Magainin Analogs Effective against Pathogenic Protozoa

C. M. HUANG,1,3 HAO-CHIA CHEN,2 and C. H. ZIERDT2

Department of Pathology, Thomas Jefferson University Hospital, 208 Pavilion, Philadelphia, Pennsylvania 19107,1 and Endocrinology and Reproduction Research Branch—National Institute of Child Health and Human Development2 and Clinical Pathology Department, Warren Grant Magnuson Clinical Center, National Institutes of Health,3 Bethesda, Maryland 20892

Received 26 February 1990/Accepted 26 June 1990

The in vitro activities of magainin analogs against Blastocystis hominis, Entamoeba histolytica, and Trypanosoma cruzi were assessed by protozoan morphological integrity and motility. The anti protozoan activities in descending order were magainin B > G > H, the same order as the α-helix contents of the analogs. Magainin B and G were effective against B. hominis, T. cruzi, and E. histolytica.

Magainins (PGS; peptides with amino-terminus glycine and carboxy-terminus serine), antimicrobial peptides of 23 amino acid residues, were isolated from the skin of the African clawed frog Xenopus laevis (4, 6). These active principles were responsible for preventing wound infection after nonsterile surgical procedures and return to microbially contaminated aquaria (6). These peptides were active against gram-positive and gram-negative bacteria and fungi (6, 7). It was previously reported that synthetic magainin analogs, after enhancement of amphiphilic helical structures, displayed an increase of up to two orders of magnitude in activity against gram-positive and gram-negative bacteria (1).

Natural magainin 2 was shown to induce osmotic lysis of several nonpathogenic protozoa (6). When Paramecium caudatum was incubated with magainin 2, the ciliate’s contractile vacuoles showed immediate swelling; subsequently, the organism was ruptured (7). The present study was undertaken to determine the in vitro efficacy of several representative magainin analogs against three pathogenic protozoa. Blastocystis hominis NAND is a bacterium-free (axenic) strain grown in egg slant medium with Locke solution and serum overlay (8). Entamoeba histolytica 200 (NIH clone) and Trypanosoma cruzi (2380-260) were kindly provided by L. S. Diamond of the National Institutes of Health and were grown as previously described (3).

Magainin analogs B, G, and H were synthesized by the standard solid-phase method and purified by preparative reverse-phase high-performance liquid chromatography as described previously (1). The amino acid sequences of these magainin analogs are shown as follows:


(The alanine, β-alanine, a, β-alanine; G, glycine; F, phenylalanine; K, lysine; L, leucine; H, histidine; S, serine; A, alanine; E, glutamic acid; V, valine; I, isoleucine; M, methionine; N, asparagine). Magainin B and G are highly active and H is inactive in inhibiting bacteria growth (1).

For assessment of anti protozoan activities, the magainin analogs were dissolved in isotonic saline solution. Final concentrations were 50, 100, 200, and 500 μg/ml. B. hominis cells were added to approximately 5.0 × 10⁷ cells per ml. An average of two organisms per field (40× objective, oil immersion) was used for E. histolytica and T. cruzi. Morphological integrity and motility were assessed at 5, 10, 20, 60, and 120 min on wet mounts by differential interference phase-contrast optics (Zeiss Photomicroscope). The percentage of injured cells was estimated. For electron microscopy, the cells of B. hominis were sedimented at 1,360 × g for 10 min after a reaction time of 5 min with magainin analogs at 500 μg/ml. The pellets were fixed for 1 h in phosphate buffer (pH 7.4) with 2% glutaraldehyde. The fixed cells were dehydrated, infiltrated with Epon-propylene oxide mixture, embedded in Epon, and sectioned.

Magainin B was the most active among the three analogs tested against B. hominis. The degree of destruction was dependent upon the peptide concentration. At 500 μg/ml, magainin B showed 100% cell destruction with release of organelles after a 5-min reaction. After 1 h of incubation, 75% of B. hominis cells had evidence of cell damage at 50 μg/ml (Fig. 1A to D). Magainin G at 500 μg/ml after 5 min showed immediate cell damage, with 50% of the cells leaking cytoplasm. Some cells became dull, with increased granularity, and the cell membrane lacked the usual glistening appearance. Cell injury was immediate; as time passed, more loss of cytoplasm and refractility occurred. At lower concentrations, membrane damage and leakage of cytoplasm were slower. Because of slow leakage, it took as long as an hour to complete the cell condensation. Regrowth was attempted with B. hominis after magainin damage by incubating the experimental tubes after the 2-h examination. Growth occurred and the culture recovered at the 100-μg/ml level, but not at 200 and 500 μg/ml. No damage was evident when B. hominis was incubated with magainin H at 500 μg/ml for 2 h.

