تأثيرات البنتوكسيفللين و أنتيجينات البويضة الذائبة متحدة مع البرازيكوانتيل على البلهارسيا المعوية في الفئران

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٢٠٠٨

EFFECTS OF PENTOXIFYLLINE AND SOLUBLE EGG ANTIGENS IN COMBINATION WITH PRAZIQUANTEL ON MURINE SCHISTOSOMIASIS *MANSONI*

A Thesis

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(Immunoparasitology)

BY

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Amina Mohamed

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LIST OF ABBREVIATIONS

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Ag	Antigen
ALT	Alanine aminotransferase
APC	Antigen presenting cells
ART	Artemether
AST	Aspartate aminotransferase
BSA	Bovine serum albumin
b.wt	Body weight
CD_4^+	Cluster differentiation 4
CAA	Circulating anodic antigens
CCA	Circulating cathodic antigens
CDC	Centers for Disease Control and Prevention
CFA	Complete freund's adjuvant
CNBR	Cyanogenbromide
cDNA	Complementary DNA
DPDx	Division of Parasitic Diseases
ECM	Extra cellular matrix
ELISA	Enzyme-linked immunosorbent assay
FUC	Fucose- containing carbohydrate structure
G	Gravity force
g	Gram
GM	Granuloma macrophage
GST	Glutathione-s-transferase
HSC	Hepatic stellate cells
hr	Hour
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
IPSE	IL-4 inducing protein
i.v.	Intravenous
KOH	Potassium hydroxide

kDa	Kilo Dalton
M	Molar
mAb	Monoclonal antibody
M.G.D	Mean granuloma diameter
mg/ Kg	Milligram per kilo gram
M.G.N	Mean granuloma number
MHC	Major histocompatibility complex
min	Minute
ml	Milliliter
mM	Millimolar
mRNA	Messenger ribonucleic acid
MSA	Major serological antigens
Mw	Molecular weight
µg	Microgram
µm	Micrometer
nm	Nanometer
OD	Optical density
OPD	Ortho- phenylenediamine
Oxa	Oxaminiquine
PIII	*S. mansoni* adult worm anionic fraction antigen
PBMC	Peripheral blood mononuclear cells
PBS-T	Phosphate buffer saline-Tween
pH	Hydrogen ion concentration
PI	Post infection
PTX	Pentoxifylline
PZQ	Praziquantel
rIrv- 5	Radiation attenuated *S. mansoni* cercariae vaccine-5
S.	*Schistosoma*
SAWA	Soluble adult worm antigen
SBSP/TBRI	Schistosome Biological Supply Program Unit/ Theodor Bilharz Research Institute
SEA	Soluble egg antigen
SEM	standard error
sm	*S. mansoni*

smp	*S. mansoni* protein
Sm28GST	*S. mansoni* 28 kDa glutathione-s-transferase
TGF	Transforming growth factor
Th	T helper cells
TIMP-1	Tissue inhibitor of metalloproteinase 1
TNF-α	Tumor necrosis factor-α
U	Unite
WHO	World health organization
WHO/TDR	World health organization special program for Research and Training in Tropical Disease.
wk	Week (s)

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ARABIC SUMMARY

ABSTRACT

Name : Amina Mohamed Ibrahim

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"Effects of Pentoxifylline and Soluble Egg Antigens in Combination with Praziquantel on Murine Schistosomiasis *mansoni*"

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Schistosomiasis remains a public health problem in the developing world. The liver is one of the main target organs affected by *S. mansoni* infection, in which eggs are deposited resulting in granuloma formation. Because liver fibrosis is a reversible process, this research program aimed to investigate the effects of the administration of the antifibrotic agent Pentoxifylline (PTX) (alone or associated with Praziquantel [PZQ]), the soluble egg antigens (SEA) (alone or with the antifibrotic agent) and the combination of the three agents (PTX, PZQ and SEA) on the course of murine schistosomiasis *mansoni*, using different parasitological, histological, immunological and biochemical parameters. Data indicated that there was no significant effect between using PZQ alone or in combination with SEA and PTX.

Keywords: Schistosomiasis; *S. mansoni*; Praziquantel; Pentoxifylline; soluble egg antigens; Liver fibrosis; Granulomas; Combined treatment; Parasitological study; Histological study; Immunological study; Biochemical study.

