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Real-time magnetic resonance imaging visualization and quantitative assessment of diffusion in the cerebral extracellular space of C6 glioma-bearing rats

Kai Li[a,b], Hongbin Han[a,b,*], Kai Zhu[a,b], Kejia Lee[c], Bo Liu[d], Fugen Zhou[d], Yu Fu[b,e], Qingyuan He[a,b]

[a] Department of Radiology, Peking University Third Hospital, Beijing, China
[b] Beijing Key Lab of MRI Device and Technique, Beijing, China
[c] The Max Planck Institute for Radio Astronomy, Bonn, Germany
[d] Image Processing Center, Beihang University, Beijing, China
[e] Department of Neurology, Peking University Third Hospital, Beijing, China

HIGHLIGHTS

- We describe a detailed and quantitative means to document drug delivery.
- We perform real-time monitoring and quantitative assessment of diffusion in brain.
- Gd-DTPA diffusion and distribution were limited in C6 gliomas.
- Gd-DTPA diffusion presented isotropic in C6 gliomas.
- Gd-DTPA diffusion presented as an anisotropic pattern in the caudate nucleus.

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ABSTRACT

Interstitial drug delivery is a promising technique for glioma treatment; however, suboptimal methodologies limit the ability to document the delivery of therapeutic agents. The present study employed magnetic resonance imaging for real-time visualization and quantitative assessment of drug diffusion in gliomas. Using gadolium-diethylenetriaminepentaacetic acid (Gd-DTPA) as a tracer, we considered diffusion in the agarose gel phantom as a reference and compared the diffusion and distribution patterns between the control group and C6 glioma-bearing rats after direct cerebral infusion. Our findings confirmed that Gd-DTPA diffusion was severely impaired in gliomas and presented in an anisotropic pattern in the caudate nucleus. The proposed method provides a new approach for the real-time monitoring of interstitial drug delivery and quantitative assessment of biophysical structural variations in diseased tissue.

1. Introduction

Gliomas are the most common type of primary neoplasms in the central nervous system and have high recurrence rates, especially for malignant glioblastoma [15]. Surgery, radiation therapy, and traditional chemotherapy often fail to significantly alter the disease course [4]. Over the past 3 decades, stereotactic techniques have been widely employed for neurosurgery, radiosurgery, radiation [13], and interstitial chemotherapy [6,28]. Combining interstitial drug delivery with stereotactic techniques has facilitated the use of interstitial drug delivery for treating primary brain tumors; it provides the most direct method of circumventing the blood–brain barrier, which is the primary obstacle to drug delivery to tumor cells [2]. In this delivery system, drug molecules infused into the interstitial fluid must diffuse through the extracellular space (ECS) to reach surrounding tumor cells distant from the injection site. However, the efficacy of many antitumor drugs delivered via this route show considerable heterogeneity regarding tumor distribution and dissemination [11]. It is believed that therapeutic efforts can be predicted by monitoring tumor drug penetration and deposition.

Various methods have been explored to qualitatively and quantitatively assess drug diffusion and penetration in the ECS of gliomas [23,25]. Given recent advancements in non-invasive imaging, great efforts have been made to investigate the use of magnetic resonance imaging (MRI) to evaluate drug deposition in tissues [5,21]. To the best of our knowledge, MRI to quantitatively evaluate both agent diffusion and distribution for direct interstitial infusion

* Corresponding author at: Department of Radiology, Peking University Third Hospital, North Huayuan Road No. 49, Haidian District, Beijing 100191, China. Tel.: +86 10 8226 6972.
E-mail address: hanhongbin@bjmu.edu.cn (H. Han).

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has not been previously performed in gliomas. Here, an extracellular contrast agent was employed to visualize diffusion in ECS. Classical diffusion theory was introduced to perform a quantitative assessment based on MRI images. The primary aim of this study was to realize real-time visualization and quantification of diffusion in the cerebral ECS of C6 glioma-bearing rats.

2. Materials and methods

2.1. Contrast agent and agarose gel phantom preparation

Gadolium-diethylenetriaminepentaacetic acid (Gd-DTPA; Magnevist; Bayer Schering Pharma AG, Berlin, Germany) was diluted to 10 mmol/L with 0.9% (w/v) sodium chloride solution. The free diffusion coefficient (D) measurement was performed on a 0.3% (w/v) agarose gel phantom at 37 °C. Agarose powder (0.3 g) was dissolved in 100 mL saline solution (0.9%), and the mixture was heated in a water bath until the powder dissolved. The solution was then solidified into a gel upon cooling to room temperature.

2.2. Experimental animal groups

All experiments were carried out on age-matched, adult male Sprague Dawley rats (200–250 g). The subjects were randomly divided into two groups: controls ($n = 8$) and rats bearing C6 gliomas ($n = 8$). The experimental protocols were approved by the Ethics Committee of Peking University Health Science Center (No. LA 2012-016).

2.3. Intracerebral glioma model

The C6 rat glioma cell line (ATCC No. CCL 107) was cultured in Dulbecco's modified Eagle medium/F12 (Invitrogen, Carlsbad, CA), containing 10% fetal bovine serum (Invitrogen), and 0.2% penicillin/streptomycin (Invitrogen), and was incubated at 37 °C in a CO_2 incubator (95% O_2: 5% CO_2).

