Parasitology International 61 (2012) 628–633

Contents lists available at SciVerse ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Sesquiterpene lactones and the diterpene 5-epi-icetexone affect the intracellular and extracellular stages of *Trypanosoma cruzi*

Esteban Lozano [a,1], Patricia Barrera [a,1], Romina Salinas [a], Israel Vega [a], Matías Nieto [b], Carlos Tonn [b], Ulrike Kemmerling [c], Renato A. Mortara [d], Miguel A. Sosa [a,e,*]

[a] Laboratorio de Biología y Fisiología Celular, "Dr. Francisco Bertini", Instituto de Histología y Embriología, Facultad de Ciencias Médicas, Universidad de Cuyo, Mendoza, Argentina
[b] INTEQUI-CONICET, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Argentina
[c] Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile
[d] Departamento de Microbiología, Inmunología e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil
[e] Instituto de Ciencias Básicas (ICB), Universidad Nacional de Cuyo, Mendoza, Argentina

ARTICLE INFO

Article history:
Received 14 December 2011
Received in revised form 5 June 2012
Accepted 18 June 2012
Available online 23 June 2012

Keywords:
Chagas disease
Trypanosoma cruzi
Natural compounds
Sesquiterpene lactones
Diterpenes

ABSTRACT

Chagas disease is a major health problem in Latin America and is caused by the parasitic protozoan *Trypanosoma cruzi*. Although many drugs have been used to alleviate the disease, these have been ineffective in the chronic phase and have also presented numerous side effects on patients. In this study we tested the effect of three sesquiterpene lactones (dehydroleucodine, helenalin and mexicanin) and a diterpene (5-epi-icetexone) on parasites (Y-strain) grown in host cells. At 48 h of treatment, the number of amastigotes inside the cells was lower than in the controls. This effect was observable at concentrations of 1.5–3.8 μM, which are of low cytotoxicity to host cells. In addition, the compounds caused a decrease in the percentage of infected cells. The treatments also reduced the presence of trypomastigotes in the extracellular medium. In all cases, helenalin was the most potent. The number of parasites per cell at 24 h indicates the occurrence of multiple infection, which would also be affected by the compounds. However, we should not discard an effect on the proliferation and survival of parasites within the host cells. On the other hand, an additional effect on the differentiation of parasites and/or the survival of extracellular trypomastigotes might be possible. We conclude that these compounds are very effective against *T. cruzi* possibly by multiple mechanisms.

1. Introduction

Trypanosoma cruzi is the causative agent of Chagas disease, or American trypanosomiasis, the prevalent health problem in Latin America and still expanding into other areas of the planet. Between 15 and 18 million people are afflicted with this potentially lethal disease (http://apps.who.int/gb/ebwha/pdf_files/EB124/B124_17-en.pdf) which affects the cardiovascular, gastrointestinal, and nervous systems of human hosts. Recent estimates indicate that between 1% and 6% of the population of Latin America is infected with *T. cruzi*, and approximately 50,000 new cases are diagnosed every year [1–4]. This monoflagellate parasite fulfills its life cycle between an invertebrate vector (a triatomine insect) and mammal vertebrates. Furthermore, depending on the host, the parasite can be found at different stages [5]. Despite the enormous efforts that have been made to alleviate the disease, there is still no effective therapy because of its ineffectiveness against the chronic phase of the disease and the many cytotoxic effects on host cells [6,7]. Current chemotherapy of Chagas disease is based on the nitroheterocyclic derivatives benznidazole and nifurtimox, although both compounds show severe side effects [7–9]. Because nifurtimox and benznidazole are far from being considered ideal trypanocidal drugs, the search for new compounds effective against *T. cruzi*, with low toxicities and increased efficacies during the indeterminate and chronic phases, still continues [reviewed in 7]. Natural compounds derived from plants have been tested against *T. cruzi* for decades, and many of them were shown to be effective against non-infective forms of parasites at very low concentrations [6,10]. It has been shown that compounds of the family of sesquiterpene lactones [11,12], and diterpenes [13] obtained from plants of different regions of Argentina are effective against *T. cruzi* epimastigotes at very low concentrations. However, there is little evidence to demonstrate that these compounds are effective against the infective forms of the parasites (trypomastigote and intracellular amastigote) [14]. Sesquiterpene lactones are now being considered for use against *T. cruzi* based on their abundance in the leaves in members of the Asteraceae (Compositae) and their potent biological activities as antimicrobial and antitumoral agents [15–18]. In this study we tested the effect of three sesquiterpene

* Corresponding author at: Laboratorio de Biología y Fisiología Celular, "Dr. Francisco Bertini", Instituto de Histología y Embriología, Facultad de Ciencias Médicas, Universidad de Cuyo, Mendoza, Argentina. Tel.: +54 261 4135000; fax: +54 261 4494117.
E-mail address: msosa@fcm.uncu.edu.ar (M.A. Sosa).
[1] Equal contribution.

1383-5769/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2012.06.005

lactones; helenalin, mexicanin and dehydroleucodine and the icetexane diterpene 5-epi-icetexone, on parasites inside mammalian cells and found that these compounds strongly reduced the number of intracellular amastigotes and extracellular trypomastigotes at 48 h of treatment.

