LECTURE

Ophthalmic NMR

Hong-Ming Cheng

*Howe Laboratory of Ophthalmology, Harvard Medical School and
the Massachusetts Eye and Ear Infirmary
Boston, MA and*

*Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology
Cambridge, MA, USA*

(Received for publication on June 29, 1988)

Abstract

There are two modes of NMR (nuclear magnetic resonance), *i.e.*, MRI (magnetic resonance imaging) and MRS (magnetic resonance spectroscopy). MRI is now a common diagnostic modality, which in many ways proves superior to X-ray CT. MRS can provide metabolic information of tissue, which is not obtainable with other diagnostic imaging means; its clinical application is in the offing. We have applied several NMR techniques to the examination of ocular tissues such as the lens, cornea, vitreous, and retina. These techniques include MRI of water protons (including NMR microscopy) and MRS of various nuclei such as phosphorus-31, carbon-13, deuterium, and proton. The results are summarized in this review.

Key words: nuclear magnetic resonance, microscopy, spectroscopy, ocular tissues, metabolism

Introduction

The phenomenon of nuclear magnetic resonance (NMR) was discovered some 40 years ago. The technique of studying magnetic resonance, NMR spectroscopy (or MRS, magnetic resonance spectroscopy), has become as major tool in chemical anal-

Reprint requests to: Dr. Hong-Ming Cheng, Howe Laboratory of Ophthalmology, Harvard Medical School, 243 Charles Street, Boston, MA 02114, USA

ysis. In recent years, with the advent of superconducting magnets and improved signal acquisition and computerized data analysis, its application has expanded to including biomedical research.¹ The development of NMR imaging or MRI (magnetic resonance imaging) is more recent, conceptualized by Paul Lauterbur in 1973.² We adopted both MRS and MRI for the study of ocular tissues in 1983. The following is a brief account of the ophthalmic NMR research conducted by us since that time.

Magnetic resonance imaging (MRI)

MRI is superior to x-ray CT for the diagnosis of soft-tissue diseases. At present, only water and lipid proton imaging are applied clinically. Imaging of other magnetic nuclei such as phosphorus-31 and fluorine-19 is still at the experimental stage.

For ocular proton imaging, it is essential that a surface coil be used to improve the filling factor. Because of the wide range of water content in various ocular tissues, it is relatively simple to obtain high-contrast images of the eye. The image resolution at a magnetic field of 1.5–2 Tesla is currently around 0.2 mm with a slice thickness of 2 mm. We have used MRI to study the following:

(1) Vitreous liquefaction:

Rabbit and cow eyes were used in our studies. Although the vitreous is composed of 1–2% protein (mostly collagen and hyaluronic acid), and the rest water, we have found substantial changes in T1 (longitudinal relaxation time) and T2 (transverse relaxation time) of water proton in vitreous digested with collagenase or hyaluronidase. The changes can be detected with MRI.^{3–5} More recently, we have found regions of liquefied vitreous in a human diabetic eye.⁶ The molecular mechanism leading to the alteration of water-proton relaxation is unclear at present time. These studies nevertheless demonstrate the capability of MRI in detecting vitreal abnormalities that are undetectable with either optical means or ultrasonography.

(2) Lens imaging:

T1 and T2 lengthened in rabbit lenses incubated in high glucose or galactose (to activate the polyol pathway). The increase correlates positively with the increase in lens hydration and the changes again can be documented with MRI.⁷ This correlation was also found in human diabetic lenses. Since the lenses in these studies appeared non-cataractous, changes in T1 and T2 can be taken as an early indication of cataractogenesis. The lengthening of T1/T2 can be attributed to a loss of the water-binding sites on lens crystallins during cataract formation. MRI can therefore be used to detect early (pre-cataractous) changes in the diabetic lens.

(3) Pathological examination of enucleated eyes:

Another utility of MRI is to examine pathologies in the enucleated eyes. We have successfully differentiated cataracts, intraocular hemorrhages, melanomas, retinal detachment, and vitreal opacification.⁸ These findings were later confirmed with the conventional pathological examinations. The advantage of MRI is its non-invasive nature, no tissue fixation or preparation is required and the eyes can later be used for other studies.

