Magnetic Resonance Imaging of Alveolar Echinococcosis Experimentally Induced in the Rat Lung

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(Received 17 May 2005/Accepted 16 September 2005)

ABSTRACT. Pulmonary alveolar echinococcosis (AE) caused by the metacestode of *Echinococcus multilocularis* is a lethal zoonosis and is a lesion secondarily induced by hematogenous dissemination from hepatic AE lesions. In the present study, a hematogenous pulmonary AE model was experimentally induced in rats by the injection of echinococcal larval tissue homogenate to the tail vein, and then the pathological and diagnostic aspects of pulmonary AE were examined by magnetic resonance imaging (MRI). Histological primary, mature and degenerated AE lesions were observed 5, 18 and 50 weeks after injection, respectively. These lesions were discriminated as signal-void, hypointense and hyperintense regions in T1-weighted MRI (T1WI), respectively. The change in signal intensity in T1WI might reflect the content of proteinaceous fluid as a result of AE cyst degeneration. Western blot analysis of sera with antibodies of two epitopes (Em18 and Em16) of *E. multilocularis* provided evidence for AE infection in the early stage. T1WI in combination with Western blot analysis could possibly become definitive and early signs of hematogenous pulmonary AE infection.

KEY WORDS: *Echinococcus multilocularis*, magnetic resonance imaging (MRI), parasitic infection, pulmonary alveolar echinococcosis (AE), Western blot.

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Alveolar echinococcosis (AE), caused by the metacestode of *Echinococcus multilocularis*, is a lethal zoonosis found in North America, Central Europe, Russia, China, Turkey and Japan [13]. Recently, in the island of Hokkaido in Japan, the infection was observed not only in humans, but also in primates having no roles in the transmission cycle. AE infection was found in the liver, the lung, the lymph node and the brain of a gorilla (*Gorilla gorilla*), a ring-tailed lemur (*Lemur catta*) [10], and an orangutan (*Pongo pygmaeus*) [14]. From the appearance of AE infection in these primates, it was postulated that the expansion of AE in endemic areas elevates the number of pulmonary AE patients because the lung is a secondary, but frequently infected site [11].

Pulmonary AE is mainly caused by hematogenous dissemination from hepatic AE lesions [4]. Though hematogenous pulmonary AE has often been observed in humans, there have been only a few reports about pathological and diagnostic observations of such patients [12]. Physical signs in pulmonary AE are hemoptysis, chest pain, cough with expectoration, and exertional dyspnea [3]. However, the pulmonary AE caused by hematogenous spread and intrapulmonary enlargement of daughter cysts is usually asymptomatic for about 10 years [15]. For diagnosis of pulmonary AE, circumstantial evidence, like primary lesions in the liver, an appropriate clinical history, a high prevalence of infection in the host’s geographical location, and laboratory findings, is employed. The cysts of cystic echinococcosis (CE) caused by *E. granulosus* can be diagnosed by typical radiological patterns, but those of AE are hard to diagnose [4]. Therefore, a direct and early diagnostic, but non-destructive method such as magnetic resonance imaging (MRI) or serological examination is required for pulmonary AE patients [1, 9].

In the present study, we tried to establish a secondary pulmonary AE model in rats injected with a homogenate of echinococcal larval tissues to the tail vein and evaluated it by use of high-resolution MRI, histologic examination, and serodiagnosis (western blot analysis) for exact and early diagnosis of hematogenous pulmonary AE.

