

ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Mgr. Katharina Zenkerová

Supervisor: Prof. Ing. Vladimír Wsól, Ph.D.

Title of diploma thesis: Effect of evobrutinib on cancer cell resistance to daunorubicin caused by carbonyl reducing enzymes

Anthracyclines (ANT) have been among the first line treatments for many types of cancer, including acute myeloid leukemia, for decades. These chemotherapeutic agents target topoisomerase II, interfere with DNA and RNA synthesis by intercalation, and induce apoptosis by forming reactive oxygen species. As with many other chemotherapeutics, administration of ANT is associated with a range of adverse effects, particularly cardiotoxicity and resistance. The culprit responsible for this cardiotoxicity is the hydroxy metabolite of ANT, formed by reduction of the carbonyl group at position 13. This metabolite is also considerably less cytotoxic; cancer cells excessively metabolize ANT, thus developing resistance to their effects. Enzymes involved in the ANT reductions are NADPH dependant carbonyl reducing enzymes, primarily from aldo-keto reductase and short-chain dehydrogenase/reductase superfamilies. These enzymes are frequently over-expressed in cancer cells and they might be an attractive target of novel chemotherapeutics. Inhibitory effects towards CRE have already been observed in some cyclin-dependent kinase inhibitors, phosphoinositide 3-kinase inhibitors, poly(ADP-ribose)polymerase inhibitors, and Bruton's tyrosine kinase (BTK) inhibitors. Following these findings, the effects of a BTK inhibitor evobrutinib (Evo) were tested on a selection of CRE *in vitro* within this thesis. Selected enzymes were incubated with substrate daunorubicin and inhibitor Evo. Using the UHPLC system and GraphPad program, the most inhibited enzymes were determined, and their IC₅₀ values were calculated. For the most inhibited enzyme (AKR1C3) K_i, type of inhibition and type of bonding were determined. Evo is a novel BTK inhibitor, non-competitively inhibiting the enzyme AKR1C3 (IC₅₀= 1,721 μM; K_i= 1,81 μM), therefore counteracting the reduction of daunorubicin to its cardiotoxic and less effective

metabolite daunorubicinol. AKR1C3 is one of the chief enzymes reducing ANT, and its inhibition may be the key to solving the problems associated with ANT administration.