TITLE: QUINOLONES CAUSE THE DEATH OF TRYPANOSOMATID PROTOZOA, AFFECTING THE MITOCHONDRIAL DNA.

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ABSTRACT:

The quinolones, as nalidixic acid, are drugs that target two bacterial enzymes of the class II topoisomerase (Topo II) family, namely gyrase and topoisomerase IV. These enzymes cleave both strands of the DNA double helix, resulting in conformational changes in this molecule, which help to control the levels of DNA under- and over-winding, as well as remove knots from bacterial chromosomes that arise in most processes of nucleic acid metabolism. Quinolones bind to bacterial TopoII, increasing the concentration of drug-enzyme-DNA cleavage complexes, which are potentially toxic, since they can be converted to permanent double-stranded DNA breaks.

TopoII are essential enzymes for the replication of mitochondrial DNA of protozoa belonging to the Trypanosomatidae family. This family comprises parasites that cause tropical diseases, such as Chagas disease and leismaniases. The mitochondrial DNA of trypanossomatids (also known as kinetoplast DNA or kDNA), presents an uncommon organization, being composed of thousands of circular DNA molecules that are interlocked forming a single network. The circles are released from this structure during replication and re-attached to the network by the action of TopoII. Since topo II inhibitors have wide clinical use, it is interesting to investigate if these drugs impair the growth of trypanosomatids. Our group previously analyzed the effects of nalidixic acid (NA) in trypanosomatids and showed that NA promoted a dose-dependent inhibition on cell proliferation and caused ultrastructural changes in the kDNA. To better understand the effect of NA on the kDNA structure, in this work we isolated networks of *C. fasciculata* and *T. cruzi* non-treated and treated with 500 µg/ml of NA for 48h and observed them using atomic force microscopy. In non-treated protozoa, the isolated kDNA appeared as an intact and massive network, composed of thousands of interlocked DNA molecules. In contrast, in drug-treated cells, we observed a great compaction of DNA strands, which were thinner on the edge and thicker at the center of the network. Measurements of thicker fibers showed that these regions correspond to points where more than 100 DNA molecules are overlapping, forming an atypical arrangement. Since the kDNA is a unique structure in the nature, topoisomerase II-based drugs, which have long been employed as antimicrobial agent, need to be further studied, since they may offer greater selectivity in drug therapy of trypanosomatid infections.

Keywords: Protozoa, Trypanosomatids, Kinetoplast DNA, Quinolones, Nalidixic acid.

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