Nanoconjugates based on cisplatin and single-walled carbon nanotubes for therapy of triple negative breast cancer

Madalina Andreea Badea[1*], Mihaela Balas[1], Florentina Gina Cojocaru[2], Daniela Ionita[3], Anca Dinischiotu[1]

[1]University of Bucharest, Faculty of Biology, Department of Biochemistry and Molecular Biology, 91-95 Splaiul Independentei, R-050095, Bucharest, Romania.
[2]University of Bucharest, Faculty of Biology, Department of Anatomy, Physiology and Biophysics, 91-95 Splaiul Independentei, R-050095, Bucharest, Romania.
[3]Politehnica University of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of General Chemistry, 313 Splaiul Independentei, 060042, Bucharest, Romania.

*Corresponding author: madalina.andreea.badea@drd.unibuc.ro

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Background and aim of the study

Background: Triple negative breast cancer has a phenotype characterized by the absence of progesterone and estrogen receptors and lack of HER2 overexpression (1). In order to find new strategies for treatment, single-walled carbon nanotubes (SWCNT) in combination with chemotherapeutics were studied and tested as new therapeutic tools (2).

Aim: The objective of this study was to evaluate the efficiency of SWCNT in the transport of cisplatin (CDDP) for improving its cytotoxic effects on MDA-MB-231 cells.

Materials and cells treatment

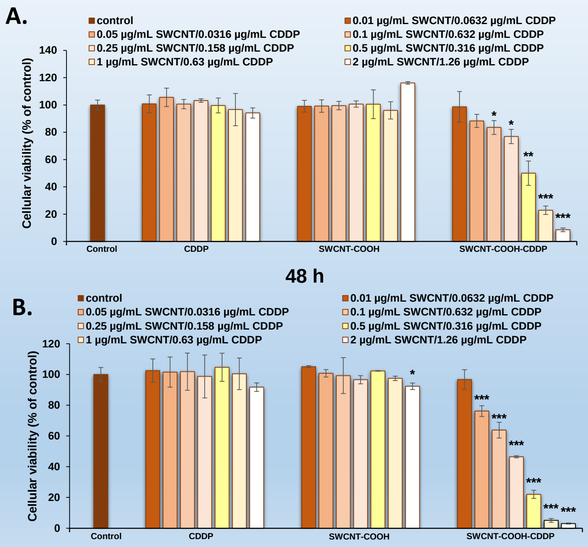
- **The nanoconjugates SWCNT-COOH-CDDP** were obtained by functionalization of SWCNT with carboxyl groups (SWCNT-COOH) and conjugation with CDDP.
- **MDA-MB-231 cell line** (triple negative breast cancer cells) was cultured in Dulbecco's Modified Eagle's Medium.
- MDA-MB-231 cells were exposed to different doses of SWCNT-COOH, SWCNT-COOH-CDDP (0.01 – 2 µg/mL) and CDDP (0.00632 – 1.26 µg/mL) for 24 and 48 h.

Methods

- **Spectrophotometric and fluorescence methods** were performed for the evaluation of cellular viability (MTT test), reduced glutathione content (GSH) and determination of reactive oxygen species (ROS) production.
- **Immunoblotting** was performed for the assessment of Nrf2, caspase-3, caspase-8 and Bid proteins expressions.
- **Wound healing assay** was performed for the evaluation of the effects of SWCNT-COOH-CDDP on cell migration.