Anesthetized rats (50 mg/kg sodium pentobarbital, intraperitoneal [i.p.]) were immobilized in a stereotactic head frame (Stoelting Co., Wood Dale, IL, USA). A scalp incision was made along the sagittal suture, and the C6 cell suspension (5×10^4 cells in 10 μL) was slowly inoculated (10 min) through a 2-mm burr hole into the right caudate nucleus (Anterior-Posterior, +1 mm; Medial-Lateral, −3.5 mm; Dorsal-Ventral, −4.5 mm). The microsyringe (Hamilton, Bonaduz AG, Switzerland) was slowly removed 5 min after inoculation, the burr hole was plugged with bone wax, and the scalp was sutured. The rats underwent MRI scanning 20 days after tumor implantation.

2.4. MRI

Rats were anesthetized (pentobarbital sodium, 50 mg/kg i.p.) and body temperature was maintained at 37 °C with a heating pad. Animals were scanned in a 3.0-T horizontal bore magnet (Magnetom Trio, A Tim System; Siemens Medical Solutions, Erlangen, Germany) interfaced to an eight-channel dedicated wrist coil. A coronal T_2-weighted turbo spin-echo sequence (repetition time, 3620 ms; echo time, 92 ms; flip angle, 120°; field of view, 80 mm; matrix, 256 × 256; slice thickness, 2 mm; number of averages, 2) was employed to demonstrate glioma formulation (Supplementary Fig. S1). Next, a three-dimensional T_1-weighted magnetization-prepared rapid acquisition gradient-echo (MP-RAGE) sequence (repetition time, 1500 ms; echo time, 3.7 ms; inversion time, 900 ms; flip angle, 9°; matrix, 512 × 96; field of view, 267 mm; voxel, 0.5 mm × 0.5 mm × 0.5 mm; number of averages, 2) was employed to visualize each tumor in coronal, axial, and sagittal planes. For the agarose gel phantom and control rats, only the MP-RAGE sequence was performed before Gd-DTPA injection.

2.5. Gd-DTPA microinjection

A volume of 2 μL Gd-DTPA solution (10 mmol/L) was microinjected at a rate of 0.2 μL/min over 10 min, followed by a 5-min waiting period to avoid dorsal reflux along the needle track. The injection sites were the centers of the agarose gel phantom, the caudate nucleus, and gliomas. For each subject, repeated scanning with T_1-weighted MP-RAGE sequence was performed at 15 and 30 min and each hour post-injection.

2.6. Diffusion parameters calculation

The concentration of a dispersed substance in the ECS follows the diffusion equation [30]

$$\frac{\partial C(r,t)}{\partial t} = D^*\nabla^2 C(r,t), \tag{1}$$

where $C(r,t)$ denotes the extracellular concentration of the diffused substance at a position r and time t, r is the radius (the distance extending from the original injection site to an arbitrary point in the Gd-DTPA distribution area), and D^* is the diffusion coefficient.

As an extracellular contrast, Gd-DTPA distributes mainly in the ECS [3]. It may leave the diffusion process by the drainage of interstitial fluid and the net number of molecules in the ECS changes over time. In the simplest case, the loss of Gd-DTPA is proportional to the local extracellular concentration, i.e., the loss process is governed by a first-order kinetic constant k'.

$$\frac{\partial C(r,t)}{\partial t} = D^*\nabla^2 C(r,t) - k'C(r,t). \tag{2}$$

According to the linear relationship between MRI signal increment and average Gd-DTPA concentration in brain tissue by using an MP-RAGE sequence within the concentration range of 0–1 mmol/L [27], the concentration of Gd-DTPA in the ECS can be calculated by

$$C(r,t) = \frac{C}{\alpha} = \frac{\Delta SI(r,t)}{\alpha k}, \tag{3}$$

where C represents the average Gd-DTPA concentration in the brain tissue, α is the volume fraction of ECS, $\Delta SI(r,t)$ is the MRI signal increment related to Gd-DTPA infusion, and k is slope of the linear equation between $\Delta SI(r,t)$ and C. It should be pointed out that a fast exchange between the intra- and extra-cellular spaces is assumed here. The slow exchange conditions, e.g., in the necrotic regions, fall outside the scope of this study.

Substituting $C(r,t)$ in Eq. (2), we can calculate

$$\frac{\partial((\Delta SI(r,t))/\alpha k)}{\partial t} = D^*\nabla^2\left(\frac{\Delta SI(r,t)}{\alpha k}\right) - k'\left(\frac{\Delta SI(r,t)}{\alpha k}\right). \tag{4}$$

Multiplying by αk, Eq. (4) can be transformed to

$$\frac{\partial(\Delta SI(r,t))}{\partial t} = D^*\nabla^2(\Delta SI(r,t)) - k'(\Delta SI(r,t)). \tag{5}$$

It's worth noting that the transformation from Eq. (2) to Eq. (4) cannot be achieved if the average Gd-DTPA concentration in the brain tissue is higher than 1 mmol/L. Considering the non-linearity of ΔSI–C relationship, $C(r,t)$ can be calculated by the traditional method based on longitudinal relaxation rate measurement.

Assuming a radial distribution in a system with spherical symmetry, Eq. (5) becomes

$$\frac{\partial(\Delta SI(r,t))}{\partial t} = D^*\frac{1}{r^2}\left(\frac{\partial}{\partial r}\left(r^2\frac{\partial(\Delta SI(r,t))}{\partial r}\right)\right) - k'(\Delta SI(r,t)), \tag{6}$$