Effects of Human Defensin-α_1 on *Trypanosoma cruzi* Trypomastigotes *in Vitro*

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Human defensin-α_1 is a biologically active peptide exhibiting a dose-dependent trypanocidal effect *in vitro* against trypomastigotes and amastigotes of *Trypanosoma cruzi* line Tulahuen. This effect is determined by fragmentation of parasite DNA reducing the capacity of passaged *T. cruzi* to invade HeLa cells.

Key Words: *defenzin-α_1; Trypanosoma cruzi; Chagas disease; trypomastigotes*

Trypomastigotes of *Trypanosoma cruzi*, a causative agent of Chagas disease (American trypanosomiasis) transmitted to humans avoid the defensive blood factors and penetrate into host cells, where they transform into amastygote forms, proliferate, and then transform back into trypomastigotes. The latter invade new targets after destruction the objects of parasitizing. The pattern of the infectious process in Chagas disease depends on number of factors, including the capacity of the agent to resist the effects of protective factors circulating in host fluids [1]. One of these factors, human defensing-α_1 (HDF-α_1), is a peptide with a molecular weight 3.5 kDa consisting of 30 amino acids. It is secreted by various immune system cells: neutrophils [3], natural killers, and B- and T-cells [2] and possesses nonspecific microbicidal activity [4,5]

Effects of HDF-α_1 on *T. cruzi* and its role in the control of invasion of the target cells were not investigated. Since preliminary experiments showed that human cells respond to *T. cruzi* infection by an increase in HDF-α_1 expression, we decided to investigate effects of this compound on trypanosomes.

MATERIALS AND METHODS

Culture of trypomastigotes and amastigotes of *Trypanosoma cruzi* (Talahuen strain) was used in experiments [6]. HDF-α_1 with amino acid sequence ACY CRIPACIAGERRYGTCIYQGRLWAFCC was obtained synthetically and purified by HPLC [9]. Mass-spectrometry showed single peak with mass-to-charge ratio 3445.07. Scrambled peptide with the sequence CACRPGCRIQYECRARLTAICIGYFAWYCG and consisting of amino acids present in HDF-α_1 was used as the control.

For evaluation of trypanocidal activity of HDF-α_1, *T. cruzi* trypomastigotes (2×10^6/ml) were cultured in DMEM in the presence of 3.7-35 μM HDF-α_1. The number of killed trypomastigotes was evaluated under a microscope. The experiments were performed in three replicates for each condition.

For evaluation of the capacity of HDF-α_1 to inhibit intracellular invasion by *T. cruzi*, HeLa cells were used as the target, since they are a well-known *in vitro* model for studying *T. cruzi* infection [8]. Trypomastigotes were incubated with sublethal doses of HDF-α_1 (3.7 μM), which is equivalent to HDF-α_1 concentration in the blood of healthy individuals. HDF-α_1 in the specified concentrations or the same amounts of control peptide were added to the culture medium. Parasite cells were washed in DMEM culture medium and added to confluent HeLa cell cultures (10 parasites per 1 target cells) for 2 h. Trypanosomes not bound to culture cells were removed by DMEM washout, fixed in methanol and stained after Geimsa and with DNA-specific dye DAPI (4',6-diamidin-2phenilindole) [7]. The number of parasites per 200 HeLa cells was estimated under a microscope [10].

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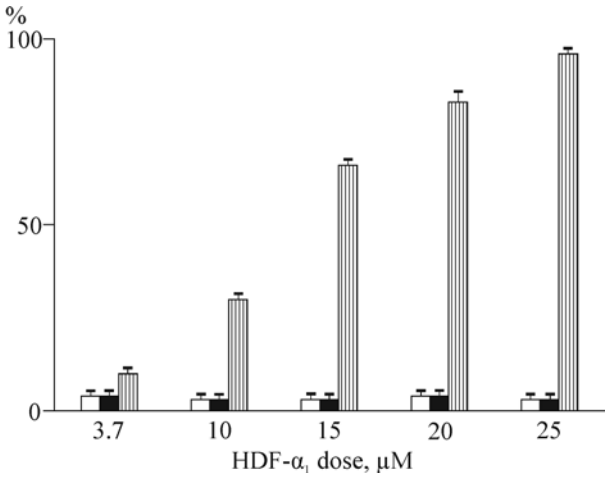

Fig. 1. Relationship between *T. cruzi* trypomastigotes death and HDF-α_1 dose. Here and in Fig. 2: light bars: culture medium, dark bars: control peptide, vertical shading: HDF-α_1.

