Tegmental Alterations in Adult *Schistosoma mansoni*
Treated with Imidazolidine Derivatives

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SUMMARY. Ultrastructural observations were made on the tegument of *Schistosoma mansoni* after 24 h *in vitro* incubation with three imidazolidine derivatives at a concentration of 120 µg/ml. Treatment with (Z)-3-(4-chloro-benzyl)-5-(4-fluoro-benzylidene)-1-methyl-2-thioximidazolidin-4-one caused slight damage to the tegument. Male worms exhibited shortening of the ridges in close proximity to the tubercles and numerous tiny protuberances emerging from the tegument; female worms exhibited peeling and focal erosion of the surface. After treatment with (Z)-3-(4-chloro-benzyl)-5-(4-nitro-benzylidene)-imidazolidine-2,4-dione, the body of male worms appeared wrinkled, there was damage to the oral sucker, a reduction in size and disorganization of the tubercles, whereas in females the most prominent alteration was severe tegumental erosion with peeling or rupture of the surface. Treatment with (Z)-5-(4-fluoro-benzylidene)-1-methyl-3-(4-phenyl-benzyl)-2-thioxo-imidazo-lidin-4-one, resulted in severe tegumental damage in male worms, including erosion with exposure of internal structures. In female worms there were numerous protuberances and deep grooves all over the tegument. The range of ultrastructural abnormalities in the S. mansoni tegument caused by imidazolidine derivatives possibly indicates that these are new antischistosomal compounds.

RESUMEN. “Alteraciones Tegmentales en Ejemplares Adultos de *Schistosoma mansoni* Tratados con Derivados Imidazolílicos”. Se realizaron observaciones ultraestructurales en el tegumento de *Schistosoma mansoni* después de 24 h de incubación *in vitro* con tres derivados de imidazolína a una concentración de 120 µg/ml. El tratamiento con (Z)-3-(4-cloro-bencil)-5-(4-fluoro-bencilideno-1-metil-2-tioxo-imidazolídin-4-ona causó un ligero daño al tegumento. Los gusanos machos exhibieron despellejamiento y erosión focal sobre la superficie. Después del tratamiento con (Z)-3-(4-cloro-bencil)-5-(4-nitro-bencilideno)-imidazolídina-2,4-diona el cuerpo de los gusanos machos apareció arrugado, hubo daño en el aparato chupador, así como una reducción de tamaño y desorganización de los tubérculos, mientras que en las hembras la aleración más prominente fue una severa erosión tegmental con despellejamiento o ruptura de la superficie. El tratamiento con (Z)-5-(4-fluoro-bencilideno-1-metil-3-(4-fenil-bencil)-2-tioxo-imidazo-lidin-4-ona provocó daño severo en los gusanos machos, incluyendo erosión con exposición de estructuras internas. En gusanos hembras hubo numerosas protuberancias y profundos surcos en todo el tegumento. El rango de anormalidades ultraestructurales en el tegumento de *S. mansoni* causado por derivados de imidazolína posiblemente indica que se trata de nuevos compuestos antiesquistosómicos.

INTRODUCTION
The tegument of *Schistosoma mansoni* is an important structure involved in the absorption of nutrients 1,2, in secretion of some products 3, and in protection from the immune response of the host 4-5. For this reason, the tegument is of crucial importance as a target for antischistosomal drugs.

Several studies have demonstrated that drugs active against schistosomiasis cause severe damage to the tegument. Praziquantel, the drug of choice for schistosomiasis, produces rapid and extensive tegumental alterations 5-7. Similar tegumental alterations have been described with the use of oxamniquine 7,8. Recently, the drug artemether, a derivative of artesminin, has been shown to cause extensive and severe damage to the tegument of adult and juvenile forms of *S. mansoni* 9,10.

Another kind of compound that displays an-
tischistosomal activity are the imidazolidine derivatives. In fact, several types of biological activity have been related to imidazolidine compounds. Nipridazol the 1-(5-nitro-2-thiazolyl)-2-imidazolidineone derivative was employed in the 1960 and 1970 as a schistosomicidal. These authors had verified activity of imidazolidine compounds in mice infected with Schistosoma mansoni. These observations led us to test new imidazolidine derivatives synthesized in the Laboratory of Design and Synthesis of Drugs, Department of Antibiotics, Federal University of Pernambuco.

