Using Atomic Force Microscopy to investigate the drug effects on the mitochondrial DNA of parasitic protozoa.

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The atomic force microscopy (AFM) has been extensively used to study biological samples, ranging from individual molecules to cells and tissues. AFM has been successfully applied to investigate the structure of nucleic acids as well as changes in DNA topology. It has been reported that the kinetoplast DNA (kDNA), the mitochondrial DNA of trypanosomatid protozoa, constitutes a potent target for chemotherapy since it is highly affected by DNA binding drugs, intercalating agents and topoisomerase inhibitors that interfere with its unique structure and replication. Our group previously analyzed the effect of nalidixic acid (a topoisomerase II inhibitor), acriflavine (an intercalating drug) and berenil (a minor-groove binding agent) on the protozoa ultrastructure using transmission electron microscopy. In this work, we used AFM to analyze the action of different classes of drugs on kDNA ultrastructural organization of *Crithidia fasciculata*, a parasite of insects, and *Trypanosoma cruzi*, the etiological agent of Chagas disease. We isolated intact kDNA of treated and non-treated protozoa and analysed the samples using AFM. In non-treated parasites, the isolated kDNA appeared as an intact and massive network presenting its typical arrangement with fibers homogeneously distributed throughout the network. The treatment with 500 µg/ml of nalidixic acid for 48h induced the formation of thicker DNA strands, while berenil (50 µM, 48h) promoted shrinkage of the network and compactation of kDNA fibers. In addition, the treatment with 50 µg/ml of acriflavine for 48 h promoted the release of minicircles from the edge of the network, culminating with kDNA fragmentation and dispersion throughout the protozoan mitochondrial matrix. Taken together, our results highlight the mechanism of action of different classes of inhibitors that target the kDNA structure, confirming that AFM is a powerful tool to study the structural organization of biological samples, including complex arrays of DNA.

Keywords: atomic force microscope, kinetoplast DNA, protozoa.

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