Longitudinal Effects on Rat Brain of Different Degrees Infection by Angiostrongylus Cantonensis

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Introduction

Angiostrongylus cantonensis (*A. cantonensis*) is a zoonotic nematode parasite residing in the pulmonary arteries and brain of rats. It was first identified and described by Chen in Canton, China [1], and was reported to cause human diseases in 1945 in Taiwan [2]. Now *A. cantonensis* is the major cause human eosinophilic meningitis in Taiwan. However, the features of the pathological changes in the brain were limited to diagnostic techniques [3-5]. Previously the diagnosis was established by immunodiagnosis, lumbar puncture and eosinophilia examination. Fourth- or fifth-stage larvae could be found in the cerebrospinal fluid (CSF) with lumbar puncture. Improper puncture and false immune response resulted in an erroneous diagnosis. Therefore, the purpose of this study was to longitudinally monitor the lesion localization, pathological changes and angiostrongyliasis characterization of rat brain infected with different numbers of *A. cantonensis* larvae by MRI techniques. The results were also verified with histopathological study. The association between the clinical features of the rats and MRI findings was also addressed.

Materials and Methods

In parasite infection, third-stage larvae of *A. cantonensis* were collected from infected *Achatina fulica* snails. A total of 24 male Wistar rats weighing 250 - 300 g (12 weeks old) were used. Rats were orally inoculated with different numbers of larvae using a metal fedding tube (7 rats for 300, 7 rats for 100, 5 rats for 50, and 5 rats for 20 *A. cantonensis* larvae). MR scans were performed after infection of *A. cantonensis*. In order to determine the permeability changes of blood-brain barrier after inflection, gadodiamide was given by intravenously injecting 367.53 mg/kg of a 0.5 M Gd-DTPA solution. T1W imaging was performed immediately after T1-shortening contrast agent (gadodiamide) administration. During the MR scanning, the rats were anesthetized with 2% isoflurane mixed with O_2, maintained with 1.5% isoflurane. Rat body temperature was maintained at 37 ºC using warm water circulation.

The experiment was performed on a 1.5T MRI system (Magnetom Sonata, Siemens Medical Systems, Erlangen, Germany). A small loop surface coil was used for RF reception. Multi-slice turbo spin echo (TSE) sequence was performed to obtain whole brain T2W images with TR/TE = 3760/114 ms; fluid attenuation inversion recovery (FLAIR) was also performed to obtain whole brain T2W images with TR/TE/TI = 8420/155/2500 ms. These sequences were performed with in-plane resolution = 195 µm x 390 µm 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. A multi-slice fast spin echo sequence was also performed to obtain whole brain contrast-enhanced T1W images in the end of scans with TR/TE = 513/46 ms, in-plane resolution = 195 µm x 390 µm and slice thickness = 1.5 mm.

Results and Discussions

Abnormal findings on MR images were observed in each rat infected with different numbers of *A. cantonensis* larvae. However, each group of the infected rats with different degrees was found to have variable pathological changes in the brain tissue. Generally, the subarachnoid size (Fig. 1a, b) and ventricle size (Fig. 1c, f) were increased. In the late phase, subarachnoid damage could be recovered (Fig. 1a, b), but ventricles were damaged permanently (Fig. 1c). Signal intensities of subarachnoid in FLAIR were increased (Fig. 1d), and mean R2 values (Fig. 1e) were decreased. The meningoencephalitis and brain injury of the rats in the group of severe infection lasted longer and became more serious than the rats in the group of mild infection. The rats in the group of mild infection survived longer than the rats in the group of severe infection (Fig. 2a, b). The result showed the difference in the rat brain disease progression with different degrees inflection by *A. Cantonensis*. Our MR results were consisted with histopathological study (Fig. 3). Moreover, a fifth-stage larva (Fig. 3a) and hemorrhage (Fig. 3b) in the rat brain parenchyma near subarachnoid space (arrow) could be found in the group of severe infection.