MATERIALS AND METHODS

The BH (BH - Belo Horizonte, MG, Brazil) strain of S. mansoni was routinely maintained at Laboratorio de Imunopatologia Keizô Asami by standard passage through Biomphalaria glabrata snails provided by the Department of Tropical Medicine (Universidade Federal de Pernambuco). Adult schistosomes were obtained by perfusion of the mesenteric and portal veins of Swiss albino mice, infected seven weeks previously.

The synthesis and physicochemical properties of (Z)-3-(4-chloro-benzyl)-5-(4-nitro-benzylidene)-imidazolidin-2,4-dione [1] have already been described. (Z)-3-(4-chloro-benzyl)-5-(4-fluoro-benzylidene)-1-methyl-2-thioxo-imidazolidin-4-one [2] and (Z)-5-(4-fluoro-benzylidene)-1-methyl-3-(4-phenyl-benzyl)-2-thioxo-imidazolidin-4-one [3] are original compounds (Fig. 1). These derivatives were synthesized by the Knoevenagel condensation reaction from benzaldehyde compounds and substituted imidazolines in the Laboratory of Design and Synthesis of Drugs, Department of Antibiotics. First 1-methyl-2-thioxo-imidazolidin-4-one was prepared using 1-methyl-glycine and thiocyanate of ammonium. Then, the 1-methyl-2-thioxo-imidazolidin-4-one reacted with 4-fluorobenzaldehyde in the DMF in the presence of sodium methoxide to form (Z)-5-(4-fluoro-benzylidene)-1-methyl-2-thioxo-imidazolidin-4-one. The alkylation of this compound was carried out in the presence of potassium hydroxide and benzyl halides in an alcohol medium. Benzyl-5-(4-fluoro-benzylidene)-1-methyl-2-thioxo-imidazolidin-4-ones (2 and 3) were thus obtained.

To study the susceptibility of adult S. mansoni worms to these drugs in vitro, we used the previously described method. Worms were maintained in a RPMI-1640 medium (Sigma) buffered to pH 7.5, supplemented with HEPES (20 mM), 10% foetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml). Incubation was carried out at 37°C in a humid atmosphere containing CO2 5%. The effect of imidazolidine compounds on the viability of the S. mansoni was examined by introduction of the drugs into the medium employed for the parasite culture. The drugs were dissolved in dimethyl sulfoxide DMSO (1.5% v/v) at 60, 80, 120, 180 and or 240 µg/ml. This solvent concentration did not affect the worms viability (i.e. motility). In all experiments, two adult worms, were placed in 2 ml of medium in multi-well plastic tissue culture dishes. The viability of the worms was observed under an inverted microscope for 5 days after the addition of the drugs. Control worms treated only with DMSO were included in every experiment. Parasites were considered dead when no movement could be detected for a 3-min period. Strictly aseptic techniques were used throughout the experiments. The drug concentration chosen for scanning electronic microscopy was 120 µg/ml, since for these imidazolidine derivatives tested in our laboratory, mortality began to be observed at this dose. The whole experiment was repeated twice, and 20 worms were observed in each.

Scanning Electronic Microscopy: at 24 h after treatment with 1, 2 and 3 at 120 µg/ml the worms were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at ambient temperature. Then, they were washed twice in the same buffer and post-fixed in 1% osmium tetroxide in phosphate buffer for 1 h at ambient temperature. All worms were dehydrated in ethanol, washed several times in 100% ethanol, critical-point-dried in CO2, mounted on stubs, coated with gold, and examined under a JEOL 250 SI scanning electronic microscope.

RESULTS AND DISCUSSION

The viability in vitro of adult worms was observed at different concentrations of the imida-
imidazoline derivatives. The effects of incubation in vitro of 1, 2 and 3 against the S. mansoni are presented in Figs. 2 and 3. The 2 and 3 compounds at a concentration of 60 μg/ml had no effect on parasite mortality. These graphs show that exposure to the imidazoline 3 resulted in the death of the parasites and this lethal effect was dose- and time-dependent. The best activity was shown with 3, the drug that contains the phenyl group in the para position of the benzyl ring. The 1 and 2 compounds at four different concentrations had little effect on parasite mortality. Control groups were not affected for up to 5 days of observation and all worms exhibited vigorous activity.