2. Materials and methods

2.1. Extraction and purification of compounds

All the compounds were isolated from plants collected in San Luis, Argentina. Helenalin and mexicanin were isolated from the aerial parts of *Gaillardia megapotamica* (Spreng.) Baker var. *radiata* (Voucher Del Vito & Petenatti 2841 deposited in the herbarium of the Universidad Nacional de San Luis, UNSL) as has been described by Giordano, O. et al. [19]. The purity of these compounds (95% for both lactones) was tested by ^{13}C-nuclear magnetic resonance, melting point analysis, and optical rotation, and the data were in accordance with those reported previously [19]. Dehydroleucodine was isolated from the leaves of *Artemisia douglassiana* Besser (Voucher Nr. 55 from the UNSL), purified, and the purity (100%) was tested as described by Giordano et al. [19]. 5-epi-icetexone (ICTX) was isolated from specimens of *Salvia gilliessi* Benth. (Voucher DPP 6087, L.A. Del Vito, from the herbarium of UNSL) and purified as described by Nieto et al. [20]. The purity (99%) was tested by thin layer chromatography and spectroscopic methods. Because the compounds were water insoluble, they were dissolved in dimethylsulfoxide (DMSO) and added to the media under concentrations in which the solvent alone did not affect the cells (not shown).

2.2. Culture of Vero cells

Vero cells were cultured in RPMI medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), penicillin/streptomycin (0.1%) and 2 mM L-glutamine, and maintained in an atmosphere of 5% CO_2 at 37 °C. For infection experiments, the cells were subcultured and seeded into 24-well plate on sterilized coverslips.

2.3. Culture of parasites

Epimastigotes of *T. cruzi* (Y-strain) (DTU *T. cruzi* II, [21]) were cultured in Diamond medium (0.1 M NaCl, 0.05 M K_2HPO_4 pH: 7.2, 0.625% triptose, 0.625% triptone, 0.625% yeast extract, 12.5 μgr/ml hemin) supplemented with 10% fetal bovine serum (Gibco) at 29 °C. To obtain trypomastigotes, the cultures were kept for 2 weeks without renewing the culture medium. Under these conditions, ~ 20% of parasites were transformed into trypomastigotes,

2.4. Infection of Vero cells and treatments with the compounds

Vero cells were grown for 24 h in 24 multiwell plates (3×10^4cells/well) containing sterile coverslips. Then, 1.5×10^6 parasites were added in 0.5 ml of medium (RPMI) and incubated for 30 min at 37 °C. Considering that approximately 20% of parasite mixture corresponds to infective forms, the multiplicity of infection (MOI) was maintained at 10 in accordance with other authors [22,23]). Subsequently, the medium was removed, to eliminate non-internalized parasites, and was replaced by fresh medium. Between 1.5 and 3.8 μM (dissolved in DMSO) of each compound under study were added to the cell cultures and incubated for 24–48 h. These concentrations were chosen because they were effective against epimastigotes and non-cytotoxic to mammalian cells [11–13]. Controls were performed with or without 0.25% DMSO. After incubation, the media were collected (for counting of released parasites), and the Vero cells were washed and processed for light microscopy. The experiment was performed in triplicate from three independent experiments.

2.5. Light microscopy

The coverslips containing the infected cells were washed twice with PBS and the cells were fixed for 5 min with pure methanol, and stained with Giemsa stain (Merck, Damstadt, Germany) (10% in water) for 15 min. After discarding the dye, the cells were washed three times with distilled water and the coverslips were mounted on slides with EUKITT mounting solution (O. Kindler GmbH & Co., Freiburg, Germany). The cells were observed under an optical microscope, and the percentage of infected cells and the number of parasites per cell were determined from 200 Vero cells counted per experiment (from fields taken randomly).

2.6. Obtention and counting of extracellular parasites

After incubations with or without compounds, the media were collected at 24 or 48 h and parasites (trypomastigotes) were fixed with 5% paraformaldehyde and counted in a Neubauer hemocytometer.

2.7. Percentage of infectivity

We calculate the percentage of infectivity based on the following equation:

$$\%I = IA \times 100/IAC,$$

where IA is the number of intracellular amastigotes in experimental conditions, and IAC is the number of intracellular amastigotes in controls, following a similar equation used by other authors for internalization assays [22].

2.8. Effect of the compounds on trypomastigotes

Bloodstream trypomastigotes were obtained from infected Albino Swiss mice by cardiac puncture, at 21 days post-infection with Y strain of *T. cruzi*. The number of parasites was adjusted with saline solution to 260 cells/μl in each tube. The compounds were then added to concentrations of 1.5 or 3 μM (MXN, ICTX), 1,5 or 3.8 μM (DhL), 1.5 μM (HLN) or 5.8 μM of benznidazole (BZN) and aliquots were collected every 30 min. The parasites were counted in a hemocytometer, assuming that the mortality is related to the disappearance of parasites.

