The Effect of a Cysteine Protease Inhibitor on *Schistosoma mansoni* : In Vitro and In Vivo Experimental Studies

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Dedication

Dedicated to my supportive and understanding ***husband****, my caring and loving* ***mother,*** *the most wonderful* ***father*** *and the sunshine of my life* ***Abdullah*** *and* ***Ali.***

Thank you for your support, really without you I would have never reached this point in my life.

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List of Abbreviations

PBS:	Phosphate buffer saline
µg/:	Microgram per milliliter
ml:	Milliliter
mg:	Milligram
CPs:	Cysteine proteases
CPIs:	Cysteine protease inhibitors
Ca^{2+}:	Calcium ion
DMSO:	Dimethyl sulphoxide
e.g.:	Example
FMK:	Fluoromethyl ketone
Fig.:	Figure
gm:	Gram
HDACs:	Histone deacetaylases
Hx and E stain:	Hematoxlin and Eosin stain
IC_{50}:	Half maximal inhibitory concentration

IL:	Interleukin
kg:	Kilogram
kOH:	Potassium hydroxide
pi:	Post infection
PVS:	Pheyl vinyl sulphone
PZQ:	Praziquental
S.:	*Schistosoma*
SBSP:	Schistosome Biological Supply Program
SmCB1:	*S.mansoni* cathepsin B1
SmCB2:	*S.mansoni* cathepsin B2
SmCD:	*S.mansoni* cathepsin D
SmCL1:	*S.mansoni* cathepsin L1
SmCL2:	*S.mansoni* cathepsin L2
SmCL3:	*S.mansoni* cathepsin L3
spp.:	Species
TBRI:	Theodor Bilharz Research Institute
Th1:	T-helper 1
Th2:	T-helper 2
TSA:	Trichostatin A

U:	International unit
VPA:	Valproic acid
VS:	Vinyl sulphones
wks:	Weeks
WHO:	World health organization
%:	Percent
>:	Greater than
<:	Less than
°C:	Degree Celsius

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Abstract

The drug of choice for treatment of schistosomiasis *mansoni* is praziquantel (PZQ) which has the possibility of development of drug resistance. Cysteine proteases play several roles in the biology of *S.mansoni*. Thereby, cysteine protease inhibitors (CPI) are possible new chemotherapeutic agents for schistosomiasis *mansoni*. The present work aimed to study the effect of phenyl vinyl sulphone (PVS), a CPI, on different stages of *S.mansoni* in an *in vitro* culture study and in experimental schistosomiasis *mansoni*, compared to PZQ. As regards the *in vitro* study, different concentrations of PVS (1µgm/ml, 2 µgm/ml, 4 µgm/ml, 6 µgm/ml, 8 µgm/ml and 10 µgm/ml) and PZQ (1µgm/ml) were assessed, by dose response studies in the form of % worm mortality for schistosomula and adults, and growth inhibition studies in the form of hemoglobin degradation by schistosomula and oviposition by adult worms. PVS caused death of schistosomula and adults in a concentration and time dependent manner. The 10 µgm/ml PVS resulted in 90% schistosmula mortality by day 1 reaching 100 % by day 2, a mortality rate higher than that by PZQ, 92 % adult worms mortality by day 5 reaching 100 % by day 6, and in arrest of hemoglobin degradation by schistosomula. No ova were detected in all culture wells containing PVS or PZQ or negative control. *In vivo* study included 8 groups of mice. Intraperitoneal PVS, subgroub a; (50 mg/kg/mouse/day for 7 days) and oral PZQ subgroup b; (500 mg/kg/mouse /day for 2 consecutive days) were assessed at different durations post infection (pi); at 1, 3, 5 and 7 weeks (wks) pi (groups I, II, III and IV, respectively), infection, PVS, PZQ, normal control (groups V-VIII) were included. The anti-schistosomal effects of PVS were assessed by parasitological parameters, histopathological parameters and haematological parameter. PVS decreased fecal egg count when given at different

durations pi, with significant decrease ($p \leq 0.05$) when given at 5 and 7 wks pi compared to infection control, and nonsignificant difference ($p > 0.05$) when given at 1, 5 and 7 wks pi compared to PZQ. PVS given at different durations of study showed a nonsignificant decrease in total worm burden compared to infection control. Each of PVS and PZQ showed significant ($p \leq 0.05$) decrease in mean egg count / gm intestine and liver when given at 1, 3, 5 and 7 wks pi compared to infection control. PVS given at 7 wks pi resulted in significant ($p \leq 0.05$) decrease in mean egg count / gm intestine and liver than when given at 1, 3 and 5 wks pi, and at 1 wk pi than when given at 3 wks pi. PVS given at 5 and 7 wks pi caused a significant ($p \leq 0.05$) decrease in mean egg count/gm intestine compared to PZQ. PVS given at 7 wks pi showed a significant increase in dead ova in intestine compared to each of infection control, and when given at 1, 3 and 5 wks pi. Each of PVS and PZQ given at 1 and 3 wks pi resulted in a nearly similar histopathological picture as infection control. At 5 and 7 wks pi, each of PVS and PZQ resulted in fibrocellular granulomas. Each of PVS and PZQ resulted in a significant decrease in mean hepatic granuloma size when given at all durations of study compared to infection control, with PVS resulting in smaller granuloma size than PZQ when each was given at 3, 5 and 7 wks pi. Each of PVS and PZQ resulted in a significant decrease in mean hepatic granuloma number when given at 3, 5 and 7 wks pi compared to infection control, and when PVS was given at each of 5 and 7 wks pi compared to each of 1 and 3 wks pi. The use of PVS increased mean hemoglobin concentration at different durations of study giving a significant ($p \leq 0.05$) increase in mean hemoglobin concentration when given at 1, 5 wks pi compared to infection control.

Keywords: *Schistosoma mansoni*, experimental schistosomiasis *mansoni* cysteine protease inhibitor, phenyl vinyl sulphone, praziquantel, *in vitro*.

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