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Development of an experimental model of schistosomal myeloradiculopathy

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ABSTRACT

Schistosomal myeloradiculopathy (SMR) is a severe form of presentation of schistosomiasis in which *Schistosoma* spp. affect the spinal cord. The aims of the present study were to develop an animal model of SMR caused by *S. mansoni*, to characterize both sensory and motor abnormalities in the infected animals, and to investigate the relationship of the sensory, motor and histological findings with the progression of the infection over time. Mechanical sensitivity and behavioral tests were performed followed by euthanasia in male *Wistar* rats divided into six groups of five animals each, on days 5, 10, 20 and 30 after infection of *S. mansoni* eggs. The controls were subjected to the same procedure but were administered phosphate-buffered saline (PBS). The spinal cord was removed and subjected to histological analysis. *S. mansoni* eggs were found in the spinal cord of 25% of the infected animals, which belonged to the groups that exhibited more significant reduction of the superficial mechanical sensitivity, thermal sensitivity and muscle strength. This model proved to be satisfactory to assess functional changes in *Wistar* rats and might be used in studies investigating the pathogenesis of SMR. To our knowledge, this is the first experimental model of SMR.

1. Introduction

Schistosomiasis is one of the most prevalent parasitic diseases worldwide (Zhang et al., 2010). Although the gastrointestinal system represents the main target of the trematode, ectopic forms may occur, including the involvement of the central nervous system (CNS). Schistosomal myeloradiculopathy (SMR) is a severe form of presentation of schistosomiasis in which the parasite, especially *Schistosoma mansoni* or *S. haematobium*, affects the CNS (Steinmann et al., 2006). Although considered rare, SMR is one of the main causes of non-traumatic myelopathy in schistosome endemic areas (Naus et al., 2003; Carod Artal et al., 2004). The pathogenesis of SMR has not been fully elucidated. The most conclusive data indicate that the deposition of parasite eggs in the nervous tissue triggers an inflammatory response that leads to the formation of granulomas, which can group resulting in expansive mass lesions. Furthermore, histological findings suggest that ischemic lesions probably caused by immune complexes-mediated vasculitis also play a role in the pathogenesis of the neurological manifestations (Ferrari, 2010).

There are very few animal models of neuroschistosomiasis and all of them refer to brain involvement. According to the review performed by Fiore and Aloe (2001), rats with neuroschistosomiasis exhibited hyperalgesia due to granuloma formation into the brain (Fiore and Aloe, 2001). The results of another study conducted by the same group suggested that a murine model of infection may be useful for the study of the mechanisms involved in human neuroschistosomiasis (Fiore et al., 1996). More recently, the authors of a study performed in China described an experimental model

Abbreviations: CNS, central nervous system; SMR, schistosomal myeloradiculopathy; CEPA/UFS, Committee of Ethics in Animal Research of Federal University of Sergipe; SBCAL, Brazilian Society of Science in Laboratory Animals; d5, day 5; d10, day 10; d20, day 20; d30, day 30; G5, group 5; G10, group 10; G20, group 10; G30, group 30.

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of cerebral schistosomiasis. After inoculating *S. japonicum* eggs into the brain of rabbits, they described the progression of the cerebral lesions and analyzed the relationship between the clinical manifestations and the morphological characteristics of the brain lesions and the pathogenesis of neuroschistosomiasis. Those authors considered their model appropriate to reproduce cerebral schistosomiasis and crucial for the development of new models (Wang et al., 2011).

The main challenge for the development of an animal model of SMR is to design a protocol of infection that represents the pathogenesis of the disease in a reliable manner. Thus, we carried out the present study whose aims were to develop an animal model of SMR by inoculating *S. mansoni* eggs into the subarachnoid space; to investigate changes in the superficial mechanical and thermal sensitivity, and muscle strength in the infected animals; to analyze the relationship of both sensory and motor changes with the progression of the infection over time; and to identify histological abnormalities in the nervous system. To our knowledge, this is the first experimental model of SMR.

2. Material and methods

The present investigation was an experimental, controlled and blinded study. It was approved by the Committee of Ethics in Animal Research of Universidade Federal de Sergipe (UFS), Aracaju, Brazil – CEPA/UFS, protocol no. 11/2013. After the Committee's approval, 50 *Wistar* male rats weighing 300–500 g were selected from the Central Vivarium of UFS. All the study procedures complied with the norms of the Brazilian Society of Science in Laboratory Animals (SBCAL).

Two experimental series were performed. The "infected" and "control" series were divided into six groups corresponding to the days in which the behavioral tests were performed followed by euthanasia; i.e., experimental days 5 (d5), 10 (d10), 20 (d20) and 30 (d30), which corresponded to groups G5, G10, G20 and G30, respectively. The preceding groups comprised five animals each (Fig. 1).

