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Antischistosomal Activity of 3-Substituted-5-(2-Aryl-2-Oxoethyl) -2, 4-Dioxo-1, 3-Thiazolidine (Ro-354)

HODA A. TAHA[1] AND MOHAMMAD I. SOLIMAN
Zoology Department, Faculty of Science, Ain Shams University, Cairo 11566, Egypt
[1]Corresponding author's e-mail: drhodataha@yahoo.co.uk

ABSTRACT

Ro-354 [3-substituted-5-(2-aryl-2-oxoethyl) -2, 4-dioxo-1, 3-thiazolidine] is a new rhodanine derivative that thought to have an antihelminthic activity. Several studies reported that different thiazolidine or rhodanine derivatives are considered as potent anthelminthic compounds. Administration of three doses to three groups of infected mice 30 day post-infection resulted in reduction of worm burden, granuloma number and diameters in infected livers of the three mice groups. Ro-354 causes severe tegumental alterations in *Schistosoma mansoni* weeks after drug administration. Swelling, vacuolization, fusion of the tegumental ridges and loss or shortening of the spines on the tubercles, erosion, cracks and peeling were observed. The range and extent of these changes differed according to the treated dose resulting in generalized deformities in these worms.

Key Words: Rhodanine derivative (Ro-354); *Schistosoma mansoni*; SEM; Liver granuloma

INTRODUCTION

Schistosomiasis is a chronic and debilitating disease that is caused by parasitic trematode worms. It continues to threaten millions of people, particularly the rural poor in the developing world (Chitsulo *et al*., 2000; Engels *et al*., 2002). Of the estimated 200 million infected people, more than half have symptoms and 20 million exhibit severe disease manifestations. There are five species of schistosomes that can infect humans, of which *Schistosoma mansoni*, *S. japonicum* and *S. haematobium* are the most important ones. While infection with the former two species is associated with chronic hepatic and intestinal fibrosis, infection with *S. haematobium* can lead to ureteric and bladder fibrosis and calcification of the urinary tract (Utzinger *et al*., 2000; Ross *et al*., 2002).

Since 1977, the pattern of schistosomiasis in Egypt changed as the prevalence of *S. mansoni* infestation increased and of *S. hematobium* decreased. This change has important public health implications, because the hepatosplenic schistosomiasis caused by *S. mansoni* is more difficult to trace and is associated with more morbidity and mortality than the urinary schistosomiasis caused by *S. hematobium* (Abdel-Wahab *et al*., 1980).

The use of antischistosomal drugs in the control of schistosomiasis still occupies a leading position, although the future of these drugs is thought to be imperiled by the possible emanation of drug resistant strains of the parasites (Coles & Bruce, 1987).

Ultrastructural studies of the tegument damage have been performed using several antischistosomal drugs such as Praziquantel (Becker *et al*., 1980; Shaw & Erasmus, 1983, 87; Shaw, 1989) Oxamniquine (Khon *et al*., 1982; Popeil & Erasmus, 1984), Hycanthone (Moore, 1977) and Ro15-5458 (Fawzi, 1999).

The only antibilharzial drug, which is effective against the three main schistosomes pathogenic for humans is praziquantel (PZQ) (Gonnert & Andrews, 1977). It has minimal side effects (Katz *et al*., 1979), but the control of schistosomiasis with PZQ at the population level faces problems, including the development of drug resistance (Liang *et al*., 2001). Reduced cure rates and the failure of treatment after PZQ treatment have been reported in patients (Fallon *et al*., 1995; Ismail *et al*., 1996; Lawn *et al*., 2003). The development and use of new effective drugs remains urgent challenges until a successful vaccine is produced.

Thiazolidinones are the derivatives of rhodanine, which belong to an important group of heterocyclic compounds. Thiazolidinones, with a carbonyl group at position 2, 4, 5 have been subject of extensive study in the recent past (Singh *et al*., 1981). The thiazolidinic nuclei represent a category of compounds that present promising biological activities: insecticidal, antimicrobial, antifungal, narcotic, sedative, anesthetic, anticonvulsive and nematocidal (Silva *et al*., 2003). Goes *et al*. (1991) showed that thiazolidine derivatives possess antifungal (against *Candida albicans* & *Newrospora crassa*) and moderate antibiotic activity (against *Escherichia coli* & *Salmonella aureus* & others).