2.9. Cytotoxicity assay

Vero cells were grown for 24 h on sterile coverslips in 24 multiwell plates (3×10^4 cells/well), as detailed above. Compounds were added at final concentrations of 1.5 or 3.0 μM. To evaluate viability, the cells were tested for exclusion of eosin at 24 or 48 h of treatment.

2.10. Statistical analysis

The data obtained were analyzed by ANOVA and Tukey–Kramer pairwise comparisons, with a significance level of $p \leq 0.05$.

3. Results

In this study we show the effect of three sesquiterpene lactones; helenalin (HLN), mexicanin (MXN) and dehydroleucodine (DhL), and a diterpene 5-epi-icetexone, (ICTX) (Fig. 1) on intracellular forms of *T. cruzi*. As seen in Figs. 2 and 3, the four compounds significantly reduced the amount of Vero cells infected with parasites at 48 h post-infection. However, as expected, at shorter periods of time (24 h) no significant differences were observed in the percentages of infected cells (Fig. 3). The compounds were effective at different concentrations ranging from 1.5 μM (HLN and MXN), to 3.0 μM (ICTX) or

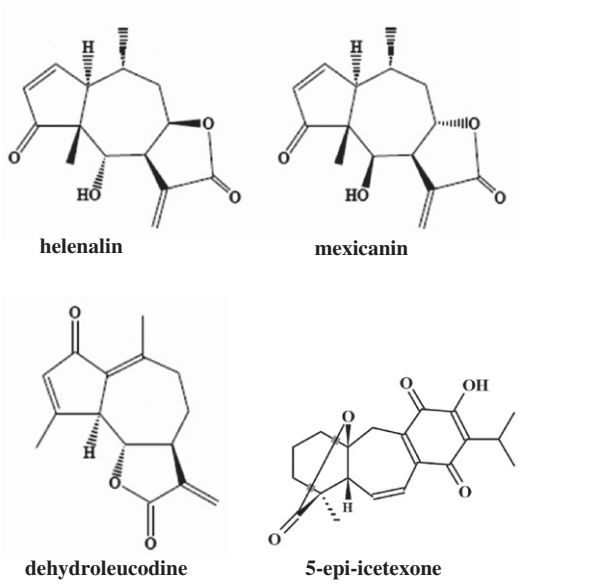

Fig. 1. Chemical structure of the natural compounds.

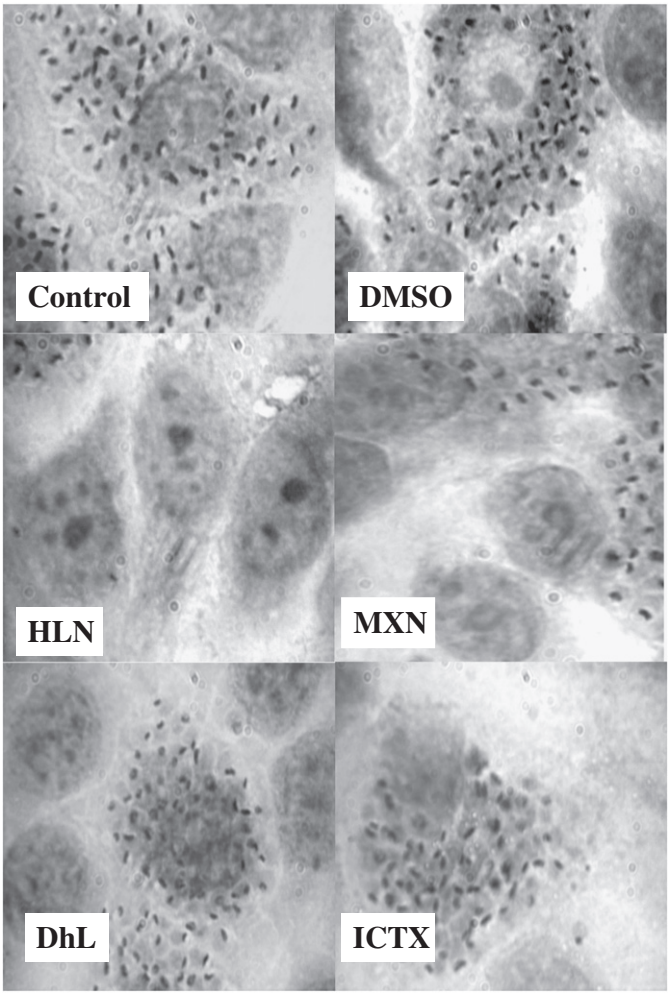

Fig. 2. Vero cells infected with *T. cruzi*, submitted to different treatments. The cells were grown in coverslips, infected with *T. cruzi* and treated with: 1.5 μM helenalin (HLN), 1.5 μM mexicanin (MXN), 3.8 μM dehydroleucodine (DhL) or 3.0 μM 5-epi-icetexone (ICTX). After 48 hour treatment with the compounds, the cells were processed for light microscopy and stained with Giemsa, as detailed in Materials and methods. DMSO: infected cells treated with solvent alone (0.25%).