(4) Microscopy:

We have recently implemented MR microscopy for the examination of ocular tissues.⁶ MR microscopy is an extension of MRI: By increasing the magnetic field strength, it is possible to increase the energy level of the magnetic nuclei so that the signals can be detected from micro-volumes of tissues. We have been able to obtain images with a resolution of 20 μ M. The ultimate goal for MR microscopy is to perform non-surgical biopsy and pathological examination in situ. This research is being actively pursued by a number of laboratories in the United States and abroad.

Magnetic resonance spectroscopy (MRS)

While MRI provides important structural, and to a lesser extent chemical-shift information, MRS can provide metabolic information on either a global or a localized basis. The most frequently observed nuclei include phosphorus-31, proton, deuterium, carbon-13, fluorine-19, and sodium-23. Ocular tissues such as the lens and the cornea are ideal for MRS studies: Because of their size (usually <10 mm) and avascularity, they can be incubated in the NMR tube for prolonged periods of time and still maintain viability. Optimal signal-to-noise is routinely obtained. We have examined ocular tissue metabolism using a variety of techniques; some examples are given below:

(1) Phosphorus-31:

Although P-31 nucleus is 100% naturally abundant, its NMR sensitivity is only 6.6% of that of proton. To improve the signal-to-noise, one can use high-field magnets, increase the number of signal acquisition, or pool tissues. P-31 MRS has yielded significant information in a number of studies:

1) "Sugar" cataracts: In lenses from diabetic rats, we have observed several-fold increase of α -glycerophosphate (GP), total loss of glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE), decrease of phosphorylcholine (PCh) and the appearance of two unknown resonances (at 5.8 and 6.6 ppm, respectively).⁹ Since these changes can be prevented by dosing the rats with aldose reductase inhibitors (ARIs), they are therefore resulted from polyol pathway activation. In lenses incubated

in high glucose (e.g. 35.5 mM), increase of GP and decrease of ATP were observed; again, these can be prevented by inclusion of an ARI in the incubating media.¹⁰ Similar, but not identical changes were noted in lenses from galactose-fed rats/rabbits or lenses incubated in high galactose. Thus, differences between models do exist. These data also demonstrate the multi-faceted nature of "sugar" cataractogenesis.

2) Vitamin A-deficient corneas: To elucidate the underlying mechanisms in keratopathy caused by vitamin A deficiency, we examined the P-31 profiles of corneas isolated from A-rats. The results showed that in the A-corneas, PCr, which was absent in the normal rat corneas, appeared. This was accompanied by an increase in inorganic phosphate and AMP. The data suggest a depressed creatine kinase activity.¹¹ The P-31 profiles returned to normal following oral vitamin A treatment.

(2) Carbon-13:

Since the natural abundance of C-13 is only 1.1%, C-13 labeled substrates can therefore be used for tracer studies. An example is the determination of the polyol pathway kinetics in the lens: We first incubated the lens in high-glucose (using C-13 glucose, labeled at the C-1 position) to allow accumulation of sorbitol, fructose, GP and lactate (the "pulse" stage), this is followed by changing the medium to that with unlabeled glucose (the "chase" stage). The rates of disappearance of C-13 labels can be used to calculate the kinetic constants.¹²⁻¹⁴ We found that up to 1/3 of total glucose was consumed by the polyol pathway when the lenses were incubated in 35.5 mM glucose. Further, the turnover of NADPH $\rightarrow$ NADP due to aldose reductase (AR) activation was 3,000%/hr. The high turnover of NADP(H) suggests that AR may compete for NADPH with glutathione reductase and other NADPH-requiring enzymes. Indeed, the lens's hydrogen peroxide detoxification is significantly impaired when incubated in high glucose.¹⁴

(3) Deuterium:

Like C-13 MRS, labeled (deuterated) substrates are used. There are several advantages of deuterium MRS: (1) the naturally abundant deuterium-enriched water (HDO) can serve as the internal standard; (2) the extremely short T1 of deuterium metabolites (<500 msec) allows rapid signal acquisition; (3) no water suppression is required; and (4) the identification of resonances is simplified because of the similarity (<0.5 ppm difference) between deuterium and the well-established proton resonances. As an example, we have shown that sorbitol resonance can be detected in rabbit lenses incubated in 5.5 or 35.5 mM deuterated glucose and the kinetics of sorbitol turnover in both cases easily followed.¹⁵

A more important utility of deuterium MRS is the measurement of Krebs cycle activity. By using deuterated glucose labeled at C-6 position as the substrate, Krebs

cycle activity can be quantitated by measuring the increase in HDO intensity, which is enriched by the release of deuterons from glucose. With this method, we have determined that Krebs cycle consumes up to 36% of total glucose in the cornea.¹⁶

(4) Proton:

Proton has the highest NMR sensitivity. However, proton spectra are dominated by the water signal (55.6 M in concentration) which obscures other proton signals. This problem can be resolved by suppressing the water signal using special pulse sequences. Proton spectroscopy can be used for the measurement of percent glucose consumed through the hexose monophosphate shunt (HMPS). This technique takes advantage of the spin-spin coupling between carbon and proton. Thus the resonances of the methyl group of C-13 lactate can be separated from that of the C-12 lactate. When the tissue is incubated in C-13 glucose labeled at the C-1 position, lactate produced from glucose that has entered the HMPS will contain no C-13, whereas that produced from glucose through the Embden-Myerhof pathway will have retained the C-13 label. The percent glucose consumed through the HMPS can be calculated according to:

percent glucose through the HMPS = $[(3 - 3r)/(3 + 2r)] \times 100$,

where $r = (\text{C-13 methyl group intensity})/(\text{C-12 methyl group intensity})$.

We have examined the HMPS in the retina,¹⁷ the cornea,¹⁸ and cultured lens epithelial cells.¹⁹ The results showed that the HMPS was activated in light-adapted retinas and in lens cells treated with t-butyl hydroperoxide (tBHP). In contrast, the cornea did not show HMPS activation by tBHP. This suggests that the HMPS is functioning at full capacity (ca. 20% of total glucose consumption). Corneal HMPS activity decreased to 10% of total glucose consumption when KCN (which inhibits oxidative phosphorylation) was added to the incubating media, suggesting a competition for glucose-6-phosphate between the HMPS and glycolysis.

Concluding remarks

The data cited above represent some of the studies conducted by us in the past 5 years. The applicability of NMR to basic and clinical ophthalmology is evident.

It should be noted that ophthalmic NMR is different from NMR of other organs/tissues: Because of the small tissue mass of the eye/ocular tissues, highly sensitive high-resolution technique must be used. To this end, we have initiated MR microscopy and more recently, spatially localized MRS using special methods such as water-suppressed proton spectroscopy, soft COSY and multiple quantum techniques. We foresee the implementation of these techniques for clinical application in the near future.

Acknowledgements: The author wishes to thank Drs. Kazuo Tsubota, Ken Hayashi, R. Gilberto Gonzalez, Stefano Miglior, James B. Aguayo, Hua Xiong and Jing Xiong for their contribution to the establishment of Ophthalmic NMR. The studies were supported by research grants EY04424 and EY07620 awarded by the National Eye Institute, National Institutes of Health, Bethesda, MD, USA.

