ELECTRO-ASSISTED REDOX SENSITIZATION OF COLON CANCER CELLS TO SN38 VIA INDUCTION OF APOPTOSIS/FERROPTOSIS AND UPREGULATION/DOWNREGULATION OF ABC TRANSPORTERS

Severina Semkova[1,2,3)*], Biliana Nikolova[(1)], Zhivko Zhelev[(1,4)], Pavel Lefterov[(5)], Elena Stoyanova[(6)], Iana Tsoneva[(1)], Ichio Aoki[(2,3)] and Rumiana Bakalova[(2,3,7)]

Aims

The main purpose of this study was to elucidate the possibilities for sensitization of colon cancer cells to the chemotherapeutic drug SN38 and investigation of mechanism of action after combination with electroporation (EP).

Abstract

Over the past few years, more experimental data suggests that the regulation and control of cellular redox-status during the chemotherapy and/or radiotherapy of malignancies could be critical to the development of new and effective therapeutic strategies. The explanation is a long-known fact one of the basic mechanisms of the anti-tumor activity of conventional chemotherapeutics is related to redox-signaling influence and induction of oxidative stress in to the cancer cells, leading to apoptosis and/or necrosis. Nowadays oxidative stress-related mechanism of action ha been reported for a number of generally used anticancer drugs.

On the other hand, the development of multidrug resistance to chemotherapeutics remains one of the major challenges in the treatment of cancers. Anticancer drug resistance is advanced by the triggering a wide range of cellular mechanisms, including: drug inactivation of the active drug substances; modification of drug target; quantitative reduction drug uptake; increased drug efflux; activation of different detoxifying/neutralizing systems; activation of DNA repair mechanisms; overcoming drug induced apoptosis/cell death; et cetera.

Materials and methods

Cell line – Colon 26 cancer cells;
Anticancer prodrug Irinotecan-SN38;
Electroporator – Chemipulse IV;

Measured parameters and Assays:

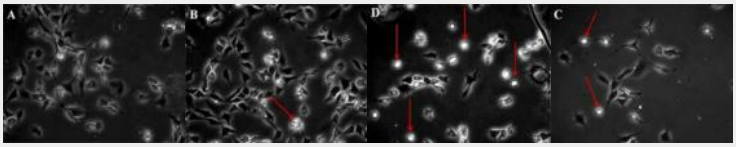
- Cell viability and proliferation -Trypan blue staining, Countess™ Automated Cell Counter (Invitrogen, USA);
- Propidium iodide & Actin staining procedures;
- Fluorescent imaging in vitro;
- Detection of intracellular superoxide - (DHE) assay, Hydroperoxides levels - (DCF) assay & Total glutathione analysis;
- Detection of apoptosis (Annexin V assay) & DNA damages.;
- Quantitative determination of ABCC1 & SLCOB1 proteins (ELISA assays)

1. Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria;
2. Department of Molecular Imaging and Theranosticsand;
3. Quantum-state Controlled MRI Group, National Institutes for Quantum and Radiological Science and Technology (QST/NIRS), Chiba, Japan;
4. Medical Faculty, TrakiaUniversity, StaraZagora, Bulgaria;
5. Faculty of Educational Studies and the Arts, Sofia University, Sofia, Bulgaria;
6. Institute of Biology and Immunology of Reproduction "Acad. KirilBratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria;
7. Medical Faculty, Sofia University, Sofia, Bulgaria;

EXPERIMENTAL RESULTS

Figure 1

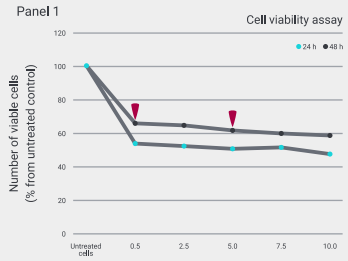
Panel 3

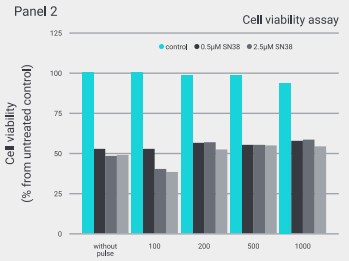


Panel1: Number of viable Colon26 cells after 24 and 48 hours of incubation with SN38 in different concentrations, presented as a percentage of control (untreated)cells. Cell viability in all samples was analyzed by trypan blue staining and was in the range 94-99%. All data are means ± SD from three independent experiments. The red arrows indicate the selected concentrations for further experiments.

