Assessment Of The Acute Phase Response In Experimental Infection Of Mice With Schistosoma Mansoni

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Citation

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Abstract

Male BALB/c mice were infected with a single cercarial dose of Schistosoma mansoni and later treated curatively with praziquantel on Day 42 post-infection. Serum from the animals was studied for changes in concentration of haptoglobin, albumin, iron, zinc and copper. The infection resulted in significant decreases in serum albumin, iron and zinc, and increases in serum haptoglobin and copper. Haptoglobin showed the most sensitive responses with a peak increase of 257% of pre-infection levels achieved. Copper, however, showed only very gradual increases, which peaked, at 16% of pre-infection concentrations. Albumin levels showed a gradual but steady decrease to reach 48.7% of pre-infection concentrations. Iron showed similar trends with maximal decrease of 48.9%. Zinc levels dropped fairly rapidly following infection and were decreased maximally to 66.4%. The post-infection changes demonstrated recovery following treatment, though pre-infection concentrations were not achieved. The protein and cation changes correlated well with the degree of tissue damage and inflammation evidenced in the histopathological studies of infected mice. The study demonstrated that elevation in serum haptoglobin and copper, and depression in serum albumin, iron and zinc concentrations occur in the acute phase of S. mansoni infection of mice, and that these changes recover with reducing tissue damage and inflammation, and are therefore good indicators of tissue pathology.

INTRODUCTION

In response to bacterial and parasitic infections, the host mounts an acute phase response which is characterized by fever, leukocytosis, increases in the erythrocyte sedimentation rate, and secretion of ACTH and glucorticoids, decreases in serum levels of iron and zinc, and increases in serum levels of copper, a negative nitrogen balance and dramatic changes in the concentrations of acute phase proteins (Heinrich et al, 1990; Baumann & Gauldie, 1994; Steel and Whitehead, 1994). These changes are attributed to the host response to tissue injury and are therefore useful indicators of the course and severity of a disease (Alsemgeest et al, 1994). The purpose of the acute phase response is to prevent further injury to the organ, to isolate and destroy the infective organism, to remove the harmful molecules and debris, and to activate the repair processes that are necessary to return the organ to its normal function (Dinarello, 1984; Baumann & Gauldie, 1994).

The acute phase response is mediated by the release of certain cytokines. It has been shown in murine intestinal schistosomiasis that a type I (Th-1 type) immune response with increased interferon-gamma (IFN-) predominates in early infection, but the type of immune response changes as egg production and tissue reaction begins (Correa-Oliveira et al, 1998). Decrease in IFN- as infection progresses is accompanied by an increase in IL-10 and granuloma formation (Coutinho et al, 2000). The earliest hepatic granulomas, therefore, form in a Th-1 environment, with down-regulation of Th-1 and up-regulation of Th-2 responses six weeks after infection (Todt et al, 2000). The granuloma is conceptualised as a Th-2 dominant reaction, but under some conditions, Th-1 granulomatous response may be predominant and damaging (Rutitzkky et al, 2001). In chronic infections, considered as 20 weeks and over, these responses are less marked (Henderson et al, 1992). Borojevic (1992) regards the chronic phase of murine schistosomiasis as predominantly Th-1 mediated. Studies in murine schistosomiasis also demonstrate that the development of fibrosis requires the production of the profibrotic cytokines IL-2 and IL-4, and is suppressed by IL-12 and IFN-. (Cheever et al, 1998; Correa-Oliveira et al, 1998). Measurement of cytokines, however, is hampered by their low and transient concentrations in plasma. Acute phase proteins and serum cations, however, are more stable in circulation and can be measured as a means of assessing the systemic cytokine response in schistosomiasis mansoni.