3.8 μM (DhL), depending on the compound used. These concentrations were chosen because they had been shown to be effective against epimastigotes of *T. cruzi* and they presented very low toxicity to mammalian cells [13,24]. At similar concentrations, benznidazole did not induce changes with respect to controls (data not shown). This difference between 24 and 48 h could be explained due to higher mortality of infected cells in the presence of compounds or to a decrease in re-infection to other cells under these conditions. However, at these periods of incubation and at the concentrations used, the compounds did not affect either the viability or growth of Vero cells (Table 1), as they grew normally and showed no morphological changes (Fig. 2). At 24 h post-infection no major differences were observed in the number of parasites per cell (predominantly in the range of 1–10, not shown), indicating that this time period was insufficient to evidence any effect of the compounds. The number of parasites per cell indicates the occurrence of multiple infections in some host cells. In controls, it was observed that the number of cells with more than 20 parasites was greatly increased at 48 h, while treatments with the compounds kept the cells with low amounts of parasites (Fig. 4). This suggests that the compounds would also inhibit the proliferation of intracellular amastigotes, and the highest inhibitory effect was observed with HLN (Fig. 4). However, the effect of HLN should be taken with some caution, since at longer incubation times (e.g. 72 or 96 h), Vero cells begin to be affected, showing an increasing mortality (from 2% to 20%) and undergoing morphological changes (data not shown). From the data presented here, we have calculated the percentage of infectivity as detailed in material and methods.

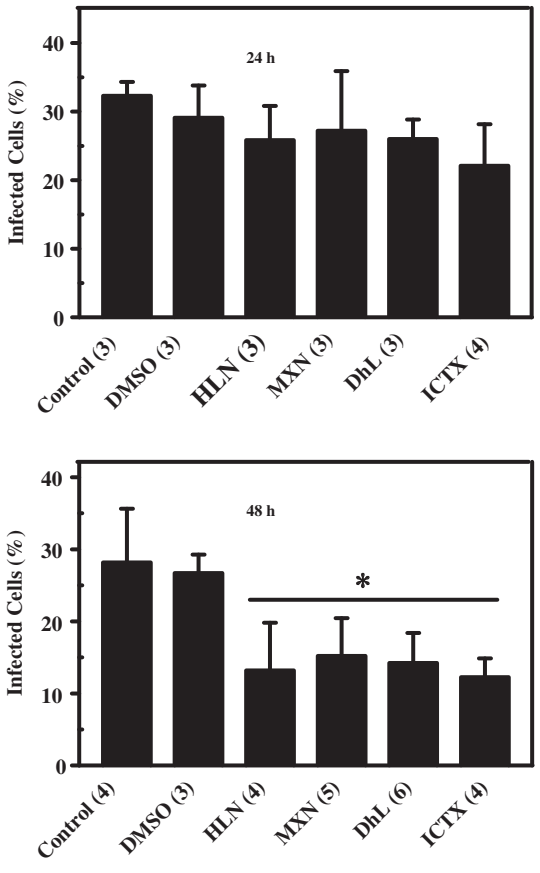

Fig. 3. Vero cells observed by light microscopy were counted for the number of infected cells. The percentage in each case was estimated from the number of infected cells/total cells counted × 100. The bars represent the means of percentages of infected cells ± SD at 24 or 48 h after treatments with the compounds, at the concentrations indicated in Fig. 2. Numbers between parentheses indicate the number of experiments for each case. DMSO: cells treated with the solvent alone; HLN: 1.5 μM helenalin; MXN: 1.5 μM mexicanin; DhL: 3.8 μM dehydroleucodine; ICTX: 3.0 μM 5-epi-icetexone. (*) Statistical difference ($p<0.001$) in relation to control groups.

Table 1
Cytotoxicity of the compounds on Vero cells.

	Dead cells (%)	
	24 h	48 h
Controls	0.40 ± 0.27	0.80 ± 0.44
DMSO	0.00 ± 0.00	0.60 ± 0.54
MXN (1.5 μM)	0.40 ± 0.27	7.25 ± 0.65
MXN (3.0 μM)	4.25 ± 0.60	13.19 ± 1.32
ICTX (1.5 μM)	0.80 ± 0.83	0.40 ± 0.22
ICTX (3.0 μM)	0.50 ± 0.25	6.80 ± 1.29
DhL (1.5 μM)	0.20 ± 0.44	0.60 ± 0.89
DhL (3.8 μM)	0.80 ± 0.83	2.00 ± 1.22
BZN (1.5 μM)	0.50 ± 0.57	0.40 ± 0.22
BZN (3.0 μM)	0.20 ± 0.44	0.80 ± 0.44

Values are expressed as the mean percentage of dead cells after 24 or 48 hour incubation with the compounds ± SD of three experiments.

As shown in Fig. 5, these percentages were significantly reduced after the treatment with the compounds, HLN being the most potent inhibitor.