MATERIALS AND METHODS

Animals: Specific-pathogen-free female Wistar rats (Slc: Wistar, 200 ± 25 g body weight, 6 to 8 weeks old, n=24) and male Mongolian gerbils (MGS/Sea, 70 ± 10 g body weight, 6 to 8 weeks old, n=6) were obtained from Japan SLC, Inc. (Hamamatsu, Japan) and Seac Yoshitomi, Ltd. (Yoshitomi-cho, Fukuoka, Japan), respectively. The rats and gerbils were housed in accordance with “The Guide for the Care and Use of Laboratory Animals, Graduate School of Veterinary Medicine, Hokkaido University”. The rats and gerbils, after *E. multilocularis* infection, were acclimated to an air-conditioned special animal room for parasite-infected experimental animals (containment room with biosafety level 3) in the Experimental Animal Facility, Graduate School of Veterinary Medicine, Hokkaido University. All animals had *ad libitum* access to food and filtered ion-
Hematogenous pulmonary animal model: The larval tissues of *E. multilocularis* from gerbils infected by intraperitoneal serial passage of the parasite originating from an infected wild vole, *Clethrionomys rufocanus bedfordiae*, in Hokkaido were obtained by a modification of a method previously described [1]. Infected gerbils were euthanized by intraperitoneal administration of an overdose of pentobarbital sodium solution (200 mg/kg). The larval tissues were carefully removed from the abdominal organs of gerbils, rinsed with sterile PBS, minced with scissors, and then filtered with a 210-µm mesh. The sediment was triply washed with minimum essential medium (MEM) and suspended at a 10% volume in MEM for injection. In this 10% homogenate, the amounts of microvesicles and protoscoleces were estimated to be 3.8 and 1.5 × 10^5/ml, respectively. Each rat (n=20) was anesthetized by intraperitoneal injection of pentobarbital sodium solution (50 mg/kg). Then 0.2 ml of the larval tissues homogenate (the sediment suspension) was intravenously injected into the left tail vein with an insulin syringe fitted with a blunt-tipped 27-gauge needle. Control rats (n=4) were prepared by injecting 0.2 ml of MEM.

**MRI:** MRI of the thoracic cavity in rats was performed 5, 18 and 50 weeks after injection of the larval tissue homogenate. The images of the thoracic cavity were initially obtained from living rats, but the movement of breathing and the heartbeat interfered with detection of AE cysts. To avoid the movement due to the breathing and heartbeat, rats were sacrificed by subcutaneous administration of an overdose of a pentobarbital sodium solution (200 mg/kg) and then MR images of the thorax were obtained [2]. MRI was performed essentially according to the method previously described [1]. In brief, MRI was carried out on a 7.05-T Varian Unity INOVA system (Varian Inc., Palo Alto, CA). Images were acquired using a 60-mm diameter quadrature radiofrequency coil. The following parameters were also employed: field of view, 6 × 6 cm²; slice thickness, 1.0 mm; T1-weighted (T1WI), T2-weighted (T2WI) and proton-density MR images (PDI) were obtained under imaging conditions of repetition/echo time = 500/20, 2000/60 and 2000/20 msec, respectively.

**Histological examination:** After MRI, lung tissues were removed and fixed with neutral-buffered 10% formalin, then embedded in paraffin wax and sectioned 5-µm thick. Each section was stained with hematoxylin/eosin or periodic-acid-Schiff (PAS) followed by hematoxylin counterstaining [1].

**Western blot analysis:** A monoclonal antibody against Em16 and serum from a Japanese AE patient, which contained antibodies to two epitopes, Em18 and Em16 (18 and 16 kDa components, respectively) of *E. multilocularis*, were prepared according to the method previously described [1]. Western blotting using antibodies to Em18 and Em16, is reported to be highly reliable for the detection of active AE [9]. Blood was collected 1, 2, 3, 4, 5, 7, 9, 13, 17, 21, 25, 31, 36, 39, 43, 47 and 50 weeks after injection. The blood was centrifuged at 1,700 × g for 10 min at 4°C. Sera were saved at –30°C until use. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transblotting were carried out using commercially available precast 4–20% gradient gels (No. 01–022, SDS-PAGE Mini, TEFCO, Tokyo, Japan). Antibodies responding to Em18 and Em16 were assessed using peroxidase-conjugated anti-rat IgG (Cappel, Cochranville, PA) at 1:1,000 dilution [1, 9].