MATERIALS AND METHODS

PARASITES

Biomphalaria pfeifferi snails were obtained from Kakuyani location, Machakos district, Kenya. They were screened by exposure to strong light to ensure that they did not have any schistosome infection. The snails were placed in aerated plastic trays in a snail room at the Institute of Primate Research, Nairobi. The room temperature was maintained at 25-28 °C with 12 hours of light /12 hours of darkness. The snails were fed on lettuce throughout the experimental period. The snails were then infected individually with 3-6 miracidia, artificially hatched from eggs of Schistosoma mansoni harvested from infected baboon faeces. The infected snails were maintained under the same conditions for four weeks, after which they were put in the dark until they were required for cercarial shedding. Cercariae for infection were then obtained by exposing the infected snails to artificial heat (28°C) and light (100 watt lamp) for 1-3 hours.

HOSTS

Seventy six-week-old male BALB/c mice, inbred at the Institute of Primate Research (Nairobi, Kenya), were used for the experiment. The animals were housed in groups of five per cage and fed on commercial pellets and provided with water ad libitum. They were kept under a natural dark: light cycle of about 12/12 hours, at an ambient temperature of 20 1°C and relative humidity of 50-60%.

The mice were each infected with approximately 110 cercariae as described by Smithers & Terry (1965). Uninfected mice served as controls. Five mice were sacrificed, under anaesthesia, at every sampling point. The mice were sampled before infection, and weekly following infection. Thirty mice were treated on Days 42 and 44 post-infection with two equal oral doses of praziquantel, at a curative dosage rate of 450mg/kg body weight. The mice were also sampled weekly thereafter.

BLOOD COLLECTION

Blood from the mice was collected by heart puncture. The blood collected at each sampling point was then pooled, allowed to clot and centrifuged. The serum collected was stored at -70°C ready for analysis.

HAPTOGLOBIN DETERMINATION

Haptoglobin (Hp) was measured using the method described by Makimura & Suzuki (1982) with modifications by Conner et al, (1988). The assay uses purified bovine Hp as standard. This test is based on the ability of Hp to bind to haemoglobin (Hb) and retain peroxidase activity at acidic pH, whereas free Hb loses its peroxidase activity. On addition of the substrate, peroxidase activity resulted in a proportionate colour change, which was read off on an ELISA plate reader at 450nm.

ALBUMIN DETERMINATION

Serum albumin was determined spectrophotometrically using bromocresol green solution as described by Varley (1964). The test uses bovine albumin as a standard. It is based on the formation of a coloured complex by albumin in citrate buffer and bromocresol green. The absorbance of this complex is proportional to the albumin concentration in the sample. Absorbance was measured at 578nm for both test samples and standard.

DETERMINATION OF SERUM CATIONS

Iron, zinc and copper concentration determinations were essentially carried out as described by Passey et al, (1985). The serum was digested with spectrosol grade concentrated nitric acid, diluted with de-ionised water and analysed by aspiration into an atomic absorption spectrophotometer. Iron concentrations were measured at a wavelength of 248.3nm and a current of 8mAmps, within a linear range of up to 5 parts per million (ppm). Zinc concentrations were measured at wavelength 213.9nm, current 3mAmps, within a linear range of up to 0.4ppm. Copper concentrations were measured at wavelength 324.7nm, current 5mAmps, within a linear range of up to 4ppm.

HISTOPATHOLOGICAL EXAMINATION OF HEPATIC TISSUES

From Day 42 post-infection and following treatment, livers from sacrificed mice were collected and fixed in 10% buffered formalin. The fixed tissue samples were dehydrated using ethyl alcohol, and then embedded in paraffin wax. Six-micrometer thick sections were cut using a rotary microtome, and stained with haematoxylin and eosin (H/E). The sections were then studied for histopathological changes under a light microscope.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using SPSS and Excel software programmes. Excel was used to graphically depict trends in the analytes measured in infected animals and in uninfected controls. The graphs were then pasted and labelled in MS Word. The data was analyzed for statistical significance at $P < 0.05$ by one-way ANOVA with

Duncan's Multiple Range Test (DMRT).

