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ORIGINAL PAPER

Immunological studies and *in vitro* schistosomicide action of new imidazolidine derivatives

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Abstract: Schistosomiasis is a major public health problem with 207 million people infected and more than 779 million at risk. The drug of choice for treating schistosomiasis is praziquantel (PZQ); however, it is inefficient against immature forms of schistosomes. The aim of this study was to test new imidazolidine derivatives LPSF/PT09 and LPSF/PT10 against adult *Schistosoma mansoni* worms. IC_{50}, cytotoxicity, immune response and cell viability assays were also available for these imidazolidines. Different concentrations of imidazolidine, from 32 to 320 µM, promoted motor abnormalities in breeding and unpaired worms, and death in 24 hours at higher concentrations. Although LPSF/PT09 and LPSF/PT10 did not affect IFN-γ and IL-10 production, they induced nitric oxide production and showed a similar behavior to praziquantel on cell death test. Thus, these new imidazolidine derivatives should undergo further study to develop schistosomiasis drugs.

Key words: *Schistosoma mansoni* , *in vitro,* imidazolidines.

INTRODUCTION

Parasites are an important group of human pathogenic organisms affecting the lives of approximately 2 billion people mostly in the tropics and subtropics in developing countries (1). Schistosomiasis is a major public health problem with 207 million people infected and further 779 million at risk. The disability caused by this chronic helminth infection was recently reassessed in order to correctly represent its previously underestimated high impact on public health (2-4).

Immunological studies in mice have described that resistance to schistosome reinfection mainly correlates with a Th1 response while pathology correlates with Th2 response (5). Cytokines such as IL-4, IL-13, TNF-α, and IFN-γ are closely linked to schistosomiasis disease and are prevalent in schistosome-associated fibrosis (6). Studies have shown that IL-10 correlates with morbidity control in humans, whereas higher levels of nitric oxide produced by macrophages display cytotoxic activity against parasites and play an important role in immune system modulation of parasitosis (7, 8).

So far, the reference drug for schistosomiasis treatment is praziquantel (PZQ), but it seems inefficient against immature forms and there are now reports of resistance in some strains which has worried world public health organizations (9, 10). Therefore many studies have been testing the effectiveness of new drugs against many strains of schistosomes (11, 12).

Imidazolidines and their derivatives comprise a substance class that has shown anti-convulsive and antiarrhythmic pharmacological activities. Furthermore, the imidazolidines have a

methylene group very reactive to carbon-5 that allows the synthesis of many derivatives through aromatic aldehyde condensation (13).

Imidazolidines have been used as anti-schistosome agents in previous studies performed by our group (14-16). Here we investigated the action of two new imidazolidine derivatives, 5-(4-chloro-arylazo)-3-(4-chloro-benzyl)-4-thioxo-imidazolidine-2-ona (LPSF/PT-09) and 3-(4-chloro-arylazo)-5-(4-chloro-benzyl)-4-thyoxo-imidazolidine-2-one (LPSF/PT-10) against adult *Schistosoma mansoni* worms. Cellular viability test, cytotoxicity and immunomodulatory activity induced *in vitro* by these new compounds on immune spleen cells were also available.

MATERIALS AND METHODS

Compounds

Imidazolidine derivatives 5-(4-chloro-arylazo)-3-(4-chloro-benzyl)-4-thioxo-imidazolidine-2-one (LPSF/PT-09) and 3-(4-chloro-arilazo)-5-(4-chloro-benzyl)-4-thioxo-imidazolidin-2-one (LPSF/PT-10) were obtained through synthesis performed at the Laboratory for Drug Design and Synthesis, Federal University of Pernambuco, Brazil, which were duly identified by nuclear magnetic resonance of hydrogen, infrared and mass spectroscopy. The praziquantel was purchase from Sigma Chemical Co. (USA – lot 044K1032).

Parasites and Intermediary Hosts

BH (Belo Horizonte city, MG, Brazil) strains of *S. mansoni* that have been maintained in the Immunopathology Laboratory Keizo Asami (LIKA) were used throughout this study. The strains were kept after they had passed through *Biomphalaria glabrata* molluscs provided by the Department of Tropical Medicine (Federal University of Pernambuco).

