is limited (Wang et al., 1999) to several millimeters. Interstitial infusion can be significantly enhanced by using convection-enhanced drug delivery (CED). This enables achieving a local drug concentration that is orders of magnitude higher than that achievable by systemic delivery (Bobo et al., 1994; Chen et al., 1999).

Real-time imaging of the convected drug enables assessment of the distribution efficiency; monitoring its extent, and avoiding high drug doses in the cerebrospinal fluid or in sensitive brain regions that may cause nonspecific neurotoxicity. It has been shown previously that such real-time imaging may be obtained by co-infusion with a low-molecular-weight tracer such as Gd-DTPA in MRI or iopamidol in computed tomography (Croteau et al., 2005; Mardor et al., 2005; Regino et al., 2008). It has also been shown that diffusion-weighted MRI can be applied for early depiction of tissue response (Mardor et al., 2001, 2005; Lidar et al., 2004).

PRX321 is a fusion toxin consisting of a circularly permuted form of interleukin-4 fused to *Pseudomonas aeruginosa* exotoxin A (Kawakami et al., 2003). PRX321 specifically binds to interleukin-4 receptors, which are overexpressed in glioblastoma (Puri et al., 1994). Thus, PRX321 was developed as a targeted FT for the treatment of glioblastoma multiforme (GBM) (Puri et al., 1996; Joshi et al., 2001).

To date, 72 patients with recurrent malignant astrocytoma or GBM have received PRX321 by intratumoral CED in four phase I/IIa studies. In the first study, nine patients with recurrent GBM received PRX321 in a single-site, open-label, dose-escalation trial (Rand et al., 2000). In the second and third studies, 31 patients received PRX321 in a dose-escalation manner. The fourth trial was a dose-escalation, single-administration in which safety and tolerability were assessed in 32 patients (Weber et al., 2003a,b).

After intratumoral infusion, there was no detectable PRX321 in the blood and no significant systemic toxicity. Grade 3 and 4 adverse events were consistent with what is commonly seen in patients with GBM and some local effects of intracerebral infusion. Significant necrosis within 3 months after treatment was noted in several patients, followed by partial and complete radiological responses.

These unequivocal radiological antitumor responses observed in a few patients indicate the feasibility of using this compound as an antglioma agent when delivered by CED. However, the lack of response in most patients suggests that inefficient distribution of the drug may be associated with treatment failure.

Similar problems of distribution by CED have been recently described in another CED-based trial using conjugated *Pseudomonas* immunotoxin (Sampson et al., 2007). Hence, one may speculate that improving the efficiency of distribution of the therapeutic agent may lead to enhanced efficacy.

We have shown recently that low-viscosity infusates tend to backflow along the catheter track, whereas high-viscosity infusates tend to form efficient convection. We have also shown that CED formation and extent may be improved by increasing infusates viscosities, thus increasing treatment effects (Mardor et al., 2005; Perlstein et al., 2008).

In the present study, we have applied MRI to assess the drug distribution and toxicity of PRX321 CED using the

infusate studied in the previous clinical trials. We then increased the viscosity of the infusate to optimize the formulation in preparation for an upcoming phase IIb trial (Mardor et al., 2008). In parallel, we studied the CED distribution efficiency of acetic acid at low and high viscosities to demonstrate the advantage of high viscosity in another convection-impeding model.

Materials and Methods

Overall Experimental Design. The PRX321 experiments were performed in 41 Sprague-Dawley male rats (300–350 g) divided into four groups of eight rats and one group of nine rats. Each group was treated by CED with one of five infusates: saline only, low- or high-viscosity infusates with PRX321 and Gd-DTPA, and low- or high-viscosity control infusates with Gd-DTPA. Rats were scanned by MRI immediately after infusion, 24 h after infusion, and 2 weeks after infusion. The distribution volumes were calculated from the immediate T1-weighted MR images. The average volumes of distribution were compared among the four groups. Procedure-related neurotoxicity was assessed by the immediate T2-weighted MR images. Early and late procedure-related and drug-related neurotoxicity was assessed by T2-weighted MR images and diffusion-weighted MR images performed 24 h and 2 weeks after infusion.

During the 2 week follow-up, rats were also weighed three times per week. At the end of the 2-week follow-up period, the rats were sacrificed and the brains were extracted and fixed in formalin. Two brains from the high-viscosity PRX321 group and two brains from the saline group were subjected to histological analysis.