Results and discussion

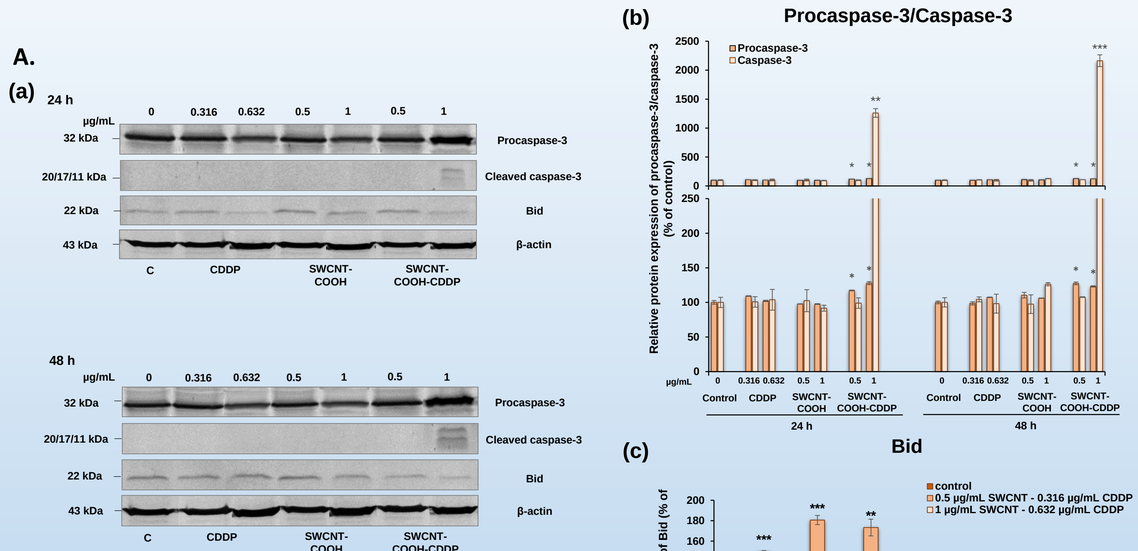
Cellular viability



- **The cellular viability decreased in a time and dose-dependent manner in the presence of nanoconjugates relative to control.**

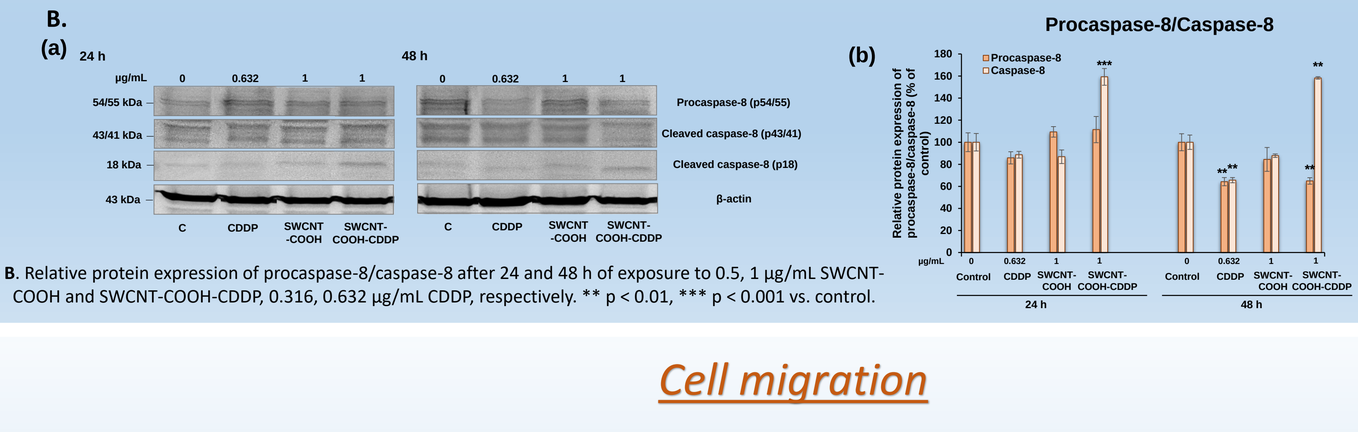
Cell viability after **(A)** 24 and **(B)** 48 h of exposure to SWCNT-COOH, SWCNT-COOH-CDDP (0.01 – 2 µg/mL) and CDDP (0.00632 – 1.26 µg/mL). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control.

