Assessing Lysosomal Pathology using Magnetic Resonance Imaging

Y. Mei[1], and R. G. Pautler[2]
[1]Department of Psychology, Rice University, Houston, Texas, United States, [2]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas, United States

Introduction:

There are many neurodegenerative diseases that cause abnormalities in the lysosome including Alzheimer's, Tay-Sachs, and Sandhoff disease. In these diseases, cellular irregularities such as oxidative stress and excessive macromolecule accumulation disrupt the membrane integrity of the lysosome, causing the organelle to lose its acidity. We hypothesize that magnetic resonance imaging (MRI) can be used to detect lysosome membrane permeabilization and loss of acidity in mouse models with lysosomal pathology. Shapiro and Koretsky showed that Mn_3O is a convertible positive T_1 contrast agent that is insoluble normally but dissolves in acid Mn_3O_4. Once in acid, it degrades into Mn^{2+} that significantly shortens T_1 recovery[1]. By incubating lysosomes with Mn_3O_4, we propose that we can visually assess the acidity and intactness of the lysosomes *in vitro* and thereby detect lysosome pathology. If successful, the results would indicate that MRI could potentially be applied to assess lysosome abnormality *in vivo*, a possible method of improving the diagnosis of many neurological disorders such as Alzheimer's disease.

Methods:

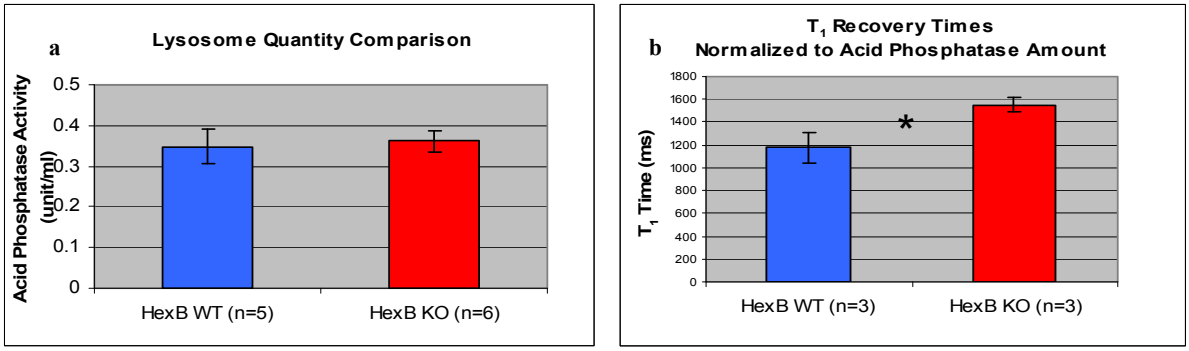
Lysosome Isolation: The animals used are the Sandhoff disease mouse model HexB-/-. HexB wildtype and HexB-/-, aged 3-5 months, were anesthesized with isofluorane and sacrificed. Brains were immediately extracted and washed with 1XPBS. After being homogenized in 1X Extraction Buffer (Sigma Inc.) with 1% protease inhibitor (1:4, w/v) using a Dounce homogenizer, the tissue was centrifuged at 1,000 x g. More 1X Extraction Buffer with 1% protease inhibitor (1:2, w/v) was added to the homogenate before the tissue was sonicated with 3 pulses. The supernatant from both centrifugations were further centrifuged at 20,000 x g. 1X Extraction Buffer with 1% protease inhibitor was added (1:0.8, w/v) to the pellet to yield the lysosome fraction. All procedures were done at 4 ºC.

Acid Phosphatase Test: The acid phosphatase test was used to determine the amount of lysosomes in each isolated fraction. P- nitrophenyl phosphate (Sigma Inc.) tablets were dissolved in citrate buffer, 0.09 M pH 4.8 (1:5, w/v), forming the substrate solution. The positive control of the acid phosphatase enzyme was made by dissolving acid phosphatase lyophilized powder (Sigma Inc.) in chilled water (1:4 w/v). The substrate solution was equilibrated at 37 ºC for 10 minutes before 30 ul of lysosome fractions and 10 uL of the positive control were added. The samples were then incubated at 37 ºC for 10 minutes while the positive control was incubated for 15 minutes. 2 ml of 0.5 NaOH was added to each sample. All samples were read at 405 nm on the spectrophotometer. The activity of the acid phosphatase was calculated using the procedure detailed by Sigma Inc.

Mn_3O_4 Incubation and T_1 Weighted Imaging: Mn_3O_4 (Sigma Inc.) was crushed using mortar and pestle for 30 minutes and suspended in 1X PBS. All lysosome fractions were incubated with 2.31 mg of Mn_3O_4 at 4 ºC overnight. Before imaging, the lysosome fractions were all centrifuged at 20,000 x g for 5 minutes at 4 ºC to remove excess Mn_3O_4. All pellets were then suspended in 300 ml of 1X Extraction Buffer with 1% protease inhibitor. All imaging protocols were completed using a 9.4 T, Bruker Avance Biospec Spectrometer, 21 cm bore horizontal imaging system (Bruker Biospin, Billerica, MA) with a 35 mm volume resonator. Data was collected using Paravision 4 (Bruker Biospin, Billerica, MA) on a Red Hat Linux computer. All phantoms were imaged utilizing a T_1 weighted protocol, using a spin echo sequence with a dynamic T_1 parameter: TR=326.38, 928.309, 1949.379, 7500 ms. Other parameters include TE=6.6 ms, FOV= 4.0 cm, slice thickness= 1 mm, averages=1, matrix=128 X 128.

Results:

Figure 1



There was no significant difference in the amount of lysosomes isolated from the HexB wildtype and Hex-/-mice (0.35 unit/ml ±0.04 unit/ml vs. 0.36 unit/ml ± 0.02 unit/ml, p=0.78) shown in Fig 1a. This indicates that the numbers of lysosomes in the two mouse groups are approximately the same. However, the results from the T_1-weighted scan showed that there was a significant difference in the properties of the lysosomes between the HexB wildtype and the HexB-/-. After normalizing the T_1 recovery times to the amount of acid phosphatase in each sample, there was a significant difference in the T_1 recovery between the HexB wildtype and HexB-/- (1174.96 ms ± 134.4 ms vs. 1547.2 ms ± 63.0 ms, p=0.03) shown in Fig 1b.

Conclusion: The approximately equal amount of lysosomes in the isolated fractions indicates that there is no significant difference in the number of lysosomes that can be isolated from both HexB wildtype and HexB -/- mice. However, a significant shortening of the T_1 weighted recovery in the HexB wildtype compared to the HexB -/- indicates that the lysosomes are much more acidic and intact in the wildtype mice. Because the lysosomes in the HexB -/- are permeabilized by the onset of Sandhoff disease, their loss of internal acidity rendered them incapable of acidifying Mn_3O_4 into Mn^{2+} ions that shorten the T_1 recovery times. The significant difference in the T_1 recovery suggests that MRI can be used to assess lysosomal pathology in mouse models. If future studies are successful, then this methodology can potentially be applied *in vivo* and used as a tool to improve current diagnostic methods for neurological disorders such as Alzheimer's disease.

Reference: 1. Shapiro, E.M and Koretsky, Alan. Magnetic Resonance in Medicine. 2008