Quantification of Fluid Accumulation in IP Space of Mice using Whole-Body NMR

Lina Avancini Colucci[1], Matthew Li[1], and Michael J. Cima[2]

[1]*Health Sciences and Technology (HST), MIT, Cambridge, Massachusetts, United States,* [2]*Materials Science and Engineering, MIT, Cambridge, Massachusetts, United States*

Introduction

The most common causes of ascites – the pathological accumulation of fluid in the intraperitoneal (IP) space – are cirrhosis, malignancies, and cardiac failure. Ascites is associated with significant morbidity and an increased mortality of 50% within 2 years[1]. Ascites is therefore an important therapeutic target in a variety of disease states. Existing methods for assessing ascites in mice involve measuring aspirated fluid volume, abdominal circumference, or body weight which are terminal, insensitive, and unspecific, respectively. The ability to accurately assess ascites in pre-clinical settings may allow for earlier compound differentiation in the therapy discovery process and a higher resolution understanding of ascites formation. This study investigated the use of whole-body NMR as a quantitative, non-invasive technique of measuring fluid accumulation in the IP space of mice.

Materials and Methods

Female Swiss-Webster mice (6-8 weeks old, $weight_{avg} = 23.9 \pm 0.8g$) were randomized into 3 groups (no saline, 2%, and 4% of body weight [BW] IP saline injection) each having n=5. Whole-body T2 relaxation time measurements were taken in a Bruker Minispec mq7.5 (0.17T) analyzer with a CPMG pulse sequence. Each measurement took about 30 seconds. A baseline T2 measurement was taken, mice were weighed, given an IP saline injection based on a percentage of their BW, immediately measured in the Minispec again and weighed. Mice that displayed the expected weight gain based on the injected fluid volume were analyzed with an inverse Laplace transform (ILT) (no injection n=5; 2% BW n=4; 4% BW n=3). Mice often urinated during T2 measurement, which offset the weight of the saline injection and meant net free fluids in the body did not change. The "no injection" mice received a sham needle stick in the IP space with no saline injection. An ILT with the CONTIN algorithm was performed on the magnetization decay data to generate a continuous T2 distribution relaxogram and extract information about the number of water compartments present in the mouse.

Results and Discussion

We found that the injection of saline into the IP space of mice resulted in an amplitude increase in the long peak (~1500ms). There was a statistically significant increase ($p<0.01$) in the amplitude of the peak of mice with both 2% and 4% BW injections compared to the mice that received a sham injection. The increase of the area under the peak was proportional to the volume of injected fluid (Fig 1).

Multi-compartmental relaxation times, as reflected by multiple peaks in a relaxogram, reflect different water environments within the mouse. The area under each peak represents the number of spins with a certain T2 value. From our own post-mortem tissue studies as well as literature findings, the 50-800ms peaks arise from intracellular and extracellular water within skeletal muscle and organs[2]. Peaks under 10ms represent hydrogen bound to macromolecules. The small peak ~1500ms represents free fluids in the body (urine, blood, etc.). Ascitic fluid, like these other free fluids, has a long relaxation time compared to other body tissues though its exact value varies based on protein content[3].

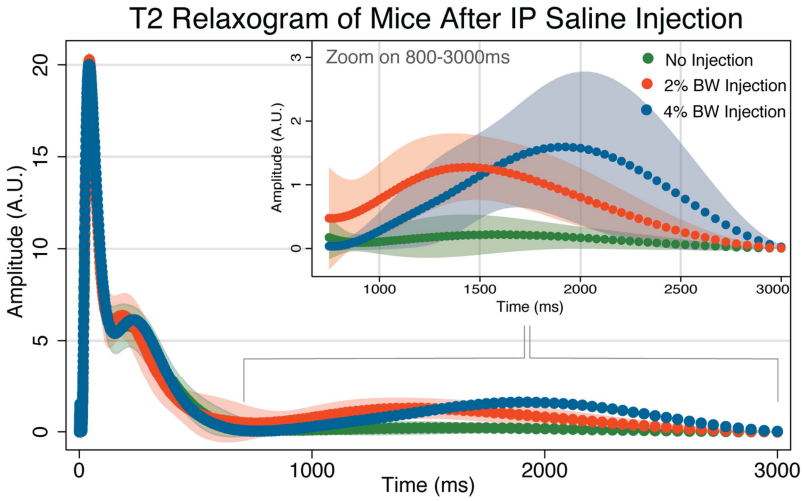

Figure 1. The whole-body T2 relaxation decay of mice was measured with a CPMG sequence. An ILT was applied to the magnetization decay data to produce a T2 relaxogram. The area under the peak ~1500ms increases proportionally to the volume of saline injected into the IP space of mice. Amplitude increase of both 2% and 4% BW injection curves are statistically significant ($p<0.01$) compared to the no injection curve. Shaded area is the standard deviation.

Ascites is not detectable via circumferential abdominal measurements in mice until several milliliters of fluid have accumulated. Ascites often occurs in a tumor mouse model meaning that the circumference increase is also due to tumor growth. Our method shows sensitivity at small fluid volume levels (2% BW injection represents less than 500uL) and provides researchers with a tool of greater resolution to understand the progression of ascites. NMR as a tool for measuring ascites is quantitative, fast, and requires no anesthesia or ionizing radiation. Future steps include exploring this method for other fluid accumulation presentations such as hypervolemia, pleural effusions, and peripheral edema. This technique could be combined with skeletal muscle analysis (the peaks between 50-800ms in Fig. 1 as shown by Saab, et al. and Tinsley, et al.[4,5]) to understand body composition changes along with ascites accumulation. Whole-body mouse relaxometry provides researchers with a more detailed understanding of fluid accumulation in the body than any of the existing methods.

References

1. Madan K, Mehta A (2011). *Int J Hepatol*, **2011**, 790232.
2. Bertram HC, et al (2001). *J Agric Food Chem*, **49**, 3092–100.
3. Brown JJ, et al (1985). *Radiology*, **154**, 727–31.
4. Saab G, Thompson RT, Marsh GD (1999). *Magn Reson Med*, **42**, 150–7.
5. Tinsley FC, Taicher GZ, Heiman ML (2004). *Obes Res*, **12**, 150–60.