TURNING UP THE HEAT: THERMAL PROTEOME PROFILING UNCOVERS HIDDEN MECHANISMS OF METRONIDAZOLE IN *GIARDIA*

ANA VICTORIA IBARRA-MENESES[1], SIMON GAGNON[1], ANTONIEL AUGUSTO SEVERO GOMES[2], JESUS D. CASTANO[3], FRANCIS BEAUDRY[3], ISABELLE DORE[4,5], DAVID LANGLAIS[6,7], MÉLANIE DIEUDÉ[5,8,9,10], NILMAR MORETTI[1], CHRISTOPHER FERNANDEZ-PRADA[1]

[1]DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY, FACULTY OF VETERINARY MEDICINE, UNIVERSITÉ DE MONTRÉAL; THE RESEARCH GROUP ON INFECTIOUS DISEASES IN PRODUCTION ANIMALS (GREMIP), FACULTY OF VETERINARY MEDICINE, UNIVERSITÉ DE MONTRÉAL, SAINT-HYACINTHE, CANADA.

[2]DEPARTAMENTO DE BIOFÍSICA E FARMACOLOGIA, INSTITUTO DE BIOCIÊNCIAS – UNIVERSIDADE ESTADUAL PAULISTA (UNESP), BOTUCATU, SÃO PAULO, BRASIL

[3]DEPARTEMENT OF BIOMEDECINE, FACULTY OF VETERINARY MEDECINE, UNIVERSITÉ DE MONTRÉAL, SAINT-HYACINTHE, QUÉBEC, CANADA

[4]DÉPARTEMENT DE MÉDECINE PRÉVENTIVE ET SOCIALE, ÉCOLE DE SANTÉ PUBLIQUE, UNIVERSITÉ DE MONTRÉAL, MONTRÉAL, CANADA

[5]CENTRE DE RECHERCHE DU CHUM, UNIVERSITÉ DE MONTRÉAL

[6]DEPARTMENT OF HUMAN GENETICS, MCGILL UNIVERSITY GENOME CENTRE, MONTREAL, QUEBEC, CANADA

[7]DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY, MCGILL RESEARCH CENTRE ON COMPLEX TRAITS, MONTREAL, QUEBEC, CANADA

[8]DEPARTMENT OF MICROBIOLOGY, INFECTIOLOGY AND IMMUNOLOGY, FACULTY OF MEDICINE, UNIVERSITÉ DE MONTRÉAL

[9]CANADIAN DONATION AND TRANSPLANT RESEARCH PROGRAM (CDTRP), EDMONTON, CANADA

[10]HÉMA-QUÉBEC, MONTRÉAL, QUÉBEC, CANADA

Giardiasis, a gastrointestinal infection caused by the protozoan parasite *Giardia*, is among the most widespread worldwide. Despite its global distribution, treatment options remain limited, and resistance to frontline drugs is an emerging concern. Metronidazole (MTZ) is the most widely used treatment for giardiasis, but relapse rates of up to 50% suggest potential resistance mechanisms. Therefore, a thorough understanding of the mode of action (MoA) of MTZ and its molecular targets is critical for developing novel therapeutic strategies. To address this challenge, we employed thermal proteome profiling (TPP), which combines cellular thermal shift assays with quantitative mass spectrometry to identify MTZ-target interactions in whole-parasite protein extracts. Bioinformatics analyses included data normalization, melting curve fitting, and identification of proteins exhibiting a temperature shift greater than 2°C upon MTZ interaction. Our approach identified 64 proteins, validating known MTZ targets like pyruvate:ferredoxin oxidoreductase (PFOR) and protein 21.1. Our study also identified novel potential targets within the *Giardia* proteome, including 14-3-3 proteins, giardins, and vacuolar protein sorting (VPS) components. Molecular docking revealed that VPS has a high binding affinity for MTZ, prompting further validation. Notably, exposure of *Giardia* cultures to MTZ resulted in significant alterations in extracellular vesicle size, providing the first evidence that VPS proteins are involved in the MoA of MTZ. Our findings offer novel insights into MTZ's molecular interactions in *Giardia* and highlight previously unrecognized drug targets. These results lay the foundation for developing new

diagnostic tools for antimicrobial resistance surveillance and the rational design of next-generation anti-*Giardia* therapies.

Support by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (RGPIN-2024-04103 to CFP) and by the Canada Foundation for Innovation (grant no. 37324 and 38858 to CFP).

Keywords: *Giardia,* Metronidazole, proteomics