As shown in Fig. 6 the number of parasites (as trypomastigotes) recovered from the extracellular medium dramatically decreased after treatment with HLN or MXN. This effect was already observable after 24 h of treatment. Meanwhile, ICTX or DhL caused a significant decrease at 48 h of treatment. This decrease caused by the compounds could be attributed either to a prevention in the differentiation and release of parasites or rapid mortality of trypomastigotes in the extracellular medium. The compounds were also tested on bloodstream trypomastigotes as described in Materials and methods. As shown in Fig. 7, this stage was very sensitive to the action of the compounds, MXN and HLN being the most powerful. In turn, all compounds used were more active than BZN (Fig. 7), whereas DMSO did not affect the parasites to the concentrations used (data not shown). In addition, the remaining parasites after treatment with MXN or HLN were immobile.

4. Discussion

Although attempts have been made to find an adequate chemotherapy against Chagas disease, only a few drugs such as nifurtimox and benznidazole are used to alleviate the disease. However, the efficiency of these drugs in the chronic phase of the disease is still controversial and they also cause many undesirable side effects on treated patients so that their use is now somewhat restricted. For decades, natural compounds have been tested against the parasite causing Chagas disease. Despite the many compounds found with trypanocidal activity, their effect has been tested mostly in vitro and therefore they cannot even be used in any therapy due to some adverse effects on host cells, and in many cases their mechanism of action is unknown. Moreover, in most cases these compounds have not been tested against intracellular parasites or in experimentally infected animals. Understanding the life cycle and metabolism of the parasites are important issues in order to find the possible targets for the action of therapeutic agents.

Terpenoid derivatives, such as sesquiterpene lactones and diterpenes, are families of molecules abundant in the plant kingdom and many of them exhibit important biological activities. In this study we showed that three sesquiterpene lactones, such as HLN, MXN and DhL, and the

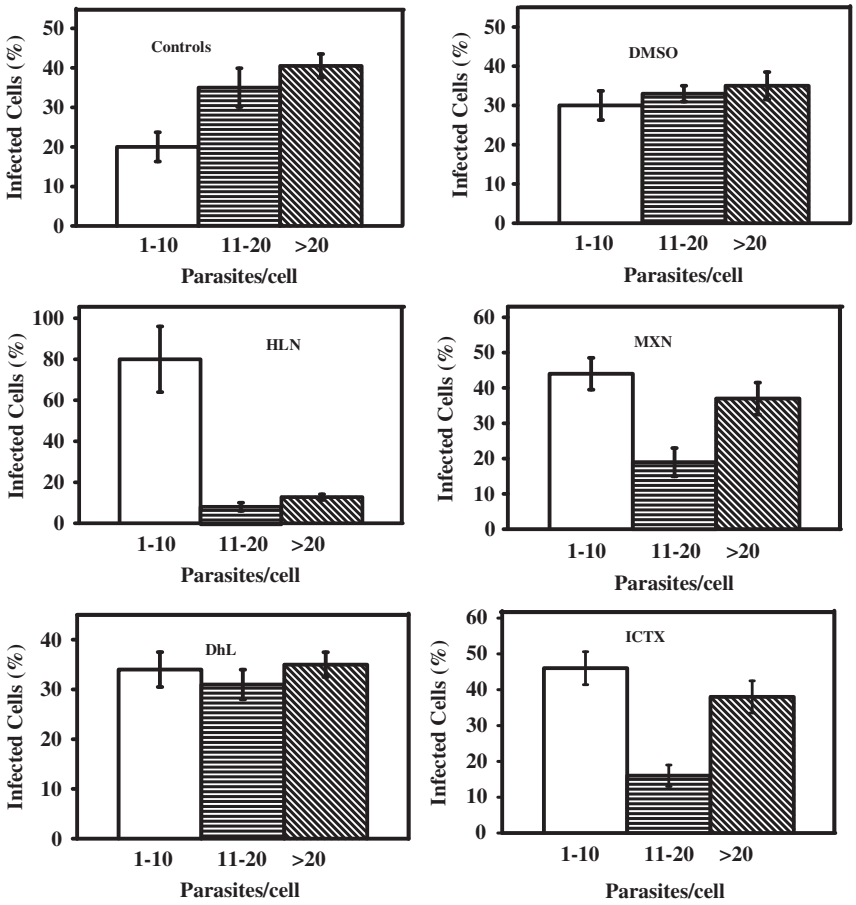

Fig. 4. Number of amastigotes per cell, counted from the infected Vero cells after 48 hour treatment with the compounds at the concentrations indicated in Fig. 2. Values represent the means of percentages from infected cells, containing: 1–10 (white bars), 11–20 (horizontal stripes), or more than 20 (diagonal stripes), parasites per cell ± SD from three experiments. DMSO: cells treated with the solvent alone; HLN: 1.5 μM helenalin; MXN: 1.5 μM mexicanin; DhL: 3.8 μM dehydroleucodine; ICTX: 3.0 μM 5-epi-icetexone.

