Murine Model of Neuroschistosomiasis Mansoni: Clinical, Histological and Magnetic Resonance Imaging Studies

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Introduction

The World Health Organization estimates that between 200 and 300 million people worldwide are infected with Schistosoma spp. and that 800 million people in the world are at risk of infection. In Brazil, approximately 2.5 million people are infected with Schistosoma mansoni and 30 million are exposed to infection [1,2].

Central Nervous System (CNS) involvement in schistosomiasis can occur during acute primary infections, but as the disease becomes chronic, neurological complications can occur as the newly forming and approximately 2% (160 people) developed cerebral complications (1950) found S. haematobium eggs in the digested brains of 56% (28 million people worldwide are infected with Schistosoma spp. and that 800 million people in the world are at risk of infection. In Brazil, approximately 2.5 million people are infected with Schistosoma mansoni and 30 million are exposed to infection [1,2].

In the year of 1944, 800 people from Asia continent were attended at Moore General Hospital (North Carolina-United States of America) and approximately 2% (160 people) developed cerebral complications attributed to schistosomiasis japonica [4-8]. In Zimbabwe, Gelfand (1950) found S. haematobium eggs in the digested brains of 56% (28 people) of 50 patients with S. haematobium infection of the bladder. Alves (1958) found that in 28% of 150 unselected autopsy cases S. haematobium eggs were detected in the brain [9-11]. It is estimated that the incidence of neurological complications varies between 0.3% and 4% of schistosomiasis mansoni [2]. The incidence of encephalic damage caused by S. mansoni in humans is unknown.

Neurological manifestations of schistosomiasis are caused by an increase in intracranial pressure, and the focal signs are triggered by the tumor masses produced by granulomas, often in the productive phase with slight fibrosis, which suggested the chronic phase of infection. The initial signs and symptoms include headache, focal or generalized seizures, ataxia, nystagmus, nausea and vomiting, intracranial hypertension and various neurological deficits [2].

For many years, we have observed evidence of brain disease (hemiplegia, spinning and urinary retention) in mice infected with S. mansoni but these mice were considered to have other diseases, such as labyrinthitis or cerebral infection [2].

Thus, neuromotor manifestations presented by infected animals should characterize neurological damage caused by S. mansoni eggs in the murine model. Therefore, the relationship between histological findings and the neurological signs of encephalic involvement, such as hemiparesia, spinning, head tilt, chest tilt, ataxia, and loss of balance could be established in this study.

Aloe et al. described eggs, both with and without granuloma formation, in CD-1 mice infected with 60 S. mansoni cercariae (Puerto Rican strain). However, the mice did not present the signs of brain disease. Additionally, Silva et al. observed very few eggs in the brains of...
Mice and infection

Swiss-webster male mice, weighing approximately 20 g, were obtained from Oswaldo Cruz Foundation (Belo Horizonte, Brazil), and maintained under standard conditions. At 6 weeks of age, mice were infected subcutaneously with 50 cercariae of a LE strain of S. mansoni, maintained at the Rene Rachou Institute (Minas Gerais, Brazil) by passage in Mus musculus albino mice and in Biomphalaria glabrata snails. The origin and maintenance of the S. mansoni used in this study have been described previously by Pellegrino & Katz (1972). All mice, infected and control animals, were provided with food and water ad libitum, under the 12 h light/12 h dark cycle, temperature 22 ± 1°C and air humidity of 40–50%.

At 88, 97 and 146 days post-infection, euthanasia procedures were performed (n=2/group), by CO2 gas chamber, according to the guidelines and principles of the Brazilian Council on Animal Care and were approved by the local Institutional Animal Care Committees at the Federal University of Minas Gerais and the Rene Rachou Research Institute (FIOCRUZ/MG, Brazil). The ex vivo samples had a catheter placed into the right heart and perfused by a fixative solution of 10% paraformaldehyde (PFA). The worm recovery was carried out as per the technique prescribed by Pellegrino and Siqueira [13-18].

Clinical monitoring

SHIRPA protocol for phenotype assessment designed as a series of individual tests that gives data regarding the integrated function of cortical arousal, cerebral locomotor control, and neuromuscular function. Such test-specific performance is directly comparable between animals, over time, and between groups. The locomotor parameters, such as head and body rotation, absence of escape reaction, paresis, loss of balance reflex, altered muscle tone, ataxia and spinning were applied [19].