The morphological features of the tegument in untreated control adult S. mansoni (Fig. 4: 1 and 2) were similar to those described by others 9,24,25.

With 1 imidazoline, 24 h after in vitro treatment, the drug-induced changes were observed on the entire surface of the body. The general changes were wrinkles on the body, damage to the oral sucker, a reduction in the size and disorganization of the tubercules (Fig. 4: 3). In female worms, the most prominent alteration was severe erosion of the tegument with peeling or rupture of the surface (Fig. 4: 4). In all worms studied, striking alterations were observed in the tubercules due to their total collapse and loss of spines and also to numerous tiny protuberances emerging from the surrounding tegument (Fig. 4: 5). Complete disappearance of sensorial structures and the formation of deep grooves were also observed (Fig. 4: 6).

With 2 imidazoline after 24 h of treatment, all worms exhibited slight damage to the tegument. Male worms exhibited shortening of ridges in close proximity of the tubercules (Fig. 5: 7). Some of the tubercules displayed a

**Figure 2.** Effect on worm viability of imidazoline derivative concentrations after 5 days.

**Figure 3.** Time-dependence of the effect on worm viability after treatment at 120 μg/ml imidazoline derivatives.

**Figure 4.** Scanning electronic micrographs of S. mansoni tegument. 1: tegument of untreated control male worm (1,050X); 2: tegument of untreated control female worm (990X). Tegumental alterations of S. mansoni 24 h after treatment with (Z)-3-(4-chloro-benzyl)-5-(4-nitro-benzylidene)-imidazolidine-2,4-dione [1], 3: surface of male showing damage to oral sucker and wrinkled body (150X); 4: surface of female showing severe extensive erosion, as well as exposure of circular muscle layer (1,500X); 5: disorganization, collapse of the tubercules (1,500X); 6: female surface with deep grooves and erosion (1,500X).
At present, praziquantel and oxamniquine are the only antischistosomal compounds readily available. However, there is an urgent need for research into and development of new antischistosomal drugs, since the existence of strains tolerant or resistant to oxamniquine and praziquantel has already been shown. In the present study, the tegmental alterations produced in female and male *S. mansoni* by imidazolidines, were assessed by means of scanning electronic microscopy. All drugs studied produced damage to the tegument, characterized by focal or general peeling and fusion of tegmental ridges. 1 and 3 were substantially more active than imidazolidine 2, producing severe and extensive damage to the tegument, as well as erosion and collapse of the damaged tegument. Therefore, the worm’s tegument is the critical target for imidazolidine derivatives, as it has been for other antischistosomal drugs studied. In vivo, the loss of the worm's integrity due to tegumental lesions and erosions, may be followed by exposure of the surface to lethal attack by the host cell.

The pattern of tegumental damage resulting from treatment with imidazolidine derivatives was similar to that previously observed in *S. mansoni* adults and schistosomules treated with praziquantel, oxamniquine, and artemether, although the damage caused by 1 and 3 imidazolines was more severe than that caused by recommended antischistosomal drugs. Comparison of the worms viability and tegumental morphology indicated that lesion may not always result in death, but killing the worms appears to be related directly to the intensity of the damage, which at all studied doses induced a lower rate of mortality, also produced discrete tegumental lesions with minute vesicles, while a delay in mortality was observed in worms exposed to the 1 derivative compared to the effect of the 3 compound. This effect could be related to the scale of damage produced in the worm’s tegument by these two drugs. Other antischistosomal drugs and host antibodies have been described as inducing surface blebbing in *Schistosomes*. This phenomenon represents an attempt to activate the repair mechanism when the worm suffers mild surface attack.

The present study showed that these (Z)-3-(4-chloro-benzyl)-5-(4-nitro-benzylidene)-imidazolidine-2,4-dione, (Z)-3-(4-chloro-benzyl)-5-(4-fluoro-benzylidene)-1-methyl-2-thioxo-imidazolidin-4-one and (Z)-5-(4-fluoro-benzylidene)-1-methyl-3-(4-phenyl-benzyl)-2-thioxo-imidazo-
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REFERENCES