Panel2: Cell viability of Colon26 cells after 48 hours of treatment with SN38 in combination with electroporation, analyzed by MTS assay. All data are means ± SD from three independent experiments. +++p < 0.001 versus cells with 100 V/cm, #p < 0.05 versus cell treated with 5 µM SN38 alone, all other variations are significant versus respective control.

Panel3: Propidium iodide staining and fluorescence imaging of Colon26 cells after electroporation: (A) Control (untreated cells); (B) 100 V/cm; (C) 500 V/cm; (D) 1000 V/cm. The red arrows indicate electroporated cells.
Magnification 63x.





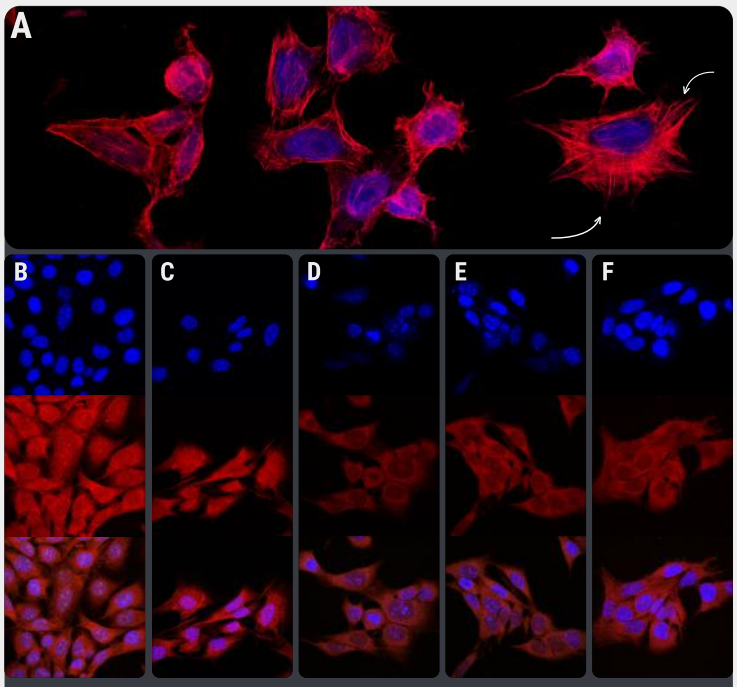
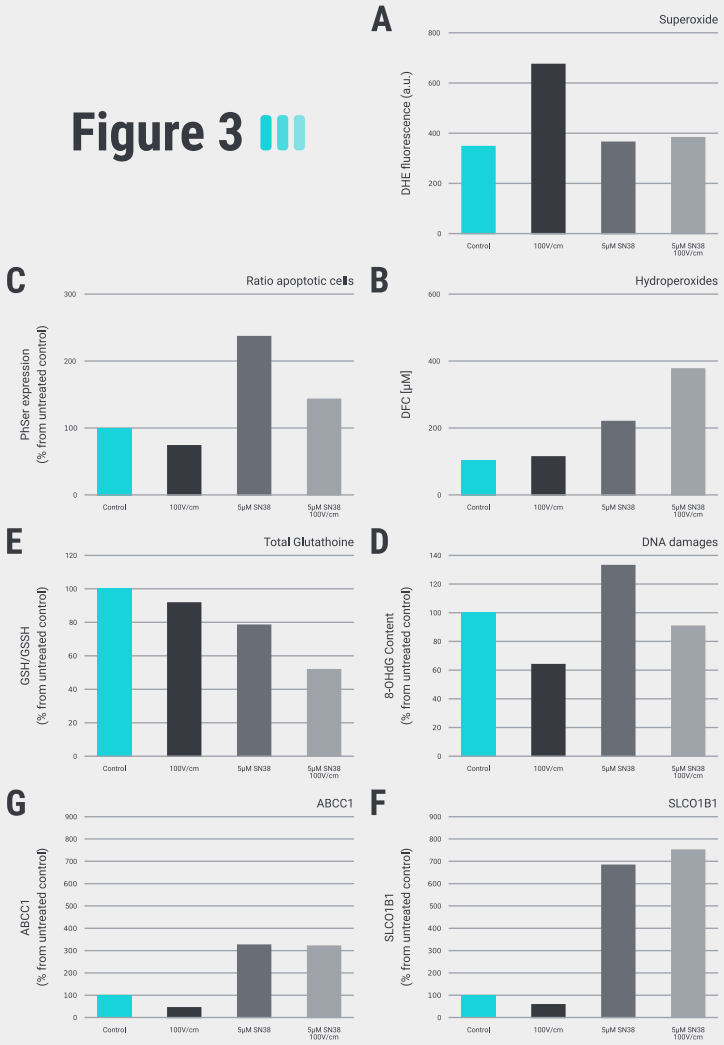