Magnetic Resonance Imaging

Mouse heads were removed and immersed in 10% PFA for more than 48 h. To preserve the shape of the brains during imaging, the brains were left inside the skull. The experiments were performed on a 7T magnetic resonance scanner (MRI System 7T/210 ASR Horizontal Bore Magnet, Agilent Technologies). Ex vivo brain images were obtained using 3D T1 GRE (TR/TE: 370 ms/5 ms, MATRIX: 128x96x96, FA: 35°, NEX: 13, FOV: 20x15x15 (mm), acquisition time: 12 h 18 min), coronal MULTI ECHO (TE/TR: 3000 ms/9 ms, 3 ECHOS, NEX: 30, MATRIX: 128x128, FOV: 15x15 (mm), SLICES: 30, SLICE THICKNESS: 0.5 mm, no GAP, acquisition time: 3 h 12 min).

For each dataset, the images were visually inspected for artifacts. For image processing, the MRicron software (http://www.mccauslandcenter.sc.edu) was used to measure and compare the lesions dimensions with findings in histopathology.

Histological analysis

Imaging was done, brain and skull was immersed in 7% nitric acid for decalcification. After 1 day (24 h), the whole skull was sectioned in 3 mm thick slices, and dove in 7% nitric acid for 24 h for complete decalcification. After that, the fragments were sectioned in 1.1 mm thick slices, each one placed in a paraffin block (10-11 blocks for each animal). Serial 4 µm sections (obtained from 50 µm intervals between each) from all paraffin blocks were stained with Hematoxylin and Eosin (HE). For each animal, 18-20 g weighing, sections were obtained using 3D T1 GRE (TR/TE: 370 ms/5 ms, MATRIX: 128x96x96, FA: 35°, NEX: 13, FOV: 20x15x15 (mm), acquisition time: 12 h 18 min), coronal MULTI ECHO (TE/TR: 3000 ms/9 ms, 3 ECHOS, NEX: 30, MATRIX: 128x128, FOV: 15x15 (mm), SLICES: 30, SLICE THICKNESS: 0.5 mm, no GAP, acquisition time: 3 h 12 min).

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The right hemisphere of each animal’s skull was stained with Nankin” ink for identification.
Brain topography

Was performed using Allen’s brain atlas, data portal (http://mouse.brain-map.org/static/atlas).

Statistical analysis

When significant differences were obtained, comparisons between groups were carried out by Fisher’s test. Analyzes were performed in the STATA 12.0 software (Stata Corporation, College Station, Texas) at 5% level of significance.

Results

Neurological alterations appeared at 88, 97 and 146 days post-infection. Euthanasia occurred immediately after the emergence of the neurological signs.

Neurological manifestations

The neuromotor alterations were head and chest tilt (to the right or left side), paresis, imbalance reflexes, ataxia and rotational motion (spinning) (Table 1) (Figure 2).

<table>
<thead>
<tr>
<th>Neurological Manifestations</th>
<th>Infected</th>
<th>Control</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinning</td>
<td>3</td>
<td>12</td>
<td>0.235</td>
</tr>
<tr>
<td>Head tilt (right side)</td>
<td>2</td>
<td>8</td>
<td>0.49</td>
</tr>
<tr>
<td>Head tilt (left side)</td>
<td>1</td>
<td>4</td>
<td>0.999</td>
</tr>
<tr>
<td>Chest tilt (right side)</td>
<td>2</td>
<td>8</td>
<td>0.49</td>
</tr>
<tr>
<td>Right hemiparesis</td>
<td>1</td>
<td>4</td>
<td>0.999</td>
</tr>
<tr>
<td>Left hemiparesis</td>
<td>2</td>
<td>8</td>
<td>0.49</td>
</tr>
<tr>
<td>Ataxia</td>
<td>3</td>
<td>12</td>
<td>0.235</td>
</tr>
<tr>
<td>Imbalance</td>
<td>3</td>
<td>12</td>
<td>0.235</td>
</tr>
</tbody>
</table>

Table 1: Neurological manifestation observed in three mice infected with 50 cercarie in FIOCRUZ/Brazil laboratory, 2016–2017.

Figure 2: A. Swiss Webster mice infected with 50 cercarie of S. mansoni. The animal presented head tilt for the left side. B. Mice not infected (control), with habitual posture.

MRI evaluation

MRI images enhanced abnormalities in encephalic parenchyma of the symptomatic animals in comparison to the control mice images. The histopathological features present in these infected mice revealed the tumor mass caused by the granulomatous reaction (Figure 3). On MRI there were signs suggesting hemorrhages inside the brain (Table 2).

Figure 3: Serial brain slices from the brain of the mouse, show in lesions due to S. mansoni. T1-weighted sequences images. Nodular lesion surrounding the parasite egg (HE), located in the piriform area.