Animals Used as Definitive Hosts

Female Swiss mice weighing 20 ± 2g were used; they were obtained and maintained at the animal facility of LIKA. Animals were infected by exposure to an *S. mansoni* cercarian suspension containing approximately 100 cercariae, using the tail immersion technique (17). Animals were housed in a controlled temperature and light environment, and were given water and commercial chow *ad libitum*. The experiments were approved by Ethics Committee for Animal Experimentation of the Federal University of Pernambuco.

Anti-*S. mansoni* Assay

For the *in vitro* test worms were maintained in RPMI-1640 medium (Sigma Chemical Co., USA) buffered to pH 7.5, supplemented with HEPES (20 mM), 10% fetal bovine serum, penicillin (100 $U.mL^{-1}$), and streptomycin (100 $\mu g.mL^{-1}$). Incubation was performed at 37°C in a humid atmosphere containing 5%CO_2. LPSF/PT-9 and LPSF/PT-10 imidazolidine derivatives were dissolved in 1.6% dimethyl sulphoxide (DMSO) and used in concentrations varying from 32 to 320 µM which were added to the medium containing the worms after a two-hour period of adaptation to the culture medium. Duplicates were carried out for each concentration. The parasites were kept for five days and monitored every 24 hours to evaluate their general condition: motor activity, alterations to the tegument, and mortality rate. The PZQ group used the same methodology, but the concentration ranged from 10 to 80 µM. Control worms were only treated with DMSO in RPMI 1640 medium.

Animals Used for Immunological, Cytotoxic, and Cellular Viability Assays

Male BALB/c mice (6 to 8 weeks old) were raised at the animal facility of the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) and maintained at the animal facility of the Aggeu Magalhães Research Center, Oswaldo Cruz Foundation, in Recife, Brazil. All mice were sacrificed and their spleens removed in accordance with the Oswaldo Cruz Foundation Commission for Experiments with Laboratory Animals (Ministry of Health, Brazil, 0266/05).

Spleen Cell Harvesting

Spleen cells were harvested according to a previous protocol (18). After killing the animal with CO_2 gas, the spleen of each mouse was removed aseptically and placed in a Falcon tube containing RPMI 1640 with fetal calf serum (complete medium). In a vertical flow, each spleen was transferred to a Petri dish where they were soaked. The cell suspensions obtained were transferred to Falcon tubes containing approximately 10 mL of incomplete medium per

spleen, centrifuged at 4°C, 200 x g for five minutes. After discarding the supernatant, distilled water was added to the sediment to promote red blood cell lysis. The supernatant, containing no cellular debris was collected and centrifuged at 4°C, 200 x g for five minutes. The resulting sediment (containing cells) was re-suspended in complete RPMI 1640. An aliquot of each cell suspension was separated, diluted in trypan blue for quantification in a Neubauer chamber and cell viability was determined.

In vitro Cytotoxicity Assay

The cytotoxicity of the compounds was determined using BALB/c mice splenocytes (6 x 10^5 cells.well^{-1}) cultured in 96-well plates containing RPMI 1640 medium (Sigma Chemical Co., USA) supplemented with 10% of fetal calf serum (FCS – Cultilab, Brazil), and 50 µg.mL^{-1} of gentamycin (Novafarma, Brazil). Each imidazolidine was evaluated at six concentrations (1, 5, 10, 25, 50, and 100 µg.mL^{-1}), in triplicate in two independent assays. Cultures were incubated in the presence of ^{3}H-thymidine (Amersham Biosciences, USA) (1 µCi.well^{-1}) for 24 hours at 37°C and 5% CO_2. The content of the plate was then harvested to determine ^{3}H-thymidine ([^{3}H] TdR) incorporation using a beta-radiation counter (β-matrix 9600®, Packard, USA). Compound toxicity was determined by comparing the percentage of ^{3}H-thymidine incorporation (as an indicator of cell viability) in imidazolidines-treated wells with untreated wells. Non-cytotoxic concentrations were defined as those where ^{3}H-thymidine incorporation was 30% lower than untreated controls. Six concentrations were also used for praziquantel (1, 5, 10, 25, 50, and 100 µg.mL^{-1}).