المستخلص

تظل البلهارسيا المعوية مشكلة عامة فى العالم النامى، و حيث أن الكبد هو أحد الاعضاء الاساسية التى تتأثر بهذا المرض فان البويضات تترسب فيه و تؤدى الى تكون الاورام الحبيبية. و نظرا لان تليف الكبد عملية قابلة للعكس لذا فان برنامج هذا البحث يهدف الى دراسة التأثيرات الناتجة عن اعطاء عامل مضاد للتليف يسمى البنتوكسيفللين (اما منفردا أو متحدا مع البرازيكواينتل) و أنتيجينات البويضة الذائبة (منفردة أو متحدة مع البنتوكسيفللين أو البرازيكواينتل) و اتحادهم الثلاثة معا من خلال الدراسات الطفيلية و الهستولوجية و المناعية والبيوكيميائية.

و قد أوضحت النتائج أن الاتحاد بين الثلاثة عوامل له نفس تأثير البرازيكواينتل منفردا، لذا ليس من المفضل عمل الاتحاد بينهم.

Introduction

Schistosomiasis is a chronic disease caused by digenetic trematodes of the genus *Schistosoma*, among which *S. mansoni*, *S. haematobium* and *S. japonicum* are the principal causative agents of the human disease (Abdel Hadi and Talaat, 2000). It continues to threaten millions of people, particularly the rural poor in the developing world (Chitsulo *et al*, 2002; Engels *et al*, 2002). It currently infects more than 200 million people in 74 countries worldwide in the endemic areas (Yosry, 2006). Dhawan *et al* (2008) reported that its prevalence is thought to be increasing.

The liver is one of the main organs affected by *S. mansoni* infection. Intestinal schistosomiasis causes severe histopathological changes and functional damage in the liver of the host (Leo and Peter, 1998). Perturbation in this tissue, caused by experimental infection with *S. mansoni,* includes the deposition of schistosome eggs and pigments, increase in organ size, extensive fibrosis, blockage of hepatic venules, reduction in portal blood flow and formation of granulomas surrounding the deposited eggs which may result in scarring, portal hypertension, haemorrhage and death (Leo and Peter, 1998; Pearce and Mac Donald, 2002; Gause *et al*, 2003).

Liver fibrosis is a wound-healing process that occurs when the liver is injured chronically (Friedman, 2003). Hepatic stellate cells (HSC) are responsible for the excess production of extracellular matrix (ECM) components (Dai and Jiang, 2001; Qing and Jiang, 2001). The activation of HSC, a key issue in the pathogenesis of hepatic fibrosis (Bartley *et al*, 2006), is mediated by various cytokines and reactive oxygen species released from the damaged hepatocytes and activated Kupffer cells. Therefore, inhibition of HSC activation and its related subsequent events, such as increased production of ECM components and enhanced proliferation, are crucial goals for intervention in the hepatic fibrogenesis cascade (Wu and Zern, 2000).

At present, chemotherapy is the most effective method for short term control of schistosomiasis (WHO, 1993). The most commonly used drug for clinical management of schistosomiasis is

praziquantel (PZQ). PZQ is the current drug of choice; single oral doses are well tolerated and show high cure and egg reduction rates against all human schistosome parasites (WHO, 2002). The development of liver fibrosis in murine schistosomiasis is prevented with early administration of the drug (El-Badrawy *et al,* 1991) and it may even be effective in reversing fibrosis in certain patients (Homeida *et al,* 1991). Martins-Leite *et al* (2008) stated that PZQ is effective in reducing the morbidity of schistosomiasis disease.

Although treatment with PZQ is effective and inexpensive (Cioli and Pica-Mattoccia, 2003), frequent problems are reported by Doenhoff *et al* (2002). Exposure of schistosomes to PZQ over generations resulted in drug resistant strains in human. Chemotherapy has little effect on already developed hepatosplenic manifestation and transmission. It also seems that chemotherapy alone is not suited for long term control, especially in endemic region (Cioli and Pica-Mattoccia, 2003). Barakat *et al* (1995) recorded diminished resistance to reinfection after treatment with PZQ which was accompanied by diminished hepatic granuloma diameter and number of $CD4^+$ T lymphocytes in the granuloma. This led to the suggestion that addition of antifibrotic chemotherapy, as an adjuvant to antihelminthic chemotherapy may be a future consideration (Wyler, 1992).