Cell death



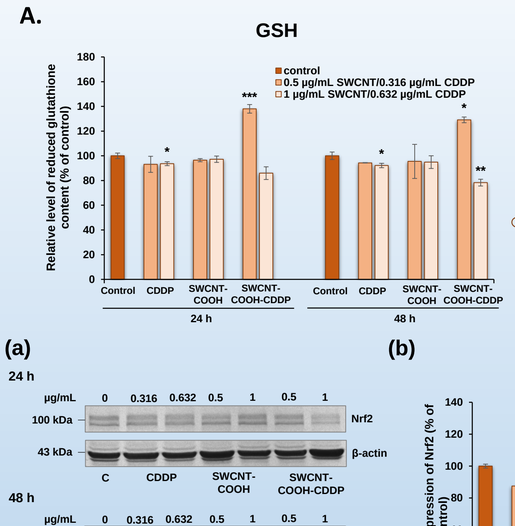
- **Caspase-3 and caspase-8 were activated in the presence of 1 µg/mL SWCNT-COOH-CDDP starting with 24 h of treatment.**
- **An increase of procaspase-3 protein expression was observed after the treatment with SWCNT-COOH-CDDP.**
- **The expression of Bid protein was upregulated after 24 h of incubation with nanoconjugates, followed by a downregulation at 48 h. A dose with 1 µg/mL SWCNT-COOH-CDDP induced downregulation of Bid protein expression at both intervals tested.**

A. Relative protein expression of (b) procaspase-3/caspase-3 and (c) Bid proteins after 24 and 48 h of exposure to 0.5, 1 µg/mL SWCNT-COOH and SWCNT-COOH-CDDP, 0.316, 0.632 µg/mL CDDP, respectively. In figure (b), the lower graph presents a magnified image of the scale range between 0–250 from the upper graph. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control.

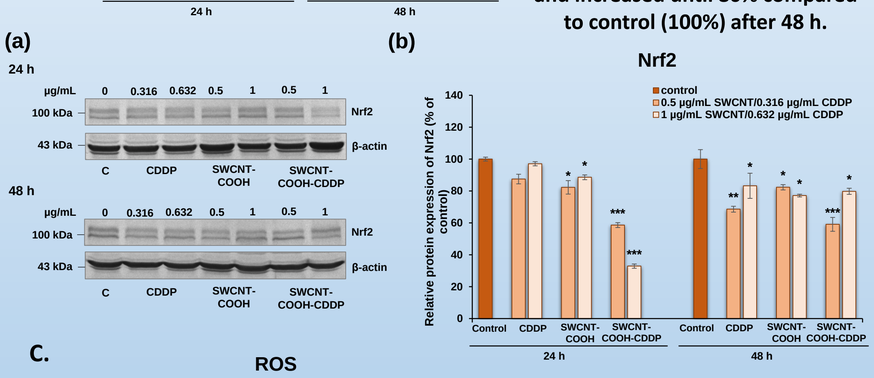


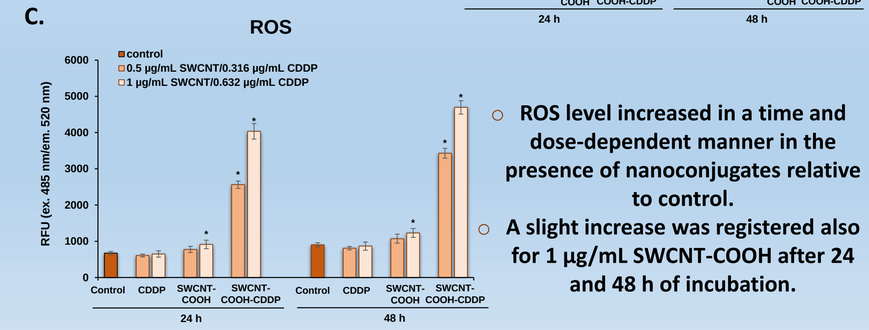
B. Relative protein expression of procaspase-8/caspase-8 after 24 and 48 h of exposure to 0.5, 1 µg/mL SWCNT-COOH and SWCNT-COOH-CDDP, 0.316, 0.632 µg/mL CDDP, respectively. ** $p < 0.01$, *** $p < 0.001$ vs. control.

