Manganese-Enhanced MRI for Preclinical Evaluation of Retinal Degeneration Treatments

Rebecca M. Schur,1 Li Sheng,1 Bhubanananda Sahu,2 Guanping Yu,1 Songqi Gao,3 Xin Yu,1 Akiko Maeda,2,3 Krzysztof Palczewski,3 and Zheng-Rong Lu1

1Department of Biomedical Engineering, School of Engineering, Case Western Reserve University, Cleveland, Ohio, United States
2Department of Ophthalmology and Visual Sciences, School of Medicine, Case Western Reserve University, Cleveland, Ohio, United States
3Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, Ohio, United States

Correspondence: Zheng-Rong Lu, M. Frank Rudy and Margaret Domiter Rudy Professor of Biomedical Engineering, Department of Biomedical Engineering, Case Western Reserve University, Wickenden Building, Room 427, 10900 Euclid Avenue, Cleveland, OH 44106, USA; zxli125@case.edu.

Submitted: January 22, 2015
Accepted: June 22, 2015

Citation: Schur RM, Sheng L, Sahu B, et al. Manganese-enhanced MRI for preclinical evaluation of retinal degeneration treatments. Invest Ophthalmol Vis Sci. 2015;56:4936–4942. DOI:10.1167/iovs.15-16522

PURPOSE. Apply manganese-enhanced magnetic resonance imaging (MEMRI) to assess ion channel activity and structure of retinas from mice subject to light-induced retinal degeneration treated with prophylactic agents.

METHODS. Abca4−/− Rdb8−/− double knockout mice with and without prophylactic retinylamine (Ret-NH2) treatment were illuminated with strong light. Manganese-enhanced MRI was used to image the retina 2 hours after intravitreous injection of MnCl2 into one eye. Contrast-enhanced MRIs of the retina and vitreous humor in each experimental group were assessed and correlated with the treatment. Findings were compared with standard structural and functional assessments of the retina by optical coherence tomography (OCT), histology, and electroretinography (ERG).

RESULTS. Manganese-enhanced MRI contrast in the retina was high in nonilluminated and illuminated Ret-NH2−treated mice, whereas no enhancement was evident in the retina of the light-illuminated mice without Ret-NH2 treatment (P < 0.0005). A relatively high signal enhancement was also observed in the vitreous humor of mice treated with Ret-NH2. Strong MEMRI signal enhancement in the retinas of mice treated with retinylamine was correlated with their structural integrity and function evidenced by OCT, histology, and a strong ERG light response.

CONCLUSIONS. Manganese-enhanced MRI has the potential to assess the response of the retina to prophylactic treatment based on the measurement of ion channel activity. This approach could be used as a complementary tool in preclinical development of new prophylactic therapies for retinopathies.

Keywords: manganese-enhanced MRI, retinylamine, retinal degeneration, efficacy evaluation
phototransduction under differing conditions\textsuperscript{20,21} and evaluated in disease models such as glaucoma,\textsuperscript{22,23} optic nerve crush,\textsuperscript{24} and diabetic retinopathy.\textsuperscript{25} With its ability to image anatomical structures and signaling activity simultaneously, we expect that MEMRI will be an effective method to assess the treatment response by measuring Ca\textsuperscript{2+} signaling activities in the retina.

In this work, we investigated the use of MEMRI in \textit{Abca4}\textsuperscript{−/−} \textit{Rdb8}\textsuperscript{−/−} double knockout mice to evaluate intense light-induced retinal degeneration and its response to prophylactic treatment with retinylamine, a potent retinoid cycle modulator, based on changes in Ca\textsuperscript{2+} channel activity in the retina. Thus, we evaluated the ability of MEMRI to assess the structure and function of healthy, drug-treated, and fully degenerated retinas in these mutant mice according to the experimental time line shown in Figure 1.

