MRI Transverse Relaxation Rate Correlates with Number of Viral Particles Expressing H-Ferritin in the CNS

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Introduction

Optimal use of transgenic animals and preclinical development of gene therapy may require methods for non-invasive visualization of gene expression (1). Recently, active research in MRI/MRS has resulted in the development of gene reporters whose expression can be coupled to genes of interest (2). The iron storage protein ferritin has been successfully used as an MR reporter gene in both vector mediated gene delivery and transgenic animal models (3,4). However, measuring the levels of gene expression non-invasively and with high spatial resolution has not yet been demonstrated with MR methods. Refining the imaging reporter systems to reflect amounts of local transcription and dose response curves requires quantification of gene expression (5).

The aim of this study is to characterize quantitatively gene expression in the mouse brain. Our present work describes an indirect method of quantifying gene expression of the heavy-chain ferritin (H-Ft) MR reporter. We targeted the central nervous system because it is particularly challenging to study due to its inherent tissue opacity and the blood brain barrier. We computed the 3D distribution of MR transverse relaxation rates in a mouse brain after transgene vector inoculation. We established that these relaxation rates correlate significantly to the number of inoculated infectious particles.

Materials and Methods

We used HSV1 vector (6) with titer $2x10^{10}$ pfu/ml coding for heavy chain ferritin and green fluorescent protein (GFP) under the Human Cytomegalovirus (CMV) promoter. We injected 5 µl of inoculum bilaterally into the cortex and striatum of C57BL/6J male mice, age 4-6 weeks (n=12). We gradually decreased the number of infectious particles in the inoculum by serial two-fold dilutions using phosphate buffer saline (PBS) until the MRI contrast produced by the gene expression was indistinguishable from the control. Control mice were injected with the same volume of PBS only. At 4 days post-injection, mice were perfused with 4% paraformaldehyde and their brains were imaged using an 11.7 T Bruker micro-imaging system. The brains were then embedded in paraffin and sectioned for histological study.

We acquired 3D spin echo (SE) images with 16 echoes, first echo TE=10 ms, an inter-echo time of 10 ms, TR=2000ms, and NEX=2. The field of view was 15x15x25 mm, matrix size 256x128x128 and voxel size 0.059x0.117x0.195 mm. In addition we acquired gradient echo (GE) images with 8 echoes, first echo TE=3 ms, an inter-echo time of 5 ms, TR=1200ms, and NEX=2. For GE we used the same field of view and matrix size. The acquired images were fit on a voxel-by-voxel basis to a single exponential relaxation decay curve by applying linear transformation using Matlab software. The procedure resulted in 3D maps of R2 and R2* transverse relaxation rates.

In order to evaluate numerically the local relaxation rates, 3D regions of interest (ROI) over the injection site were drawn on the second TE image using Amira software. The ROIs were then transferred to the R2 and R2* maps, and the mean relaxation rates and the standard deviation were extracted from histograms of the ROI. A linear regression model was applied to correlate the mean relaxation rate with the amount of infectious viral particles inoculated using the statistical package R and Matlab software.

Results and Discussion

The transgene vector mediated expression of H-Ft resulted in an increase of the contrast on GE and SE images (Figure 1A). The R2 and R2* maps showed a pronounced increase of relaxation rate at the site of vector inoculation (Figure 1B). We quantified the local relaxation rates in the mouse striatum and established that they correlate significantly with the number of viral particles at the site of inoculation ($p=0.0023$ for SE and $p=0.0008$ for GE, Figure 1C).

Our study confirms that quantitative MR imaging of gene expression in intact animals is possible. In addition our relaxation rate measurements can be coupled with dose dependent inducible promoters making MRI quantification of gene expression even more accurate (2, 5). Our results may be useful for future studies assessing quantitatively the molecular events in the central nervous system of living animals.

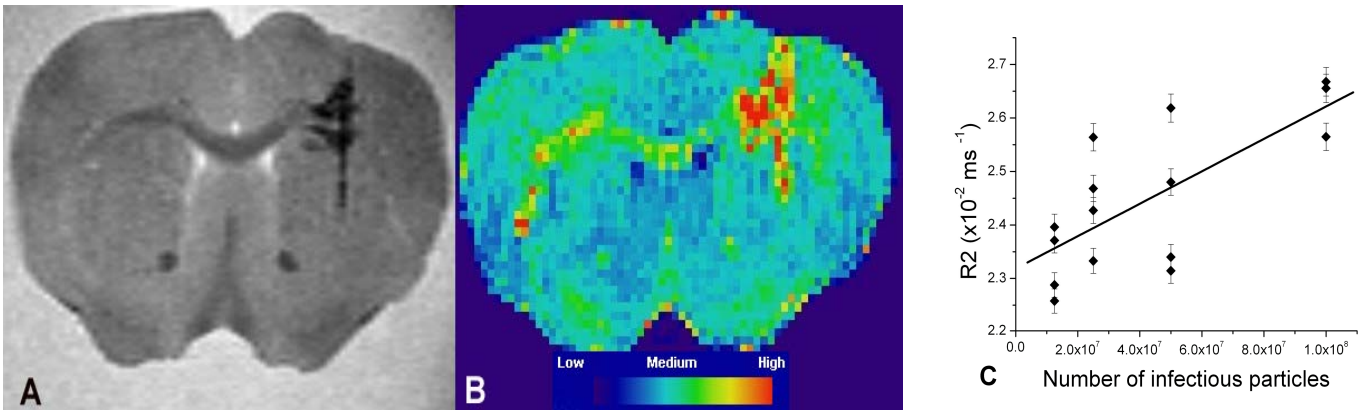

Fig. 1. Representative data of mouse brain inoculated with the HSV1 vector containing H-Ft transgenes. (A) T2-weighted single-slice spin echo coronal image (TE=20 ms) showing the site inoculated with $5x10^7$ viral particles. (B) R2* map generated from the multi-echo data shows the same brain region. (C) Linear correlation plot between ROI relaxation rate and the number of injected viral particles coding for the transgene HFt

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