Oxidative stress



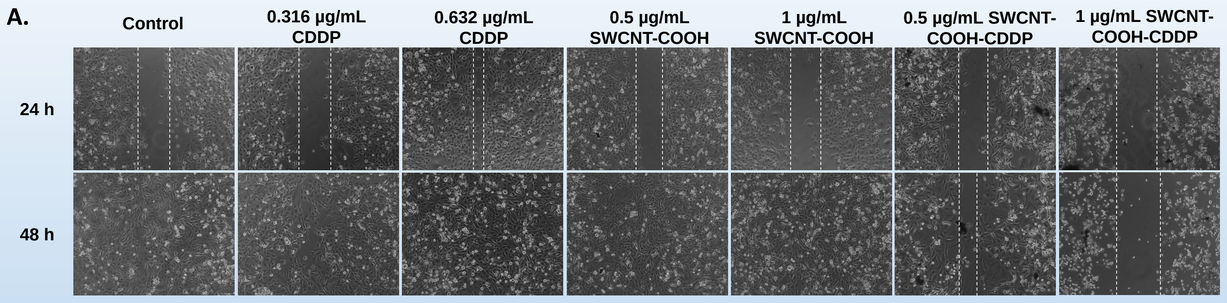
- **The level of GSH raised after 24 and 48 h of exposure to 0.5 µg/mL SWCNT-COOH-CDDP, while a decrease until 78.31% was recorded after 48 h in the presence of 1 µg/mL nanoconjugates.**
- **The expression of Nrf2 decreased until 33% after 24 h of treatment with 1 µg/mL SWCNT-COOH-CDDP and increased until 80% compared to control (100%) after 48 h.**

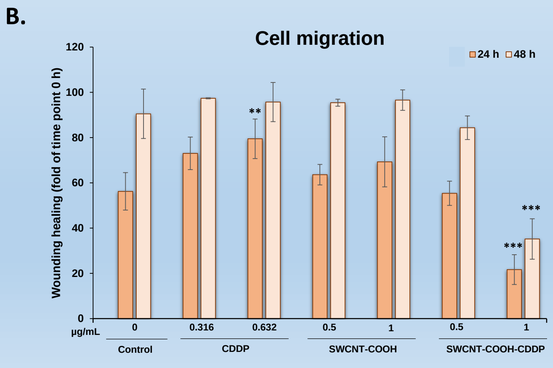




- **ROS level increased in a time and dose-dependent manner in the presence of nanoconjugates relative to control.**
- **A slight increase was registered also for 1 µg/mL SWCNT-COOH after 24 and 48 h of incubation.**

Cell migration





- **The inhibition of the cell migration was observed after 24 and 48 h of exposure with 1 µg/mL SWCNT-COOH-CDDP.**
- **A dose of 0.632 µg/mL increased the migration capacity of MDA-MB-231 after 24 h of treatment.**

Cell migration (scratch wound healing assay). **(A)** Bright-field images presenting the MDA-MB-231 cell culture after 24 and 48 h of wounding and incubation with 0.5, 1 µg/mL SWCNT-COOH and SWCNT-COOH-CDDP, 0.316, 0.632 µg/mL CDDP, respectively. **(B)** Quantification of presented images.

Conclusions: these nanoconjugates induced apoptosis in MDA-MB-231 cells, probably by both intrinsic and extrinsic pathways, by triggering the oxidative stress mechanisms, and inhibited their migration potential.

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