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Abstracts

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10. Cancer Pharmacology

10.001 **Evaluation of the Cytotoxicity and Molecular Effects of Theranostic Nanosystems in 3D Breast Cancer Cell Model.** Melo GB¹, Kawassaki RK^{1,2}, Garnique ADMB¹, Guimarães RR², Araki K², Lopes LB¹ ¹ICB USP, Dpt of Pharmacology ²IQ USP, Dpt of Fundamental Chemistry

Introduction: Breast cancer stands as a major cause of women's death worldwide¹. Despite the availability of treatment and diagnostic tools, these methods still face significant limitations². In this scenario, nanomedicine has been brought to light as a means to enhance cancer targeting and reduce toxic effects of non-selective drugs³. In view of this, we developed a theranostic nanosystem for delivery into breast ducts (intraductal) to enable local treatment, facilitate diagnostic and minimize toxic systemic effects often observed with traditional procedures. Ethosomes were selected as nanocarrier for the co-encapsulation of the antineoplastic drug methotrexate (MTX) and ultrasmall paramagnetic nanoparticles (UPNs), aiming to combine both treatment and diagnostic functions. **Methods:** Ethosomes (Et) were synthesized using the ethanol injection method, and their size was determined through Dynamic Light Scattering (DLS) analysis. The breast cancer cell lineage MCF-7 was employed to obtain spheroids using the liquid overlay technique. These spheroids were then treated with unloaded Et, Et containing MTX and UPN (Et MTX+UPN), or a solution of MTX with drug concentrations ranging from 0.77 to 482 μM for encapsulated MTX, which showed an encapsulation efficiency of 43.9%, and 8 to 5,000 μM for MTX in solution. After 48 hours of treatment, cell viability was assessed using two methods: resazurin assays and double staining for viability and death markers (Hoechst and Propidium iodide, PI, respectively). To investigate whether nanoencapsulation mediated changes in the pharmacodynamic effects of methotrexate, the expression of selected proteins (PARP1, Bax, acetyl- α -tubulin and α -tubulin proteins) was investigated after protein extraction using Western Blot. **Results:** DLS analysis showed a size range of 90 to 100 nm among all synthesized formulations and confirmed nanometric size. Resazurin viability assay showed that Et MTX+UPN treatment reduces IC_{50} by 23.7- and 9.9-fold comparing to MTX solution and unloaded Et, respectively. This finding was confirmed by the double staining assay, which revealed a rising increase of death marker staining (PI), indicating development of necrotic core and an increase in cell death due to treatment. Cell treatment with Et MTX+UPN and MTX solution increased the proapoptotic Bax expression (33.3- and 35.5-fold, $p < 0.05$) compared to the untreated control. For acetyl- α -tubulin, the most significant rise (1.9-fold, $p < 0.05$) was reported with Et MTX+UPN treatment when comparing to Et containing only UPN. No difference was observed in PARP expression after treatment. **Conclusion:** Viability assay findings suggest a more pronounced cytotoxicity effect in spheroids mediated by the co-encapsulation of MTX and UPN into ethosomes, when comparing to the MTX solution. These results are corroborated by Western Blot assays. The significant increase in acetyl- α -tubulin and Bax expression after Et MTX+UPN treatment also indicates an increased cytotoxicity promoted by the co-encapsulation. **Financial Support:** São Paulo Research Foundation (FAPESP, grants #2023/13424-5; #2018/13877-1; #2019/02151-2). National Council for Scientific and Technological Development (CNPq, SisNANO-USP grant #442599/2019-6). **References:** 1. Wilkinson, L. *Br J Radiol*, v. 95, 2022. 2. Mahoney, ME. *Clin. Breast Cancer*, vol. 13, p. 280-286, 2013 3. Ramos, AP. *Biophys Rev*, v. 9, p. 79–89, 2017.