**Materials and Methods**

**Animals and Treatment**

\textit{Abca4}\textsuperscript{−/−} \textit{Rdb8}\textsuperscript{−/−} double knockout mice were generated as previously described.\textsuperscript{6} All mice used of either sex in this study were homozygous for the Leu450 allele of \textit{Rpe65} as determined by a genotyping protocol described earlier,\textsuperscript{26} and the mice were also free of \textit{Crb1}/\textit{rd8} \textit{Pde6}/\textit{rd1} mutations.\textsuperscript{27} This mouse model is light-sensitive, and retinal degeneration can be accelerated by intense light exposure.\textsuperscript{3,28,29} Animals were housed and bred in the Animal Resource Center at Case Western Reserve University (CWRU; Cleveland, OH, USA) and cared for according to an approved protocol by the CWRU Institutional Animal Care and Use Committee and in compliance with recommendations from the American Veterinary Medical Association Panel on Euthanasia and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The drug treatment, light illumination (LI), and evaluation schedule is shown in Figure 1B. Untreated, LI (\textit{n} = 5) and treated, LI (Ret-NH\textsubscript{2}-LI, \textit{n} = 5) mice were illuminated with 10,000 lux yellow light for 60 minutes while contained in a white bucket with their pupils dilated with 1% tropicamide. Animals were housed in the dark for 6 days prior to ERG. Animals were then kept in conditions of ambient lighting for optical coherence tomography (OCT) imaging and Mn\textsuperscript{2+} injection. Nonilluminated (NIL, \textit{n} = 6) mice not exposed to light served as healthy controls. Retinylamine was obtained as previously described\textsuperscript{6} and administered to the treatment group by oral gavage 16 hours prior to light illumination (50 mg/kg dissolved in 100 \textmu L 10% dimethylsulfoxide (DMSO) in vegetable oil).

**Optical Coherence Tomography**

Ultra-high resolution spectral-domain optical coherence tomography (SD-OCT Envisu C2200; Bioptigen, Irvine, CA, USA) was used to image the structure of mouse retinas in vivo. Animals were anesthetized by intraperitoneal injection of a cocktail (15 \textmu L/g body weight) comprised of ketamine (6 mg/mL) and xylazine (0.44 mg/mL) in PBS buffer (10 mM sodium phosphate, pH 7.2, and 100 mM NaCl). Pupils were dilated with 1% tropicamide for imaging. Four images acquired in the B-scan mode were averaged to construct each final SD-OCT image. The outer nuclear layer (ONL) thickness was quantified with Bioptigen software at 150, 300, and 450 \textmu M from the ocular nerve head in the temporal-nasal and superior-inferior directions.

**Electroretinography**

Electroretinograms were acquired as previously described\textsuperscript{30} 24 hours after OCT imaging to allow the animals sufficient time to recover from anesthesia. Mice were anesthetized by the same procedure used for OCT imaging and experiments were performed in a dark room. Three electrodes were placed on the animal: a contact lens electrode on the eye, a reference electrode underneath the skin between the ears, and a ground electrode underneath the skin of the tail. Electroretinograms were recorded with the universal electrophysiologic system UTAS E-3000 (LKC Technologies, Inc., Gaithersburg, MD, USA). Light intensity calibrated by the manufacturer was computer-controlled. Mice were placed in a Ganzfeld dome, and scotopic responses to flash stimuli were obtained from both eyes simultaneously.

**Manganese-Enhanced MRI**

Mn\textsuperscript{2+} was administered by intravitreous injection into the right eye of the mouse in ambient lighting conditions (2.4 \textmu L, 5 mM MnCl\textsubscript{2} per mouse; Sigma-Aldrich Corp., St. Louis, MO, USA). The noninjected left eye was used as a contralateral control. Mice were anesthetized by the same procedure used for OCT imaging and allowed to wake up before being reanesthetized with 2% isoflurane immediately prior to MRI experiments. Magnetic resonance images were acquired on a preclinical 7T scanner (Bruker BioSpin, Billerica, MA, USA) equipped with a volume coil. A series of scouting scans was performed to locate the eyes in the scanner. Two hours after injection of MnCl\textsubscript{2}, a high signal-to-noise ratio (SNR) scan was performed with a two-dimensional (2D) coronal multislice, multiecho spin echo acquisition (TR = 400 ms, TE = 10.6 ms, FOV = 2.5 \times 2.5 cm, resolution = 98 \times 195 \mu m, number of averages = 16, total imaging time = 14 minutes).

