ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department od Biochemical Sciences

Candidate: Bc. Robin Klieber

Supervisor: PharmDr. Anna Jirkovská, Ph.D.

Title of thesis: The use of RNA interference for the modification of DNA topoisomerase II levels in cancer cells and its influence on the antineoplastic effect of anthracyclines.

Topoisomerase II (TOP II) is an enzyme that alters the topological state of the DNA double helix during physiological processes through the formation of transient DNA double strand breaks. Two TOP II isoforms are known: TOP IIα is essential for proper separation of chromosomes in mitotic cells, whereas TOP IIβ is primarily associated with gene transcription. Anthracycline antibiotics (ANT) belong to the group of topoisomerase poisons that stabilize the covalent complex of TOP II and DNA. This prevents the religation of the DNA double strand breaks and thus causes irreversible DNA damage leading to programmed cell death. Although ANTs are frequently administered in various antineoplastic protocols (hematooncological malignancies, hormone-dependent tumors and others), the therapy still possess a high risk of irreversible cardiotoxicity. The mechanism of cardiotoxicity remains unraveled. However, it has been previously discussed that TOP IIβ inhibition could play a key role in this process.

The practical aim of this work was to optimize methodology of RNA interference using siRNA molecules targeting the TOP IIβ isoform in human tumor suspension cell line HL-60. The transfection was performed by the electroporation. Evaluation of TOP IIβ was accomplished both on mRNA level by RT-qPCR and protein level via immunoblotting. Furthermore, antiproliferative effect of the Daunorubicin as a model ANT drug was investigated in TOP IIβ depleted cells.