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BRIEF REPORT

Effect of Zinc-Treated *Entamoeba histolytica* on the Human Polymorphonuclear Respiratory Burst

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One of the mechanisms that *Entamoeba histolytica* uses to evade host immune response is inhibition of the polymorphonuclear (PMN) leukocyte respiratory burst. In studies previously conducted in a model used in our laboratory, we observed that when treating trophozoites with different zinc concentrations certain amebic functions are inhibited while significantly limiting development of hepatic abscess in golden hamsters (*Mesocricetus aureatus*). We carried out an *in vitro* study using a chemoluminescent method to assess the effect zinc-treated amebic trophozoites exercise on respiratory burst in human PMNs. We measured response of PMNs incubated with *E. histolytica* trophozoites from cultures with TYI-S33 medium alone and with zinc. Zinc concentrations between 0.1 and 1.0 mM did not affect amebic trophozoite viability, and PMNs in contact with these in a zinc-free medium had an oxidative response similar to that obtained with zymosan and significantly greater ($p < 0.05$) than that generated by cells co-incubated with amebas cultured in TYI-S33 medium alone. These results suggest that zinc alters the amebic mechanism that inhibits the oxidative function of human polymorphonuclear leukocyte.

Key Words: Entamoeba histolytica, Ameba, Protozoa, Parasitic, Chemoluminescence, Respiratory burst, Amebic function, Polymorphonuclear, Zinc.

Introduction

Entamoeba histolytica is a parasite with great invasive and evasive potential due to its lithic activity, which allows it to penetrate and displace itself through tissues, as well as due to the mechanisms it has developed to evade host immune responses. In this respect, it is known that the ameba, by means of a surface lectin, inhibits the complement membrane attachment complex. The parasite is capable of redistributing the antibodies on its surface and the attached complement in a phenomenon known as capping. Caps can be freed into the medium or internalized, protecting the ameba from damage caused by these components (1). Additionally, by means of different mechanisms it induces an increase in calcium in the white cell (2), altering its function immediately and thus modifying important cellular processes including certain host immunity effectors. During acute phase of invasive amebiasis, the following changes have been found in patients: decrease in helper T lymphocyte count (CD4) and increase in cytotoxic T lymphocytes (CD8), as well as decrease in proliferative response of T lymphocytes to amebic antigens (3).

Other *in vitro* assays have shown that amebic trophozoites are able to inhibit polymorphonuclear respiratory bursts (4–7) and to destroy them (8). In a previous study, we observed that zinc (0.1 and 1 mM) inhibited replication and adherence of *E. histolytica* and significantly diminished development of amebic hepatic abscesses in hamsters (9). Under normal conditions, the ameba alters phagocytic functions and induces death of human leukocytes, suppressing

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one of our main defense mechanisms. It is then necessary to evaluate other mechanisms that could be disturbed by zinc; therefore, our objective was to study the effect that *E. histolytica* treated with zinc may have on the respiratory bursts in PMNs.

Materials and Methods

Parasite Culture

E. histolytica HM1-IMSS trophozoites were axenically grown in TYI-S33 medium (10). Amebas (1×10^5) (live trophozoites) were placed in tubes with 10 mL TYI-S33 culture medium supplemented with zinc sulfate (0.1 and 1.0 mM) and were incubated at 37°C for 72 h. Amebas were harvested by chilling on ice for 10 min and were subsequently washed three times in cold phosphate-buffered saline solution (PBS) 0.15 M pH 7.4 to eliminate any residual zinc.

Polymorphonuclear Leukocytes

Twenty-milliliter samples of heparinized venous blood were obtained from healthy adult donors. The blood, diluted 1:2 with PBS, pH 7.2, was placed over 5 mL of Polymorphoprep d = 1.113 (Nycomed Pharma AS, Oslo, Norway). The suspension was centrifuged at $400 \times g$ for 40 min at 20°C and interphase cells were collected and washed three times with PBS. Remaining erythrocytes were lysed by hypotonic shock with NH_4Cl 155 mM and the button was washed twice with PBS. A total of 97% of these cells were PMNs and the remainder, lymphocytes. For respiratory burst assays (measured using chemoluminiscence), PMNs were adjusted to a concentration of 1×10^7 cells per mL and used immediately.

Viability

Amebic trophozoite and PMN leukocyte viability were 95 and 90%, respectively, as determined using trypan blue exclusion technique. Nonstimulated PMN leukocytes were co-incubated for 3 h at 37°C with control (wild-type) amebic trophozoites (cell ratio 10:1) or amebic trophozoites previously cultured with zinc (0.1, 1.0 mM). Aliquots of these combinations were dyed and placed on slides for microscopic observation and viability count.

Chemoluminescence

Cells were placed in 3-mL polystyrene vials (LKB-Pharmacia, Uppsala, Sweden) with 1×10^6 PMN in 100 µL PBS, or 100 µL PBS with 5×10^3 amebas (grown for 72 h in culture medium TYI-S33 alone or added with zinc and washed). Vials were incubated for 15 min at 37°C, and the following were added: 700 µL luminol (Eastman Kodak, Rochester, NY, USA) 1×10^{-6} M and 200 µL opsonized zymosan (12.5 mg/mL). Vials were continuously counted for 30 min using a BioOrbit 1250 luminometer (LKB-Pharmacia) and results were registered in mV and expressed as the area under the curve or as an average number ± standard error (SE) of 1×10^6 mV/10^6 cells in 1 min at maximum chemoluminescence peak.

Statistical Analysis

Results were analyzed using Fisher exact test (11) for variance and nonparametric Mann–Whitney *U* test (12).

Results

Cell Viability

At 180 min PMN incubated alone had a 95% viability, amebic trophozoites alone were 90% alive. The different co-incubation conditions are summarized in Table 1.

Entamoeba histolytica *vs. PMN*

The observed mortality of PMNs co-incubated with control *E. histolytica* trophozoites (10:1) during 3 h was 47%, significantly greater ($p < 0.05$) than in trophozoites pre-treated with zinc (0.1 and 1.0 mM), which was 28 and 21%, respectively.

PMNs vs. Entamoeba histolytica

Amebas pre-treated with zinc (0.1 and 1.0 mM) on interacting with PMNs had a mortality rate of 19 and 25%, respectively, significantly greater ($p < 0.001$) than that observed with control ameba (10%), which did not vary during contact with PMNs.

Chemoluminiscence

Zymosan-stimulated PMNs emitted luminescence equivalent to 40.3 ± 6.5 mV/10^6 (Figure 1), which diminished significantly ($p < 0.05$) in cells co-incubated with control

Table 1. Mortality

Co-incubation	15 min		180 min	
PMN + *E. histolytica* control	PMN	5%	PMN	47%
	Amebas	10%	Amebas	10%
PMN + *E. histolytica* pre-treated with 0.1 M Zn	PMN	10%	PMN	28%
	Amebas	12%	Amebas	19%
PMN + *E. histolytica* pre-treated with 1.0 M Zn	PMN	10%	PMN	21%
	Amebas	12%	Amebas	25%