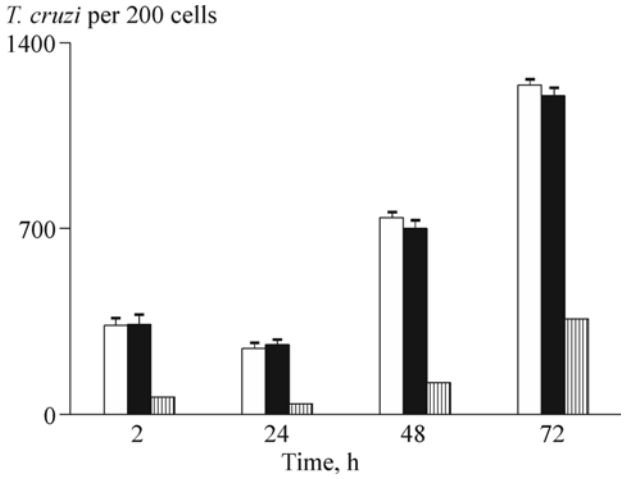

Fig. 2. Decrease in the number of HeLa cells infested with trypanosomes after incubation with 3.7 μM HDF-α_1.

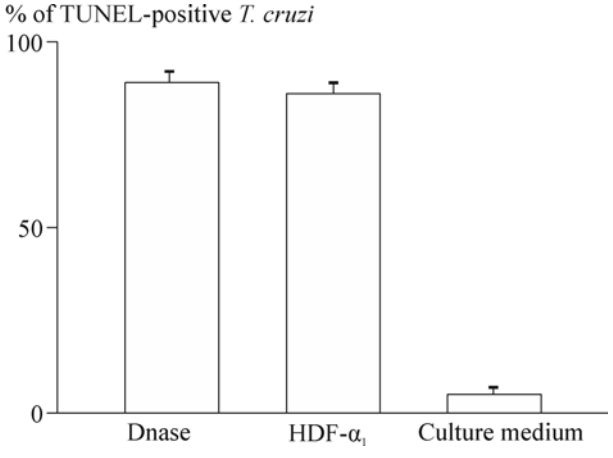

Fig. 3. Induction of *T. cruzi* DNA fragmentation under the influence of HDF-α_1.

Monitoring of *T. cruzi* DNA fragmentation was performed using an approach based on specific labeling of DNA ends with biotinylated dUTP (TUNEL-analysis) extensively used for detection cell death *in situ* (Roche Diagnostics Inc., Indianapolis) [7]. *T. cruzi* trypomastigotes (10^6/ml) were cultured with 10 μM HDF-α_1 for 5 min at 37°C in DMEM culture medium without phenol red (Invitrogen Inc.). *T. cruzi* incubated in culture medium without HDF-α_1 or with HDF-α_1 pretreated with DNase from bovine pancreas (No. 6101, Promega, Madison) were used as controls. TUNEL assay was performed under a fluorescent microscope (Nikon Labophot, Nikon).

The data were processed statistically using Graphpad Prism software. The differences were considered to be significant at $p<0.05$ (Student's t test).

RESULTS

HDF-α_1 was established to exhibit dose-dependent trypanocidal activity against *T. cruzi* trypomastigotes and amastigotes. The effect of the agent corresponded to the saturation kinetics. The control peptide did not affect parasite viability (Fig. 1).

After 2-h incubation with 3.7 μM HDF-α_1, *T. cruzi* trypomastigotes demonstrated reduced binding and penetration into the host cells. These trypomastigotes replicated in the form of amastigotes 48-72 h after infection of HeLa cells. Their number in the samples treated with HDF-α_1 was significantly lower than in samples treated with the control peptide and medium (Fig. 2).

HDF-α_1 is capable of inducing DNA fragmentation in *T. cruzi* trypomastigotes. TUNEL assay showed that incubation of *T. cruzi* trypomastigotes with 10 μM HDF-α_1 led in the appearance of 98% of TUNEL-positive parasites (compared to control DNase-treated samples).

These findings led us to a conclusion that HDF-α_1 exhibits a trypanocidal effect on *T. cruzi* and initiates parasite DNA fragmentation, which results in trypanosome death and reduction of invasion of cultured human tumor cells.

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