Foye and Tovivich (1977) recorded antibacterial and antiviral activities of N-glucopyroanosyl-5-aralkylidemerhodanines. They recorded also some effects on blood sugar levels with several rhodanines.

Studies related to acute toxicity (LD_{50}) of some rhodanine derivatives [N-tryptophyl-5-(3, 5-di-tert-butyl-4-hydroxybenzylidine) rhodanine and N-tryptophyl-5 (3, 5-di-tert-butyl- 4 -hydroxybenzylidine) -2, 4 thiozalidinedione] in a range of oral doses of 25, 50, 100, 250, 500 and 1000

mg/kg body weight did not indicate any toxicity (Silva *et al*., 2003).

The present work was designed to assess the validity of 3-substituted-5-(2-aryl-2-oxoethyl)-2, 4-dioxo-1, 3-thiazolidine (Ro-354) as a new compound used as antischistosomal drug.

In the present study, the effect of treatment with Ro-354 on the tegument of adult *Schistosoma mansoni* worms has been examined in mice infected and treated 30 days post-infection using scanning electron microscopy. In addition, the effect of drug on the worm burden and granulomatous tissues in liver of different experimentally infected mice groups was investigated.

MATERIALS AND METHODS

Experimental animals and infection. Fourty albino CD1 male mice were supplied by the Schistosome Biological Supply Program (SBSP) at Thoedor Bilharz Resaerch Institute, Giza, Egypt. Each mouse was experimentally infected with 80 cercariae of *Schistosoma mansoni* by tail immersion method. Mice were randomly assigned into four equal groups of 10 animals each and housed with controlled temperature and light environment and fed on standard diet and normal drinking water.

Treatment regimes and drug. Ro-354 (3-substituted-5-(2-aryl-2-oxoethyl) -2, 4-dioxo-1, 3-thiazolidine) (Fig. 1) was prepared in Chemistry Department, Faculty of Science, Ain Shams University.

Thirty days post-infection, mice were divided into four equal groups, Group I was the infected Non-treated group, group II infected and treated with 50 mg/kg body weight of Ro-354, group III was treated with 100 mg/kg body weight, while group IV was treated with 200 mg/kg body weight. The above doses were chosen based on the LD_{50} of thiazolidine derivatives that previously investigated as 100 mg/kg body weight, so we tested the half and double values of the LD_{50}. For oral administration the compound was suspended in distilled water and given in a single dose of 50 mg/Kg body weight, 100 mg/Kg body weight and 200 mg/Kg body weight.

Worm recovery. Two weeks later, the worms were portally perfused from un-treated and drug treated mice by the perfusion technique described by Smithers and Terry (1965).

Histological preparation. The liver tissues were fixed in aqueous Bouin's solution and traditional paraffin sections were prepared. Hepatic granuloma counting and diameter measurements were done according to the method of Mahmoud and Warren (1974).

Scanning electron microscopy. Worms were fixed in 2.5% glutraldhyde in phosphate buffer for 24 h and post-fixed in 1% osmium tetroxide for 1 h. They were dehydrated through a graded series of ethanol, dried in critical point dryer. After drying, they were mounted and coated with gold in Ion sputtering apparatus. They were examined and photographed in Jeol scanning electron microscope at 60 kV.

Statistical analysis. The data of granuloma diameters and numbers were analyzed by one-way ANOVA test using a computer program named Graph Pad Prism Viewer.

RESULTS

The mean number of worms recovered from treated groups (II, III & IV) was lower than in the infected un-treated group I. In the treated groups, the lowest worm burden was obtained from mice treated with 50 mg/kg body weight of Ro-354 (7.5), followed by those treated with 200 mg/kg body weight (11.5). The highest worm burden from the treated groups was recorded in mice treated with 100 mg/kg body weight (12). The percentage of worm reduction was 53.1%, 25% and 28.1 in groups II, III and IV, respectively (Table I).

The mean number and diameter of liver granuloma (Fig. 2 & 3) were significantly decreased in comparison with those of un-treated group, while the differences among the treated groups were insignificant. The greatest decrease was recorded in mice treated with 50 mg/kg body weight of "Ro-354", followed by those treated with 100 mg/kg body weight and finally those treated with 200 mg/kg body weight. (Fig. 4 a-d).

