EVALUATION OF DNA DAMAGE AFTER BLEOMYCIN ELECTROTRANSFER TO CHO AND MX-1 CELL LINES BY USING COMET ASSAY

Aistė Rimgailaitė, Paulius Ruzgys, Saulius Šatkauskas

Faculty of natural sciences, Vytautas Magnus university, Lithuania
aiste.rimgailaite@stud.vdu.lt

Electroporation is a process, when applied electric field is a cause for an increased cell membrane permeability, leading to increased uptake of exogenous molecules such as anticancer drugs. Therefore, this method was applied to cancer treatment, as anticancer drugs can be easier transported into electroporated tumour cells. Currently, the combination of anticancer drug (mainly bleomycin) and the method of electroporation is used in clinics and termed as electrochemotherapy. Electroporation enables to reduce working concentration by 3 orders of magnitude, yet at constant cancer treatment effect.

Once inside the cell, the anticancer drug bleomycin induces the cellular DNA cleavage, that in turn leads to cell death. To the best of our knowledge, there is no published research that present cellular DNA damage after BLM transfer to electroporated cells. Consequently, it is unknown, whether the BLM has the same effect in healthy cells as compared to tumour cells and vice versa. Therefore, in this *in vitro* study, the evaluation of DNA damage after bleomycin electrotransfer to breast cancer tumour (MX-1) and chinese hamster ovary (CHO) cell lines was done by using comet assay (a single cell gel electrophoresis (SCGE)) technique.

CHO and MX-1 cell lines were used for bleomycin electrotransfer experiments. The anticancer drug bleomycin was used for electrotransfer experiments in the concentrations ranging from 0.2 to 20000 ng/ml. Cells with bleomycin were suspended in electroporation medium (conductivity 0.1 S/m, osmolarity 270 mOsm, pH 7.1). Then electroporation was performed by using combination of 8 electric pulses that induced electric fields at the amplitude of 1400 V/cm for the duration of 100 µs. Afterwards, comet assay was performed to evaluate DNA damage. In addition, clonogenic assay was done to evaluate cell viability.

Electroporated cells were resuspended in low melting agarose (0.5 %), was put on objective glass and covered with cover slip, 70 min after electroporation. Afterwards, cells were kept in lysis buffer for 24 hours. Thereafter, electrophoresis for 30 min in alkaline buffer (pH 13) was performed with voltage at 0.74 V/cm and 300 mA current. Fluorophore ethidium bromide (1 µg/ml) that binds to DNA was used to obtain visualization of the DNA damage under fluorescent microscope. Obtained cell DNA\Ethidium bromide complex fluorescence images were processed with open access software ImageJ plugin OpenComet v1.3.1.

Obtained results indicate a significant DNA damage caused by BLM electrotransfer in both used cell lines, as compared to incubation with BLM without effect by electric field. Notable, that significantly higher DNA damage as a result of BLM electrotransfer was done in MX-1 cell line when comparing to incubation with BLM without effect by electric field. Nevertheless, the tendency of dealt DNA damage was observed to be higher in CHO cell line as compared to MX-1, when using same BLM concentrations were used. As compared to MX-1, a higher DNA damage was observed in CHO cell line, which lead to decreased cell viability, approximated by evaluating cell ability to form a colony.