To study the effect of infusate viscosity on the distribution efficiency of another convection-impeding substance, an additional experiment was performed with acetic acid in 16 additional rats divided into two groups of eight rats. One group was treated with a low-viscosity infusate consisting of acetic acid and Gd-DTPA. The second group was treated with a high-viscosity infusate, consisting of acetic acid, Gd-DTPA, and sucrose. The study was performed in accordance with the guidelines of The Animal Care and Use Committee of Sheba Medical Center, which is recognized by the Israeli authorities for animal experimentation.

PRX321 Infusates. PRX321 was studied in the concentration found optimal in previous clinical trials (1.5 $\mu\text{g}/\text{ml}$). Solutions of PRX321 were prepared in two different infusate formulations: 0.02% human serum albumin (HSA) in saline, as was used in the previous clinical protocols; and 3.0% HSA in saline for increased viscosity. Gd-DTPA was added in a concentration of 1:70 (in volume) dilution to all infusates. Treatment infusates consisted of PRX321 dissolved in the solutions. Controls consisted of the infusates with no PRX321. A group infused with saline only was added as baseline for toxicity. Therefore, the following infusates were tested: infusate 0 ($n = 8$), saline; infusate 1 ($n = 8$), low-viscosity PRX321; infusate 2 ($n = 8$), low-viscosity control; infusate 3 ($n = 9$), high-viscosity PRX321; and infusate 4 ($n = 8$), high-viscosity control.

Acetic Acid Infusates. Acetic acid was studied at a concentration of 0.5% in saline, pH 3.0. In this case, sucrose was used to increase viscosity: infusate 5 ($n = 8$), low-viscosity acetic acid and infusate 6 ($n = 8$), high-viscosity acetic acid. Table 1 provides a list of all infusates.

CED Procedure. Under general anesthesia, a midline scalp incision was made to identify the bregma. A 1-mm burr hole was drilled on the right, 3 mm anterior and 2 mm lateral to the bregma. A 33-gauge needle attached to a 1000- μl syringe (gastight; Hamilton Co., Reno, NV) was placed stereotactically into the striatum to a depth of 5 mm. The infusion was performed using a BASI syringe pump (Bee Hive; BASI, West Lafayette, IN) at a rate of 1 $\mu\text{l}/\text{min}$ for duration of 40 min up to a total volume of 40 μl .

MRI Monitoring. Immediate assessment of CED formation and extent was performed using a specially designed animal volume coil

TABLE 1

Infusate ingredients and the average CED volume for each infusate, as calculated from T1-weighted MR images acquired immediately after infusion

Infusate No.	Solvent	Viscosity	Drug	Gd-DTPA	No. of Rats	Avg. Distribution Volume <i>mm</i> ³
0	Saline alone	Low	None	None	8	Notmeasured
1	0.02% HSA	Low	PRX321(1.5μg/ml)	1:70	8	14.9 ± 4.4
2	0.02% HSA	Low	None	1:70	8	29.5 ± 3.4
3	3% HSA	High	PRX321(1.5μg/ml)	1:70	9	43.7 ± 9.0
4	3% HSA	High	None	1:70	8	45.6 ± 8.7
5	0.5% Acetic acid	Low	None	1:70	8	15.4 ± 11.2
6	0.5% Acetic acid + 12% sucrose	High	None	1:70	8	39.4 ± 19.7

(5 cm in diameter) on a clinical 0.5 T interventional MRI machine (Signa SP/i; GE Medical Systems, Waukesha, WI) with the LX operating system and gradients intensity of up to 1 Gauss/cm. Early assessment of tissue response was performed using a phased array knee coil on a clinical 3.0 T MRI machine (GE Medical Systems) with the 10.4 M LX operating system, gradients intensity of up to 4.3 Gauss/cm, and the line scan diffusion-weighted imaging (Gudbjartsson et al., 1996) acquisition software package.

MRI acquired immediately after infusion consisted of T1-weighted MRI for depiction of infusate distribution and T2-weighted MRI for depiction of anatomy and procedure-related toxicity. MRI acquired at 24 h and 2 weeks after infusion consisted of T1-weighted MRI for depiction of hemorrhage and T2-weighted and diffusion-weighted MRI for depiction of toxicity.

Calculation of CED Extent. The volume (in cubic millimeters) of distribution was calculated from the T1-weighted MR images acquired immediately after infusion. 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.

Results

PRX321 Drug Distribution

The average CED volume, as calculated from the immediate T1-weighted MR images for each infusate is listed in Table 1. Examples of poor, moderate, and efficient CED distributions are shown in Fig. 1.

