Acta Tropica 170 (2017) 149–160

Contents lists available at ScienceDirect

Acta Tropica

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

# Chaetocin—A histone methyltransferase inhibitor—Impairs proliferation, arrests cell cycle and induces nucleolar disassembly in *Trypanosoma cruzi*

Aline Araujo Zuma[a], Jean de Oliveira Santos[a], Isabela Mendes[b], Wanderley de Souza[a], Carlos Renato Machado[b], Maria Cristina M. Motta[a,*]

[a] Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21491-590, Rio de Janeiro, RJ, Brazil
[b] Laboratório de Genética Bioquímica, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil

ARTICLE INFO

*Article history:*
Received 12 August 2016
Received in revised form 12 January 2017
Accepted 6 February 2017
Available online 6 February 2017

*Keywords:*
*Trypanosoma cruzi*
Chaetocin
Histone methyltransferase inhibitor
Proliferation
Cell cycle
Ultrastructure

ABSTRACT

The Trypanosomatidae family includes pathogenic species of medical and veterinary interest. Chagas disease is endemic in Latin America, and about 8 million people are infected worldwide. There is a need for more effective drugs for the acute, undetermined and chronic phases of the disease that, in addition, do not cause side effects, stimulating the search for identification of new drug targets, as well as new chemotherapeutic targets. Trypanosomatids contain characteristic structures, such as the nucleus that undergoes a closed mitosis without chromosome formation and variations of chromatin packing in the different protozoa developmental stages. The nuclear DNA is condensed by histones that suffer post-translational modifications, such as addition of methyl groups by histone methyltransferases (MHT) and addition of acetyl groups by acetyltransferases. These processes modulate gene expression and chromatin organization, which are crucial to transcription, replication, repair and recombination. In the present study, the effects of chaetocin, a HMT inhibitor, on *T. cruzi* epimastigote proliferation, viability, ultrastructure and cell cycle were investigated. Results indicate that chaetocin promoted irreversible inhibition of protozoa growth, evident unpacking of nuclear heterochromatin and intense nucleolus fragmentation, which is associated with parasite cell cycle arrest and RNA transcription blockage. Taken together, data obtained with chaetocin treatment stimulate the use of histone methyltransferase inhibitors against pathogenic trypanosomatids.



## 1. Introduction

Trypanosomatids are found in several countries of different continents and are classified as heteroxenic, which are pathogenic; or monoxenic, which only inhabit invertebrate hosts during their entire life cycle. Although pathogenic species are a minority in this family, they raise medical and veterinary interest since they are agents of lethal diseases in men, as well as in animals and plants of economic interest. In Latin America and in other developed countries, millions people are affected by Chagas disease, whose etiological agent is *Trypanosoma cruzi*; whereas in Africa, diseases like nagana (in animals) and sleeping sickness (in humans) are caused by *Trypanosoma brucei*. In underdeveloped countries, numerous cases of leishmaniasis are reported and attributed to several species of the *Leishmania* genus (De Souza, 2002; Jensen and Englund, 2012).

Trypanosomatids contain typical eukaryotic organelles, such as the endoplasmic reticulum, the Golgi complex and the nucleus. The latter presents the heterochromatin close to the nuclear envelope and around the nucleolus, which is localized in the central region of the nucleus. The nucleolus presents characteristic domains, such as the fibrillary center and the granular region, but is less organized when compared to that observed in upper eukaryotes (Motta et al., 2003). Throughout the cell cycle, chromatin organization and distribution are more dispersed during the interphase and becomes more condensed in the beginning of the closed mitosis, when the nucleus is more elongated and the nucleolus disorganizes. At the end of mitosis, chromatin migrates to the polar region and the nucleus

* Corresponding author.
*E-mail address:* cristinamotta@hotmail.com (M.C.M. Motta).

http://dx.doi.org/10.1016/j.actatropica.2017.02.007
0001-706X/

divides during cytokinesis, however condensed chromosomes are never observed (Ogbadoyi et al., 2000; Elias et al., 2002; De Souza, 2002).

Chromatin is constituted by DNA, which is associated to histones and non-histone proteins, forming repetitive units, the nucleosomes. Each nucleosome is composed by a DNA fragment and an octameric structure containing the histones H2A, H2B, H3 and H4 (Monneret, 2005; Martínez-Iglesias et al., 2008; Legartová et al., 2013). Histones are basic proteins rich in lysine and arginine residues. They present a C-terminal domain, located inside the nucleosome, and an outside N-terminal tail containing lysine residues (Monneret, 2005). Several post-translational modifications are observed in histone tails, playing an important role in epigenetic control of gene expression. Methyl groups added to histones by histone methyltransferases (MHT) may alter chromatin condensation, while addition of acetyl groups to histones by acetiltransferases relaxes the DNA fibrils. Such processes influence the access of proteins to DNA, thus modulating the condensation state of chromatin, which is crucial for transcription, replication, repair and recombination (Monneret, 2005; Legartová et al., 2013).

Although histones are considered one of the most conserved proteins in eukaryotes, in trypanosomatids they are quite divergent compared to other organisms, especially in the N-terminal region, that contains alternative sites for post-translational modifications (revised by Figueiredo et al. (2009)). Lysine acetylation and methylation, arginine methylation and serine phosphorylation have been observed in trypanosomatid histones. Histones H4 and H2A N-terminus are frequently acetylated, while histones H3 and H2B are preferentially methylated. In *T. brucei* and *T. cruzi*, histone H4 is acetylated in the N-terminal portion at lysine residues 4, 10 and 14, whereas lysine 18 is methylated (Chagas da Cunha et al., 2006; Janzen et al., 2006; Mandava et al., 2007; Elias et al., 2009).