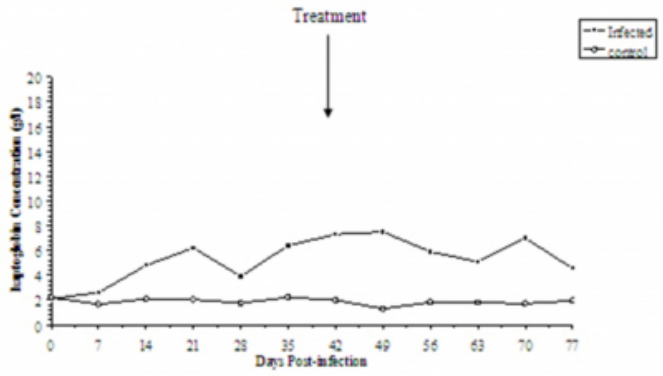
RESULTS

CHANGES IN PROTEIN CONCENTRATIONS

Following infection of the mice, there was a gradual increase in mean serum haptoglobin concentrations, as shown in Figure 1. The increase was observed from Day 7 post-infection, where levels rose from pre-infection concentrations of 2.10g/l to 2.6g/l. The increase was sustained until Day 42, when mean concentrations of haptoglobin reached 7.30g/l. Following curative treatment with praziquantel on Day 42, the haptoglobin levels continued to increase until Day 49 post-infection, reaching a peak of 7.5 g/l. Thereafter, there was a gradual decline in levels to reach 4.6g/l on Day 77 post-infection. Post-infection concentrations, however, remained significantly different from those of uninfected controls at $P<0.05$.

Figure 1

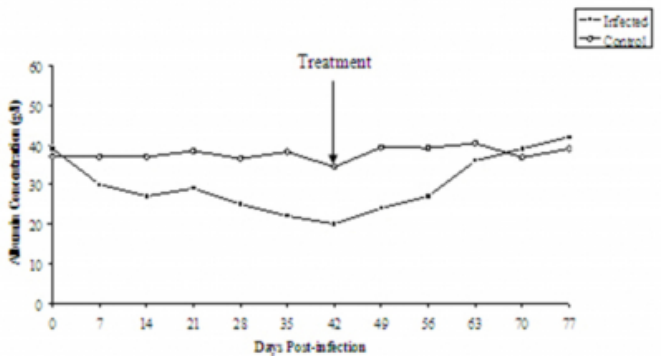
Figure 1: Mean Serum haptoglobin concentrations in control and infected mice



Changes in mean albumin concentrations of mice infected with S. mansoni and uninfected controls are depicted in Figure 2. Following infection, there was a gradual decline in albumin concentrations from a mean pre-infection concentration of 39g/l. This decline persisted until Day 42 post-infection when the mean albumin concentration was down to 20g/l. Following treatment at Day 42 post-infection, the albumin levels gradually increased to reach levels of 40g/l by Day 77 when the experiment was terminated. Albumin concentrations in the infected animals differed significantly ($P<0.05$) from those of uninfected controls, until Day 63 post-infection when concentrations rose to 36g/l.

Figure 2

Figure 2: Mean Serum albumin concentrations in control and infected mice

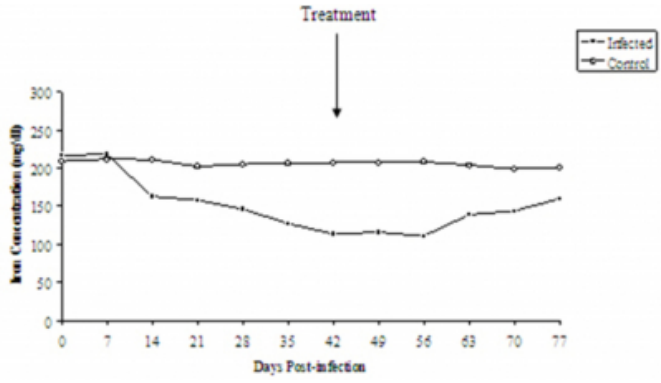


CHANGES IN CATION CONCENTRATIONS

Changes in mean iron concentrations of mice infected with S. mansoni and uninfected controls, are depicted in Figure 3. The mean iron concentrations in the infected animals decreased gradually from pre-infection levels of 217 g/dl to reach levels of 114 g/dl by Day 42 post-infection, when the mice were curatively treated with praziquantel. The iron levels remained around treatment concentrations for two weeks after which they gradually increased to 160 g/dl by Day 77 post-infection. Iron concentrations in the infected animals remained significantly different, at $P<0.05$, from those of the uninfected controls throughout the experiment.