**RESULTS**

**MRI and histological examination 5 weeks after injection:** Figure 1 shows PDI (Fig. 1A), T1WI (Fig.1B), and T2WI (Fig. 1C) 5 weeks after injection. One abnormal spot approximately 1 mm diameter was visualized in the left lung near the heart in PDI and T2WI (arrows in Figs. 1A and 1C). No spots were visualized at the same position in T1WI (arrow in Fig. 1B). Figure 1D shows macroscopic observation of the lung. Two white nodules approximately 1 mm diameter were observed in the middle of the left lung lobe (white and black arrows). Figure 1E shows a section of the left lung lobe. Two cystlike nodules were observed at the edge of lung lobe section (white and black arrows). The arrows in Figs. 1D and 1E show the same cyst. The hydatid fluid contents in these cavities might be responsible for the hyperintensity in PDI and T2WI shown in Figs. 1A and 1C. Figure 1F shows its histological examination. The hydatid fluid was macroscopically observed in this cavity. The cyst wall consisted of two layers, an outer layer formed by the lung tissues (arrowheads) and inner layers formed by parasitic organs. In the outer layer, hyperplasia in the granulation tissue was observed. The outer layer contained no calcified tissue (arrowheads). In the inner layer, the primordia of protoscoleces and brood capsules were observed (arrows and open arrowheads, respectively). From these results, this abnormal spot corresponded to a primary AE cyst because the hydatid fluid (water) has an abundance of protons coupled to the elongation of T1 and T2 relaxation times and is generally observed with hyperintensity in PDI and T2WI and with hypointensity or a signal-void region in T1WI [7].

**MRI and histological examination 18 weeks after injection:** Figure 2 shows PDI (Fig. 2A), T1WI (Fig. 2B), and T2WI (Fig. 2C) 18 weeks after injection. The hyperintense region in the lung was visualized in all MR images (arrows in Figs. 2A, 2B and 2C). The signal intensity in T1WI was the same as that of the pectoral muscle (Fig. 2B). Figure 2D shows macroscopic observation of the lung. Several white nodules exceeding 2 mm in diameter were observed on the surface of the lung lobe. The abnormal signal region (arrow in Figs. 2A-2C) was considered to correspond to the nodule indicated by the arrow in Fig. 2D. Figure 2E shows the section of this nodule filled with a large amount of white material and a small amount of hydatid fluid. This forms the cellula structure (arrows in Fig. 2E). Figure 2F shows its histological examination. This cyst exhibits multivesiculation. The development of immature protoscoleces was observed in it (arrows), and the number of immature protoscoleces was more than that at 5 weeks after injection. A large amount of immature protoscoleces and the existence of the
fibrous tissue and laminated layer might contribute to the increase in the signal intensity in the T1WI. Incrassate granulation and fibrous tissues surround this cyst (open arrowheads), but calcification was observed around it.

MRI and histological examination 50 weeks after injection: Figure 3 shows PDI (Fig. 3A), T1WI (Fig. 3B), and T2WI (Fig. 3C) 50 weeks after injection. Hyperintense regions of various sizes (1–4 mm) were observed in the thoracic cavity in PDI (Fig. 3A). These regions were also imaged as various signal intensities in T1WI and T2WI.
The relative signal intensities in the regions indicated by open arrowheads in Figs. 3A-3C were similar to those in Figs. 1A-1C. White arrows indicate the hyperintense regions in all MR images. A large number of white nodules were observed on the lung lobe surface. The region indicated by arrows in Figs. 3A-3C corresponds to the white nodule indicated by the arrow in Fig. 3D. Figure 3E shows a section of the region indicated by the white arrow in Figs. 3A-3D. The white arrow in Fig. 3E indicates a cyst containing hydatid fluid and white material. The black arrow indicated a cyst containing mainly white material. Open arrowhead indicates a cyst containing mainly hydatid fluid. Bar=3 mm. (F) Histological examination of the region indicated by white arrows in Figs. 3A-3C. The white and black arrows indicate cysts containing hydatid fluid, protoscoleces and calcareous corpuscles. HE. Bar=3 mm.

