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Summary of the dissertation submitted for the degree of Doctor in Biocybernetics and Biomedical Engineering

Title: Design, manufacturing and characterization of nanopharmaceuticals containing disulfiram.

Disulfiram is a commercially available drug used in treatment of chronic alcoholism. In recent years its anti-cancer activity had been proved but its clinical use is limited due to high dosages toxicity when taken orally. Literature data suggest that other routs of administration and/of formulation should be considered. The use of nano-pharmaceuticals increases drug concentration in the tumor tissue and reduces the systemic toxicity due to the enhanced permeability and retention effect. In addition, multi-drug resistance can be overcome with nano-pharmaceuticals by the simultaneous administration of two drugs in a single carrier.

Hepatocellular carcinoma (HCC) is a primary liver tumor that is responsible for over one-third cancer related deaths worldwide. Despite many years of research the effective treatment is still not available. HCC is usually diagnosed in advanced stage because of lack of the effective screening test. For patients with advance diseases the only therapy approved by FDA is sorafenib, a multikinase inhibitor that inhibits tumor cell proliferation and tumor angiogenesis. However, the response rate of sorafenib is very low and the median survival time is 11 months in comparison to 6 months for patients treated with placebo. Extension of the survival time is low because HCC cells rapidly acquire the multi-drug resistance. Literature data shown that disulfiram can reduce drug resistance and can be used as adjuvant or as primary drug in the anti-cancer therapy.

In thesis I present the strategy to deliver a nano-pharmaceutical containing disulfiram to hepatocellular carcinoma. This strategy is based on the difference in vessel structure between healthy and tumor tissue and the natural function of the liver to remove the nano-agregates along with the hydrophobic toxins. According to my design nano-pharmaceutics should be no larger than 150 nm in diameter, have a slight negative zeta potential and have a short circulation time. Liposomes were selected as a model of a nano-pharmaceutical because they are

biocompatible, biodegradable and have ability to compartmentalize and solubilize both hydrophilic and hydrophobic compounds.

To design the most effective liposomal carrier containing disulfiram it was necessarily to determine the localization of disulfiram in the lipid bilayer. The obtained results show that disulfiram is located in the middle of the lipid bilayer. The encapsulation efficiency of disulfiram in liposomes of different lipid composition was also studied. The results show that there is no correlation between the parameters of lipid shape (critical packing parameter) and the disulfiram encapsulation capacity. It had been found that, there was a negative correlation between the short-range lipid packing and the encapsulation efficiency. Moreover, it was found that not only the amount of double bonds but also their position in the lipid molecule has an effect on the encapsulation efficiency. Based on experimental tests, it has been determined that the DOPC liposomes are the most efficient lipid system to passively encapsulate disulfiram. They showed a high degree of the encapsulation efficiency (~5mol%) and was a simple one-component system.

The liposome interior can be used to encapsulate hydrophilic substances. To encapsulate disulfiram inside liposomes it was necessarily to increase its solubility through the formation of complexes with hydroxypropyl-β-cyclodextrins. To make this process more efficient a new method for preparing liposomes was tested. The extrusion of lipid gel is a method, in which very high concentration of lipids is used for the formation of liposomes. This approach results with very high encapsulation efficiency of the hydrophilic substance within the liposomes aqueous phase. However, obtained results show that the encapsulation efficiency does not differ from that when there are no cyclodextrin present. It has been suggested that this may be due to competitive association of disulfiram and lipid fatty acids with cyclodextrins.

Literature data show that anti-cancer activity of disulfiram requires the presence of zinc or copper ions. This means that the nanoparticle should be able to deliver simultaneously disulfiram and copper ions to the cancer cell or to its surrounding, preferably in the form of the active complex. To form liposomes containing both cupper ions and disulfiram the gradient of copper ions across the lipid bilayer was used to actively load disulfiram to liposomes. This approach allows formation of nano-carrier containing disulfiram crystals precipitated in the inner liposomal aqueous space. The effect of lipid composition, magnitude of copper ions gradient and the method of the gradient generation was investigated. The results show that the method of copper ions gradient generation by the chelation of none-encapsulated ions is more

efficient than removing those ions by ultrafiltration. In addition, it has been found that the encapsulation efficiency of a copper-disulfiram complex depends on the lipid composition of liposomes and it is higher in the presence of cholesterol in lipid bilayer.

Cytotoxicity of disulfiram in liposomes and that in DMSO was compared. The obtained results show no significant difference in pharmacological efficacy of tested disulfiram formulation and disulfiram alone.

In conclusion, the paper presents a comprehensive research methodology aimed at developing liposomal drug carrier for physico-chemically complex molecules such as disulfiram. As a result of the presented work, a new liposome formulation of disulfiram has been developed and thoroughly characterized.