Measurement of Cytokine Levels in Splenocyte Supernatants

Spleen cells were cultured in 24-well culture plates (TPP) at a density of 10^6 cells.well^{-1}. Cytokines were quantified in 24-, 48-, 72-hour, and six-day supernatants from cultures stimulated with concanavalin A (Con A – 2.5 µg.mL^{-1}) and phytohemagglutinin (PHA – 5 µg.mL^{-1}) mitogens and PT-9 and PT-10 (1 µg.mL^{-1}), or maintained only in culture medium (control). Levels of IL-10 and IFN-γ were measured by sandwich ELISA, according to manufacturer's protocols. The monoclonal antibodies used were from an OptEIA® Kit (BD Biosciences, USA), having previously been titered. Plates with 96 wells (Nalge Nunc International Corporation, Denmark) were sensitized with specific anti-cytokine antibodies (according to manufacturer's instructions) and incubated overnight at 4°C. Standard cytokines were added after serial dilution from their initial concentration (16000 pg.mL^{-1}). After washes, 50 µL of all samples and standards were added in duplicate and the plate incubated for two hours at room temperature. Subsequently, the specific antibodies were combined with biotin (according to manufacturer's instructions) and incubated for 90 minutes at room temperature. Revealer solution was added containing 2.2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS). Reaction was blocked with 1M sulphuric acid and the reading taken in a spectrophotometer (Bio-Rad 3550®, Hercules, USA) at 415 nm. Sample concentrations were calculated in the linear region of the titration curve of cytokine standard curves, and final concentrations were expressed in pg.mL^{-1}, using Microplate Manager® Version 4.0 software (Bio-Rad Laboratories, USA).

In vitro Nitrite Analysis

Mice spleen cells were used to evaluate nitrite concentration, when treated with Con A (2.5 µg.mL^{-1}), PHA (5 µg.mL^{-1}), LPSF/PT-9 and LPSF/PT-10 (1, 10, and 100 µg.mL^{-1}), and after 24, 48, 72 hours and six days of incubation. Culture media were carefully collected for subsequent measurement by Griess colorimetric method (19). NO concentration was estimated by the standard curve (3.12 – 100 µmol.mL^{-1}).

Analysis of Cellular Viability Using Annexin V-FITC and Propidium Iodide Staining

Immune spleen cells were treated with praziquantel (PZQ), LPSF/PT-9, or LPSF/PT-10 all at a 1 µg.mL^{-1} concentration. These treated cells were maintained in culture in 24-well plates (TPP) for 24, 48 and 72 hours, each then analyzed for cellular viability. Cells without treatment were used as negative control. Subsequently, lymphocytes were centrifuged at 4°C, 450 x g for ten minutes. After discarding supernatant, 1 mL of 1x PBS was added to the sediment and centrifuged at 4°C, 450 x g for ten minutes. After again discarding supernatant, the resulting pellet was re-suspended in a binding buffer [10

mM HEPES (pH7.4), 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1.8 mM $CaCl_2$] and annexin V conjugated with fluorescein isothiocyanate (FITC) (1:500) and propidium iodide (PI, 20 μg.mL^{-1}; 106 cells) was added to each cytometer tube.

Flow cytometry was performed on a FACSCalibur® (Becton Dickinson Biosciences, USA) and analyzed using Cell Quest Pro® software (Becton Dickinson Biosciences, USA). Analysis of results was performed on graphs by dot plot. Double-positive cells (Annexin-FITC+/PI+) were considered spleen cells in the late stage of apoptosis, whereas only PI+ cells were necrotic cells. Annexin-FITC+/PI- represented splenocytes in the early stage of apoptosis. Double-negative results were considered as viable cells.

Statistical Analysis

Data were analyzed using non-parametric tests. To detect differences between groups, the Mann-Whitney *U* test and Tukey *t* test were used. All results were expressed by mean values of groups ± standard deviations and were analyzed considering $p < 0.05$ as statistically significant.