Figure 3

Figure 3: Mean Serum iron concentrations in control and infected mice

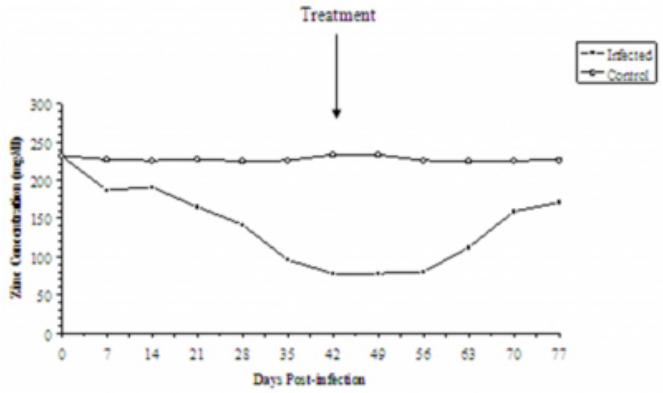


Changes in mean zinc concentrations of mice infected with S. mansoni and uninfected controls, are depicted in Figure 4. Zinc concentrations in the infected mice gradually declined from pre-infection concentrations of 232 g/dl in the initial two weeks This was followed by a drastic decline where concentrations dropped to reach 78 g/dl by the time of treatment. Following curative treatment with praziquantel, zinc concentrations remained at treatment levels for about

two weeks after which the levels drastically increased to 171 g/dl at 77 days post-infection, when the experiment was terminated. Though post-treatment levels demonstrated drastic increases, they were still significantly lower ($P<0.05$) than levels in the uninfected controls.

Figure 4

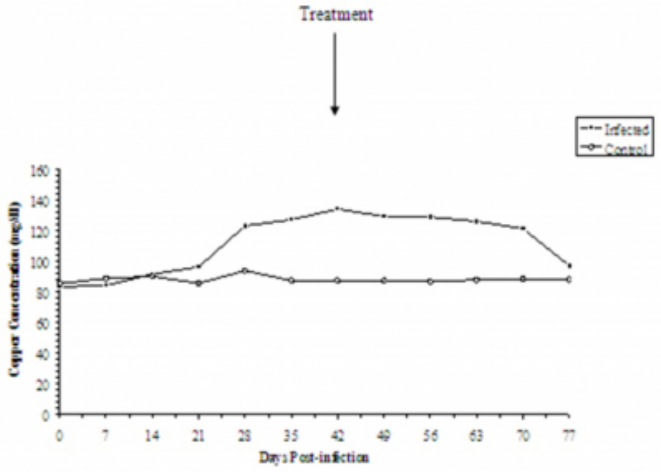
Figure 4: Mean Serum Zinc concentrations in control and infected mice



Changes in mean copper concentrations of mice infected with S. mansoni and uninfected controls, are depicted in Figure 5. Copper concentrations in the infected animals increased gradually following infection until Day 21, from pre-infection concentrations of 83.2 g/l to 96.0 g/l. After 21 days post-infection, the copper levels showed a drastic increase to reach a peak concentration of 134.0 g/l, 42 days post-infection. Following curative treatment with praziquantel on Day 42 post-infection, copper levels showed a slow but steady decline until the completion of the experiment on Day 77 post-infection, when mean concentrations were down to 97.0 g/l. Significant differences ($P<0.05$) between copper concentrations in the infected animals and in the uninfected controls were demonstrated from Day 14 post infection, and remained so throughout the experiment.