Scanning electron microscope examination. The tegumental surface of male *S. mansoni* recovered from un-treated infected mice 30 day post-infection was examined by SEM. It was provided with numerous large tubercles bearing spines the areas between the tubercles (intertubercular matrix) were devoid from spines (Fig. 5a).

Administration of the three doses to the three groups of infected mice 30-day post infection resulted in ultrastructural alterations, which were apparent 2 weeks after drug administration. The range and extent of these changes differed according to the treated dose resulting in generalized deformities in these worms.

Worms of group II revealed a variety of changes in the tegumental surface. The tubercles on the dorsal surface showed extensive loss of spines. Spines may be partially or completely disappeared in some worms (Fig. 5b, c). Some tubercles lost their normal architecture and fused together forming bubble-like lesion and few host inflammatory cells were observed (Fig. 5d). A complete destruction of some tubercles, numerous blebs were also observed (Fig. 5e). The ventral sucker of this group showed normal structure, but numerous host inflammatory cells were observed around it (Fig. 5f).

The worms recovered from group III (100 mg/kg) showed moderate alterations. Tubercles lost some spines, the papillary pores show abnormal widening, numerous blebs and some inflammatory host cells were observed (Fig. 6a & b). The ventral sucker show abnormal structural alterations included lobulation, retraction, lost of spines and aggregation of host inflammatory cells (Fig. 6c).

Table I. Mean worm burden, granuloma diameter and number in different groups untreated and treated with "Ro- 354"

Mice Groups	male	Female	total	Reduction percent	Granuloma diameter (M±SE)	Granuloma number (M±SE)
Group I	8	8	16	-	0.20±0.039	8.8±0.37
Group II	4	3.5	8	53.1	0.10±.008*	3±0.31*
Group III	6	6	12	25	0.187±0.027	5.8±106
Group IV	5	6.5	11.5	28.1	0.186±0.030	6.4±0.92

*significant where P value<0.005

Fig. 1. Ro-354 (3-substituted-5-(2-aryl-2-oxoethyl)-2, 4-dioxo-1, 3-thiazolidine)

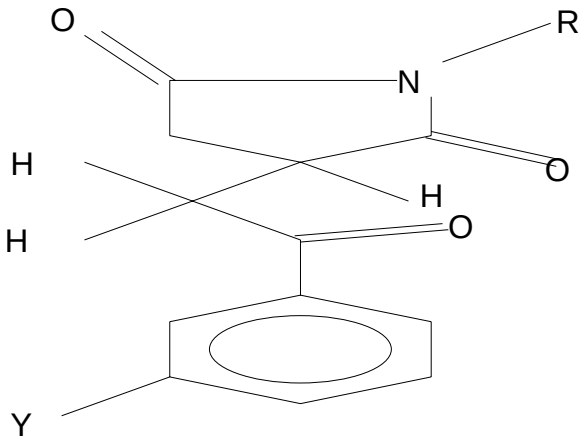
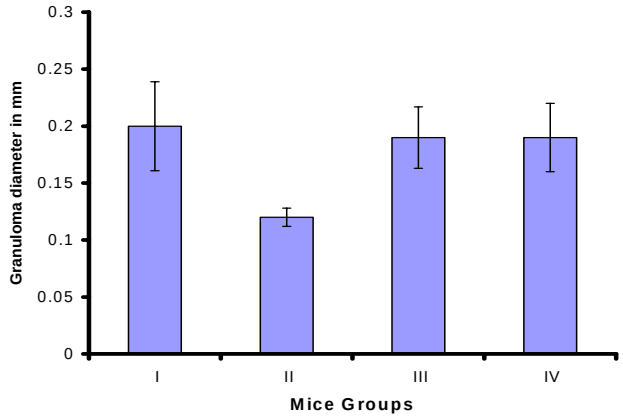
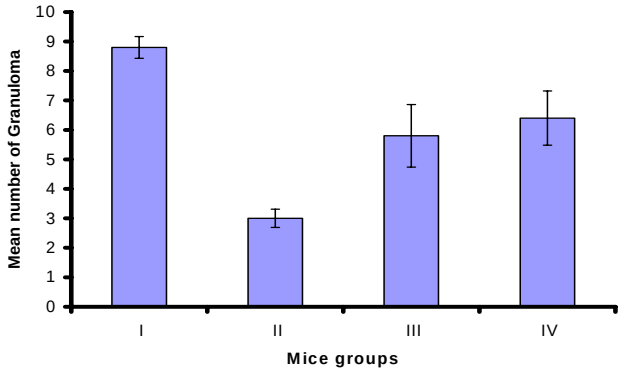


