Decreased ADC precedes cellular swelling in N-Methyl-D-Aspartate (NMDA) treated mouse retina

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Introduction

Acute excitotoxicity causes cytotoxic edema that precedes neuron cell necrosis and apoptosis in neurological diseases[1]. Apparent diffusion coefficient (ADC) decreases early reflecting cytotoxic edema in various brain injuries[2]. Our recent study demonstrated that high-resolution diffusion MRI detected three retinal layers of distinct ADC[3]. In this study, we examined the feasibility of detecting N-methyl-D-aspartate (NMDA) induced retinal excitotoxic injury in mice using *in vivo* diffusion MRI.

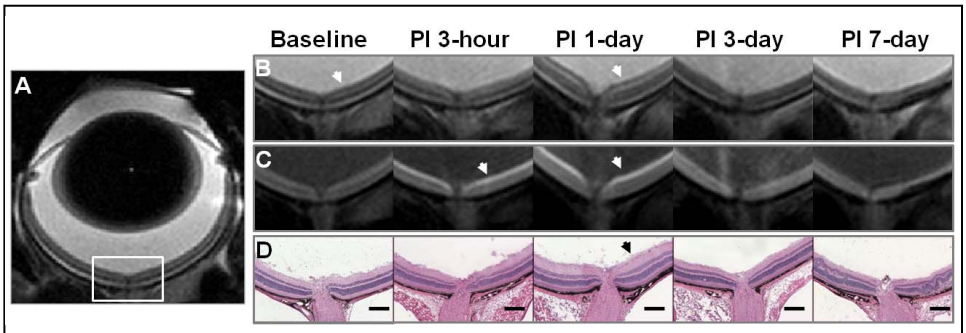

Fig. 1 (A) A representative of non diffusion-weighted image of a normal mouse eye. The highlighted box shows central retina close to the optic nerve head. **(B)** T_2-weighted MR images of mouse retina at baseline and PI 3-hour, 1-, 3-, 7-day. **(C)** Diffusion weighted MR images of the same mouse eyes. **(D)** H&E staining of retina from the same mouse. Scale bar, 200 μm. PI – post injection.

Materials and Methods

Animal Model: Eight-week old male C57BL/6 mice (~25 g) received 0.5 μl of 5 mM NMDA/saline through intravitreal injection. *In vivo* diffusion MRI was performed at 3-hour (n=6), 1- (n=5), 3- (n=3), and 7-day (n=4) post injection (PI). Baseline measurements were performed on age-matched C57BL/6 male mice without NMDA treatment (n=4). *In vivo MRI:* Diffusion weighted imaging (DWI) was performed on 11.74 T utilizing a standard spin-echo diffusion-weighted sequence. Three pairs of diffusion weighted images with orthogonal diffusion weighting directions and opposite gradient polarity and a non-diffusion weighted image were acquired for each mouse eye[3]. MRI acquisition parameters were: TR 2 sec, TE 34 ms, Δ 15 ms, δ 5 ms, b = 0 and 955 s/mm^2, slice thickness 400 μm, FOV 12 × 12 mm^2, in-plain resolution 47 × 47 μm^2 (23 × 23 μm^2 zero filled), and number of average 1. Mice were euthanized at the conclusion of MRI. Eyes were enucleated and snap frozen for histology. *Data Analysis:* Calculation of ADC map and identification of region of interest were performed as previously reported[3]. Starting from the vitreous, the three MR-detected retinal layers were assigned to NFL/GCL/IPL (dark) – INL/OPL (bright) – ONL/IS/OS† (dark). At PI 1-day, there were three, two extra layers resulting from NMDA toxicity, MR-detected layers in the middle and were tentatively assigned as INL/OPL. *Histology:* Eight-μm thick tissue sections bisecting the optic nerve were collected for hematoxylin and eosin (H&E) staining to examine the changes of retinal layers. *Statistics:* One-way ANOVA was performed to test ADC difference between NMDA-treated groups and baseline.

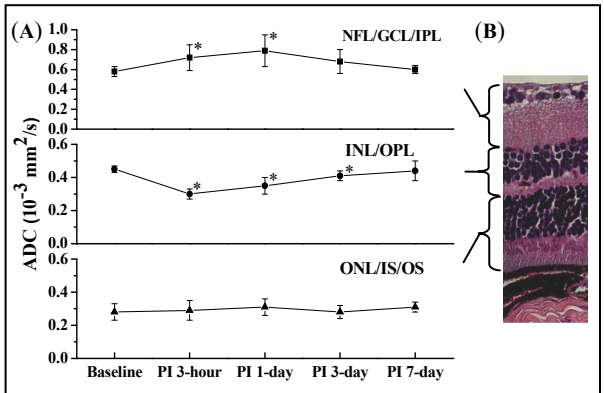

Fig. 2 (A) Diffusion weighted MRI determined ADC in NFL/GCL/IPL, INL/OPL, and ONL/IS/OS of mouse retina at baseline and PI 3-hour, 1-, 3-, and 7-day. (**B**) H&E stained mouse retina shows corresponding retinal cell layers. *, $p < 0.05$ compared to baseline.

Results

Figure 1 shows MR images and the matched H&E staining of mouse retina with or without NMDA treatment. A hyper-intense inner retinal layer between NFL and OPL was seen in DWI as early as at PI 3-hour. At PI 1-day, retinal swelling was evidenced by the increased number of MR-detected middle layers and the increased retinal thickness in H&E staining. At PI 3-day and 7-day, both the inner retinal hyperintensity and retinal swelling subsided, possibly as a result of tissue loss. Figure 2 shows DWI determined ADC in respective MR-detected retinal layers. A transient decrease of ADC in INL/OPL that peaked at PI 3-hour (~30%) was observed. In contrast, ADC in ONL/IS/OS exhibited no change at all times. Additionally, ADC in NFL/GCL/IPL increased at PI 3-hour and 1-day.

Discussion and Conclusion

Intravitreal injection of NMDA caused a transient decrease of ADC in INL/OPL at PI 3-hour, the earliest measurement in our hands, suggesting the excitotoxicity induced edema in these retinal layers[4]. ADC in ONL/IS/OS was not affected by NMDA treatment, reflecting the resistance of pre-synaptic photoreceptors to NMDA excitotoxicity[5]. The transient increase of ADC in NFL/GCL/IPL at PI 3-hour and PI 1-day could be attributed to the acute necrosis of ganglion cells which are sensitive to excitotoxicity[6]. Overall, current results support that decreased ADC as a biomarker of cytotoxic edema providing an early measure of retinal excitotoxicity injury before retinal swelling.

†, Abbreviations of retinal layers: NFL – nerve fiber layer; GCL – ganglion cell layer; IPL – inner plexiform layer; INL – inner nuclear layer; OPL – outer plexiform layer; ONL – outer nuclear layer; IS – inner segment; OS – outer segment.

References

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