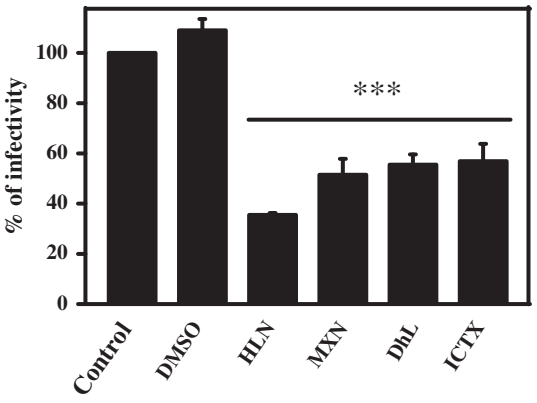

Fig. 5. The percentage of infectivity estimated from the data in Figs. 4 and 3 of the corresponding experiment (n = 3), by the equation detailed in Materials and methods. Bars represent the means ± SD. (***) Significant differences in relation to control groups ($p<0.001$). DMSO: cells treated with the solvent alone; HLN: 1.5 μM helenalin; MXN: 1.5 μM mexicanin; DhL: 3.8 μM dehydroleucodine; ICTX: 3.0 μM 5-epi-icetexone.

diterpene ICTX appear to be effective against intracellular parasites, as they inhibit the growth of amastigotes and possibly affecting their differentiation into trypomastigotes in the host cells. These compounds were previously tested in vitro against epimastigotes, affecting their viability and growth at very low concentrations [11–13]. After 48 h of treatment with compounds, the percentage of Vero cells infected with parasites, as well as the number of parasites per cell, were significantly lower than in controls, and the most powerful effect was due to HLN. This lactone (HLN) and derivatives have been previously tested successfully against trypomastigotes of *T. cruzi* [13], using a different strain of parasites (Tulahuen). Moreover, it appears that the spatial orientation of a hydroxyl group could be crucial for the activity of these lactones, and justify the differences between HLN and MXN observed by us and by Schmidt et al. [13].

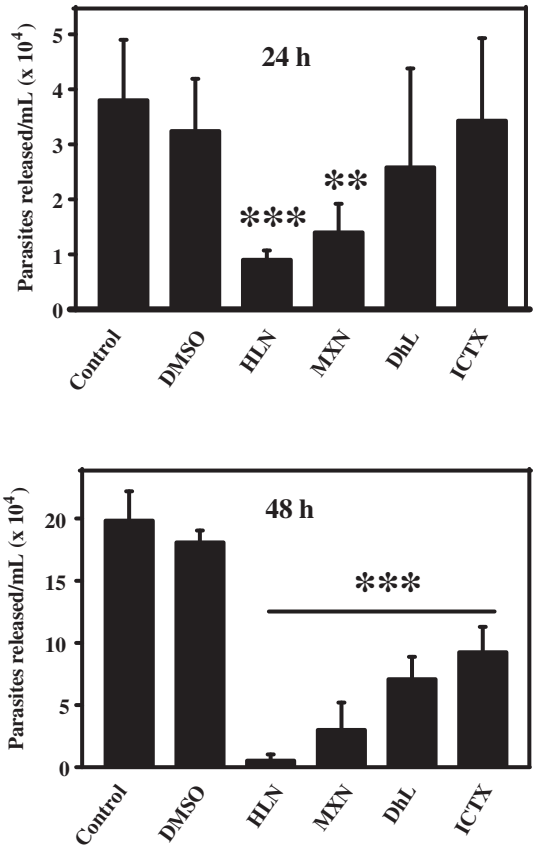

Fig. 6. Number or parasites released at 24 or 48 h after treatment with the compounds at the concentrations indicated in Fig. 2. Values represent the means of released parasites/ml rescued from the medium ± SD from three independent experiments. (**) and (***), significant differences in relation to control groups ($p<0.01$ and $p<0.001$ respectively). DMSO: cells treated with the solvent alone; HLN: 1.5 μM helenalin; MXN: 1.5 μM mexicanin; DhL: 3.8 μM dehydroleucodine; ICTX: 3.0 μM 5-epi-icetexone.

It is known that after several rounds of replication in the form of amastigotes, parasites differentiate into trypomastigotes and are released to the extracellular medium to infect new cells [25]. Our results show that the compounds significantly reduced the number of extracellular parasites (trypomastigotes), presumably as a result of low numbers of amastigotes or the compounds may also affect the differentiation into trypomastigotes. However, this phenomenon could also be a consequence of high mortality of amastigotes inside the cells, which has not been tested in this work. Another possibility is that the trypomastigotes are killed immediately by the compounds, as soon as the parasites are released into the extracellular environment. In support of this, an increased sensitivity of trypomastigote forms to certain drugs [26–28] has been reported, and the compounds tested here were also shown to be toxic to the trypomastigotes, even at very short exposure times. We ruled out that the effects are caused by the solvent used, since controls with DMSO did not differ significantly from controls without solvent, at least at the concentrations used in the trials. The effect of sesquiterpene lactones on intracellular *T. cruzi* has also been tested by others [14], with apparent different toxicities on parasites. However, these authors probed the compounds on other host cells and parasite strain, and this could explain the observed differences.

