

Cardiotoxicity of Anthracyclines – The Influence of Metal Ions on the Properties of Annamycin and Doxorubicin

Anthracyclines, such as doxorubicin (DOX), rank among the most effective chemotherapeutic agents; however, their clinical utility is severely limited by dose-dependent cardiotoxicity, which may lead to irreversible myocardial damage. The primary mechanism of DOX action is inhibition of topoisomerase II enzymes (Top2 α and Top2 β). Nevertheless, recent data suggest that cardiotoxicity—traditionally attributed to Top2 β activity in cardiomyocytes—may not be solely mediated through this pathway. Notably, Annamycin (ANN), a novel DOX analogue currently under clinical evaluation, exhibits significantly reduced cardiotoxic potential despite being a stronger Top2 β inhibitor than DOX. This paradoxical observation points to the involvement of alternative mechanisms of cardiac injury, particularly oxidative stress induced by redox-active drug–metal complexes.

Anthracyclines are well-documented for their ability to form complexes with transition metal ions—especially Fe³⁺—which catalyze the generation of reactive oxygen species (ROS), exacerbating oxidative stress, particularly in cardiomyocytes characterized by high metabolic demand and limited antioxidant capacity. While the role of iron in this process is well established, the contributions of other metal ions such as Cu²⁺ and Zn²⁺ remain poorly defined, despite their biochemical properties suggesting a potentially significant role in modulating anthracycline-induced toxicity. The selection of these three metal ions is based on their distinct yet complementary mechanisms of action: Fe³⁺ and Cu²⁺ are involved in redox cycling (e.g., Fenton and Haber-Weiss reactions), enhancing ROS production, whereas Zn²⁺, although redox-inactive, plays a key role in modulating oxidative stress responses and stabilizing antioxidant and DNA-repair proteins. Given growing doubts regarding the central role of Top2 β and an expanding body of evidence implicating oxidative mechanisms in anthracycline cardiotoxicity, there is a compelling need for a renewed and comprehensive investigation of metal ion involvement in this phenomenon.

The proposed project comprises four complementary research modules: (1) physicochemical characterization of Annamycin complexes with Fe³⁺, Cu²⁺, and Zn²⁺; (2) cheminformatic modeling of conformation, binding affinity, and redox properties; (3) *in vitro* biological assays using human cardiomyocyte cell lines (AC10 and AC16); and (4) application of zebrafish (*Danio rerio*) embryos and larvae as a high-throughput, predictive *in vitro* model. The physicochemical component will utilize advanced analytical techniques including UV-Vis, FT-IR, and resonance-enhanced Raman spectroscopy, X-ray diffraction, and quantum mechanical (DFT) modeling to enable in-depth structural and redox profiling of anthracycline–metal complexes. Biological models will be used to assess cytotoxicity, cell migration, expression of apoptosis- and ferroptosis-related proteins, ROS generation, as well as cardiac morphology and function in zebrafish exposed to metal ions. The biological component will employ cytotoxicity assays (MTT, XTT, CCK-8), Western blot analysis of protein expression, ELISA-based ROS quantification, and digital assessment of cardiac features using DanioScope software.

The project aims to elucidate the molecular mechanisms underlying the differential cardiotoxicity of anthracyclines and to define the role of selected metal ions in modulating their redox behavior. The anticipated outcomes will enhance our understanding of structure–toxicity relationships in anthracyclines and lay the groundwork for the rational design of analogues with improved therapeutic indices.

This is a multidisciplinary initiative that integrates the expertise of research teams across several domains: chemists and physicists from Białystok University of Technology, pharmacology and toxicology specialists from the Medical University of Białystok, and a world-renowned expert in medicinal chemistry—Prof. Waldemar Priebe from University from Texas MD Anderson Cancer Center in Houston, the original developer of Annamycin. This collaborative framework ensures a comprehensive approach to the research question, combining precise physicochemical analysis with biological validation of anthracycline mechanisms of action.