The average distribution volume of infusate 2 (low viscosity, no drug) was larger than the average distribution volume of infusate 1 (low viscosity, with drug) by a factor of 1.98 ($p < 0.02$; unpaired two-tailed t test), suggesting that PRX321 impedes the convection process.

The average distribution volume of infusate 3 (high viscosity, with drug) was larger than the average distribution volume of infusate 1 (low viscosity, with drug) by a factor of 2.93 ($p < 0.015$; unpaired two-tailed t test). There was no significant difference between the volumes of distribution of infusates 3 (high viscosity, with drug) and 4 (high viscosity, no drug), suggesting that high viscosity increases the distribution efficiency of PRX321 by nearly a factor of 3, resulting in similar efficiency to that of a high-viscosity placebo.

Acetic Acid Distribution

The average distribution volume of infusate 2 (low-viscosity HSA) was larger than the average distribution volume of infusate 5 (low-viscosity acetic acid) by a factor of 1.92 ($p < 0.02$; unpaired two-tail t test), suggesting that acetic acid impedes the convection process.

The average distribution volume of infusate 6 (high-viscosity acetic acid) was larger than the average distribution volume of infusate 5 (low-viscosity acetic acid) by a factor of 2.56

Full backflow - 0 mm³

Moderate distribution: 24.2 mm³

Efficient distribution: 87.9 mm³

Fig. 1. Examples of poor, moderate, and efficient infusate distributions in the rat brain, as depicted by T1-weighted MRI acquired immediately after CED with Gd-containing infusates. The rat brain presented in the top image was treated with infusate 1; the rats presented in the middle and bottom images were treated with infusate 4.

($p < 0.01$; unpaired two-tailed t test), suggesting that high viscosity increases the distribution efficiency of acetic acid by nearly a factor of 2.5.

PRX321 Procedure-Related Toxicity

Mortality. Two rats were found dead within 24 h after infusion: one rat treated with solution 1 and one rat treated with solution 2. This mortality rate (less than 10%) is consistent with procedure-related mortality during CED experiments in rat models in our laboratory. Because one rat was treated with the drug and the other without drug, there is no statistical difference between the groups in means of mortality.

Radiological Toxicity. Three types of treatment-related toxicity and tissue damage were observed in the immediate and follow-up MR images: hydrocephalus, minor tissue damage along the catheter insertion path, and bleeding in the striatum. A detailed summary of these findings is listed in Table 2. All the radiological changes observed seem to be procedure-related. There was no significant difference in

TABLE 2

Radiological toxicity of rats treated with infusates 0 to 4

Toxicity Type	Radiological Change	Infusate 1, No. of Rats	Infusate 2, No. of Rats	Infusate 3, No. of Rats	Infusate 4, No. of Rats
Hydrocephalus	Depicted in T2 immediately after infusion and remained stable, except for one rat (hydrocephalus resolved on the second follow-up MRI)	1	1	1	1
Minor tissue damage along catheter insertion path	Depicted on T2 MRI and DWMRI	2	1	1	1
Bleeding in the striatum	Depicted on T2 and DWMRI in the first follow-up and was recovered by the second follow-up MRI		1		1

DWMRI, diffusion-weighted magnetic resonance imaging.

their occurrence between the four infusates or between drug-treated rats versus controls. An example of hydrocephalus with no tissue toxicity as depicted in the follow-up MR images acquired 24 h after infusion is shown in Fig. 2.

Weight Changes. There was no significant difference between the average weights of different infusates (infusate 1 versus infusate 2, $p < 0.66$; infusate 3 versus infusate 4, $p < 0.43$; infusate 1 versus infusate 3, $p < 0.49$; and infusate 2 versus infusate 4, $p < 0.72$) or between drug-treated rats versus placebo-treated rats (infusates 1 + 3 versus infusates 2 + 4, $p < 0.98$; infusates 1 + 2 versus infusates 3 + 4, $p < 0.72$). These results are consistent with no drug-related or viscosity-related toxicity.

Histological Examination. Two representative brains treated with the high-viscosity drug infusate (infusate 3) and two representative brains treated with the saline (infusate 0) were subjected to histological analysis, consisting of slicing, staining with hematoxylin and eosin, and a detailed review by a neuropathologist with previous experience in preclinical and clinical CED. Tissue damage, edema and hemorrhage, macrophages with hemosiderin, and reactive astrocytes were found to some degree in all rats at the catheter entrance site and up to 1 to 2 mm into the cortex; maximal width, 0.25 mm at the cortex surface.

