Chapter 3: Difference from prior studies of the drug combination in liposomes

3.0 Difference from prior studies of the drug combination in liposomes

3.1 Dual drug nanocarriers of Doxorubicin and Vincristine

The proposed drug combination in the study, Doxorubicin (DOX) and Vincristine (VCR) have been previously reported for their efficacy when formulated in a single nanocarrier. The various studies performed using this drug combination have been mentioned below: -

- Abraham et al., tested the opportunity of using the drug combination of doxorubicin and vincristine to augment the therapeutic potential mediated through nanocarrier platforms. They co-encapsulated the drugs using metal ion gradient and pH gradient-based drug loading techniques. Liposomes were prepared using manganese sulphate and gradient was created using ionophore A23187 for loading of Doxorubicin while vincristine was loaded using pH gradient. The drug elimination studies indicated a change in the weight ratio of the drugs in the nanocarrier from 4:1 (injected) to 20:1 (plasma transit) of Doxorubicin: vincristine over 24 hours of study. The tumor xenograft models were established using MDA435/LCC6 cell line in SCID-RAG-2M mice. However, the prepared formulation did not have a significant difference in the tumor load reduction as compared to liposomal vincristine. These results were verified using in-vitro cytotoxicity assay and evaluation using Chou-Talalay method indicated antagonism of the combination at the tested weight ratio of the drugs (1).

- Li et al. developed thermosensitive dual drug loaded liposomes for evaluation against drug resistance observed in breast cancer. The drugs (1:4 wt/wt of vincristine: doxorubicin) were actively co-loaded in DPPC: DSPE-PEG$_{2000}$: MSPC liposomes against pH gradient generated using citric acid and subsequent change of the acidic external pH using sodium carbonate. The formulation presented high encapsulation of both drugs (>95%) and particle size of approximately 90 nm while exhibiting time-dependent release profiles at 37°C (normal body temperature) and 42°C (mild hypothermia) and being stable at -20°C for 6 months. The MTT assay of formulation in PANC and SW-620 cell lines indicated improved cellular killing properties as compared to naïve and single drug liposomes. Further, cellular uptake studies in HT-1080 cell lines indicated the improved uptake of the dual loaded formulation. The formulation presented improved bio-distribution, pharmacokinetic profile and tumor reduction when tested in conjunction with mild hyperthermia against MCF-7 xenograft model in female nude mice. Their results indicated the efficiency of the drug combination when formulated in single nanoliposome and with assisted delivery using mile hyperthermia (2).

- Dong et al. encapsulated both the drugs (1:1 wt/wt ratio) in COMPRITOL-888ATO, cremophor ELP as well as stearic acid based solid lipid nanocarrier vehicle for evaluation of the efficacy in B-cell lymphoma. The prepared formulation had a particle size of 95 nm, zeta potential of 21 mV and encapsulation efficiency of more than 83% for both the drugs. This formulation presented a controlled release profile over 24 hours of both the drugs and showed significantly improved IC_{50} values when tested in LY1 cell line as compared to the drug solutions and the single drug nanocarriers. The formulation presented significantly improved tumor reduction when tested in LY1 xenograft model in BALB/c mice confirming the anti-tumor efficacy of the formulation in B-cell lymphoma (3).

- Zhang et al. attempted to deliver the drug combination (4:1 w/w of doxorubicin: vincristine) by circumventing the blood brain barrier using dual targeting ligands against glioma. The ligands used for surface modification of the HSPC, cholesterol and mPEG-2k-DSPE liposomes were T7 (ligand for transferrin surface receptor) as well as DA7R (targeted against surface overexpression of vascular endothelial growth factor receptor 2) peptides. These ligands were first conjugated to DSPE-PEG_{2000}-Maleamide and then incorporated in the drug loaded liposomes using post-insertion technique. The formulation presented an encapsulation efficiency of more than 85% for both the drugs and a particle size of approximately 93 nm. The in-vitro cytotoxicity studies in C6 cells indicated improved cellular killing as compared to drugs solutions, single drug liposomes and non-targeted liposomes. Additionally, the cellular uptake studies in bEnd.3/C6 co-culture BBB model presented significantly improved cellular uptake as compared to the other formulations. The anti-tumor efficacy studies in orthotropic model of C6 cells in female ICR mice further presented improved anti-glioma activity of the prepared formulation (4).

3.2 Differences of the proposed study with the earlier reported studies

The above-mentioned studies presented various short comings which include lack of determination of the combinatorial index for the intended disease, lack of efficacy, lack of physicochemical stability, unfavourable drug release profiles and non-scalability in manufacturing. Importantly, none of these approaches have been tested favourably in NSCLC and TNBC.

The aim of the current study was to develop a stable, scalable co-loaded formulation using the design of experiments (DOE) optimization technique, similar in physicochemical and biochemical characteristics to the clinical standard (PEGylated Liposomal Doxorubicin hydrochloride) while improving the therapeutic efficacy of the formulation against TNBC and NSCLC. The combinatorial index of the drugs against TNBC and NSCLC were intended to be determined using the Chou-Talalay method and the ratio presenting the best efficacy in both the cancers would be selected for further encapsulation in the PEGylated liposome. Both the drugs would be actively co-loaded with comparative physicochemical and biochemical characterization with the clinically standard. Comparative in-vitro biological activity of the formulations would be evaluated using cellular uptake studies, cell proliferation assay along with its mechanistic evaluation using cell cycle analysis, apoptosis assay and wound healing. The effect of co-encapsulation would further be evaluated in-vivo in both cancer models by first determining the maximum tolerated dose, followed by the tumor regression study with finally determining the pharmacokinetic and tissue distribution profiles. The current study is aimed at improving the therapeutic efficacy of PEGylated liposomal doxorubicin in TNBC and NSCLC.

3.3 References

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