ABSTRACT

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Title of thesis: The detection of protein covalent complexes with DNA using fluorescent microscopy

Anthracycline antibiotics are present one of the most potent antineoplastic drugs. The mechanism of their action is complex. They are reported to intercalate to DNA, form DNA adducts and interact with topoisomerase II (TopII) as its poisons. Catalytic cycle of TopII is interrupted when anthracyclines stabilize the covalent complex of DNA and TopII and that causes cell damage. However, using of anthracyclines is limited by several adverse effects e. g. myelotoxicity and cardiotoxicity. The mechanism of cardiotoxicity is still unclear but may be associated with poisoning of the TopIIβ isoform. Unlike the TopIIα, TopIIβ is present mostly in quiescent cells as cardiomyocytes. Furthermore, the only clinically approved cardioprotective drug dexrazoxane belongs to TopII catalytic inhibitors. Nevertheless, the details of the dexrazoxane-afforded protection are unclear.

This thesis was aimed to optimize the TARDIS (trapped in agarose DNA immunostaining) assay to detect and quantify covalent cleavage complexes, compare different ways for analysis of the complexes and finally choose an appropriate type of statistical analysis. For our study human leukemic cell line HL-60 and primary rat neonatal ventricular cardiomyocytes (NVCM) were used. After incubation with the selected drug, the cells were „trapped“ in agarose and lysed. Subsequently the complexes were labeled with anti-TopII primary antibody and Alexa Fluor conjugated secondary antibody. The acquired signal was then analysed by Cell Profiler program. Using this optimized method, we tested the ability of various types of TopII inhibitors (etoposide, daunorubicin, XK-469, dexrazoxane and BNS-22) to stabilize cleavage complexes or prevent them, respectively.