References

1. Gadian, D. G.: Nuclear magnetic resonance and its applications to living systems, Clarendon Press, New York, 1982
2. Lauterbur, P. C.: Image formation by induced local interactions: Examples employing nuclear magnetic resonance. *Nature* 242: 190-191, 1973
3. Gonzalez, R. G., Cheng, H.-M., Barnett, P. A., Aguayo, J. B., Glaser, B., Rosen, B., Burt, T. and Brady, T.: Proton magnetic resonance imaging of the vitreous body. *Science* 223: 399-400, 1984
4. Aguayo, J., Glaser, B., Mildvan, A., Cheng, H.-M., Gonzalez, R. G. and Brady, T.: Study of vitreous liquefaction by NMR spectroscopy and imaging. *Invest. Ophthalmol. Vis. Sci.* 26: 692-697, 1985
5. Miglior, S., Kain, H. L., Libondi, T., Gonzalez, R. G., Barnett, P., Krauss, J. M. and Cheng, H.-M.: Early vitreous changes in experimental proliferative vitreoretinopathy. *Arch. Ophthalmol.* 104: 1618-1624, 1985
6. Aguayo, J. B. and Cheng, H.-M.: Magnetic resonance microscopy of ocular tissues. *Med. Sci. Res.* 15: 1059-1060, 1988
7. Cheng, H.-M., Yeh, L. I., Barnett, P. A., Miglior, S., Eagen, J. C., González, G. and Brady, T. J.: Proton magnetic resonance imaging of ocular lens. *Exp. Eye Res.* 45: 875-882, 1987
8. TerPenning, B. J., Cheng, H.-M., Barnett, P. A., Seddon, J., Sang, D., Latina, M., Aguayo, J., Gonzalez, R. G. and Brady, T.: Imaging enucleated human eyes at 1.4 tesla. *J. Comput. Assist. Tomogr.* 10: 551-559, 1986
9. Gonzalez, R. G., Miglior, S., von Saltza, I., Buckley, L., Neuringer, L. J. and Cheng, H.-M.: ³¹P NMR studies of the diabetic lens. *Magn. Reson. Med.* 6: 435-444, 1988
10. Gonzalez, R. G., Barnett, P. A., Cheng, H.-M. and Chylack, L. T., Jr.: Altered phosphate metabolism in the intact rabbit lens under high glucose conditions and its prevention by aldose reductase inhibitor. *Exp. Eye Res.* 39: 553-562, 1984
11. Hayashi, K., Cheng, H.-M., Wolf, G. and Kenyon, K.: Metabolic changes in the corneas of vitamin A deficient rat. (in preparation)
12. Gonzalez, R. G., Barnett, P. A., Aguayo, J. B., Cheng, H.-M. and Chylack, L. T., Jr.: Direct measurement of polyol pathway activity in the ocular lens. *Diabetes* 33: 196-199, 1984
13. Barnett, P. A., Gonzalez, R. G., Chylack, L. T., Jr. and Cheng, H.-M.: The effect of oxidation on sorbitol pathway kinetics. *Diabetes* 35: 426-432, 1986
14. Cheng, H.-M. and Gonzalez, R. G.: The effect of high glucose and oxidative stress on lens metabolism, aldose reductase and senile cataractogenesis. *Metabolism* 35 (Suppl. 1): 10-14, 1986
15. Aguayo, J. B., McLennan, I. J., Aguiar, E. and Cheng, H.-M.: The study of diabetic cataractogenesis in the intact rabbit lens by deuterium NMR spectroscopy. *Biochem. Biophys. Res. Commun.* 142: 359-366, 1987
16. Aguayo, J. B., McLennan, I. J., Graham, C. and Cheng, H.-M.: Dynamic monitoring of corneal carbohydrate metabolism using high-resolution deuterium NMR spectroscopy. *Exp. Eye Res.* (in press)
17. Aguiar, E., Cheng, H.-M. and Lam, D. M.-K.: Real-time hexose monophosphate shunt activity in light- and dark-adapted rabbit retinas. *Ophthalmic Res.* 19: 298-302, 1987

18. Serdahl, C. L., Willis, J. A., Cheng, H.-M., Aguayo, J. B. and Kenyon, K. R.: NMR studies of the hexose monophosphate shunt in the rabbit cornea. *Med. Sci. Res.* 15: 1169-1170, 1987
19. Cheng, H.-M., Aguiar, E., Ford, J. J., Kelleher, P. and Lam, D. M.-K.: Proton NMR spectroscopy of glucose consumption by cultured lens epithelial cells. *J. Ocular Pharmacol.* 2: 319-324, 1986