Figure 5

Figure 5: Mean Serum Copper concentrations in control and infected mice



PATHOLOGICAL FINDINGS

GROSS PATHOLOGY

The infected mice begun to lose body condition by Day 42 post-infection. They continued to deteriorate with progress of the disease, until after curative treatment with praziquantel. They also showed no visible granuloma on gross examination up to Day 49 post-infection, when their livers revealed presence of moderate-to-severe hepatic granuloma. By Day 56 post-infection animals had very poor body condition and numerous liver granuloma. The treated animals, however, appeared to have improved body condition and their livers had fewer granuloma. By Day 63 post-infection, the treated animals had regained good body condition. They showed only few granuloma, but still showed hepatomegaly and splenomegally. The treated animals at Day 70 post-infection generally had good body condition. They showed very few liver granuloma. However, they had pronounced enlargement of the liver, spleen, and appendix, with marked ascitis. On Day 77 post-infection, all treated animals had ascitis, enlarged livers and spleens.

HISTOPATHOLOGY

Hematoxylin and eosin (H & E) stained liver sections from the animals 42 days post-infection showed many trapped eggs with little tissue reaction around them. There were also some adult worms trapped in the tissues. There was minimal cellular infiltration by leukocytes and eosinophils within the hepatic tissue and around the blood vessels.

Day 49 animals showed trapped eggs with little cellular infiltration, similar to those seen in Day 42 sections. In addition, numerous pronounced granuloma with massive

tissue reaction around them were observed.

By Day 56 post-infection, there was massive tissue fibrosis, but the granuloma had started to resolve. Moreover, there was evidence of cellular infiltration, with the predominant cell-type changing from the eosinophils seen in previous sections to lymphocytes. Numerous pockets of lymphocytes were seen amidst normal hepatic tissue. In addition, unlike in the previous week, periportal fibrosis was observed.

By Day 63 post-infection, sections showed a slight reduction of fibrosis within the hepatic tissue, though massive periportal fibrosis and lymphocyte infiltration persisted. There was also evidence of massive bile duct hyperplasia.

Compared to Day 63, tissue fibrosis was significantly decreased by Day 70 post-infection, although periportal fibrosis was still present. Bile duct hyperplasia was still evident, with little cellular infiltration. A few adult worms were still trapped in blood vessels.

By Day 77 post-infection, fibrosis was greatly decreased, with most of the hepatic tissue having normalised. There was very little cellular infiltration. Only a few resolving granuloma were seen. No bile duct hyperplasia was evident, but periportal fibrosis could still be seen.

DISCUSSION

The present study demonstrated that the pathogenetic process of S.mansoni could be faithfully produced in mice models. Histopathological studies of hepatic tissue of infected mice revealed that granuloma formation peaks around weeks 6-7 post-infection. The development and modulation of granuloma, and eventual fibrosis in the liver that was experimentally induced in the mice is similar to that seen in humans infected with S. mansoni. Portal space enlargement and the preliminary stages of “pipestem” fibrosis were also demonstrated in the infected mice. This deducedly led to portal hypertension and the subsequent hepatomegally and splenomegally observed. Hepatic fibrosis may also be credited for increased pressure on the bile ducts, leading to the bile duct hyperplasia observed in the livers of some mice. The development of “pipestem” fibrosis agrees with studies done on mice infected with S. mansoni (Coutinho, 2004).

Following treatment with a curative dose of praziquantel, the histopathological effects of the schistosomal inflammatory processes regressed. By the termination of the experiment, most of the hepatic tissue fibrosis, bile duct hyperplasia and granuloma had resolved. However, there was still some peri-portal fibrosis. This could explain the continued hepatomegally, splenomegally and ascitis observed in the treated animals. Furthermore, these complications mitigated the inability to attain pre-infection levels seen in the various analytes. This study collaborates Zwingenberger et al's (1990) findings of cessation of the fibrogenetic processes and resolution of fibrosis following specific treatment of hepato-splenic schistosomiasis mansoni with praziquantel. Furthermore, slight regression of portal pathology was observed by the time the experiment was terminated. It may be inferred that with time, keeping in mind that this study covered a period up to only 5 weeks following treatment, more obvious regression of the periportal fibrosis may have been evident.

