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Development of Liposomal Formulations of Zinc Diethyldithiocarbamate for Colorectal Cancer Treatment

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SUMMARY

$(DDC)_2Zn$, disulfiram (DS) metabolite, has shown promising anticancer effects in vitro but further investigations in vivo are limited by its poor water solubility. In this study, liposomes are assessed as a delivery system for $(DDC)_2Zn$. Liposomes were prepared by the thin-film hydration method, followed by high-pressure homogenisation (HPH) for size reduction. The nano-liposomes were then characterised by size, polydispersity index (PDI), zeta potential (ZP), drug loading and encapsulation efficiencies (DLE% and EE%), and MTT cytotoxicity assay. The HSPC-based (PBS) liposomes showed a nano-range of sizes (< 200nm), good PDI (<0.5) but moderate EE% (<40%). However, $(DDC)_2Zn$ liposomal formulations showed enhanced cytotoxic activities toward colorectal cancer cells. Therefore, liposomal formulations of $(DDC)_2Zn$ with improved DLE% and EE% might have immense potential in cancer therapy.

INTRODUCTION

Cancer is one of the leading causes of death worldwide. Since anticancer drug development has been slow, scientists are using drug repurposing strategies to identify new anticancer indications for approved drugs i.e., disulfiram (DS). DS, a drug used worldwide for anti-alcoholism therapy, has shown strong anti-cancer activities in vitro. However, the clinical application of DS is limited by its rapid degradation in the bloodstream. Interestingly, the cytotoxicity of DS towards cancer cells is dependent on its chelation with bivalent metals such as zinc (Zn) and Copper (Cu), and the formation of its active metabolites $(DDC)_2$ Zn/Cu. $(DDC)_2Zn$ complex has shown strong anticancer activity but its poor solubility in water as well as in ethanol has been a hurdle to further development.

Lipid nanocarriers, such as liposomes, have been widely used to increase the stability, therapeutic index, efficacy and biodistribution of the encapsulated drug. In this study, nanoliposomes of $(DDC)_2Zn$ were developed to assess their potential as druggable formulations for anticancer treatment.

MATERIALS AND METHODS

The liposomes were prepared via the thin-film hydration method reported by (Dyondi, Sarkar, and Banerjee, 2015) with slight adjustments; The drug was added in 10 mol% of the lipid phase which consisted of HSPC or DPPC and cholesterol (50:50). The lipid film was hydrated by PBS or water to obtain a final lipid phase concentration of 10mg/ml. Size reduction was performed by HPH (10cycles). The characterisation and cytotoxicity studies of the

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liposomes were performed as reported by (Najlah, et al., 2019).

RESULTS AND DISCUSSION

The phospholipid: cholesterol in 1:1 molar ratio was used, to produce suitable lipid carriers for highly hydrophobic anticancer drugs (Najlah, et al., 2019). Table 1 shows that the HPH reduced the size of the liposomes to below 300 nm, which has been reported to be ideal for extravasation into tumours (Danhier, Feron, and Preat, 2010).

Table 1. *particle size (nm), polydispersity index (PDI), and zeta potential (ZP) of formulations after HPH (n=3 ± SD).*

Formulation	Size (nm)	PDI	ZP (mV)
HSPC:Ch (L) (DW)*	226 ± 44.1	0.459 ± 0.08	-19.3 ± 3.7
HSPC:Ch (E) (DW)	144 ± 4.3	0.443 ± 0.02	-9.0 ± 2.5
DPPC:Ch (L) (DW)	199 ± 16.0	0.402 ± 0.08	-14.8 ± 2.0
DPPC:Ch (E) (DW)	142 ± 7.6	0.446 ± 0.02	-11.4 ± 1.4
HSPC:Ch (L) (PBS)	174 ± 11.2	0.428 ± 0.09	-15.2 ± 6.6
HSPC:Ch (E) (PBS)	147 ± 15.6	0.384 ± 0.01	-11.7 ± 1.7
DPPC:Ch (L) (PBS)	287 ± 48.4	0.403 ± 0.04	-13.7 ± 2.4
DPPC:Ch (E) (PBS)	205 ± 7.6	0.465 ± 0.03	-14.6 ± 1.5

** Drug Loaded formulation (L), Empty formulation (E), Distilled water (DW), Phosphate Buffer Saline (PBS).*

As seen in table 1, the homogeneity of the formulations was within the acceptable PDI range (<0.7). The breadth of the PDI was independent of phospholipid type. However, the Empty HSPC: Ch liposomes (PBS) had a significantly narrower PDI (0.384 ± 0.01) than the Empty HSPC: Ch (distilled water) (0.443 ± 0.02), which could indicate the ionic strength of the medium may have impacted the bilayer arrangement in the empty formulations. The same significant difference was seen in the ZP of the mentioned formulations (-11.7 ± 1.7 and -9.0 ± 2.5 respectively), which could indicate that the ions in PBS had also influenced the surface charge of the liposomes.

As shown in figure 1, the phospholipid type or the hydrating medium had no influence on the DLE% and EE% of the liposomes (no significant difference), which was in accordance with previous findings by the group (Najlah, et al, 2019). Although the EE% and DLE% of the HSPC: Ch (PBS) formulation seemed generally higher than the other formulations in this study, they are still lower than what has been reported in the literature for DS liposomes (Najlah, et al,. 2019) (DLE of 64% and EE of 84%). This might be due to molecular conformational differences between DDC-Zn and DS.

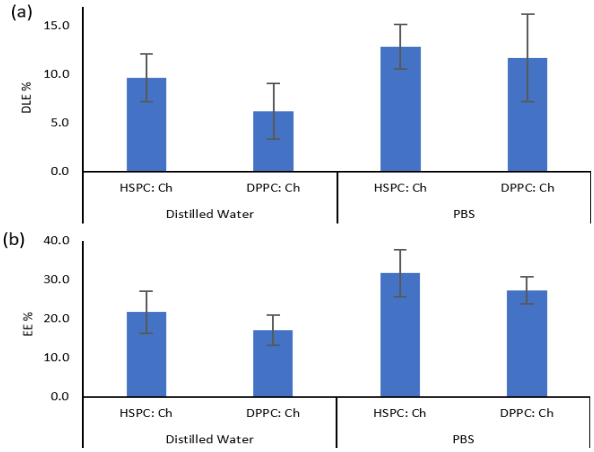

Fig. 1. *(a) Drug Loading Efficiencies (DLE%), (b) Encapsulation Efficiencies (EE%) of the formulations (n=3 ± SD).*

Our preliminary studies have shown that liposomal $(DDC)_2Zn$ had significantly higher cytotoxicity towards colorectal cancer cells than free $(DDC)_2Zn$ (data not shown) and further cancer molecular biology studies are currently under investigation.

CONCLUSIONS

The DLE% and EE% of the formulations were not affected by the lipid type or the hydrating medium, however, the HSPC: Ch (PBS) liposomal formulation showed to have the most ideal characteristics for anticancer treatment against colorectal cancer cells in vitro. However, further modifications must be carried out to improve the EE% of the liposomes.

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