In Vivo Characterization of Rabbit Eosinophilic Meningitis Caused by Angiostrongylus Cantonensis

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Introduction

Angiostrongylus cantonensis (*A. cantonensis*) is a parasitic nematode which causes Angiostrongyliasis, the most common cause of eosinophilic meningitis in Southeast Asia and the Pacific Basin [1]. The roundworm commonly resides in the pulmonary arteries and hearts of rats, giving it the nickname the rat lungworm. Snails are the primary intermediate hosts, where larvae develop until they are infective. Humans are incidental hosts and may become infected after ingestion of the worms in raw snails from contaminated vegetables [2]. The typical clinical presentation is acute meningitis with an eosinophilic pleocytosis frequently accompanied by encephalopathy and other symptoms of central nervous system (CNS). Patients usually present an insidious or sudden onset of excruciating headache, neck stiffness, nausea, vomiting and paraesthesia.

The diagnosis of eosinophilic meningitis can be arrived at through detection of elevated cranial pressure and increased volumes of eosinophils. The diagnosis of the cause of eosinophilic meningitis and the presence of *A. cantonensis* is remarkably more difficult. A spinal tap, or a sample of cerebrospinal fluid (CSF), must be taken to search for *A. cantonensis* worms or larvae. *A. cantonensis* is virtually undetectable in the CSF of half of the infected individuals. Current methods of detecting specific antigens associated with *A. cantonensis* are also unreliable [3, 4]. Therefore, the purpose of this study was to determine the longitudinal effects of rabbit brain infected with 700 larvae of *A. cantonensis* by advanced MRI techniques, and the results were verified by histopathological study.

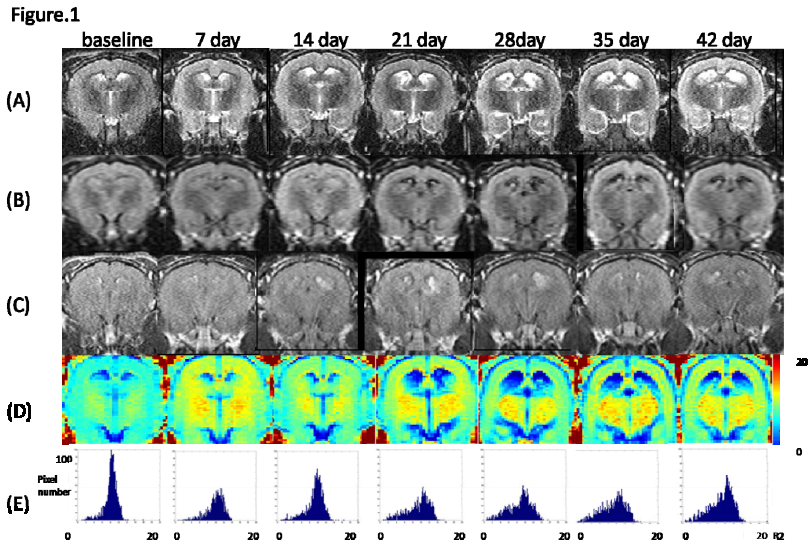
Materials and Methods

Each of four male New Zealand white rabbits was weight about 2.5 kg and was infected by the 700 3rd-stage larvae of *A. cantonensis* though the metal fedding tube. The 3rd-stage larvae of *A. cantonensis* were collected from Taichung, Taiwan. The rabbits were anesthetized with 3-4% isoflurane mixed with O_2, and maintained with 1-2% isoflurane during the MRI scanning. The warn water circulation system was used keeping rabbit body temperature about 37°C in the MRI room. MR scans were performed before and each week after infection of *A. cantonensis*. In order to determine the degree of inflammatory after infection, gadodiamide was given by intravenously injecting 151 mg/kg of a 0.5 M Gd-DTPA solution.