The catheter tract could be seen in some of the rats in the subiculum and up to the border with the striatum, accompa-

nied by hemosiderin-laden macrophages with minimal damage to surrounding tissue. In one of the control rats, the needle tract was seen also in the striatum (pass through the hippocampus), 5.25 mm from the surface. It was accompanied by inflammatory cells, including macrophages. The maximal distance of tissue changes from the catheter path in the striatum was ± 0.2 mm.

No tissue damage or inflammatory response was seen in any of the rats beyond the catheter track. No additional abnormalities were identified in the retrostriatal or striatal area (in which the drug was distributed). An example of a histology slide of a rat brain treated with PRX321 is shown in Fig. 3.

Discussion

We have presented previously the use of co-infusion of Gd-DTPA during CED and MRI for immediate assessment of drug distribution. T2-weighted and diffusion-weighted MRI was used for early assessment of cytotoxic tissue response to the distributed drug. In this report, we applied these imaging methodologies to test the distribution of various infusates with and without PRX321 to assess their relative drug distributions characteristics and the associated tissue toxicity.

The efficient formation of convection depends on many

Fig. 2. MRI acquired 24 h after CED, depicting significant bilateral hydrocephalus and unilateral occipital liquid accumulation. The diffusion-weighted MRIs and the calculated apparent diffusion coefficient maps (on the right) show no tissue toxicity.

Fig. 3. Histology slide of a rat treated with CED of PRX321. Hemosiderin-laden macrophages with no architectural disruption can be seen along the catheter path in the cortex (a). Structural changes can be seen in the hippocampus (b). No abnormalities in the striatum (c).

parameters, including patient-related parameters, such as tissue consistency and catheter positioning (Lidar et al., 2004; Sampson et al., 2007). The response rate of the few convection-based trials published so far is promising, although some drugs seem to be more efficient than others. We have suggested previously that some drugs may be less efficient than others due to their low viscosity, hindering efficient formation of convection and hence lacking therapeutic effect. We have shown that by increasing the viscosity of infusates (which may be simply attained by increasing the solvent concentration, such as HSA or sucrose) it is possible to significantly increase the efficiency of CED formation and extent. We have also shown that the increased distribution obtained by this approach indeed resulted in increased treatment effects (Mardor et al., 2005).

In the current study, we found that PRX321 impedes convection. This conclusion is based on the results of the low-viscosity infusates 1 and 2, showing that the low-viscosity infusate containing the drug produced significantly lower distributions than the control low-viscosity infusate. This result may explain the low number of responding patients in the previous clinical studies, which used the same low-viscosity infusate.

Increasing the viscosity of the infusate, by simply increasing the concentration of the solvent (HSA), increased the average volume of distribution by nearly a factor of 3, bringing it to be similar to that of the high-viscosity solution without the drug. These results suggest that increasing the solvent concentration increase the drug distribution efficiency significantly, which may lead to a larger number of responding patients.

This study is the first to demonstrate the feasibility of applying increased viscosity to reverse a convection-impeding infusate into an efficiently distributing infusate. We demonstrate this phenomenon in one study using PRX321 as the convection-impeding substance and HSA as the means to

increase viscosity, and in another study using acetic acid as the convection-impeding substance and sucrose as the means to increase viscosity.

No drug-related toxicity was observed. Two rats died within 24 h after infusion. One rat was treated with the drug (infusate 1) and one rat without the drug (infusate 2). This mortality rate (less than 10%) is consistent with what we expect from treatment-related mortality of CED experiments in rat models in our laboratory regardless of the agent used for convection. No evidence of drug-related toxicity was observed in any of the MR images acquired after convection, and there was no significant difference between the treatment-related radiological changes observed in the drug-treated rats versus the placebo-treated rats.

There was no significant difference between the average weights of drug-treated rats versus placebo-treated rats, and the histological examination of representative brains chosen from the group treated with the high-viscosity drug infusate (infusate 3) and from the group treated with the saline infusate (infusate 0) showed no significant differences between rats treated with the drug versus rats treated with saline and no abnormalities in the striatum region in which the drug was distributed.

In summary, these results suggest that PRX321 impedes convection, resulting in small volumes of drug distribution with the current formulation. These results are consistent with the presumed poor distribution of PRX321 in the early clinical studies. Fortunately, reformulation to increase viscosity resulted in tripling of the volume of distribution of the drug, suggesting an expected enhanced treatment effect. The results of this study will be implemented in an upcoming phase IIb clinical trial.

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Address correspondence to: Dr. Yael Mardor, The Advanced Technology Center, Sheba Medical Center, Tel-Hashomer 52621, Israel. E-mail: yael.mardor@sheba.health.gov.il