In conclusion, we demonstrate here that the compounds under study, as well as being active against epimastigotes [11–13], would also effect invasiveness to mammalian cells, and/or proliferation of intracellular parasites and the survival of trypomastigotes. All this could indicate that the compounds act by multiple mechanisms and possibly on different targets at each stage of the parasite. Their mechanisms of action remain to be elucidated, and if they only affect proliferation or the viability of the parasites inside mammalian cells, and/or differentiation into trypomastigotes. Although we have not used the typical host cells such as macrophages, because of serious difficulties to infect these cells in vitro, we believe that testing with Vero cells could represent in some way the mechanism of infection, since at least the processing cycles trypomastigote–amastigote–trypomastigote are completed in these cells. The findings presented here could be considered as an approach in the study of new drugs on models that could somehow simulate events in the chronic phase of Chagas disease [22].

5. Conclusions

We conclude that sesquiterpene lactones, such as helenalin, mexicanin and dehydroleucodine are active against infective forms of *T. cruzi*. These compounds and the diterpene 5-epi-icetexone diminished the percentages of infected Vero cells and the number of parasites per cell. Moreover, the compounds reduced the number of trypomastigotes in the medium. It remains to be discovered whether the effect of these compounds is on proliferation and/or the vitality of the intracellular parasites.

Acknowledgments

Dr. Miguel Sosa is a career researcher of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina). This work was supported by funds from the University of Cuyo (SECyT, 06/J280). We kindly acknowledge Mr. Tirso Sartor (IHEM-CONICET) and Mrs. Solange da Silva Hernandes (UNIFESP, Escola Paulista de Medicina,

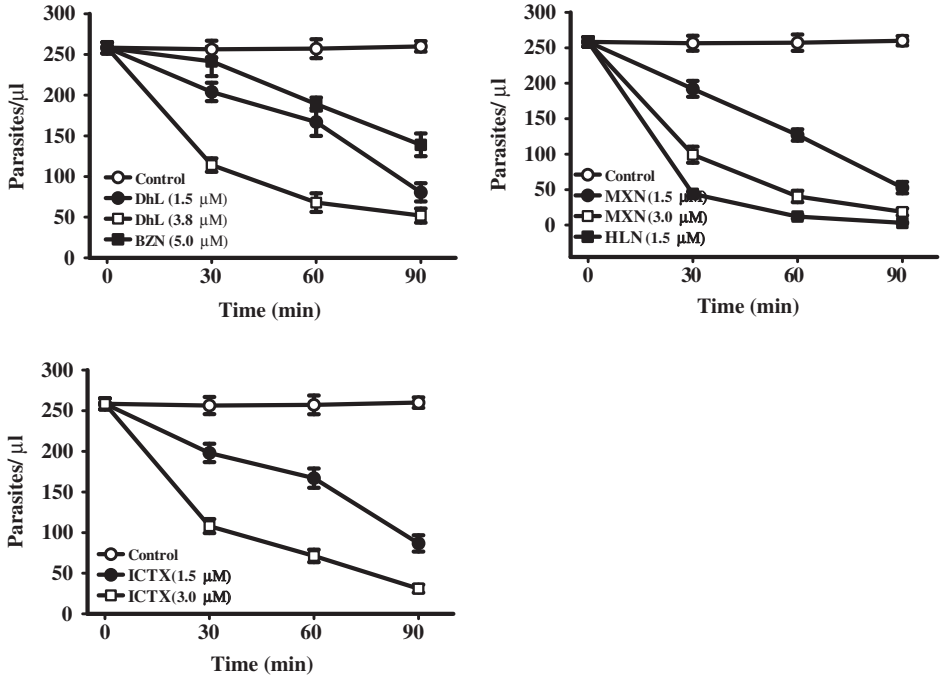

Fig. 7. Effect of the compounds on bloodstream trypomastigotes of *T. cruzi*. The assays were carried out with parasites obtained from the blood of infected mice, as detailed in Materials and methods. After the addition of compounds, to the indicated concentrations, the parasites were counted every 30 min. The values represent the means of two independent experiments ± SD. DMSO: cells treated with the solvent alone; HLN: helenalin; MXN: mexicanin; DhL: dehydroleucodine; lCTX: 5-epi-icetexone; BZN: benznidazole at the indicated concentrations.

São Paulo) for their valuable technical assistance. Mr. E. Lozano and Mrs. P. Barrera were partially supported by AMSUD/Pasteur.

