In vivo MRI assessment of hepato-splenic disease in a murine model of schistosmiasis

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Target audience: basic scientists, physicians, pharmaceutical companies

Purpose: Schistosomiasis or bilharzia, a chronic tropical helminthic disease affecting more than 240 million people,[1] is still far from eradication. The hepatosplenic form of the disease caused by infection with trematodes of the *Schistosom*a genus (ie *Schistosoma mansoni*) is associated with severe morbidity due to schistosoma migration into the liver and development of progressive liver fibrosis. Hepatic fibrosis may eventually lead to esophageal varices and portal hypertension that can be lethal. Disease burden is often difficult to assess, therefore non-invasive markers for the diagnosis and the follow-up of schistosiomasis-related morbidity are needed. In this study, we characterized the hepatosplenic disease in a mouse model of schistosomiasis by *in vivo* MRI and investigated the role of the transverse relaxation time (T_2) to assess liver fibrosis.

Methods: Seven-week old female CBA/J mice (n=12) were infested percutaneously with 30 cercariae of the venezuelan strain of *S. mansoni* maintained in their intermediate host (the snail) whereas 12 non-infested mice were used as controls. Mice were imaged at 11.75T (Bruker AVANCE 500 WB, Germany) under isoflurane anesthesia. A first group of animals (controls n=6, infected mice n=6) was examined 2 and 6 weeks after infection with *S. mansoni,* while a second group (controls n=6, infected mice n=6) was imaged 10 weeks after infection. Transverse MR images (FOV 24 × 24 mm^2, slice thickness 0.5 mm) were acquired using prospective respiratory gating (PC-SAM, Small Animal Instruments Inc., NY). T_2 maps (TR ≥ 9 s; 12 equally spaced echoes at TE = 7.5 to 120 ms; matrix 64 × 64, 2 accumulations, acquisition time 20 minutes) were acquired in a single slice 0.5 mm caudal of the portal vein bifurcation. Contrast enhanced anatomical imaging (2D spin-echo sequence, TR ≥ 448 ms; TE = 14 ms, matrix 240 × 240, 4 accumulations, acquisition time < 20 minutes) was started 15 minutes after ip injection of 50 µl of 0.5 M gadoteric acid (DOTAREM®, Guerbet, France). The number of contiguous slices varied from 40 to 60 (2 to 3 repeated acquisitions with different slice position) to entirely cover the liver and spleen in diseased animals. The cross-section of the portal vein 0.5 mm caudal of the level of bifurcation was measured to assess portal hypertension. After MRI examination at 6 (first group) and 10 weeks (second group of mice), livers were stained with hematoxylin eosin to assess egg load, granuloma formation, necrosis and portal inflammatory infiltrates, and sirius red to visualize collagen fibers and thus evaluate the percentage of fibrotic tissue. Mann-Whitney test was used to compare control and infested animals in volumetric studies (significance set to P<0.05). Spearman test was used for correlation between T_2 and the percentage of fibrosis obtained with sirius red staining.

Figure 1:

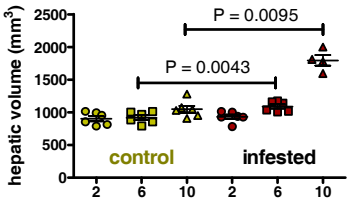


Figure 2:

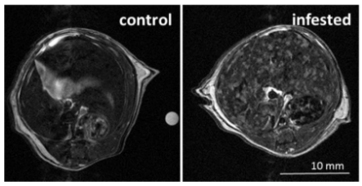


Figure 3:

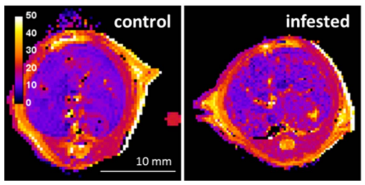


Figure 4:

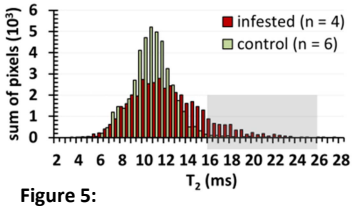
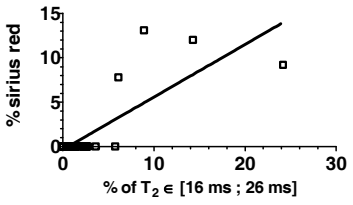


Figure 5:



Results: Organomegaly and alteration of the portal vein section were the first signs of the disease visible on MRI. Indeed, hepatomegaly (Figure 1), splenomegaly and portal hypertension as assessed by MRI were significant at 6 weeks (+19%, +52% and +60%, respectively) and 10 weeks post infestation (+72%, +170% and +139%, respectively). Multifocal contrast enhancement of the liver appeared later and was clearly visible on MRI in 4 mice at 10 weeks post infestation (Figure 2). These lesions were also characterized by a T_2 > 16 ms while liver parenchyma has T_2 values well below 14 ms (Figures 3 and 4). According to histology they correspond to granulomatous reactions to eggs, hepatocellular necrosis, inflammatory infiltration, and fibrosis. Histology confirmed the absence of lesions in the two remaining mice sacrificed at 10 weeks in which the infestation had failed (no parasite detected in the liver). Sirius red staining was negative at 6 weeks after infestation, whereas at 10 weeks collagen rich areas could be visualized in infected livers. The liver fraction with 16 ms < T_2 < 26 ms (Figure 4) correlated with area fraction of fibrosis stained with sirius red (Figure 5).

Discussion: The MRI results on this animal model are in accordance with the known features of the human disease (hepato-splenomegaly, portal hypertension and liver fibrosis).[1] Structural changes suggestive of liver fibrosis appeared at a late time-point of the follow-up (10 weeks after infestation) and were detectable by both MRI and histology. Lesions were also easily distinguishable on T_2 maps as spots with a T_2 increase of > 14%. Although *ex vivo* studies at 11.75T describe a T_2 decrease in a mouse model of hepatic fibrosis,[3] a T_2 increase of 17 - 22% with increasing fibrosis has previously been observed *in vivo* at 7T.[2] The T_2 increase might be explained by inflammatory activity accompanied by edema, but in our exploratory study, despite the small number of animals, the area fraction of increased T_2 in the liver was significantly correlated with the area fraction affected by fibrosis. This result suggests that T_2 mapping is a reliable non-invasive marker for disease monitoring.

Conclusion: This multimodal MRI approach can monitor hepatosplenic disease progression and assess liver fibrosis non-invasively even at early stages with a sensitivity similar to that of histology. These findings could be useful in preclinical studies to evaluate novel therapeutics for the treatment of schistosomiasis disease.

References:

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3. Anderson SW, Jara H, Ozonoff A, O'Brien M, Hamilton JA, Soto JA. Effect of disease progression on liver apparent diffusion coefficient and T_2 values in a murine model of hepatic fibrosis at 11.7 Tesla MRI. J Magn Reson Imaging. 2012 Jan;35(1):140-6.