An MRI study of rat model of Cryptococcal Meningo-Encephalitis

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Introduction

CNME is the most common fungal opportunistic infection associated with death in AIDS patients. Approximately one quarter of AIDS patients die within two weeks of CNME diagnosis in sub-Saharan Africa. Current antifungal management strategies are too complex to benefit resource limited countries that face the burden of the HIV pandemic. Animal models that mimic the pathology of this disease are vital to the design of novel treatment approaches. Existing animal models of CNME require intra-cisternal inoculation and do not mimic the pathology noted in AIDS patients. Given that *C. neoformans* can easily traverse the blood-brain barrier, we hypothesized that intravenous inoculation is a viable method of inducing CNME in the rat. One of the measures of success for an inoculation method could be determined by the ability to demonstrate intracerebral lesions due to *C. neoformans* infection. A second challenge was be to find a non-invasive modality that would allow us to follow the intracerebral lesions longitudinally over time and allow testing the efficacy of new drug therapies developed for the treatment of *C. neoformans* infection. MRI, a relatively non-invasive imaging modality provides excellent soft tissue contrast and meets all the requirements for this project. Thus, there were two aims of the study, first, to evaluate the success of intravenous inoculation method by visualizing using MRI, intracerebral lesions due cryptococcal infection in response to various doses of the inocula and second, to follow the course of lesions over a period of 14 days and compare the MRI results with the histopathological findings.

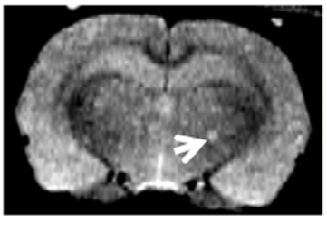
Materials and Methods

The study was approved by the Local Animal Research Committee and conformed to the NIH guidelines for the use of animals for research. Nine 250 -300 g male Fischer (F344) rats were divided into three groups and were inoculated via tail vein injection with 10^3, 10^5, and 10^7 colony forming units(CFU) of *C. neoformans*. The inoculum was generated by using a 48 hour culture of *C. neoformans var grubii* [Strain H99, ATCC 208821] to create a 1.0 Mcfarland standard in phosphate buffered saline. Animals were maintained in 12h dark- 12h light cycle vivarium with free access to food and water with weight and behavior recorded daily. On day 7 post infection, one animal was randomly selected from each group, transported to the MRI suite and imaged in a 4.7T dedicated research MRI scanner (Bruker Biospin®, Billerica, MA). During the imaging experiment, animals were anesthetized using isoflurane gas (induction dosage 2-3%; maintenance dose 1.5-2%). Real time monitoring of physiological parameters (heart rate and respiratory rate) was performed during the entire duration of the study. After the initial localizer images, multi-slice T2w (anatomical) and diffusion-weighted images (DWI) were acquired in these animals with the following parameters: T2w- TR/TE 4s/65ms, FOV 3.2cm x 3.2 cm, matrix 256x128, number of slices 20, slice thickness 2 mm, #averages 22; DWI- TR/TE 2s/50ms, # averages 15, d = 5ms, D = 20ms, b=0 and 927 s/mm^2. The DWI images were slice matched to the T2w images and slices were prescribed to obtain whole brain coverage. At the end of the MRI experiments, the raw MRI data was transferred to an offline workstation. Apparent Diffusion Coefficient (ADC) maps were reconstructed from DWI using commercial software MRVision 1.67 (MRVision, Winchester, MA). Region of Interest (ROI) were drawn on ADC maps in anatomical regions corresponding to the lesions identified on anatomical T2w images. Given data that revealed formation of brain lesions on day 7, the rat infected at 10^7 was reimaged by MRI on day 10 to assess disease progression. Animals that underwent MRI were sacrificed and the brains harvested for histology (Hematoxylin and Mucicarmine). The animals that were not imaged were sacrificed on day 15 and brain fungal burden enumerated using standard methods.

Results and Discussion

MR images acquired on day 7 post infection demonstrated multiple diffuse well circumscribed intracerebral lesions that appeared hyperintense on T2w images. An increase in ADC ($1.05 \pm 0.06 \times 10^{-3}$ mm^2 s^{-1}) was observed in the cryptococcal brain lesions on the ADC maps suggesting that the intracerebral lesions were cystic lesions. Intracerebral lesions were only visible on T2w images in animals that were inoculated with the highest CFU i.e. 10^7 colony forming units (CFU) of *C. neoformans*. There was very good correlation between the brain lesions seen on T2w images and histopathology staining method (see Figure) suggesting that MRI is a reliable method for longitudinal evaluation of intracerebral cryptococcal lesions in a rat model. Additionally, MRI results are in agreement with the clinical MRI findings related to intracerebral distribution of cryptococcal lesions from patients with CNME [2]. The brain fungal burden was below the limit of detection (10^2 CFU/g) in rats infected with 10^3 CFU, and was detectable in 1 rat infected with 10^5 CFU. In contrast, all rats infected with 10^7 CFU had fungal burdens that exceeded 10^4 CFU/g. Furthermore, necropsy data revealed that the lung and liver were grossly infected in rats infected with 10^7 CFU. No overt brain infection was noted in rats infected with 10^3 or 10^5 CFU, which is in agreement with MRI findings. In contrast, that rat infected with 10^7 CFU had multiple brain lesions that were filled with *C. neoformans* (mucicarmine stained). These preliminary data indicate that intravenous inoculation is a viable route for induction of disseminated CNME in the rat as confirmed using MRI and histopathology staining methods. The pathologic features of the disease closely resemble severe CNME in AIDS patients. Future work involves using perfusion MRI technique using Gd-DTPA to further characterize the intracerebral lesions and use MRI to test the sensitivity of novel pharmaceutical molecules for treatment of CNME.

References: [1] Segal BH and Steinbach WJ, Expert Rev Anti Infect Ther. 2007. 5:883-892. [2] Chang, KH. et al, AJR 1989. 154:809.



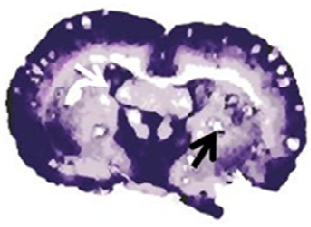


Figure shows a T2w image (left) and a slice matched tissue of the CNME infected rat brain (right). Intracerebral lesions appear as regions with high signal on the T2w images, are well circumscribed and diffusely distributed over the entire brain tissue. There was a good correlation between MRI results and findings from histopathological staining method (arrow shows a matching lesion). An increase in ADC was also observed in these lesions.