Fig. 2. Histogram showing the mean diameter of granuloma in different groups of mice infected with *S. mansoni*



In group IV (200 mg/kg), a serious damage has been observed everywhere on the bodies of treated male worms. Tubercles showed extensive loss of spines (Fig. 6d). Some tubercles were completely destructed or eroded (Fig. 6e & 7a). Numerous abnormal cracks were observed through intertubercular spaces (Fig. 6d, e). Semi- and complete fusion of tubercles were also noticed (Fig. 7a). Moreover, tremendous alterations occurred in group IV, where large swollen parts (diameter range 4.16 - 60 µm) were observed

Fig. 3. Histogram showing the mean number of granuloma in different groups of mice infected with *S. mansoni*



in different regions of the males bodies, especially on the edges of gynaecophoric canal (Fig. 7b, c) and on dorsal surface (Fig. 7d). The ventral sucker showed swollen bodies, erosion in some parts and tremendous loss of spines (Fig. 7e).

DISCUSSION

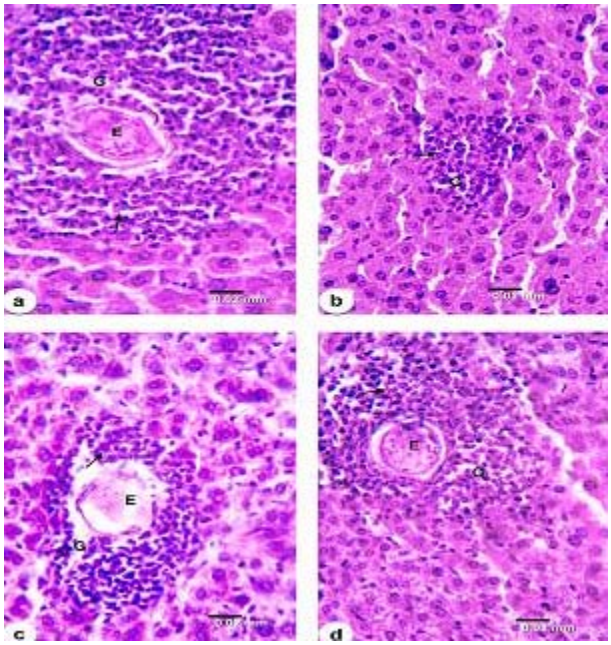
The tegument of *S. mansoni* is an important interface between the parasite and its intravascular environment in the host. Through this specialized tegument, adult worms perform three basic activities for their survival: assimilate blood nutrients from the host, are able to escape from the immune response of the host against their presence (McLaren, 1980; Kalapothakis *et al.*, 1988; Abath & Werkhavser, 1996) and regenerate from induced lesions (Popiel *et al.*, 1985). So the tegument is considered as an important target for antischistosomal drugs.

The present study is the first to document tegumental alterations in *S. mansoni* induced by a new derivative of rhodanine also the tissue reactions of treated mice infected with *S. mansoni* were investigated.

The worm burden, number and diameter of granuloma were reduced in infected mice treated with Ro-354; but the lowest values were recorded in group II (50 mg/kg body weight). The reduction in the previous parameters was considered by several authors as a strong evidence of the efficiency of anti-schistosomal drugs. Thus the reduction of the worm recovery may be correlated with the findings of Filho *et al.* (2002), Utzinger *et al*. (2002) and Suleiman *et al.* (2004), who treated schistosomiases with oxamniquine, arthemether and praziquentel, respectively.

The reduction of granuloma diameter was reported by Badawy *et al.* (1991), Mahmoud *et al.* (2002) and Mostafa (2005) in their studies on the effect of praziquantel and black-seed oil, black seed oil and/or sedr honey, respectively on the hepatic schistosomiasis. Mostafa (2001) mentioned that the reduction of granuloma diameter could be due to the reduction of type II procollagen, which is responsible for the granuloma formation.