**Histology**

Mice were euthanized immediately following MRI experiments. Eye cups were harvested, fixed in 4% paraformaldehyde overnight, and embedded and frozen in OCT compound (Tissue Tek, Torrance, CA, USA). Slides, 12-\textmu m thick, were prepared and stained with hematoxylin and eosin (H&E; Vector Laboratories, Burlingame, CA, USA).

**Data/Statistical Analysis**

Manganese-enhanced MRI images and ERG traces were visualized and analyzed with MATLAB (Mathworks, Natick, MA, USA). Signal enhancement in MRI was quantified as the percentage change of signal between the contrast-injected eye and the contralateral control. The retina was delineated by manually-defined regions of interest. A-waves and b-waves were calculated from ERG traces using a custom MATLAB script. Statistical analyses were performed using one-way ANOVA with a \textit{P} value of less than or equal to 0.05 considered significant.

**Results**

**Structural Evaluation of the Retina**

Representative OCT images of the retina of \textit{Abca4}\textsuperscript{−/−} \textit{Rdb8}\textsuperscript{−/−} double knockout mice in NIL, retinylamine-treated (Ret-NH\textsubscript{2}-LI) and/or LI groups are shown in Figure 2A. The treatment with Ret-NH\textsubscript{2} protected the retina from light-induced degeneration, while light illumination destroyed the retinas of the mice without the treatment. Mice in the Ret-NH\textsubscript{2}-LI group retained an intact retina structure as did those without illumination. Histologic analyses of the retina were in good agreement with the OCT results. An intact retinal structure with a thick ONL was present in NIL and retinylamine-treated animals (Fig. 2B),
FIGURE 1. Use of MEMRI to assess retinal integrity and function. Mn\(^{2+}\) was injected directly into the vitreous humor of the right eye. (A) Actively signaling photoreceptor cells take up and retain Mn\(^{2+}\) through voltage-gated calcium channels. This phenomenon is detected as a bright signal in the retina on T\(_1\)-weighted MRI. (B) Treatment and experimental schedule for drug-treated, illuminated Abca4\(^{-/-}\) Rdh8\(^{-/-}\) double knockout mice (Ret-NH\(_2\)-LI), LI mice, and NLI mice. Retinylamine-treated animals were protected from rapid retinal degeneration due to intense light exposure, whereas untreated LI animals underwent this degeneration. Nonilluminated animals did not undergo retinal degeneration and served as healthy controls.

FIGURE 2. Structural evaluations of retinal degeneration. (A) Representative OCT images of the retinas of the mice with no treatment and NLI, with retinylamine treatment and light illumination (Ret-NH\(_2\)-LI), and with light illumination and no treatment (LI). (B) Hematoxylin and eosin staining of the eye cups. (C) Outer nuclear layer thickness quantified by OCT in the nasal-temporal (top) and superior-inferior (bottom) directions as a function of distance from the optic nerve head in the NLI (black squares), Ret-NH\(_2\)-LI (red circles), and LI (blue triangles) mice. Error bars represent ± SD. Scale bars: 50 μm.
MEMRI to Assess Retinal Degeneration

**ERG Measurements of Retinal Function**

Electroretinograms revealed retinal function in response to Ret-NH$_2$ treatment with representative traces shown in Figure 3A. In LI untreated mice only a minimal response was detected. Electroretinogram responses in the treatment group and NLI mice exhibited a light response under all scotopic and most photopic conditions, with higher b-waves in NLI controls than in untreated LI mice without Ret-NH$_2$ treatment. The signal in the retina remained unchanged with no Mn$^{2+}$ enhancement in LI animals but limited responsiveness in untreated, LI mice. The higher signal in the vitreous of the retinylamine-treated animals suggests a slower transport of Mn$^{2+}$ out of the eyes relative to the other experimental groups.