The effect of magainin analogs on T. cruzi is seen in Fig. 1E to G. After a 5-min incubation at 500 μg of magainin B per ml, motility was absent. Immediate killing of T. cruzi was also seen with magainin B at 100 μg/ml. At 50 μg/ml, 75% of T. cruzi cells had some degree of cell damage, including loss of motility and granulation of the cytoplasm. Magainin analog H showed slight damage only at 50 μg/ml, but clumps of T. cruzi were resistant.

E. histolytica was very susceptible to magainin B; 100% cell death occurred at 500 μg/ml after a 5-min incubation. At 50 μg/ml, membrane leakage was seen in 90% of E. histolytica cells after a 1-h reaction. No pseudopods were seen in the presence of this peptide (Fig. 1H to J). Magainin G

* Corresponding author.
FIG. 1. (A to D) *B. hominis* with indicated amounts of magainin B. (A) 500 µg/ml. Total cell destruction was apparent within 10 min, with leakage of cell contents, including mitochondria and nuclei, into the surrounding medium. (B) 200 µg/ml. Cells show leakage and loss of refractility. (C) 100 µg/ml.Collapsed as well as intact cells are evident. (D) Magainin-free control. (E to G) *T. cruzi* with indicated amounts of magainin B. (E) 500 µg/ml. Total destruction followed membrane rupture. Flagellum is the only recognizable feature; motility is gone. (F) 100 µg/ml. Result is the same as that in panel E. (G) Magainin-free control. A clump of *T. cruzi* with flagellar movement is shown as a blur at the periphery. (H to J) *E. histolytica* with indicated amounts of magainin B. (H) 100 µg/ml. *E. histolytica* exhibited cessation of motility, loss of membrane refractility, and leakage of cell contents. (I) 50 µg/ml. The large amoeba shows the same cytopathology as in panel H. (J) Magainin-free control: refractile cell, actively motile. (K and L) Electron micrographs of *B. hominis*. (K) Normal *B. hominis* cells. (L) *B. hominis* treated with magainin B (500 µg/ml for 5 min). Loss of membrane integrity is evident.
showed similar effects against *E. histolytica*. Again, magainin H had no apparent effect on *E. histolytica* at 500 μg/ml.

The effect of magainin B on *B. hominis* as examined by electron microscopy is shown in Fig. 1K and L. Cell damage, with destruction of the cell membrane and release of organelles, was observed within 5 min of incubation. This finding was consistent with injuries seen by differential interference phase-contrast optics. *B. hominis* cells showed similar damage caused by magainin G, whereas there was no detectable damage by magainin H at 500 μg/ml.

The effect of analogs B and G on these protozoa was seen immediately or within minutes, as membrane leakage resulted in cell death. The mechanism of antiprotozoan activity of the magainin analogs is elusive. Matsuzaki et al. (5) demonstrated that magainin 1 induced leakage of a fluorescent marker, calcein, entrapped in lipid vesicles consisting of acidic and neutral phospholipids; this finding suggests that electrostatic interactions are of prime importance in interaction with the membrane. In the present study, *B. hominis* membrane leakage led to cell death; the leakage was clearly shown with differential interference phase-contrast optics and by an electron microscopy study. Therefore, it appears that the interaction of magainin analogs with the lipid components of the protozoan cell membrane is the cause of cell leakage and death.

We have shown previously (1, 2) that magainin analogs with high α-helical contents increase antimicrobial activity against gram-positive and gram-negative bacteria. In this study, the antiprotozoan activities, shown in descending order, were magainin B (61% α-helix calculated from circular dichroism spectra) > magainin G (52%) > magainin H (35%) (1). The substitution of D-Ala (magainin H) at three positions of the peptide sequence yielded no appreciable antiprotozoan activity. Therefore, a similar relationship holds between the potential of helix conformation and the antiprotozoan activity. In conclusion, magainin analogs B and G were shown to be effective against *B. hominis*, *T. cruzi*, and *E. histolytica* in vitro. An additional in vivo study is needed to establish their potential as a new class of antiprotozoal drugs.

**LITERATURE CITED**