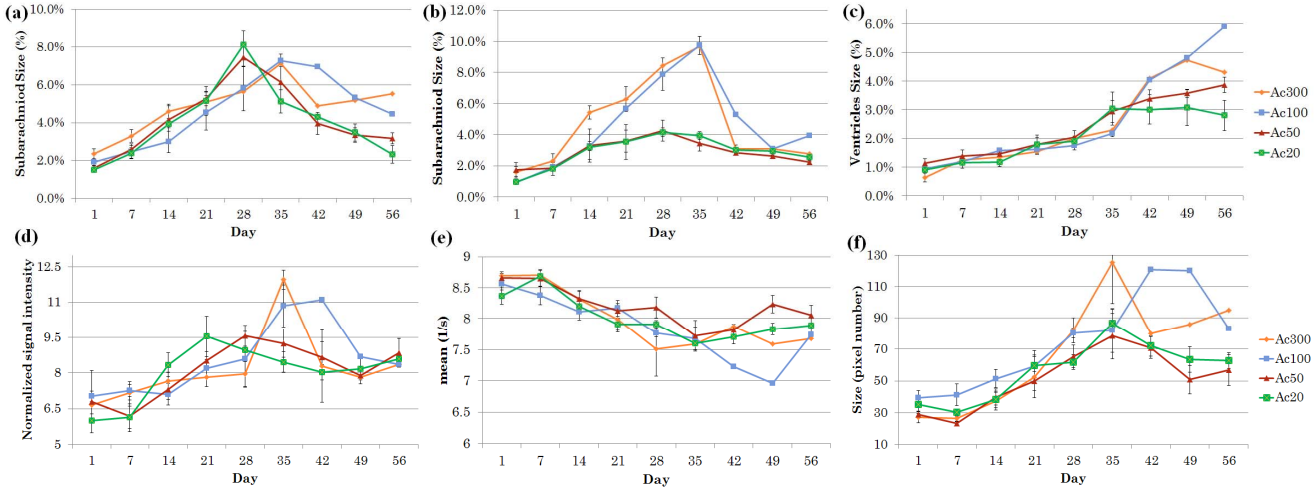

Fig. 1 (a) Subarachniod size in T1WI, (b) subarachniod size in T2WI, (c) ventricles size in T2WI, (d) subarachniod signal intensity in FLAIR, (e) mean value of R2 mapping, and (f) pixel numbers in R2 mapping (size of subarachniod and ventricles) of rat brains infected with 20-300 *A. cantonensis* larvae after 1 to 56 days.

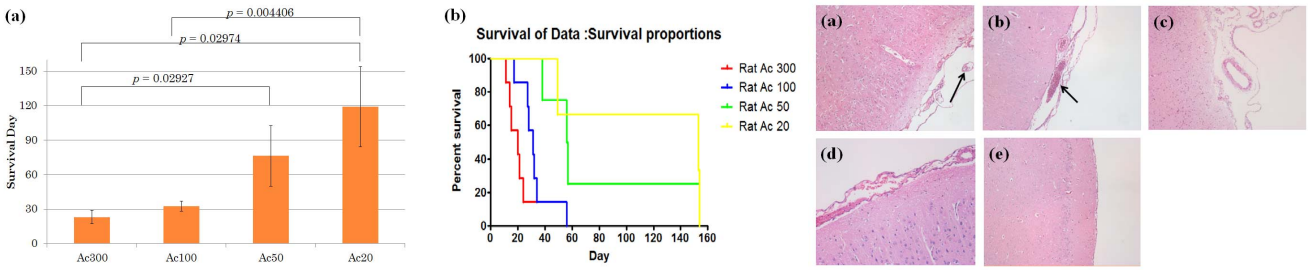

Fig. 2 (a) Survial day and (b) survival rate of rat brains infected with 20-300 *A. cantonensis* larva.

Fig. 3 Microphotograph of histopathology of rat brains infected with (a) 300, (b) 100, (c) 50, and (d) 20 *A. cantonensis* larva, and (e) is control baseline.

Conclusions

Our results showed the difference in the rat brain disease progression and survival rate with different degrees inflection by *A. Cantonensis*, and they were consisted with histopathological study. MRI was sensitive in showing tissue change and edema, and provided higher tissue contrast and superior sensitivity in the detection of lesions. Therefore, MRI was suggested to be a non-invasive technique in localizing and characterizing lesions during the acute as well as late phase of angiostrongyliasis due to *A. cantonensis*.

References [1] Chen H-T. Annals of Parasitology, 1935; 13: 312-317. [2] Nomura S. et al., Formosan Medical World 1945; 3: 589-592. [3] Alicata JE. Advances in Parasitology, 1965; 3: 223-248. [4] Tsai H-C. et al., Am. J. Trop. Med. Hyg., 2003; 68(3): 281-285. [5] Wang L-C. et al., J Antimicrob Chemother, 2006; 57: 294-300.