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Development of liposomal formulations of selected substances in order to obtain effective immunotherapy of colorectal cancer

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SUMMARY

Colorectal cancer is the third most common cancer worldwide diagnosed among men and women. It is the fourth most common cause of cancer death [1-3]. At the same time, they are one of the main cause of the number of deaths in the world. In 2018, 1.8 million new cases and 881 thousand deaths were recorded. Based on the demographic assessment, an increase in the number of cases is expected, which in 2030 will reach over 2.2 million new cases and 1.1 million deaths [1-3]. It is the most common type of cancer in Europe, accounting for the highest incidence rate in the world [4]. Despite of using modern surgical techniques, new chemotherapeutic drugs and advanced radiotherapy, the mortality rate among patients with colorectal cancer is still high, therefore alternative treatments are sought. During the past decades, immunotherapy has become one of the promising strategies on par with the above-mentioned therapies. Despite the wide application of immunotherapy in the treatment of patients with haematological cancers, the development of effective immunotherapies in the treatment of solid cancers are required, due to the lack of an immune response and the presence of TME [5]. Among the mechanisms related to the low immunogenicity of cancer cells, was identified the resistance to the mechanisms of innate immunity of the immune system.

The effectiveness of phagocytosis depends on pro-phagocytic signals "eat me" and anti-phagocytic signals "do not eat me" on the surface of normal and cancer cells. One of the ”do not eat me” signals is the transmembrane protein CD47 on the surface of tumor cells. CD47 is responsible for inhibiting the phagocytosis of cancer cells by interacting with the transmembrane regulatory protein SIRPα on the macrophage surface. One of the directions in the development of new forms of immunotherapy is searching for the processes causing immunogenic phagocytosis such as ADCP combined with the use of *Toll*-like receptors agonists and ICD. One of the well-known immunomodulator is imiquimod - an agonist of the TLR7 receptor. Berberine is a natural compound potentially enhance the phagocytosis of cancer cells as a result of ICD and macrophage stimulation. The use of the above substances is associated with many side effects and their low bioavailability. The use of nanocarriers allows for the effective delivery above compounds that stimulate the immune cells with simultaneous accumulation in the cancer tissue, contributing to the reduction of cytotoxicity to host cells. Liposomes - artificially obtained lipid vesicles, are a promising nanocarrier for targeted therapies with controlled drug release.

SUMMARY

The aim of this doctoral dissertation was to develop liposomal formulations of imiquimod and berberine and checking their biological activity responsible for obtaining effective immunotherapy of colorectal cancer. The first part describes the basic parameters allowing for active encapsulation of substances using selected gradients, such as the encapsulation efficiency (EE%), the influence of the substance/lipid ratio on EE%, the influence of external pH on EE%, and the kinetics of substance entrapment inside the liposomes. These studies allowed for the development of optimal conditions for the encapsulation of berberine and imiquimod inside liposomes. In subsequent experiments long-term stability of the obtained liposomal formulations of both substances was tested, demonstrating their stability. Next, the stability of the developed formulations was tested in the presence of human plasma *in vitro*. In the case of berberine, its physical state inside the liposomes was also determined using Cryo-TEM microscopy.

The second part of the dissertation included the analysis of the biological activity of the developed liposome formulations of berberine and imiquimod. In the case of the conducted experiments, it can be concluded that the liposomal formulations of berberine were highly cytotoxic towards colon cancer cell lines, with the simultaneous lack of biological activity towards the normal colon line. The developed liposome formulation containing berberine closed with a vitamin C gradient was responsible for generation of ROS, decrease in the GSH level, leakage of Ca^{2+} ions, the reduction of the MMP as a consequence leading to a decrease in the ATP level with a simultaneous lack of caspase activity and increasing the amount of CRT on the surface of cancer cells.

The described mechanism of action of the liposomal form of berberine was most likely associated with the induction of ICD, contributing to the increased number of phagocytic cancer cells. Moreover, it was shown that the treatment of macrophages with the above liposomal formulation significantly increases the phagocytosis of cancer cells in comparison to the used imiquimod and anti-CD47 antibody. Based on the macrophage cytotoxicity tests and phagocytosis, it can be concluded that the developed liposomal form of imiquimod shows the same properties as the free drug.

The obtained results provide new information on the role of berberine in eliciting the immune response against colorectal cancer. It was shown that the amount of macrophage-phagocytosed tumor cells treated with the liposomal form of berberine was greater as compared to the untreated control. Moreover, macrophages treated with the liposomal form of berberine were characterized by an increased phagocytic activity of tumor cells.

SUMMARY

The obtained results provide new insights into the role of berberine in maintaining the immune response against colorectal cancer. The findings presented here demonstrate that it significantly increases the number of phagocytic cancer cells by treated macrophages. These conclusions may contribute to the development of new forms of immunotherapy of colorectal cancer (especially for the stage accompanied with metastasis), which in this thesis was presented as an example of newly described combinations of compounds influencing the phagocytosis of cancer cells.

BIBLIOGRAPHY

[1] Araghi M., Soerjomataram I., Jenkins M., et al. *Global trends in colorectal cancer mortality: projections to the year 2035*. Int. J. Cancer: 144, 2992–3000 (2019).

[2] Goodarzi E., Beiranvand R., Naemi H., et al. *Worldwide incidence and mortality of colorectal cancer and human development index (HDI): an ecological study*. WCRJ 2019; 6:e1433 DOI:10.32113/wcrj_201911_1433.

[3] Granados-Romero JJ., Valderrama-Trevino AI., Contreras-Flores EH., et al. *Colorectal cancer: a review.* Int J Res Med Sci. 2017 Nov;5(11):4667-4676.

[4] Colorectal cancer *A guide for journalists on colorectal cancer and its treatment* https://www.roche.com/dam/jcr:8e08ab40-4ae0-4c61-99dd-a139cce45edc/en/med-colorectal-cancer.pdf ostatni dostęp: 19.02.2021r.

[5] Jiang Q., Zhang C., Wang H., et al. (2019) *Mitochondria-Targeting Immunogenic Cell Death Inducer Improves the Adoptive T-Cell Therapy Against Solid Tumor*. Front. Oncol. 9:1196. doi: 10.3389/fonc.2019.01196.