Fig. 4a. Photomicrograph of a section of liver of mice infected with *S. mansoni* and untreated used as control group showing large fibrocellular granuloma (G) surrounding the egg (E) and with large number of inflammatory cells (arrow). Bar = 0.02 mm. (b). Photomicrograph of a section of liver of mice infected with *S. mansoni* and treated with 50 mg/kg body weight of Ro-354 showing marked reduction in size of granulmatous tissue (G) and cellularity. Bar = 0.02 mm. (c). Photomicrograph of a section of liver of mice infected with *S. mansoni* and treated with 100 mg/kg body weight of Ro-354 showing small granulmatous tissue (G) and cellularity with central egg (E). Bar = 0.02 mm. (d). Photomicrograph of a section of liver of mice infected with *S. mansoni* and treated with 200 mg/kg body weight of Ro-354 showing moderately fibrocellular granuloma (G) with a central egg (E). Bar = 0.02 mm

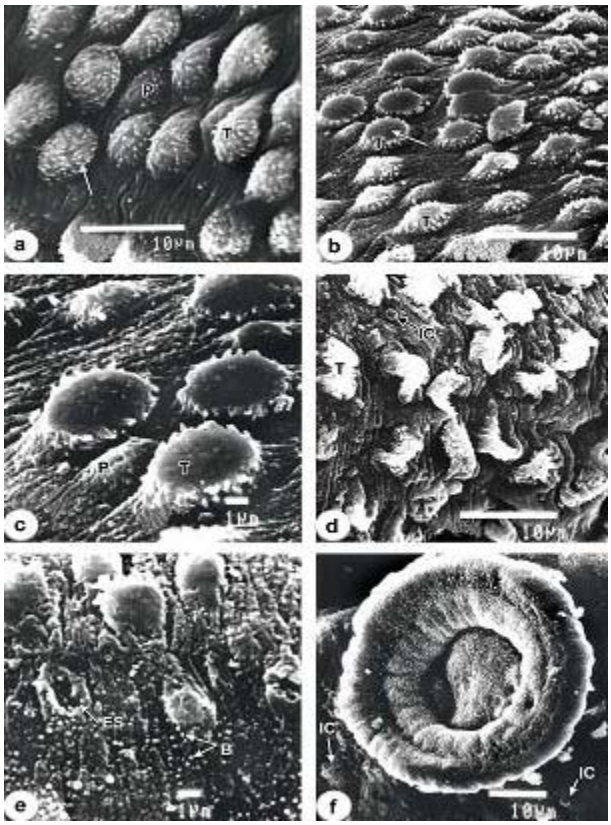


Alterations in the surface ultrastructure of schistosome worms were investigated by several authors for the evaluation of antischistosomal drugs (Mohamed & Fawzi, 1997; El-Sayed & Allam, 1997; Fawzi, 1999; Mostafa & Soliman, 2002; Jiraungkoorskul *et al.*, 2005; Soliman & Ibrahim, 2005; Shaohong *et al*., 2006). Moreover, the alterations caused by antischistosomal drugs were more pronounced in the male tegument than in that of female (Shaw & Erasmus, 1987; Shalaby *et al*., 1991). This may be explained by the fact that most of the female's body is enclosed with the host's microenvironment (Mostafa & Soliman, 2002; Mostafa, 2005). Therefore, the present study examined the surface topography of male worms to determine the antischistosomicidal effect of "Ro 354".

The three doses of the compound induced extensive alterations in the tegument surface, with swelling,

Fig. 5a. SEM of the tegumental surface of male *S. mansoni* recovered from control infected mice showing numerous spines (arrow) covering the tubercles (T), small papilla (P) with minute pore is observed. Bar = 10 µm. (b-f). Scanning electron micrographs of mature males *S. mansoni* recovered from mice treated with a single dose of Ro-354 (50 mg/kg body weight).