Figure 2

Immunofluorescence imaging of actincy to skeleton: (A) Control; (B) 100 V/cm; (C) 1000 V/cm; (D) 5 µM SN38; (E) 5 µM SN38 and 100 V/cm; (F) 5 µM SN38 and 1000 V/cm. The white arrows indicate stress fibers. Magnification – 63x.

Figure 3



(A) Intracellular levels of superoxide, analyzed by a DHE-assay. The data were normalized to 1×106 cells/mL. All data are means ± SD from three independent experiments; +++p < 0.001 versus cells treated with 100 V/cm, all variations are significant versus control.

(B) Intracellular levels of hydroperoxides, analyzed by a DCF-assay. The data were normalized to 1×106 cells/mL. All data are the means ± SD from three independent experiments. +++p < 0.001 versus cells with 100 V/cm, ###p < 0.001 versus 5 µM SN38.

(C) Induction of apoptosis in SN38/EP-treated Colon26 cells, analysed by phosphatidylserine (PhSer) expression on the cell surface. The data were normalized to 1×106 cells/mL. All data are means ± SD from three independent experiments: +p < 0.05 versus cells with 100 V/cm, #p < 0.05 versus 5 µM SN38, all other variations are significant versus control.

(D) Oxidative DNA damages in SN38/EP-treated Colon26 cells, analyzed by an OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation). All data are the means ± SD from three independent experiments: +p < 0.05 versus cells with 100V/cm, #p < 0.05 versus 5µ MSN38, all other variations are significant versus control.

(E) Intracellular levels of total glutathione, analyzed by OxiSelect™ Total Glutathione (GSSG/GSH) Assay. The data were normalized to 1×106 cells/mL. Data are shown as a percent from untreated control and are the means ± SD from three independent experiments: +p < 0.05 versus cells with 100 V/cm, +++p < 0.001 versus cells with 100 V/cm, ###p < 0.001 versus 5µ MSN38, all variations are significant versus control.

(F) Level of SLCO1B1 protein expression (as a % from untreated control) in Colon26 cells after 48 hours of incubation, analyzed by Human Solute Carrier Organic Anion Transporter Family Member 1B1 (SLCO1B1) ELISA Kit. The data were normalized to 1×106cells/mL and total protein quantitation (Bradford protein assay). All data are the means ± SD from three independent experiments: +p < 0.05 versus cells with 100 V/cm, all other variations are significant versus control and versus cells with 100V/cm.

(G) Levels of ABCC1 protein expression, (as a % from untreated control) in Colon26 cells after 48 hours of incubation, analyzed by Human Multidrug Resistance-Associated Protein 1 (ABCC1) ELISA Kit. The data were normalized to 1×106 cells/mL and total protein quantitation (Bradford protein assay). All data are means ± SD from three independent experiments: all variations are statistically significant versus control and versus cells with 100V/cm.

CONCLUSIONS

The anticancer effect of combined treatment is related to changes in the redox-homeostasis of cancer cells, leading to activation of signal pathways for induction of cell death via apoptosis and/or ferroptosis and influence on multidrug resistance proteins.

Thus, the electroporation has the potential to increase the sensitivity of cancer cells to conventional anticancer therapy with SN38.

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Article

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