Besifloxacin is active against *Toxoplasma gondii* infection *in vitro*

MILENA RIBEIRO PECLAT DE ARAUJO[1]; CARLLA ASSIS DE ARAUJO E SILVA[1]; WANDERLEY DE SOUZA[12]; ROSSIANE CLAUDIA VOMMARO[12].

[1] Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho - Universidade Federal do Rio de Janeiro.

[2] Instituto Nacional de Ciência e Tecnologia em Biologia Estrutural e Bioimagens, INBEB – CENABIO, Brazil

Ocular toxoplasmosis (OT) is a disease caused by *Toxoplasma gondii*, a protozoan belonging to the Apicomplexa phylum. Currently, the standard treatment for OT involves a combination of trimethoprim and sulfamethoxazole with corticosteroids. However, this therapy is only effective during the acute phase of the disease and can cause hypersensitivity reactions and severe adverse effects. Recently, antimicrobial repositioning has garnered significant attention as a promising therapeutic strategy, particularly for drugs targeting essential pathways located within the apicoplast. This is a prokaryotic-derived organelle that is important for the survival and replication of *T. gondii*. Besifloxacin (BSX) is a fluoroquinolone in Besivance® eye drops, previously used for bacterial conjunctivitis. Previous studies have shown that fluoroquinolones impair endodyogeny of *T. gondii*, leading to the formation of masses of parasites non individualized. Thus, we investigated the cellular effects of the antibacterial BSX on *T. gondii* infection *in vitro* to evaluate its potential as an alternative drug for OT. Cytotoxicity assays showed CC_{50} values > 100 µM in retinal epithelial (ARPE-19), kidney epithelial (LLC-MK2) and fibroblast (HFF) cell types. In antiproliferative assays using tachyzoites from the RH strain, BSX demonstrated high selective activity against *T. gondii*, with IC_{50} values of 5 µM and 2.8 µM after 24h and 48h of infection, respectively. Lysis plate analyses demonstrated a 65% reduction in HFF monolayer damage after 20 µM (IC_{90}) of BSX treatment, even post-removal on day 5. Immunofluorescence revealed BSX induced parasite mass formation, apicoplast loss/fragmentation, centrosome dispersion, and mitochondrial alterations in ARPE-19 cells infected with RH-ACP-YFP. Transmission electron microscopy confirmed endodyogeny arrest and failed daughter cell individualization in BSX-treated cells. These findings suggest BSX has potential for in vivo testing and repositioning for OT treatment.

Supported by FAPERJ, CNPq, CAPES

Keywords: Toxoplasma gondii; drug repurposing; besifloxacin