**DISCUSSION**

Here we have demonstrated the use of MEMRI to assess the efficacy of prophylactic treatment with retinylamine by measuring Ca$^{2+}$ channel activity in the retina. Following intravitreous injection of Mn$^{2+}$, healthy retinas appeared bright in T$_1$-weighted MRIs, while damaged retinas appeared dark. The health of the retina for each group was confirmed with OCT, histology, and ERG. MEMRI results reported here accurately stratified the mice into groups with no Mn$^{2+}$ enhancement in the NLI controls, whereas in LI mice without Ret-NH$_2$ treatment, signal in the retina remained unchanged. Manganese-enhanced MRI enhancement in the retina was quantified by the percent change of signal in the retina of the injected eye relative to that of the contralateral control. Contrast enhancement due to the Mn$^{2+}$ injection was statistically significant for NLI and retinylamine-treated mice compared with the LI mice. Clearance of Mn$^{2+}$ from the injection site was assessed by MRI signal enhancement in the vitreous humor and was quantified according to the same procedure used for the retina. The vitreous signal was highest after retinylamine treatment, lower in NLI mice, and negligible in untreated LI controls. The higher signal in the vitreous of the retinylamine-treated animals suggests a slower transport of Mn$^{2+}$ out of the eyes relative to the other experimental groups.
MEMRI to evaluate the efficacy of retinylamine for treatment of the latter.

The Abca4<sup>−/−</sup> Rdh8<sup>−/−</sup> mouse shows a phenotype similar to that of human Stargardt disease and AMD. Moreover, mutations of ABCA4 are associated with both diseases. Accelerated retinal degeneration following exposure to intense light permits an examination of retinal pathology and therapeutic effects within a short period of time. In previous work, it was demonstrated that the primary cause of retinal degeneration in Abca4<sup>−/−</sup> Rdh8<sup>−/−</sup> mice is a delay in clearance of all-trans-retinal after light perception, such that buildup of atRAL resulted in photoreceptor cell death characteristic of retinal degenerative diseases in humans. To prevent the toxic effects of atRAL, a visual cycle modulator, retinylamine, was shown to decrease the production of atRAL, allowing its clearance and preventing photoreceptor damage in both wild-type and Abca4<sup>−/−</sup> Rdh8<sup>−/−</sup> mice.

Based on Ca<sup>2+</sup> signaling in retinal photoreceptor cells, MEMRI was used in this work to study the effects of retinylamine in protecting against retinal degeneration in Abca4<sup>−/−</sup> Rdh8<sup>−/−</sup> double knockout mice. In NLI and retinylamine-treated mice, the bright MEMRI signal reflected normal ion channel activity in the structurally and functionally intact photoreceptor cells. Using custom-built surface coils, investigators were able to monitor the function of the visual cycle. Based on Ca<sup>2+</sup> channel activity, this technique can be used in Abca4<sup>−/−</sup> Rdh8<sup>−/−</sup> double knockout mice to assess retinal degeneration and the therapeutic effects within a short period of time.

In future work, we will expand our imaging protocol to study other components of the visual system in addition to the retina. Using a volume coil with a large field of view, we can modify our MRI sequence to perform 3D volume imaging of the whole brain, including the optic nerve tract. This extension will allow the use of imaging to explore the full effects and mechanisms of drugs on the visual system in the whole brain. The same methodology will also be applied to evaluate other therapeutics for retinopathies and animal models of retinal dysfunction.

**CONCLUSIONS**

Manganese-enhanced MRI is an effective imaging modality for evaluating the function of the visual cycle. Based on Ca<sup>2+</sup> channel activity, this technique can be used in Abca4<sup>−/−</sup> Rdh8<sup>−/−</sup> double knockout mice to assess retinal degeneration and the therapeutic effects within a short period of time.
prophylactic therapeutic efficacy of retinylamine. Manganese-enhanced MRI complements the established methods currently employed for this purpose and could be expanded to explore and understand the effects and mechanisms of new therapeutics under drug development.

Acknowledgments

The authors thank Ed Wu, PhD, of the University of Hong Kong for his valuable suggestions. Supported by grants from the Interdisciplinary Biomedical Imaging Training Program, National Institutes of Health (Bethesda, MD, USA) T32EB007509 administered by the Department of Biomedical Engineering, Case Western Reserve University. This work was supported in part by funding from the National Eye Institute of the National Institutes of Health Grant R24EY0211260. Disclosure: R. M. Schur, None; L. Sheng, None; B. Sahu, None; G. Yu, None; S. Gao, None; X. Yu, None; A. Maeda, None; K. Palczewski, None; Z.-R. Lu, None.

References