Pentoxifylline (PTX) is an immunomodulatory and antifibrotic agent (Reis *et al,* 2001; Raetsch *et al*, 2002). The main pharmacological action of PTX is to protect hepatocytes from excessive cytokines (transforming growth factor (TGF) - βI and II and platelet - derived growth factor) that are responsible for activation of HSC, by inhibiting them. Therefore, inhibition of HSC activation and its related subsequent events are crucial goals for the intervention in the hepatic fibrogenesis cascade.

Xiong *et al* (2003) concluded that high dose of PTX treatment could reduce significantly the content of hepatic TGF- β, type 1 and type 11 collagen, in schistosomiasis *japonica* mice with liver fibrosis, thus, playing its role of antifibrosis.

El-Lakkany and Nosseir (2007) assumed that prolonged treatment with PTX has a potent anti-fibrogenic role, especially, when used in early stages of infection, with limited toxic effects on schistosome worms and eggs. Thus, PTX can be used as an adjuvant therapeutic tool with anti-helminthic drugs in treatment of human schistosomiasis.

Since the pathology in schistosomiasis consists of granuloma formation around the parasite eggs (Williams *et al*, 2005; Abath *et al*, 2006), specific immunosupression with schistosome antigen via induction of immunological unresponsiveness was attempted (Weigle, 1973). Pacifico *et al* (2006) stated that vaccination of mice infected with *S. mansoni* with adult worm tegument antigen induced a Th0 type of immune response and protective immunity. Moderate reduction of granuloma size has been achieved in adult mice by intraperitoneal injection of large doses of soluble egg antigen (SEA) alone (Mc Curley *et al,* 1986). Recently, Bethony *et al* (2008) stated that the control of schistosomiasis depends not only on the drug administration but also on the development of vaccines. Earlier, Bergquist *et al* (2005) has recommended the combination of chemotherapy and vaccines to control schistosomiasis.

Aim of the work

This research program aims to investigate the effects of the administration of the antifibrotic agent PTX (alone or associated with PZQ), the soluble egg antigen (SEA) (alone or with the antifibrotic agent) and the combination of the antifibrotic agent PTX, PZQ and SEA on the course of murine schistosomiasis *mansoni*, using different parasitological, pathological and immunological parameters.

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1-Life Cycle

Schistosomiasis is an infectious disease caused by trematode flatworms of the genus *Schistosoma.* A large number of schistosomes are known; however, only five appear to be primarily affecting man, these include *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. mekonji* and *S. haematobium* (Ross *et al*, 2002).

The three species of *Schistosoma*, *S. mansoni, S. haematobium and S. japonicum* have similar life cycle. Schistosomes are dioecious and the females neither attain sexual maturity nor migrate out of the liver until the male clasps it in the gynaecophoric canal and hence they reproduce sexually. The life cycle includes two hosts, a definitive host (man), where the parasite undergoes sexual reproduction, and a single intermediate snail host (El-Ansary and Al-Daihan, 2006), where a number of asexual reproductive stages develop. Adults of *S. mansoni* reside in the inferior mesenteric veins where the female worms produce eggs that are deposited in the liver, intestine or other tissues, depending on the infecting species and released in excreta to complete the cycle (Bergquist *et al*, 2005). Some eggs pass through the intestinal wall to be deposited with the feces or are carried by the blood flow into the liver, where they induce vigorous granulomatous response (Capron and Dessaint, 1992). These mature eggs hatch only in freshwater, releasing a ciliated larval stage the miracidium. This free living larval stage must penetrate a compatible freshwater snail within a period of several hours (hr) for the life cycle to continue (Dunne and Vennervald, 2003). The liberated miracidium penetrates the snail host (*Biomphalaria alexanderina* for *S. mansoni*) (Yoshino *et al*, 2001), loses its cilia and transforms into a saculated primary sporocyst which in turn develops into daughter sporocysts. These migrate to the snail's digestive gland where they grow and multiply, eventually releasing many unisexual cercariae through out the snail tissues into the water (Bergquist *et al*, 2005). When cercariae locate their definitive host, they penetrate its skin with the aid of the lytic proteinase. This process is quite rapid and then cercariae transform into schistosomula, which