EFFECTS OF ARACHIDONIC ACID ON *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA HAEMATOBIUM* WORMS

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ABSTRACT

Name: **Marwa Mohsen Abou Eldahab**

Title of the thesis: **Effects of Arachidonic Acid on *Schistosoma mansoni* and *Schistosoma haematobium* Worms.**

Key Words: ***Schistosoma mansoni, Schistosoma haematobium,* Unsaturated Fatty Acids, Arachidonic Acid, Schistosomiasis.**

Development of arachidonic acid (ARA) for the treatment of schistosomiasis *mansoni* and schistosomiasis *haematobium* is a novel approach. It is based on the break down of the life cycle of schistosomes by the activation of the neutral sphingomyelinase (nSMase) bounded to the parasite tegument, using unsaturated fatty acids, such as ARA, which leads to the exposure of lung stage larvae surface membrane antigens to antibody binding, as well as, eventual attrition of developing schistosomula and adult worms.

5 mM ARA was found to lead to irreversible killing of ex vivo 3-, 4-, 5-, and 6-week-old *S. mansoni* and 8-, 10-, and 12-week-old *Schistosoma haematobium* worms within 3 to 4 h, depending on the parasite age. ARA-mediated lethal action was prevented by nSMase inhibitors, such as CaCl2 and GW4869. Scanning and transmission electron microscopy revealed that ARA-mediated worm killing was associated with spine destruction, membrane blebbing, basal vacuolation associated with the basal lamina, fragmentation of muscles and disorganization of the apical membrane structure.

ARA-mediated *S. mansoni* and *S. haematobium* worm attrition was reproduced *in vivo* in different experiments using pure ARA oil, ARA capsules, ARA oil/ARA infant milk formula (ARA milk) or ARA capsule/ARA infant milk formula (ARA milk). Total worm burden and egg counts were significantly reduced (30 to 60%) in *S. haematobium* infected hamster groups,

and this reduction was seen in both *S. mansoni* and *S. haematobium* by adding ARA-Nestle infant milk formula. *S. haematobium* was more susceptible to ARA as antischistosomal drug than *S. mansoni* worms. This was supported by detecting significantly higher antibody titres against schistosomal antigens in *S. haematobium* infected hamster groups. Electron microscopy revealed *in vivo* destructive tegumental effect on the worms.

Tumour necrosis factor-α mRNA was expressed in *S. mansoni* treated and infected control groups in addition to interferon-γ in infected control and ARA oil treated groups indicating the persistence of a highly pro-inflammatory Th1-like response beyond the acute phase of infection. Transforming growth factor-β m RNA was also detected in ARA oil and ARA capsules treated hamster groups, which has a down regulatory effect. These results were supported by the significant decrease ($P < 0.01$) in granuloma diameter by 43-39% in ARA oil and ARA capsule treated groups, respectively.

ARA is already marketed for human use in the United States and Canada for proper development of newborns and muscle growth of athletes; thus, ARA has potential as a safe and cost-effective addition to antischistosomal therapy.

AIM OF THE WORK

Long Term Objective:

Development of a novel oral treatment for schistosomiasis *mansoni* and schistosomiasis *haematobium* based on a natural source (arachidonic acid ARA).

Short Term Objectives:

***In vitro* studies** were carried out to assess the effect and mechanism of ARA on *S. mansoni* and *S. haematobium* juvenile and adult stages by testing:

- The effect of ARA concentration.
- The effect of fetal calf serum (FCS).
- The effect of ARA inhibitors.
- ARA effect on juvenile and adult worms' tegument by EM (Scanning and Transmission).

***In vivo* studies** were carried out to examine the efficacy of different formula of ARA as a novel drug on both *S. mansoni* and *S. haematobium.* Determine the best regimen and its efficacy by different parasitological and immunological techniques.

CONTENTS

LIST OF ABBREVIATIONS

ADP	Adenosine-5-diphosphate.
ATP	Adenosine-5-tiphosphate.
AP	Aminophenazone.
ARA	Arachidonic acid.
A-SMase	Acid sphingomyelinase.
bp	base pair.
BSA	Bovine serum albumin.
$CaCl_2$	Calcium chloride.
cDNA	Complementary DNA.
dATP	Deoxyadenosine triphosphate.
dCTP	Deoxycytidine triphosphate.
dGTP	Deoxyguanosine triphosphate.
dTTP	Deoxythymidine triphosphate.
DAP	Dihydroxyacetone phosphate.
DHA	Docosahexaenoic acid.
DMSO	Dimethyl sulphoxide.
DNA	Deoxyribonucleic acid.
EDTA	Ethylenediamine tetra-acetic acid.
ELISA	Enzyme-linked immunosorbent assay.
FA	Fatty acids.
FCS	Fetal calf serum.
FGS	Female genital schistosomiasis.
For.	Forward.
G3P	Glycerol-3-phosphate.
GPD	Glycerol phosphate dehydrogenase.
GSH	Tripeptide glutathione.
GW4869	Inhibitor of nSMase.
h	hour(s).
HIV	Human immunodeficiency virus.
H_2O_2	Hydrogen peroxide.
IDT	Integrated DNA Technologies.
Ig	Immunoglobulin.
IF	Immunofluoresence.
IFN-γ	Interferon-gamma.

IL	Interleukin.
kDa	kilo Dalton.
LPL	Lipoprotein lipase.
MBCD	Methyl- β-cyclodextrine.
$MgCl_2$	Magnesium chloride.
$Mn(OAc)_2$	Manganese acetate.
Na_2CO_3	Sodium carbonate.
NH_4Cl	Ammonium chloride.
$NaHCO_3$	Sodium bicarbonate.
Na_2CO_3	Sodium carbonate.
NaH_2PO_4	Dibasic sodium phosphate.
Na_2HPO_4	Monobasic sodium phosphate.
N-SMase	Neutral sphingomyelinase.
Oxa	Oxamniquine.
OZs	Secondary ozonides (Trioxolanes).
PAF	Platelets-activating factor.
PBMCs	Peripheral blood mononuclear cells.
PBS	Phosphate buffered saline.
PBS-T	Phosphate buffered saline-Tween.
PKC	Protein kinase C.
PLC	Phospholipase C.
POD	Peroxidase.
PZQ	Praziquantel.
RA	Radiation-attenuated cercariae.
Rev.	Reverse.
RNA	Ribonucleic acid.
r.p.m	round/minute.
RPMI medium	Rosewell park memorial institute medium.
RT-PCR	Reverse transcription-polymerase chain reaction.
SBSP	Schistosome Biological Supply Program.
SD	Standard deviation.
SEM	Scanning electron microscopy.
SM	Sphingomyeline.
SMase	Sphingomyelinase.
SMP	Surface membrane antigens.

Sup	Supernatant.
TBE	Tris / Boric Acid / EDTA.
TBRI	Theodor Bilharz Research Institute.
TEM	Transmission electron microscopy.
TGR	Thioredoxin glutathione reductase.
TGF-β	Transforming growth factor-beta.
Th1	T helper cell type 1.
Th2	T helper cell type 2.
TNF-α	Tumour necrosis factor-alpha.
TPA	Phorbol esters.
UV	Ultra Violet.
WHO	World Health Organization.

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