

Summary of the Master thesis NO 27008 Faculty of Veterinary Medicine, Urmia University.

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Title of thesis: Anticancer evaluation of nitazoxanide loaded in exosomes derived from mesenchymal stem cells treated with lipopolysaccharide on breast cancer cell line (4T1)

Summary

Introduction: Breast cancer is one of the most common and lethal malignancies in women, and with its high mortality rate and frequent development of drug resistance, it remains a major challenge in standard treatments. Limitations of conventional chemotherapy, such as systemic toxicity and multidrug resistance, have emphasized the need for the development of novel and targeted drug delivery strategies. In this context, the use of exosomes derived from mesenchymal stem cells as natural drug carriers has attracted significant attention due to their high biocompatibility, targeting capability, and ability to cross biological barriers. Nitazoxanide, an anti-parasitic drug with anticancer potential, as a small molecule with low side effects, can exert significant antitumor effects through signaling pathways involved in apoptosis and cell proliferation. Furthermore, treating mesenchymal stem cells with lipopolysaccharide can modify the exosomal secretion profile and enhance the efficacy of the loaded drugs.

Materials and methods: Mesenchymal stem cells were extracted from the bone marrow of Balb/c mice, and after treatment with LPS, the exosomes of mesenchymal stem cells were isolated by the sedimentation method and characterized using DLS and flow cytometry techniques. The concentration of exosomal proteins was also measured by the Bradford method. Then, nitazoxanide was loaded into the exosomes of LPS-treated mesenchymal stem cells by ultrasound and incubation. Breast cancer cells (4T1) were cultured and treated with different concentrations of empty exosomes, nitazoxanide-loaded exosomes, and nitazoxanide alone. The cytotoxicity of the different treatments was evaluated by MTT and LDH assays. The incidence of apoptosis and necrosis was examined using flow cytometry analysis (Annexin V/PI staining) and fluorescence microscopy (AO/PI staining). To investigate the ability to colonize, a clonogenic assay was performed. Caspase-3 enzyme activity was measured using a specific kit and recording optical absorption at a wavelength of 405 nm. In order to evaluate the changes in the expression of apoptosis-related genes (BAX and BCL2), total RNA from treated and control cells was extracted and after cDNA synthesis, gene expression was determined using qRT-PCR and the GAPDH gene was used as a reference gene. After isolation and characterization of MSC-derived exosomes (with and without LPS treatment) and loading with nitazoxanide, the physicochemical properties, surface markers, and drug encapsulation efficiency were evaluated. Particle sizes ranged from 124 to 190 nm, with drug loading efficiency of approximately 58–62%. MTT assay revealed that

nitazoxanide-loaded exosomes from LPS-treated MSCs (NTZ-EXO-LPS) exhibited the highest cytotoxicity, especially after 48 hours, with the lowest IC₅₀ compared to other treatments. LDH assay confirmed these findings, with NTZ-EXO-LPS inducing the highest LDH release. Apoptosis assessment via AO/PI staining and flow cytometry showed that NTZ-EXO-LPS induced the highest level of late apoptosis, while exosomes without the drug had minimal effects. In the clonogenic assay, NTZ-EXO-LPS demonstrated the strongest inhibitory effect on colony formation. Caspase-3 activity was significantly increased in all treatment groups, with the highest level observed in NTZ-EXO-LPS. qRT-PCR analysis revealed a marked upregulation of the pro-apoptotic gene BAX and a relative downregulation of the anti-apoptotic gene BCL2, resulting in a significantly higher BAX/BCL2 ratio in NTZ-EXO-LPS-treated cells. Overall, loading nitazoxanide into LPS-treated exosomes produced the most potent anticancer effects by inducing apoptosis, inhibiting proliferation, and reducing the viability of 4T1 cancer cells. However, further laboratory studies and clinical trials are necessary to confirm these results and fully investigate the therapeutic potential of this formulation.

Key words: Mesenchymal stem cell exosomes, Nitazoxanide, Cancer cells, Apoptosis