<table>
<thead>
<tr>
<th>Topography</th>
<th>Infected</th>
<th>Control</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limbic lobe</td>
<td>2</td>
<td>8.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sensoriaimotor area (cerebral cortex)</td>
<td>3</td>
<td>12.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Visual area (cerebral cortex)</td>
<td>2</td>
<td>8.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Auditory area (cerebral cortex)</td>
<td>1</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Fissures of cerebellar cortex</td>
<td>2</td>
<td>8.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Brainstem</td>
<td>2</td>
<td>8.0</td>
<td>0.0</td>
</tr>
<tr>
<td>III Ventricle</td>
<td>1</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>IV Ventricle</td>
<td>1</td>
<td>4.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 2: MRI topography of encephalic lesions attributed to S. mansoni eggs and granulomatous reaction, of three infected mice subcutaneously, CENABIO/UFRJ*, 2016–2017.

Histological brain topography

Morphological examination of serial coronal brain sections showed S. mansoni eggs or granulomas found in the olfactory tract, limbic lobe, cerebral cortex, cerebellum cortex, III and IV ventricles, and brainstem (Table 3). The granulomas were localized in proximity to arteries, suggesting that the ova gain access to the CNS via arterial system. Red blood cells were also observed in most brain areas.
Histological analysis

Eggs of *S. mansoni* were disseminated in the right and left cerebral hemispheres, in regions of the leptomeninges, cerebral and cerebellar parenchyma and brainstem. In the most of samples, the eggs reached the encephalon by arteriolar system (Figure 4).

Epithelioid granulomatous infiltrate were most frequent in the leptomeninges, fissures and ventricles, in the brain and cerebellum. *S. mansoni* eggs with low inflammatory reaction were common in the cerebral and cerebellar parenchyma. All the eggs observed had miracidium, proving recent deposition in the encephalon. Macrophages, eosinophils, lymphocytes identified the inflammatory cells. Fibroblasts and areas with collagen were also present (Figure 4).

Discussion

In 25 *Swiss-webster* mice subcutaneously infected with 50 cercariae of the *S. mansoni* (LE strain), three mice presented the following neurological manifestations: spinning, hemiparesis, head and chest tilt (to the right or left side), ataxia, loss of balance and body contortion. MRI sagittal, coronal and axial T1w images of brain demonstrate in encephalon bilateral focal hypersignal, indicating morphological changes. Histology confirmed the lesions in the brain. The samples presented viable eggs of *S. mansoni* near arterioles and red blood cells.

Animals were observed until 160 days post-infection. At that time, 3 mice (12%) presented neurological manifestations suggestive of brain injury. The first symptomatic animal was sacrificed at 88 days post-infection, the second at 97 days and the third at 146 days. The control animals of the respective symptomatic did not present neurological alterations.

Qualitative SHIRPA protocol was applied based on gradations by scores to track basic neurological functions [19]. Such protocol integrates the functions related to skeletal striated muscle, sensory systems, motor neuron, autonomic system and spinocerebellar pathways. The neurological manifestations are consistent with characteristic changes of Central Nervous System (CNS) functional damages.

Histo-topographic survey of the mice brain identified granulomatous lesions disseminated in the cerebral hemispheres, in Sensory-motor, visual and auditory cerebral cortex areas, cerebellum fissures, olfactory tract, III and IV ventricles, limbic lobe and...
brainstem. Associated to granulomatous lesions were observed leptomeningeal thickening in cerebral and cerebellum cortex. The microscopic picture is characterized by granulomas constituted by macrophages, eosinophils, lymphocytes and giant cells around the ova. Fibroblasts and focal collagen are also present (Figure 5).

Aloe et al. [3] described schistosome eggs in histological sections from the brain of mice and reported that mice with S. mansoni periovular granulomas in the brain showed decrease of nervous growth factor expression [21]. Based on such findings, Fiore et al. reported that mice infected with S. mansoni exhibited behavioral disturbances, probably associated with modifications in the levels of nerve growth factor and cytokines induced by granulomas [3,21,22]. These two reports did not indicate how frequently S. mansoni eggs reach the CNS of the mouse. There was also no sign of neurologic involvement.

The MRI image suggested haemorrhages in our samples. There are no reports about MRI image that correlate with brain lesions by encephalic neuroschistosomiasis mansoni in the murine model (Figure 4). Haemorrhages in the subarachnoid and intraparenchymal in the cerebral and cerebellar regions were identified by histological samples. The proximity of eggs and granulomas of S. mansoni to the arterioles suggests that the penetration into the CNS in the murine model occurred via the arteriolar route. Often the arteriole wall presented an inflammatory pattern (arteritis) of fibrinoid necrosis (Figure 3). Human brains were identified within the cerebral arteries S. mansoni eggs surrounded by mononuclear inflammatory cells. Herein we observed segmental ruptures and alterations of the epithelium with arterial fibrinoid necrosis [23-24].

Conclusions

In summary, the present study demonstrates that the subcutaneous infection in Swiss-webster mice by S. mansoni cercariae develops a neurological disease quite similar to the neuroschistosomiasis described in humans.

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