2.1. Procedure for infection with S. mansoni eggs

The infected group was anesthetized with thiopental sodium via intraperitoneal injection (50 mg/kg). A total of 20 μL of a suspension of *S. mansoni* eggs (concentration: 25,000 eggs/mL) was injected into the subarachnoid space of each animal using a 1-mL syringe (Hylden and Wilcox, 1980; Dalton et al., 1997). The control animals were subjected to the same procedure but were injected with sterile phosphate-buffered saline (PBS).

2.2. Behavioral tests

The animals injected with *S. mansoni* eggs and control animals were subjected to mechanical and thermal sensitivity and muscle strength tests on d0, d5, d10, d20 and d30. The investigator who performed the tests was blinded to the animals' experimental series.

Mechanical sensitivity was assessed by means of a digital anesthesiometer (*Insight*®, Ribeirão Preto, SP, Brazil). A tip coupled to the anesthesiometer was pressed five consecutive times on the plantar surface of the hind paws and removed as soon as the animals withdrew the corresponding paw in response to the stimulus. The arithmetical mean of the results of the five applications was recorded as the secondary mechanical threshold, and the difference among the values obtained at the various time-points was considered in the assessment of the threshold variation (Gopalkrishnan and Sluka, 2000).

The thermal threshold was assessed using a *Hot Plate* (*Insight*®, Ribeirão Preto, SP, Brazil). The response latency was defined as the time that the animals remained on a heated metal plate (50 ± 0.5 °C) until reacting to the thermal stimulus, which was manifested by withdrawing or licking the paws (Tita et al., 2001). This assessment provided information on latency because the tail withdrawal reflex is characterized by medullary and supramedullary integration (Mitchell and Hellon, 1977).

The possible motor effects of the inoculation of *S. mansoni* eggs were assessed using the *Grip Strength Meter* device (*Insight*®, São Paulo, SP, Brazil). The grip strength test is a non-invasive and widely used method to assess the paw strength of rats. It is based on the natural tendency of the animals to grasp a bar or grid when hanging by the tail. A force transducer measures the peak force (in grams). The aim of the test is to assess the neuromuscular tonus and/or muscle strength of the fore- and hind limbs simultaneously or separately. Changes in the grip strength are considered to represent an increase or reduction in the muscle strength (Ishiyama et al., 2004; Mahmood et al., 2010). The test was performed with one animal at a time, and three measurements were recorded for each animal at five-minute intervals; the highest value was selected for analysis.

2.3. Histological analysis

Animals from both the infected and control groups were euthanized on d10, d20 or d30 by the administration of 150 mg/kg of thiopental sodium via intraperitoneal injection according to the norms established by CEPA/UFS. The spinal cord with its bone sheath and paravertebral muscles was removed and stored in flasks containing 10% formalin.

Spinal cord segments from T10 to L5 were cut in sections on the sagittal plane at five-μm intervals using a cryostat; the sections were fixed on glass slides and stained with hematoxylin-eosin. They were assessed regarding the presence of eggs, granulomas and aggregates of inflammatory cells.

2.4. Statistical analysis

The non-parametric Mann-Whitney test was used to compare the results of the neurofunctional tests between the two groups. When the control and infected groups exhibited statistically significant values ($p < 0.05$) in individual analysis, Friedman's analysis of variance (ANOVA) followed by Dunn's test was used to analyze the intragroup differences.

3. Results

Some of the animals in infected groups G10 and G20 exhibited spasmodic activity within the first two hours after intrathecal injection. Infected group G20 exhibited statistically significant differences in all the neurofunctional tests applied compared with the control group. The tactile sensitivity test performed with the digital anesthesiometer demonstrated a reduction in sensitivity evidenced by increases in the sensitivity threshold from 37.2 g to 52.0 g in the right paw and from 37.0 g to 49.1 g in the left paw (Table 1 and Fig. 2A). Thermal hyposensitivity, characterized by latency values varying from 4 to 9.8 s, was observed in infected group G20 (Table 2 and Fig. 2B). The average values exhibited by infected group G20 on the muscle strength test were 2,079.6 g to 1014 g, which means that there was a reduction in muscle strength (Table 3 and Fig. 3).

Fig. 4 depicts a *S. mansoni* egg in the anterior white commissure of the spinal cord at the level of the conus medullaris. Histological analysis of the segments T10 to L5 detected the presence of eggs in 25% of the animals, all of which belonged to the groups that exhibited significant clinical manifestations (G20 and G30). Eggs