Haptoglobin has been described as a sensitive, specific and efficient disease marker in several animal species including cattle, sheep and dogs. It is also less likely to give false positive and negative results in comparison to other indicators such as haematology (Skinner & Roberts, 1994). These findings have been echoed in the present study, where serum haptoglobin levels were seen to respond significantly to S. mansoni infection and subsequently to recovery following curative treatment.

Slower responses, however, were observed in serum albumin levels. This could be due to the fact that the liver has large reserves of albumin synthetic capacity. In addition, albumin generally has a long plasma half-life (20 days in humans). These attributes could account for the slow responses observed.

The kinetics observed in serum iron during the study agrees with a study by Laudage & Schirp (1996), who found that schistosomiasis was a rare cause of iron-deficiency anaemia. This could partially be credited to the increase in haptoglobin observed in the study, as the haemoglobin-haptoglobin complex binds iron and preserves it, thus preventing iron deficiency anaemia. Serum iron has also been found to decline substantially in the acute phase response of a number of infections (Alsemgeest et al, 1994; Hayes, 1994; Mwangi et al, 1995).

Zinc also was seen to drop fairly rapidly following infection with S. mansoni. This hypozincemia occurs because of a sequestration of zinc within hepatocytes, thymocytes and marrow cells. The sequestration of zinc is the secondary consequence of the induced expression, caused by pro-inflammatory cytokines, of metallothionein 1 and 2 genes

within these responding cells (Vallee & Falchuk, 1993; Taylor, 1996).

The post-infection increase in serum copper observed in the study may be concluded to have been concomitant with increase in ceruloplasmin, as ceruloplasmin normally carries about 95% of the circulating copper (Danks, 1995; Taylor, 1996). A study by Mikhail & Mansour (1982), found plasma copper in patients with active S mansoni infection was significantly elevated and zinc was significantly depressed, and the degree of this correlated with the associated hepato-splenic complications of the disease. At clinical recovery, levels of copper and zinc were significantly improved in all the patients from pre-treatment values, but were within the normal range only in patients without complications. This indicated that complications associated with schistosomiasis could delay normalisation of copper and zinc levels beyond the clinical cure stage. These observations made by Mikhail & Mansour (1982), were echoed in this study when complications, including hepatomegally, splenomegally and ascitis, were seen to hinder attainment of pre-infection concentrations of the acute phase reactants.

The initial evidence of active S. mansoni disease is the Katayama syndrome. The incubation period of the Katayama syndrome ranges generally from 3-7 weeks in humans, and is associated with the onset of egg deposition. The acute phase reactants monitored in this study begun to demonstrate detectable changes in serum concentrations long before the onset of egg deposition, from as early as one week following infection, and remained elevated with the presence of residual pathology. This demonstrated the potential of the reactants as early indicators of inflammation.

The acute phase reactants monitored in the infected mice demonstrated changes that could be directly linked to the extent of tissue damage. The changes appeared to be in direct response to either regression or improvement of tissue damage. Haptoglobin, albumin, iron, zinc and copper, all showed maximum changes in serum concentration between week 6 and 8 post-infection. The histopathological studies of hepatic tissues of the mice revealed that it was in this period that there was peak granuloma presence, massive tissue fibrosis and periportal fibrosis. Thereafter, the effects of curative treatment with praziquantel begun to be evident as the granuloma and tissue fibrosis resolved. These improvements in the damaged hepatic tissues were also favourably reflected in the serum concentrations of the acute phase reactants. The reactants showed recovery to normal serum concentrations with the healing of tissue damage. They however did not achieve pre- infection concentrations, an indication of residual tissue pathology. This was supported by the continued hepatomegally, splenomegally and ascitis seen in the gross pathology of the mice.

The study demonstrated that there are significant changes that occur in serum proteins and cations during the acute phase of murine S. mansoni infection. The study has also indicated that the diagnosis, prognosis and monitoring of treatment in S. mansoni infection can be enhanced by monitoring changes of haptoglobin, albumin, iron, zinc and copper.

The application of measurements of acute phase reactants in diagnostics and in determination of disease prognosis would be of great value because the reactants are not species-specific, they are easy to measure and are not adversely affected by physiological variations. They are also good indicators of early disease and residual pathology.

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