The experiment was performed on a 1.5T MRI system (Magnetom Sonata, Siemens Medical Systems, Erlangen, Germany). Double loop array coils were used for RF reception. Multi-slice turbo spin echo (TSE) sequence was performed to obtain whole brain T2W (TR/TE = 4330/114 ms, in-plane resolution = 0.39×0.19×1.5 mm^3 and slice thickness = 1.5 mm) and FLAIR images (TR/TE/TI=8630/155/2500ms, in-plane resolution = 0.78×0.39×2 mm^3 and slice thickness = 1.5 mm). To improve detection sensitivity over the full extent of T2 changes caused by the infection of *A. cantonensis*, image data for R2 mapping were acquired. To obtain R2 mapping, single-slice multi-echo spin echo sequence with half spatial resolution was performed to acquire 32 sets of images corresponding to 32 different TEs, ranging from 15 to 480 ms, to sample along the decay of transverse magnetization. In the end, T1W imaging (TR/TE = 570/46ms, in-plane resolution = 0.39×0.19×1.5 mm^3 and slice thickness = 1.5 mm) was performed immediately after T1-shortening contrast agent (gadodiamide) administration. The data were analyzed by Mathematica and Matlab.

Results and Discussions

Abnormal findings on MR images were observed in each rabbit infected with 700 *A. cantonensis* larvae. Our MR images showed the ventricles of each infected rabbit gradually became dilatation over time (Fig. 1). In T2W images, the ventricles size increased from 1.896% ± 0.333% to 5.710% ± 1.228% in the brain of the rabbit infected after 42 days (Fig. 1A, 2A). In FLAIR, it increased from 1.183% ± 0.185% to 3.368% ± 0.584% in brain of the rabbit infected in the same period (Fig. 1B, 2B). The ventricular dilatation was obvious 14 days after the infection. The results implied that hydrocephalus was caused by *A. Cantonensis* 14 days after the infection. In addition, the mean R2 values (R2=1/T2) of whole rabbit brain decreased from 8.66 ± 0.74 to 8.98 ± 0.33 ms^{-1} in R2 mapping (Fig. 1D, 1E). The signal intensity of the ventricle changed was also found in T1W images 21 days after the infection (Fig. 1C). It implied CSF content changed, and may be due to hemorrhage or inflammatory cells. The size of ventricular choroid plexus increased gradually from 0.978% ± 0.122% to 5.620% ± 0.622% in T1W images (Fig. 2C). This implied ventricular choroid plexus hyperplasia was induced by *A. Cantonensis* infection. More subjects were needed to improve statistic significance. In the future, the non-invasive MRI platform developed in rabbits can be extended for early diagnosis in humans.



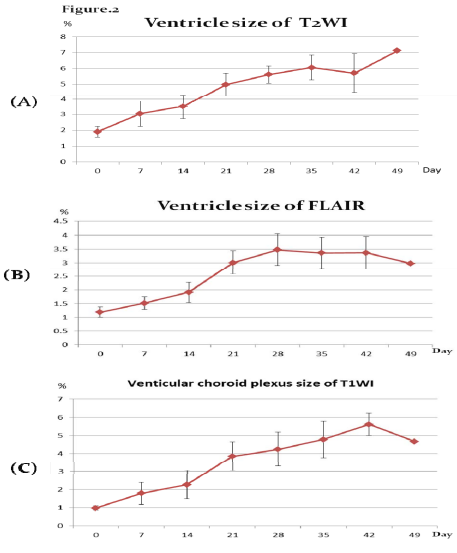


Fig. 1 (A) T2W images, (B) FLAIR, (C) contrast-enhanced T1W images, (D) R2 mapping and (E) histogram of rabbit brains infected with 700 *A. cantonensis* larvae after 0 to 42 days.

Fig. 2 Changes of rabbit brains infected with 700 *A. cantonensis* larvae after 0 to 49 days. (A, B) ventricular size increased in T2W and FLAIR images. (C) The ventricular choroid plexus size increased in contrast-enhanced T1W images.

Conclusions

Our MRI results showed pathological changes of brain, including ventricular dilatation and choroid plexus enlargement, with time in the rabbit infected with 700 *A. cantonensis* larvae. Therefore, MRI was suggested to be a non-invasive technique in detecting eosinophilic meningitis resulted from *A. cantonensis.* In the future, the non-invasive MRI platform developed in rabbits can be extended for early diagnosis in humans.

References

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