RESULTS

PZQ Cytotoxicity Was Superior to Imidazolidine Derivatives

For the purpose of determining selectivity, imidazolidine derivative actions were tested against spleen cells from BALB/c mice (an effective method which for evaluating specific T lymphocyte cytotoxicity), using praziquantel as the standard drug. Cytotoxicity assays using immune mouse spleen cells showed that LPSF/PT-9 presented nontoxic effects at 10, 5, and 1 μg.mL^{-1} concentrations and LPSF/PT-10 did not exhibit cytotoxicity at 5 and 1 μg.mL^{-1} concentrations (Table 1). However praziquantel showed higher toxicity against splenocytes at all tested concentrations (Table 2). LPSF/PT-9 and LPSF/PT-10 also were tested against *S. mansoni* adult worms and results expressed in terms of IC_{50} (μM) values. PZQ also was used as the reference schistosomicide drug. *In vitro* adult worm mortality was observed at different imidazolidine derivative concentrations. Imidazolidine derivatives did show activity against adult *S. mansoni* worms. LPSF/PT-09 induced 100% mortality at 320 μM concentration in the first 48 hours. The same behavior was present at 100 μM concentrations after 72 hours. LPSF/PT-10 induced 100% worm mortality in 24 hours at 320 μM concentration. The same behavior was seen at 200 and 100 μM concentrations after 72 hours. Oviposition by adult worms was also not seen at any of these imidazolidine concentrations. The control group remained viable throughout the whole observation period (Table 1).

LPSF/PT-9 and LPSF/PT-10 Did Not Produce IFN-γ and IL-10 Cytokines

For immunological assays we investigated IL-10 and IFN-γ cytokines and nitric oxide production on supernatants from cultures of splenocytes stimulated *in vitro* with LPSF/PT-9 and LPSF/PT-10 at 1 μg.mL^{-1} concentrations. The immunological properties of mitogens phytohemagglutinin (PHA at 5 μg.mL^{-1}) and concanavalin A (Con A at 2.5 μg.mL^{-1}) were used as positive controls, and cells plus medium (without stimulus) were used as negative control. Although statistically higher values were produced for Con A and PHA mitogens at all experimental culture times (data not show), neither experimental imidazolidine was efficient in producing IL-10 and IFN-γ from culture supernatants.

LPSF/PT-9 and LPSF/PT-10 Induce Higher Nitrite Production Levels

The effects of NO-induced production in murine spleen cells were measured on *in vitro* cultures treated with LPSF/PT-9 and LPSF/PT-10 at 100, 10, and 1 μg.mL^{-1} concentrations. Statistically higher values were observed for both LPSF/PT-9 and LPSF/PT-10 at 100 μg.mL^{-1}. At 24 hours, LPSF/PT-9 on 100 and 1 μg.mL^{-1} concentrations showed higher values than control, and LPSF/PT-10 at 100 μg.mL^{-1} was superior to control. Also at 24 hours, LPSF/PT-10 was statistically superior to LPSF/PT-9 at 100 μg.mL^{-1} (Figure 1 – A). After 48 hours, LPSF/PT-9 and LPSF/PT-10 at 100 μg.mL^{-1} were statistically superior to control and among themselves (Figure 1 – B). Although LPSF/PT-9 presented higher values, only LPSF/PT-10 at 100 μg.mL^{-1} showed statistical difference to control at 72 hours (Figure 1 – C). At the final culture time, LPSF/PT-9 and LPSF/PT-10 at 100 μg.mL^{-1} showed statistical difference to control. At six days we also observed

Table 1. *In vitro* effects of LPSF/PT-09 and LPSF/PT-10 against *Schistosoma mansoni* adult worms

		Concentration (μM)							
		320	200	100	50	32			
	Time (h)	Mortality (%)					Observations (worms)	Cytotoxicity (μg.mL^{-1})[a]	IC_{50} (μM)[b]
LPSF/PT-09	24	74	79	80	19	0	Not paired, no sucker adherence, absence of eggs, tegument morphology altered	10	51.74
	48	100	80	83	44	0			59.19
	72	100	100	100	63	0			49.52
	96	100	100	100	88	6			45.32
	120	100	100	100	88	18			43.06
		Concentration (μM)							
		320	200	100	50	32			
	Time (h)	Mortality (%)					Observations (worms)	Cytotoxicity (μg.mL^{-1})[a]	IC_{50} (μM)[b]
LPSF/PT-10	24	100	65	54	0	0	Not paired, no sucker adherence, absence of eggs, appearance of bubbles, tegument morphology altered	5	115.8
	48	100	100	85	0	0			95.31
	72	100	100	100	0	0			76.82
	96	100	100	100	0	0			76.82
	120	100	100	100	0	0			76.82

[a] Expressed as the highest concentration tested that was not cytotoxic for BALB/c mice splenocytes. Values in μM are showed in parentheses.
[b] Calculated at five concentrations using data from at least three independent experiments, with SD less than 10% in all cases.