The roles of histone modifications on *T. cruzi* cellular processes have been elucidated. Histone acetylations in lysines 10 and 14 are required for DNA replication and transcription, as well as for chromatin organization and remodeling (Ramos et al., 2015). Such post-translational modifications may also be involved in trypanosomatid differentiation: in epimastigotes, phosphorylation of serine 23 in H2B and methylations of lysine 76 in histone H3 predominate, while trypomastigotes mainly present lysine acetylations in histone H2A and methylation of lysine 23 in histone H3. Furthermore, the replicative stage contains more histone modifications than the trypomastigote form (De Jesus et al., 2016). Histone modifications and distribution are directly related to the protozoan cycle progression. H1 phosphorylation, for example, is concentrated in the nucleolar region during the G1/S phase, but occupies the entire nuclear space in mitoses, when phosphorylation is maximized (Gutiyama et al., 2008). Moreover, histone H4 acetylated at lysine 4 is found in the condensed chromatin, while histones acetylated at 10 or 14 residues are distributed throughout the nucleus (Nardelli et al., 2009; Elias et al., 2009).

Considering that epigenetic regulation in pathogenic trypanosomatids affects parasite life cycle and virulence, drugs that target enzymes involved in histone methylation have been employed to clarify how the post-transcriptional modifications of histones influence gene expression (Marks et al., 2004).

Histone methyltransferases inhibitors have been used with success against tumor cells, since they promote proliferation inhibition, cell cycle arrest and apoptosis (Marks and Xu, 2009). Chaetocin, which is produced by yeast belonging to the genus *Chaetomium*, is a histone methyltransferase (HMT) inhibitor (Lai et al., 2015). In tumor cells, this compound promotes changes in nuclear organization, such as strong chromosome condensation, and considerably reduces cellular proliferation and viability (Isham et al., 2007; Illner et al., 2010). In this context, it is important to study the effects of HMTs in lower eukaryotes, such as fungi and protists.

In the present study, we showed for the first time the effects of chaetocin in a trypanosomatid species. This inhibitor impaired cell proliferation, reduced cell viability and blocked cell cycle on G2/M phase of *T. cruzi* epimastigotes. Chaetocin also promoted nucleolar disassembly, which seems to be induced by the reduction in rRNA transcription, an effect that has never been described for an inhibitor that has methyltransferases as a target. Furthermore, reversibility assays showed that parasites were not able to re-stablish proliferation after drug removal, indicating that HMT inhibitors may be exploited in therapeutic treatments against trypanosomatid diseases.

## 2. Material and methods

### 2.1. Protozoa culture and drug treatment

Epimastigote forms of Y strain *T. cruzi* were cultivated for 24 h at 28 °C in liver infusion tryptose (LIT) medium (Camargo, 1964) supplemented with 10% fetal calf serum.

Chaetocin was diluted in dimethyl sulfoxide (DMSO) to a concentration of 10 mM and evaluated in concentrations of 1, 5, 10, and 50 μM. Cells were collected every 24 h for counting in a Neubauer chamber. To compare the control and the treated groups, paired *t*-tests were applied to the results using a 95% confidence interval (GraphPad Prism version 5.00 for Windows; GraphPad Software Inc., San Diego, CA, USA).

To evaluate the reversibility effect of cell proliferation, parasites were treated for 2 days, cultures were washed with LIT to remove the drug from the medium and subsequently incubated with LIT and fetal calf serum up to 168 h.

### 2.2. Cell viability

Parasites were analyzed by the MTS/PMS method (Henriques et al., 2011), which is based on mitochondrial dehydrogenase enzyme activity, and also by propidium iodide (PI) incorporation, which is based on plasma membrane integrity. In the MTS/PMS method, parasites were incubated with the MTS/PMS solution for 4 h. Untreated parasites were fixed with 0.4% formaldehyde for 10 min and used as negative control. The percentage of viable parasites was obtained using a spectrofluorometer (Molecular Devices Microplate Reader, SpectraMax M2/M2$^e$, Molecular Devices) at a wavelength of 490 nm. For PI incorporation, parasites were ressuspended in PBS with 10 μg/ml PI. As negative controls, parasites were permeabilized with 1% Triton X-100 for 15 min and then incubated with PI. The samples were analyzed using a BD Accuri C6 flow cytometer (BD Biosciences, USA), considering 10.000 events, and the data were analyzed using BD Accuri C6 software.

### 2.3. Transmission electron microscopy

Treated and non-treated parasites were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 1 h and were washed in the same buffer. The cells were post-fixed for 1 h in 0.1 M cacodylate buffer containing 1% $OsO_4$ and 0.8% potassium ferricyanide. Parasites were washed in the same buffer, dehydrated in a graded series of acetone, and embedded in Epon (Electron Microscopy Sciences, Hatfield, PA, USA). Ultrathin sections were stained with uranyl acetate for 45 min, then with lead citrate for 5 min and observed using a Zeiss 900 transmission electron microscope (Zeiss, Oberkochen, Germany).