References

[1] Moloney A. Trial renews interest in Chagas' disease. The Lancet 2009;374:1490.
[2] Morris K. After 100 years, time to break silence on Chagas' disease. The Lancet Infectious Diseases 2009;9:468.
[3] The Lancet Editorial. Chagas disease: a neglected emergency. The Lancet 2009;373:1820.
[4] Chagas disease: the forgotten American neuroinfection. The Lancet Neurology 2009;8:501.
[5] De Souza W. Basic cell biology of *Trypanosoma cruzi*. Current Pharmacology Design 2002;8:269–85.
[6] Sepúlveda-Boza S, Cassels BK. Plant metabolites active against *Trypanosoma cruzi*. Planta Medica 1996;62:97–162.
[7] Duschak VG. A decade of targets and patented drugs for chemotherapy of Chagas disease. Recent Patents on Anti-Infective Drug Discovery 2011;6:216–59.
[8] Amato Neto V. Etiological treatment for infection by *Trypanosoma cruzi*. Memórias do Instituto Oswaldo Cruz 1999;94:337–9.
[9] Maya JD, Repetto Y, Agosin M, Ojeda JM, Tellez R, Gaule C, et al. Effect of Nifurtimox and Benznidazole upon glutathione and trypanothione content in epimastigote, trypomastigote, and amastigote forms of *Trypanosoma cruzi*. Molecular and Biochemical Parasitology 1997;86:101–6.
[10] Salem MM, Werbovetz KA. Natural products from plants as drug candidates and lead compounds against leishmaniasis and trypanosomiasis. Current Medicinal Chemistry 2006;13:2571–98.
[11] Brengio SD, Belmonte SA, Guerreiro E, Giordano OS, Pietrobon EO, Sosa MA. The sesquiterpene lactone dehydroleucodine (DhL) affects the growth of cultured epimastigotes of *Trypanosoma cruzi*. The Journal of Parasitology 2000;86:407–12.
[12] Jimenez-Ortiz V, Brengio SD, Giordano O, Tonn C, Sanchez M, Burgos MH, et al. The trypanocidal effect of sesquiterpene lactones helenalin and mexicanin on cultured epimastigotes. The Journal of Parasitology 2005;91:170–4.
[13] Sanchez AM, Jimenez-Ortiz V, Sartor T, Tonn CE, García EE, Nieto M, et al. A novel icetexane diterpene, 5-epi-icetexone from *Salvia gilliessi* is active against *Trypanosoma cruzi*. Acta Tropica 2006;98:118–24.
[14] Schmidt T, Brun G, Willhun G, Khalid SA. Trypanocidal activity of helenalin and some structural related sesquiterpene lactones. Planta Medica 2002;68:750–1.
[15] Picman AK. Biological activities of sesquiterpene lactones. Biochemical Systematics and Ecology 1986;14:255–81.
[16] Jisaka M, Ohigashi H, Takegawa K, Huffman MA, Koshimizu K. Antitumoral and antimicrobial activities of bitter sesquiterpene lactones of *Vemonia amygdalina*, a possible medicinal plant used by wild chimpanzees. Bioscience, Biotechnology, and Biochemistry 1993;57:833–4.
[17] Quintero A, Pelcastre A, Solano JD. Antitumoral activity of new pyrimidine derivatives of sesquiterpene lactones. Journal of Pharmacy and Pharmaceutical Sciences 1999;2:108–12.
[18] Dirsch VM, Stuppner H, Vollman AM. Helenalin triggers a CD95 death receptor-independent apoptosis that is not affected by overexpression of Bcl-XL or Bcl-2. Cancer Research 2001;61:5817–23.
[19] Giordano OS, Guerreiro E, Pestchanker MJ, Guzman J, Pastor D, Guardia T. The gastric cytoprotective effect of several sesquiterpene lactones. Journal of Natural Products 1990;53:803–9.
[20] Nieto M, Garcia EE, Giordano OS, Tonn CE. Icetexane and abietane diterpenoids from *Salvia gilliessi*. Phytochemistry 2000;53:911–5.
[21] Zingales B, Andrade SG, Briones MR, Campbell DA, Chiari E, Fernandes O, et al. New consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends Tcl to TcVI. Memórias do Instituto Oswaldo Cruz 2009;104:1051–4.
[22] Fernandes MC, Cortez M, Yoneyama KAG, Straus AH, Yoshida N, Mortara R. Novel strategy in *Trypanosoma cruzi* cell invasion: implication of cholesterol and host cell microdomains. International Journal of Parasitology 2007;37:1431–41.
[23] Pires SF, Da Rocha WD, Freitas JM, Oliveira LA, Kitten GT, Machado CR, et al. Cell culture and animal infection with distinct *Trypanosoma cruzi* strains expressing red and green fluorescent proteins. International Journal of Parasitology 2008;38:289–97.
[24] Barrera P, Jimenez-Ortiz V, Giordano O, Tonn C, Galanti N, Sosa MA. Natural sesquiterpene lactones are active against *Leishmania mexicana* possible by multiple effects. The Journal of Parasitology 2008;94:1143–9.
[25] Burleigh BA, Woosley AM. Cell signalling and *Trypanosoma cruzi* invasion. Cellular Microbiology 2002;4:710–1.
[26] Sülsen V, Frank FM, Cazorla SI, Anesini CA, Malchiodi EL, Freixa B, et al. Trypanocidal and leishmanicidal activities of sesquiterpene lactones from *Ambrosia tenuifolia* Sprengel (Asteraceae). Antimicrobial Agents and Chemotherapy 2008;52:2415–9.
[27] Karioti A, Skaltsa H, Kaiser M, Tasdemir D. Trypanocidal, leishmanicidal and cytotoxic effects of anthecotulide-type linear sesquiterpene lactones from *Anthemis auriculata*. Phytomedicine 2009;16:783–7.
[28] Uchiyama N. Antichagasic activities of natural products against *Trypanosoma cruzi*. Journal of Health Science 2009;55:31–9.