Table 2. *In vitro* effects of praziquantel against *Schistosoma mansoni* adult worms

		Concentration (μM)						
		80	40	20	10			
	Time (h)	Mortality (%)				Observations[a] (worms)	Cytotoxicity[b] (μg.mL^{-1})	IC_{50} (μM)[c]
PZQ	24	60	45	36	30	Less movement and contractions, absence of eggs, tegument morphology altered	<1	37.02
	48	66	56	50	45			35.08
	72	76	67	57	53			33.91
	96	100	100	100	92			15.36
	120	100	100	100	92			15.36

[a]Control group paired adults worms without apparent morphological change, presence of eggs.
[b]Expressed as the highest concentration tested that was not cytotoxic for BALB/c mice splenocytes. Values in IM are showed in parentheses.
[c]Calculated at seven concentrations using data obtained from at least three independent experiments, with SD less than 10% in all cases

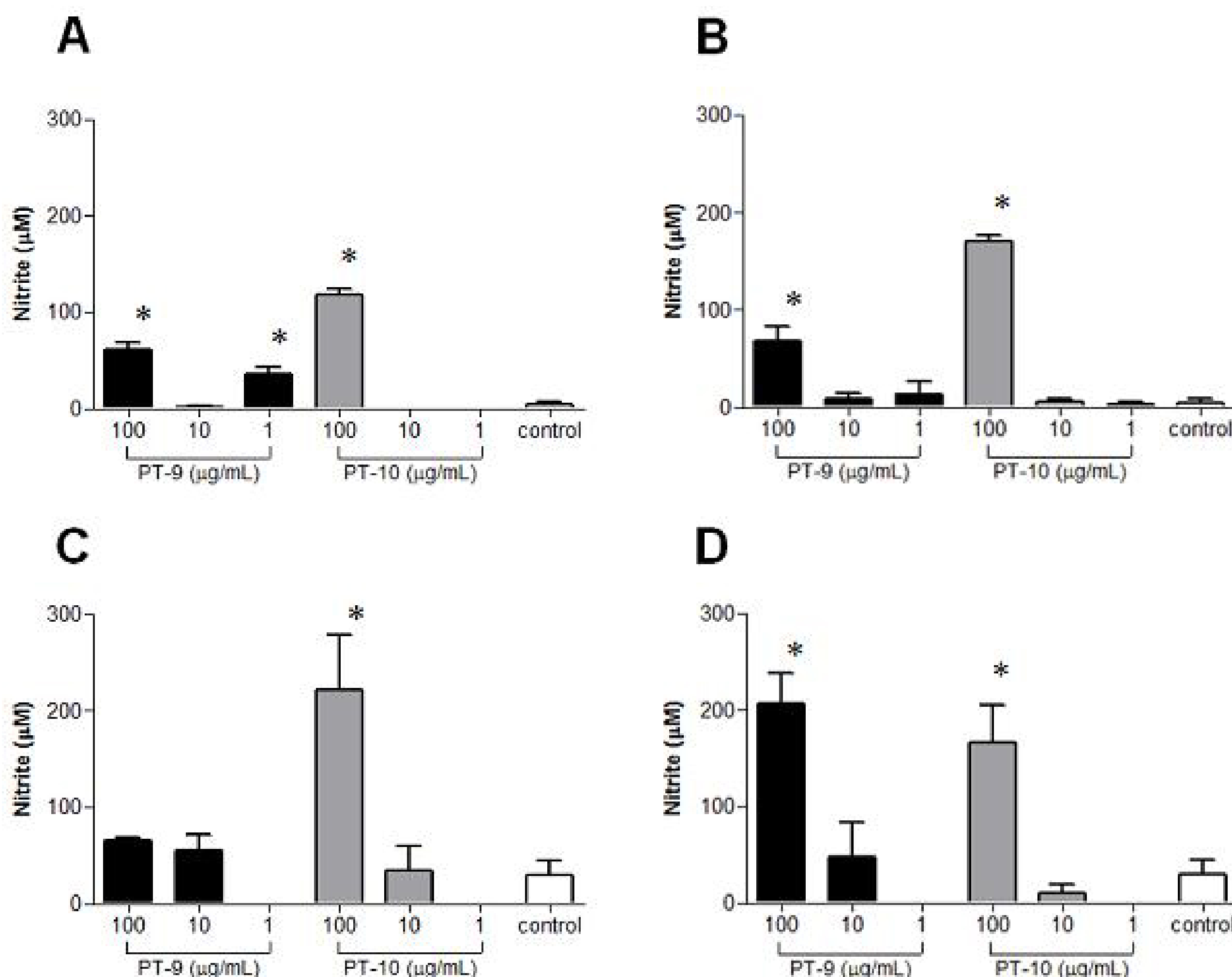

Figure 1. Nitrite production in immune spleen cell cultures from BALB/c mice comparing experimental imidazolidines. Control is culture without stimulus. (**A**) and (**B**): Twenty-four and 48 hours of assay, respectively. At these two experimental times, LPSF/PT-9 and LPSF/PT-10 showed higher values than the control and among themselves. (**C**) Seventy-two hours of assay. LPSF/PT-10 at 100 µg.mL^{-1} did show statistical difference to control. (**D**) LPSF/PT-9 and LPSF/PT-10 at 100 µg.mL^{-1} did show statistical difference in relation to control and among themselves at six days of assay. Horizontal bars represent the average of three independent experiments per group. * $p < 0.05$.