**DISCUSSION**

AE cysts are generally smaller than cystic echinococcosis (CE) cysts caused by *E. granulosus* [5], and pulmonary AE cysts do not have markedly calcified tissues, which are a definite sign of hepatic AE in CT and MRI [6, 11]. If calcification occurs in pulmonary AE lesions, these lesions might not be detected in MRI because calcified tissues have short T1 and T2 relaxation times leading to MR images with low signal intensity [7]. Furthermore, the normal lung is also imaged with hypointensity because of low proton densities [2]. Therefore, it might be difficult to diagnose hematogenous pulmonary AE lesions with MRI. Fortunately, in the
The present study histological examination of pulmonary AE lesions showed that no remarkable calcification influencing the MRI signal intensity occurred. This result was consistent with a report that calcification in pulmonary cysts is rare (0.7% of cases) [11] and the fact that there are only a few reports about the diagnosis of pulmonary AE patients with radiography [12]. The present study showed that MRI signal intensity was reflected in each stage of pulmonary AE lesions consisting of various cysts: 1) the primary AE cyst was shown as a signal-void region in T1WI, and hypointense in T2WI and PDI (Figs. 1A-1C), 2) the mature AE cyst showed isointensity in T1WI and hyperintensity in T2WI and PDI (Figs. 2A-2C), and 3) the degenerated AE cyst was hypointense in all images (Figs. 3A-3C). Histological examination also revealed that 1) the primary AE cyst mainly contained hydatid fluid (Fig. 1F), 2) the mature AE cyst contained protoscoleces, granulation tissue, fibrous tissue and hydatid fluid (Fig. 2F), and 3) the degenerated AE lesion contained fibrous tissue, calcareous corpuscles, hydatid fluid and granulation tissue (Fig. 3F). The increase in signal intensity of AE cysts in T1WI might reflect the amount of proteinaceous fluids from AE cyst degeneration, because proteinaceous fluid has a short T1 relaxation time and short or intermediate T2 relaxation time depending on the protein content [7]. These results indicated that the progression of hematogenous pulmonary AE infection without calcification could be diagnosed by the changes in MR signal intensity in T1WI.

The utility of serodiagnosis by Western blot analysis using specific antibodies of two epitopes, Em18 and Em16, for hepatic AE infection has been demonstrated in a rat model and human patients [8, 9]. In the present study, the band of Em18 was detected in the early stage (9 weeks after injection) (Fig. 4). MRI alone cannot distinguish secondary pulmonary AE infection from other granulomatous lung diseases in the early stage [4]. Hence a combination of both MRI and serodiagnosis would be effective for definitive diagnosis of pulmonary AE infection. Indeed, the present study demonstrated that MRI and the Em18 band diagnosed the pulmonary AE infection at 5 and 9 weeks after injection, respectively (Figs. 1A-1C and Fig. 4).

MRI-diagnosis of human pulmonary AE infection will become possible if TR time will be synchronized with the periods of breaths and heartbeats. We confirmed that MRI signal intensity was reflected in each stage of pulmonary AE lesions consisting of various cysts, and concluded that the progression of hematogenous pulmonary AE infection without calcification could be diagnosed by the changes in MR signal intensity in T1WI. The combination of T1WI and immunoblot analysis of the Em18 band could possibility become definitive and early signs of hematogenous pulmonary AE infection.

ACKNOWLEDGMENT. This work was supported, in part, by Grants-in-Aid for Encouragement of Young Scientists (No. 15780200, T.A.), Scientific Research (B) (No. 15380199 and 17380178, O.I.) and (C)(No. 17580275, T.A.), and Exploratory Research (No. 17658126, M.K.) from the Ministry of Education, Science, Sports and Culture of Japan.

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