Form No.5

Abstract of Thesis/Dissertation

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Title : Studies on the evaluation of potential antigens for an antigen and antibody-based serodiagnostic assays for *Schistosoma japonicum* infection

(抗原及び抗体の検出に基づく血清診断法の開発のための日本住血吸虫抗原の評価に関する研究)

Abstract

Schistosomiasis is considered the most devastating human parasitic disease next only to malaria. It is endemic in 78 countries around the world including China, the Philippines and parts of Indonesia. Official estimates show that about 240 million people are affected, of which 20 million people suffer from severe illness. The Asian schistosomiasis caused by *Schistosoma japonicum* was discovered in Japan in 1904. World Health Organization has designated this disease as Neglected Tropical Diseases for its active control. Initially, eggs are passed out into fresh water from the feces of an infected human or animal, which subsequently hatch into a free swimming miracidia. At this stage, the parasite actively seeks to infect the semi-aquatic intermediate snail host: *Oncomelania hupensis hupensis* in China and *O. h. quadrasi* in the Philippines. Inside the snail, the parasite undergoes clonal replication and develops into numerous

cercariae, the infective stage of this parasite.

The peculiarity of *S. japonicum* to infect several mammalian species including domestic animals sets it apart from other *Schistosoma* species infective to man. Animals in close association with humans, such as cattle, water buffaloes, dogs and pigs are most susceptible to the infection. This has made the control of the disease particularly complicated. Infection with *S. japonicum* is characterized by marked hepatic portal fibrosis leading to ascites. Aberrant eggs may also cross the blood brain barrier causing seizures as in the case of cerebral schistosomiasis. The huge number of eggs laid by a single female contributes to the higher potential of this disease to spread in the environment and cause pathology associated with egg deposition localizing in vital organs such as the liver, spleen and brain.

Diagnosis plays a crucial role for ensuring sound treatment and surveillance of the disease targeted for elimination. Prior to treatment, an accurate diagnosis should be first met. And re-testing should be done after treatment regimen to confirm the absence of the disease. Several decades employing numerous control measures that include mass drug administration (MDA) with praziquantel resulted to the decrease in prevalence in the endemic areas. This, however, led to a decrease in sensitivity of stool microscopy, which is the gold standard and the most commonly used diagnostic test for *S. japonicum* infection. In order to compensate for this, collection of stool samples should be done for three consecutive days, which makes the test more laborious and results in the decrease of compliance from patients being tested.

Detection of parasite circulating antigens provides informative result as it can indicate the true status of infection. At present, the only available antigen-based testing

for schistosomiasis is based on the circulating cathodic antigen of *S. mansoni.* This test has been successfully applied in numerous endemic areas in Africa. However, this does not hold true for *S. japonicum* infection where the testing has been evaluated in a proof-of-concept study. The ongoing need for a reliable *S. japonicum* specific antigen-based serodiagnosis has led to the evaluation of several antigen targets from the excretory and secretory products of the parasite, which have been shown to be potential biomarkers for schistosomiasis diagnosis. The *S. japonicum* thioredoxin peroxidase-1 (SjTPx-1) has multiple biological functions, including its main function as a key enzyme that combats reactive oxygen species. Immunohistochemistry demonstrated the presence of SjTPx-1 in all life stages of *S. japonicum* as well as its extensive distribution on the surface tegument of the parasite, and the tissues surrounding the egg in the liver. Additionally, this enzyme was recently identified as a component of the excretory and secretory products of the adult worm, thus making SjTPx-1 a promising candidate for an antigen-based serodiagnosis.

In this study, SjTPx-1 was evaluated for its potential use as target antigen for antigen-based serodiagnosis of *S. japonicum* infection. Recombinant SjTPx-1 (rSjTPx-1) was expressed as a hexahistidine-tagged fusion protein. Rabbit polyclonal antibody (pAb) and mouse monoclonal antibody (mAbs) were raised against the recombinant protein. The antibodies produced against the rSjTPx-1 can detect the native form of the antigen in crude adult worm lysate with Western blotting. This confirms that the recombinant antigen produced was antigenically similar to the naturally existing SjTPx-1 from the parasite. Since TPx-1 is conserved throughout the animal kingdom, the specific binding of mAbs to parasite TPx-1 but not to mammalian orthologues was

also verified. The mAbs were highly specific to SjTPx-1 characterized by a positive reaction only to the parasite TPx-1 but not to the mammalian orthologues, represented by *Mus musculus* Prx-1 and *Homo sapiens* Prx-1. After verification of the specific binding of the antibodies to be used, a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was developed. The rabbit pAb was used as a capture antibody while the mouse mAb was used as a detection antibody. This method was able to detect at least 1 ng/ml of rSjTPx-1 antigen comparable to the detection limit reported in previous studies using different circulating antigen targets. In addition, this method was able to detect the antigen from all serum samples of experimentally infected rabbit and mice at 11 and 8 weeks after the cercariae challenge. The diagnostic potential of SjTPx-1 in human clinical samples was also evaluated, in which 4 out of 10 serum samples collected from stool-confirmed patients had detectable levels of SjTPx-1. The results suggest that this antigen has the potential to be used as a biomarker for *S. japonicum* infection.

The emergence of new schistosomiasis endemic foci is a growing concern mainly attributed to human and animal migration. The lack of an effective screening method which is both sensitive and specific is urgently needed in order to prevent the introduction of this disease in areas where the snail intermediate host is present. Molecular means of disease diagnosis such as indirect ELISA are known to be highly sensitive and specific as well as able to detect early infection. Early disease diagnosis is beneficial such that prompt medical attention is given to patients thus preventing disease progression. In the animal health sector, improved animal quarantine and treatment is expected. Thus, the development of a sensitive and specific method of diagnosis is

required.

S. japonicum cathepsin B (SjCatB) is a protease expressed throughout the life stages of the parasite. This enzyme is highly expressed in the infective cercariae giving it an early exposure to the host immune system. In this study, the full length coding sequence of SjCatB was cloned and expressed as a fusion protein containing a hexahistidine tag on the N-terminus. This recombinant antigen (rSjCatB) was tested for its performance in an indirect ELISA format. rSjCatB showed a sensitivity of 100 % and a specificity of 95.0% in the ELISA against the serum samples collected from experimentally infected mice at >8 weeks post-infection. As early as 6 weeks post-infection, 2 out of 4 mice were detected positive in the ELISA. The recombinant antigen marked a sensitivity of 86.7 % and a specificity of 96.7 % in the ELISA against the serum samples collected from stool-confirmed patients. In addition, serum samples collected from humans infected with other parasites had minimal cross reactivity with rSjCatB. Data in this study suggests that SjCatB is a highly sensitive and specific diagnostic antigen capable of early detection of *S. japonicum* infection.

By taking advantage of the strengths of both antigen and antibody based serodiagnostic assays, I envision that these tools will improve the surveillance efforts done in assessing the effective implementation of control strategies that will lead to the successful elimination of *S. japonicum* infection in endemic areas.

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