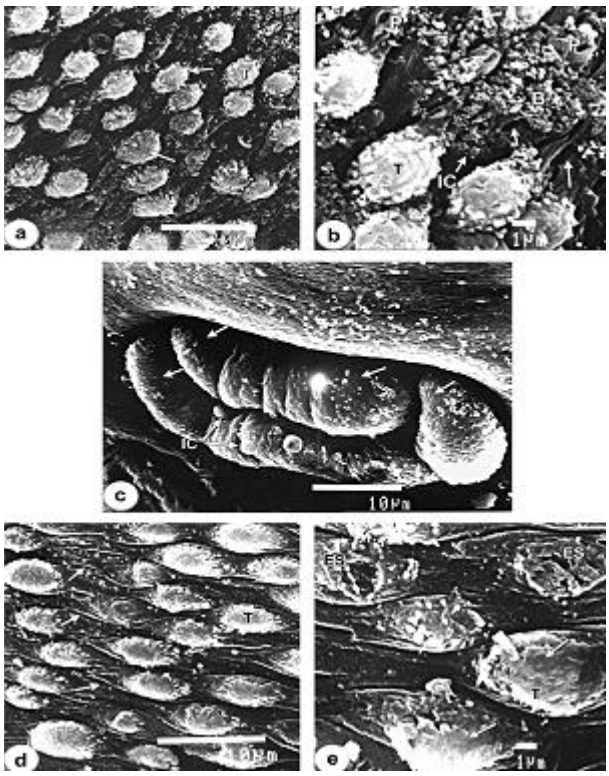
(b). Showing tubercles (T) with extensive loss of spines (arrow). Bar = 10 µm. (c). High magnification of figure 8. Bar = 1 µm. (d). Tubercles (T) lost their normal shape and fused together forming bubble-like lesion. Note some inflammatory host cells (IC). Bar = 10 µm. (e). Another area of treated worm, showing erosion (ES) and tubercles (T) with complete loss of spines. Note also small blebs (B) scattered in the intertubercular spaces. Bar = 1 µm. (f). Showing normal architecture of the ventral sucker with numerous spines, but several host inflammatory cells (IC) were observed around it. Bar = 10 µm



vacuolization or blebbing, fusion of tegumental ridges. The observed morphological alterations could be a mechanism for the killing of the worms by this compound. The alteration in ventral sucker must result in a loss of ability to adhere to blood vessels rendering ingestion of nutrients from blood more difficult (Xiao *et al*., 2001). The damage to the tegument along the worm's body would have impaired the function of the tegument and destroy the defense system of the worm, so that it could easily be attacked by the host's immune system (Xiao *et al.*, 2001). More or less similar changes were produced in response to different chemical drugs. Voge and Bueding (1980) presented a detailed study

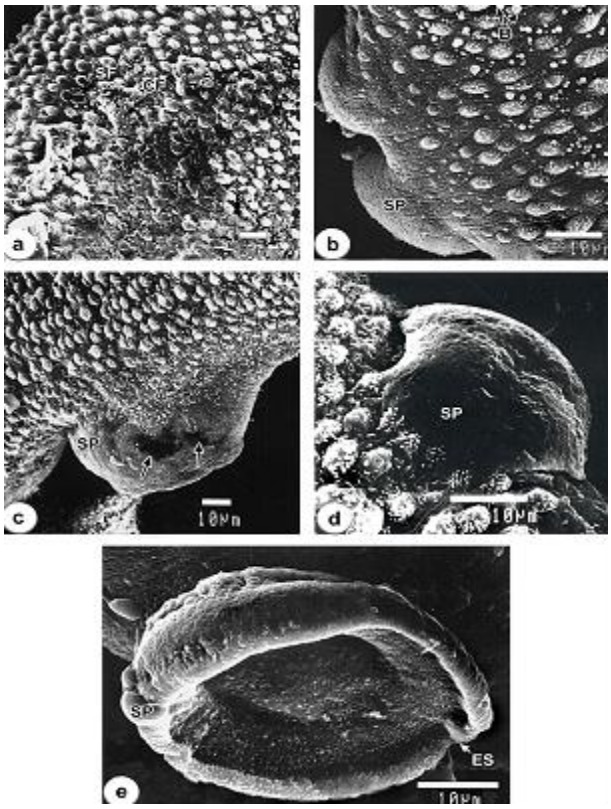
Fig. 6 (a-c). Scanning electron micrographs of mature males *S. mansoni* recovered from mice treated with a single dose of Ro-354 (100 mg/kg body weight). (d & e). Scanning electron micrographs of tegumental surface of the mature males *S. mansoni* recovered from mice treated with a single dose of Ro-354 (200 mg/kg body weight).

