ABSTRACT

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Title of Doctoral Thesis	Modulation of cancer cell resistance through inhibition of selected human carbonyl reductases by tyrosine kinase inhibitors

In cancer therapy, developing resistance to anthracycline antibiotics (ANTs), highly effective chemotherapeutic agents, poses a significant challenge. Besides other mechanisms, ANT resistance is mediated through the activity of carbonyl reducing enzymes (CREs) from the superfamily of aldo-ketoreductases (AKRs) and short-chain dehydrogenases/reductases (SDRs). These enzymes convert ANTs into less active alcohol metabolites, reducing their therapeutic efficacy. In addition, ATP-binding cassette (ABC) efflux transporters contribute to resistance by actively pumping ANTs out of cancer cells, thereby reducing their intracellular concentrations.

Bruton's tyrosine kinase inhibitors (BTKis) represent a novel approach to overcoming ANT resistance. In clinical trials, BTKis have been tested alone or in combination with ANT-containing standard chemotherapy. Our research focuses on evaluating the potential of BTKis (acalabrutinib, ACA; ibrutinib, IBR; tirabrutinib, TIR; zanubrutinib, ZAN) to inhibit selected CREs (CBR1, AKR1A1, 1B1, 1B10, 1C3, 7A2), and to potentiate the effects of daunorubicin (DAUN) in cancer cells.

In the experimental part, the inhibitory potential of selected BTKis on the activity of the respective recombinant CREs and the reductive conversion of DAUN to its metabolite daunorubicinol (DAUN-OL) was investigated. Of all the CREs studied, all BTKis showed the highest inhibitory potential for AKR1C3, whereas the other studied reductases were inhibited insignificantly. This inhibitory effect was verified at the cellular level using the HCT116 cell line transiently transfected with AKR1C3. All BTKis effectively inhibited AKR1C3 activity in intact cells. Subsequent studies investigated the effect of combining DAUN with selected BTKis at the level of cancer cell lines with transient or endogenous expression of AKR1C3. After quantitative analysis of dose response relationships, all selected BTKis exhibited a synergistic effect with DAUN, significantly enhancing the cytotoxic effect of DAUN on cancer cells transiently overexpressing AKR1C3. This synergistic effect was verified in the A549 cell line with endogenous expression of AKR1C3. Combinations of DAUN with BTKis (ACA, IBR, ZAN) led to a decrease in the effective dose of DAUN and an improvement in the synergistic parameters as a function of the dose of BTKi. To assess whether the synergistic effect of combining DAUN with selected BTKis (TIR, ZAN) is affected by changes in AKR1C3 expression, potential alterations in AKR1C3 mRNA levels were investigated. The results indicated no upregulation of AKR1C3 mRNA. Furthermore, the effect of selected BTKis (TIR, ZAN) on the efflux activity of ABC transporters was investigated. Both BTKi significantly inhibited ABC transporters (ABCB1, C1, and G2), suggesting an additional mechanism to combat ANT resistance.

Our findings suggest that BTKis may effectively modulate ANT resistance by targeting multiple resistance mechanisms. Combination therapy represents a promising approach to improving clinical outcomes in ANT-based cancer treatment.