that LPSF/PT-9 at 100 µg.mL^{-1} was statistical superior to LPSF/PT-10 at 10 µg.mL^{-1} (Figure 1 – D).

Low Cellular Death Induced by LPSF/PT-9 and LPSF/PT-10 Imidazolidines

Cellular viability was performed to measure cellular damage induced by imidazolidine derivatives; all compounds were used at a 1 µg.mL^{-1} concentration. After 24 hours, the number of apoptotic cells was higher than cells in late apoptosis and necrotic cells. At this time, PZQ was statistically superior to LPSF/PT-9 and LPSF/PT-10 (Figure 2 – A), but not higher than control cells (without treatment). In late apoptosis, we observed that LPSF/PT-10 was superior to LPSF/PT-9 and PZQ, but not to controls. LPSF/PT-10 demonstrated higher quantities of necrotic cells than controls, PZQ, and LPSF/PT-9 (Figure 2 – B). At 48 hours, PZQ showed higher values than the other compounds. PZQ was superior to LPSF/PT-9 (Figure 2 – A) and LPSF/PT-10 at apoptosis, late apoptosis, and necrosis, but only in necrosis were these values statistically superior to controls (Figure 2 – B). The only time that presented a statistical difference in relation to controls for all composites tested was 72 hours. At apoptosis PZQ, LPSF/PT-9, and LPSF/PT-10