(a). Showing effect of Ro-354 on the tubercles (T) that lost some of their spines (arrow). Bar = 10 μm. (b). High magnification of figure 13. Note numerous blebs (B), some host inflammatory cells (IC) and abnormal large papillary pores (P). Some cracks (arrow) were observed in the intertubercular spaces. Bar = 1 μm. (c). Showing abnormal shape of the ventral sucker which divided into swollen lobes (arrows). Some attached host inflammatory cells (IC) were observed. Bar = 10 μm. (d). Showing tubercles (T) lost most of their spines while numerous abnormal cracks were observed through the intertubercular spaces (arrows). Bar = 10 μm. (e). High magnification of figure 16 showing destruction with complete loss of spines of the tubercles (arrows), erosion (ES) of some tubercles (T) was also noticed. Bar = 1 μm



on *S. mansoni* tegumental surface alterations induced by subcurative doses of the schistosomicide amoscanate. They observed swelling, wrinkling, constriction, collapse of sensory bulbs and erosion of large areas of the surface. Mehlhorn *et al*. (1981) reported that the primary effect of praziquantel that eventually lead to the death of *S. mansoni*, the disruption of the tegument. Tegumental changes induced by oxamniquine treatment of adult *S. mansoni* included marked oedema, wrinkling, distortion, complete disorganization of suckers, destruction of tubercles and collapse of sensory bulbs, as observed by Amin and Mikhail (1989). Also Ro15-5458 was recorded to cause tegumental

Fig. 7a. Another worm showing severe lesion on the dorsolateral area. Note semi (SF) and complete fusion (CF) of tubercles. Bar = 10 μm. (b). Showing large swollen parts (SP) of the tegument at the edge of gynaecophoric canal. Note numerous blebs (B) scattered through intertubercular spaces. Bar = 10 μm. (c). Another large swollen part (SP) showing lateral erosions (arrows). Bar = 10 μm. (d). Showing swollen part (SP) on the dorsal surface. Bar = 10 μm. (e): showing severe effect of Ro-354 on the ventral sucker. Note swollen parts (SP), eroded parts (ES) and lost most spines of the sucker. Bar = 10 μm



damage of *S. mansoni* in the form of vacuolation (Fawzi, 1999). Mostafa (2005) noted loss of normal surface architecture, erosion of the tegument and loss of spines in *S. mansoni* after using Sedr honey and/or black seed oil. Jiraungkoorskul *et al*. (2005) recorded severe swelling, vacuolization, fusion of the tegumental ridges and loss or shortening of the spines on the trabeculae, collapse and peeling due to administration of artesunate against *S. mekongi*. Shaohong *et al*. (2006) observed similar effects of artesunate against *S. mansoni*.

Kusel *et al*. (1989) reported some of the functions of glycoproteins in parasite surface: “they act as receptors for growth substances, as a physical or immunochemical barrier to cells and antibodies of the host immune system and maintain the structure of the surface membrane”. Therefore, the tegumental changes induced by Ro-354 could have

exerted a profound effect upon the metabolic activities of the parasite. Moreover, the alterations produced in the tegumental surface make the worms vulnerable to the host immune system and attacked by the host's inflammatory cells. This explains the aggregations of host inflammatory cells around the destructed parts of the tegument in the present study. Melhorn *et al*. (1981) reported that after treatment of *S. mansoni* with praziquantel, the leucocytes of the host attacked the damaged surface and penetrated to the interior tissues of the parasite.

It is well known that male worms use tubercles and spines in holding to the wall of blood vessels, since the treatment with "Ro-354" causing partial or complete destruction to these structures, the worms can be drifted with blood stream.

In the present investigation, the surface of male worms obtained from mice treated with 50 mg/kg body weight of "Ro-354", showed extensive loss of spines. Moreover, tubercles lost their normal shape and fused together forming bubble-like lesion in some areas. On the other hand, the lowest diameter and number of granuloma and worm burden were recorded in the same group. This indicates that the dose of 50 mg/kg body weight, which represents the half of LD 50, is the most effective dose on the parasite. Moreover, it could be used as a curative dose for the host. So the dose of 50 mg/kg body weight is considered the optimal dose used as antischistosomal drug.

In conclusion, the tegumental alterations caused by thiazolidinine derivative [3-substituted-5-(2-aryl-2-oxoethyl) -2, 4-dioxo-1, 3-thiazolidine] gives the possibility to be used as potent antischistosomal drug.

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