SUMMARY

Dominik Lipka

New liposomal formulations of the anticancer anthracycline antibiotic

The development of more effective anti-cancer therapy is now one of the biggest challenges posed to modern science. It seems that one of the most obvious direction to increase the efficiency of existing treatments is the development of drug nanocarriers which allow for their efficient delivery to tumor. First approved nano-drug was Doxil® being a liposomal formulation of doxorubicin.

Liposomes are artificial lipid vesicles built of the lipid bilayer enclosing in its interior aqueous solution. Liposomes can encapsulate in its interior, inter alia, anticancer drugs.

The aim of the research was to develope a new method of active loading weak bases in to the liposomes. For this purpose, a new method based on a gradient of ammonium ascorbate and ascorbic acid was developed. This new method of active loading allowed to obtain a liposomal formulation of epirubicin which is an anthracycline antibiotic with anticancer properties.

In first part of the research I investigated kinetics of drug loading, optimal external pH, time and drug-to-lipid ratio for the purpose of remote loading, and in vitro stability was investigated. This study were designed to develop optimal conditions for encapsulating the drug within the liposomes. In next step stability of newly developed liposomal formulation of epirubicin was assessed.

Next, the stability of newly developed liposomal formulation of epirubicin was measured in the presence of 50% human serum *in vitro.* The physical state of the drug inside the liposomes was assessed using the Cryo-TEM microscope and circular dichroism method. These parameters were compared to the liposomes prepared by the use of currently available methods which ensure encapsulating of epirubicin inside the liposomes. ie. the methods based on ammonium sulfate and ammonium versenate.

The cytotoxic activity of new liposomal formulation *in vitro* towards the murine and human breast cancer cell lines was investigated. The biological activity of the new formulation was compared with other formulations. The correlation of the physical state of the drug inside the liposomes with its biological activity *in vitro* was also performed.

The new developed formulations were also examined for their antitumor activity *in vivo* in a mouse model of breast cancer 4T1 and compared with the activity of the free drug. Also pharmacokinetic profile for the encapsulated drug was designated.

In the second part, the additional active targeted liposomal formulations have been developed by attaching folic acid residues on the terminal groups of the pegylated phosphatidyletanoloamine. I examined the basic parameters of targeted formulations, such as long-term stability under accelerated conditions and stability in the presence of human plasma in vitro. The effect of density of folic acid on liposomes surface on the antitumor activity of the liposomes and the effect of linker length on its activity was also examined. I investigated the localizations of the drug inside the cells *in vitro* when targeted and non-targeted formulation was used were also examined. In the end, pharmacokinetic profile for the encapsulated drug inside the active targeted liposomes was designated.