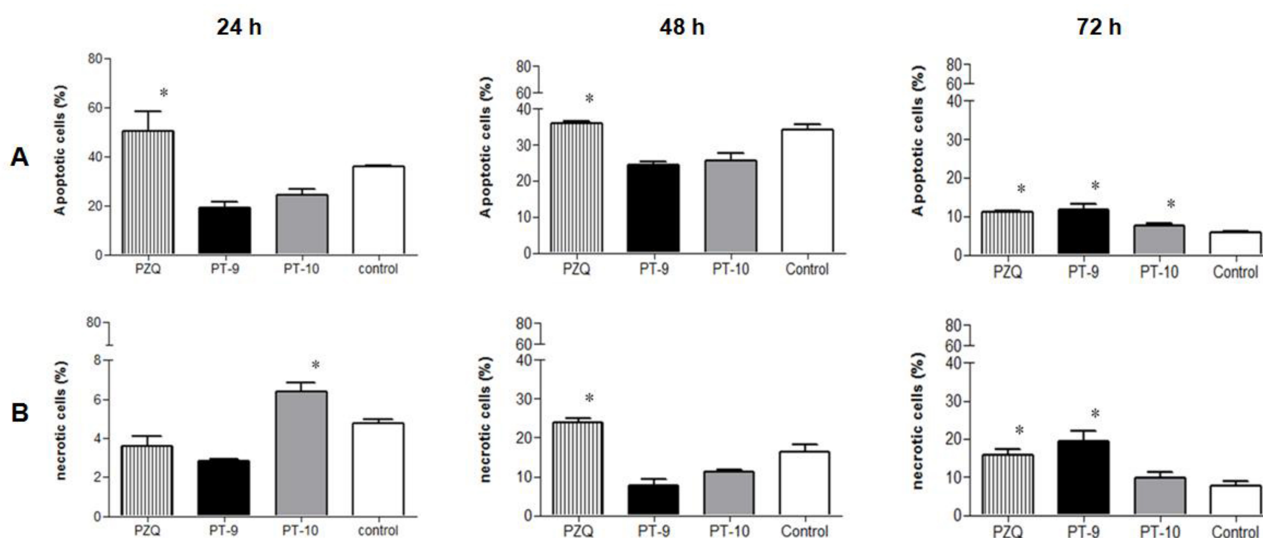

Figure 2. The effects of imidazolidine derivatives on immune spleen cell viability. (**A**) Apoptosis investigated using annexin V at 24, 48 and 72 hours assay. PZQ was statistical superior to PT-9 and PT-10 but not to controls at 24 hours. At 48 hours, PZQ was superior to LPSF/PT-9 and LPSF/PT-10, but not to controls. PZQ, LPSF/PT-9, and LPSF/PT-10 were all superior to control, and PZQ was superior to PT-10 at 72 hours. (**B**) Necrosis assay using propidium iodide at 24, 48 and 72 hours. PT-10 demonstrated a higher quantity of necrotic cells than controls, PZQ, and LPSF/PT-9 at 24 hours. PZQ was superior to LPSF/PT-9 and LPSF/PT-10 and controls at 48 hours. PZQ and LPSF/PT-9 were superior to controls and LPSF/PT-10 at 72 hours. Horizontal bars represent the average of three independent experiments per group. * $p < 0.05$.

were superior to controls and PZQ was superior to LPSF/PT-10 (Figure 2 – A). PZQ and LPSF/PT-9 induced more late apoptosis than controls, and both were statistically superior to LPSF/PT-10. This behavior was similar in the necrosis assay, i.e., PZQ and LPSF/PT-9 were superior to controls and LPSF/PT-10 (Figure 2 – B).

DISCUSSION

Imidazolidine derivatives have been studied by several research groups. The main activities assigned to these compounds are antibacterial, antiamebic, anti-*T. cruzi* and anti-*Schistosome* (16, 20-22). We analyzed the cytotoxicity and immunological properties of two new imidazolidine derivatives, LPSF/PT-9 and LPSF/PT-10, against *Schistosoma mansoni* worms.

LPSF/PT-9 and LPSF/PT-10 were less cytotoxic and induced superior mortality in adult worms than praziquantel. But in relation to the absence of oviposition by adult worms and motor abnormalities, imidazolidines were similar to PZQ. Many drugs have been compared with praziquantel and their cytotoxicity and ability to kill adult *S. mansoni* worms has been shown as similar to praziquantel (23, 24). However, equally to praziquantel, the mechanism by which imidazolidines exert schistosomicide activity *in vitro* is still unclear. Many studies have demonstrated that low PZQ concentrations, *in vitro*, appear to impair the function of the worms' suckers and at higher concentrations increases the contraction (irreversibly at very high concentrations) of the worms' strobila (chain of proglottids), cause irreversible focal vacuolization with subsequent cestodal disintegration at specific sites of the cestodal integument (25-28). As well as these aspects, imidazolidines act on apoptotic cells with melanoma; their route was identified in *S. mansoni* and should be investigated as to their role in the death of these parasites (15, 16, 29).

Reports have shown a Th1 and Th2 response balance in schistosomiasis patients. Before treatment, patients in the acute phase, as well as those with the severe hepatosplenic form of the disease, have high levels of pro-inflammatory cytokines in their serum (IL-1, IL-6 and IFN-γ). On the other hand, patients with the intestinal clinical form have higher levels of IL-10 (5, 30). Furthermore, these same studies strongly suggest that resistance to infection is multifactorial and

that it can not be clearly correlated with a single immune mechanism. Stephaniel (31) affirm that low concentrations of IL-10, IL-4, and TGF-β inhibit macrophage larvicidal activity, as well as nitric oxide (NO) production, in a synergistic mechanism. The induction of T-lymphocyte proliferation by mitogens involves both cell-cell interaction and molecular communication. One of the changes observed after stimulation of lymphocytes by mitogens like PHA or Con A, are increased ion fluxes and transport of other substrate across the plasma membrane. The consequences of increased K^+, for example, include increased intracellular levels of Na^{2+} and Ca^{2+} in addition to decreased Mg^{2+} and K^+ in PHA stimulated lymphocytes. This fact shows the requirement for calcium in lymphocyte activation and its participation in the process (32). The central event in PZQ activity on schistosomes may well be the phenomenon of calcium influx into the tegument which is observed within a couple of minutes of drug exposure. However, the mechanisms leading to this alteration in calcium homeostasis are not clear (33, 34).

In our study, imidazolidine derivatives did not induce significant production of IFN-γ or IL-10. However, the potent anti-schistosomiasis agent, nitric oxide, was strongly stimulated. Nitric oxide has been shown to only affect targets located very close to its source. This behavior is important to avoid toxicity to surrounding tissues and indicates that the larvicidal action of NO does not require interaction with other effector cell products (31). Against parasitosis, there is ample direct and indirect evidence that NO can act as an antischistosomal and, more broadly, antiparasitic molecule (35-37). NO produced by human white cells has been shown to kill larval schistosome parasites and has recently been shown to kill a multitude of eukaryotic and prokaryotic organisms, against which no other defense mechanism has, to our knowledge, been reported (35, 38, 39). In addition, studies have shown that compounds which induce nitric oxide release possess potential immunomodulatory properties (40). Here, we observed that LPSF/PT-9 and LPSF/PT-10 imidazolidines stimulated NO production by immune spleen cells and this behavior may indicate a possible immunostimulant activity induced by imidazolidine derivatives.

The two types of cellular death, apoptosis and necrosis, differ fundamentally in morphology, biochemistry and biological relevance. Apoptotic cells are generated in vast quantities in the central lymphoid organs, such as the thymus and bone marrow, and are removed daily by phagocytic cells for homeostasis maintenance of these tissues. On the other hand, cellular death by necrosis occurs, generally, as a response to injuries suffered by these cells that have ruptured the plasmatic membrane leading to homeostasis loss and a constituent release that starts an inflammatory response (41). However, the distinction between initial apoptosis, late apoptosis, and necrosis is still largely under discussion.

Damage to cell morphology in *in vitro* assays has been demonstrated by many drugs. Malheiros *et al*. (42) using trifluoperazine (TFP), dibucaine (DBC), and praziquantel observed cytotoxic effects, such as hemolysis and the release of membrane lipids, in human erythrocyte membranes in a dose-dependent mechanism. In our cellular death test, PZQ showed higher values at all experimental times and some results were superior to the imidazolidines when tested on immune spleen cells. But behavior between experimental composites was very similar, especially at 72 hours assay, indicating that similar routes may be activated in the induction of cell death for the compounds evaluated and the reference drug PZQ. However, we believe that more assays are needed to answer this question.

LPSF/PT-9 and LPSF/PT-10 imidazolidine derivatives displayed relevant antischistosomal activity *in vitro*, induced higher nitric oxide production by immune spleen cells, and showed similar behavior to praziquantel in the cellular death test. It is therefore possible that these new imidazolidine derivatives can be future candidates for schistosomiasis drugs, but further studies are needed to elucidate the mechanisms induced for this response. Understanding the mechanisms that mediate the effects these compounds have on the immune system will provide information that will make these compounds future candidates for schistosomiasis drugs.

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There are no conflicts of interest.

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ETHICS COMMITTEE APPROVAL
This study was approved by the Ethics Committee for Animal Experimentation, Federal University of Pernambuco (process n. 007639/2007-12), in accordance with law 9.605, article 32, decree 3179, art 17.

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