P-083	**Estrogen receptor targeted delivery of an anticancer agent for breast cancer therapy**

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INTRODUCTION AND OBJECTIVES

The encapsulation of anticancer agent in carrier system protects healthy tissues from its cytotoxic effects. Further, conjugation of site-directing ligands to nanocarrier encapsulating active moieties, targeted to over-expressed cell surface receptors is a promising approach for delivery of therapeutic agents to tumor cells (Rai S et al. 2007). In the present study estrogen receptor targeted liposomes encapsulating doxorubicin was designed to enhance the efficiency of liposomal formulation with the added advantage of delivery of doxorubicin to its destination site i.e. cancerous cells over-expressing estrogen receptors (ERs).

MATERIAL AND METHODS

Estrone coupled simple liposomes and stealth liposomes were prepared with the composition of egg phosphatidylcholine/cholesterol/ dipalmitoyl phosphatidylethanolamine-estrone (PC/CHOL/DPPE-ES) and other with the composition of egg phosphatidylcholine/cholesterol/ dipalmitoyl phosphatidylethanolamine-polyethylene glycol-estrone (PC/CHOL/DPPE-PEG-ES) (Lasic DD et al. 1992). DPPE-PEG-ES was synthesized using the method described by Zalipsky *et al*. 1983 with modification as shown in figure 1 (Zalipsky S et al. 1983).

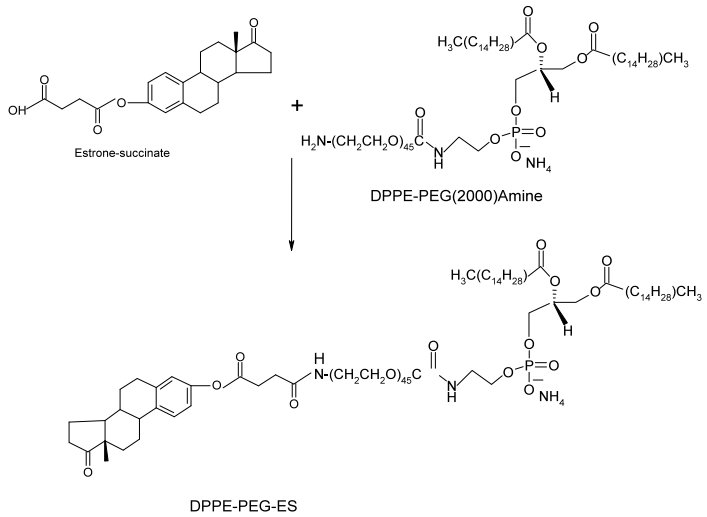

Figure 1: Scheme of synthesis of DPPE-PEG-ES

Formulations were evaluated for size, shape, entrapment efficiency (EE %) and polydispersity index (PI). The *in vitro* drug release is performed by dialysis tube method. *In vitro* MTT cytotoxicity assays were conducted on MCF-7 and MDA-MB31 cells for one hour with various concentrations of formulation and free drug to evaluate the advantages of estrogen receptor targeting. Biodistribution study was performed with the fluorescent dye 6-carboxyfluoroscein. Quantitative estimation of drug biodistribution was done on female albino rats by HPLC method (Rose LM et al. 1988). For tumor regression study, in female balb/c mice tumor was induced by administering DMBA and treated with doxorubicin formulations *via* i.v. route once a week. Tumor volumes were measured at timed intervals.

RESULTS AND DISCUSSION

The average vesicle size of the estrone-appended liposomes was found to be 208±24 and 258±18 nm for estrone-appended stealth liposomes, EE is greater than 80% in all cases and PI is less than 1 (Table.1).

Table 1: Size, Entrapment efficiency and Polydispersity index of different formulations

Formulation	Size (nm)	(EE%)	PI
Simple Liposomes	193±11.8	89.5±3.8	0.13
Estrone Coupled Liposomes	207±8.4	85.2±4.1	0.15
Stealth Liposomes	218±12.1	88.3±6.7	0.18
Estrone coupled stealth Liposomes	243±4.6	84.9±9.4	0.16

The TEM showed spherical nature of the prepared formulation (Fig.2).

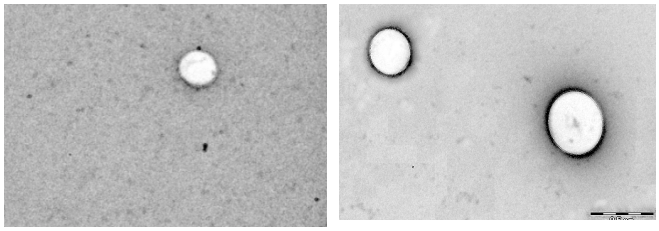

Figure 2: TEM of (A)Estrone coupled liposomes, (B) Estrone coupled stealth liposomes

The fluorescent microscopy studies showed efficient uptake by tumor tissues (Figure 3).

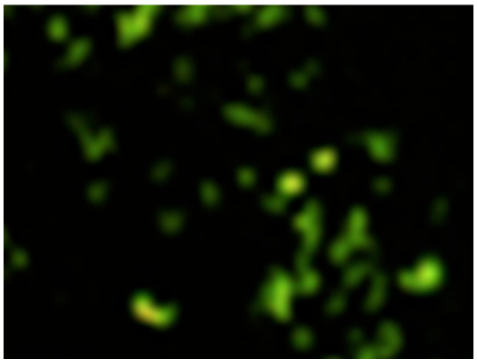

Figure 3: Liposome uptake by tumor tissues

Plasma profile of DOX encapsulated liposomes was shown in figure 4 (Speth PA et al. 1988).

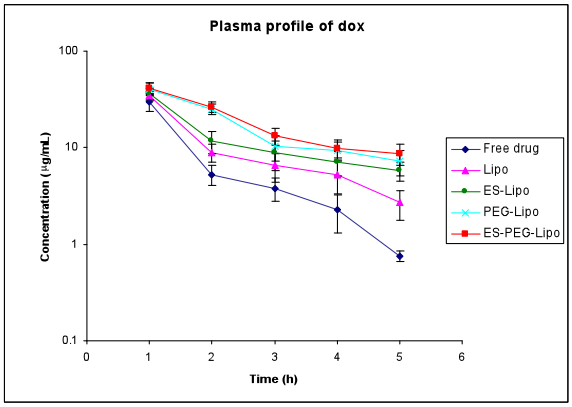

Figure 4: Plasma profile of DOX encapsulated liposomes

In vitro uptake studies the doxorubicin liposomes showed a greater extent of intracellular uptake against estrogen-receptor-positive cancer cells than estrogen-receptor-negative cells. They also exhibited more potent cytotoxic effect on MCF-7cells than free doxorubicin. The concentration of doxorubicin after 1 hr of administration in various tissues was 4.6±1.12 µg/g in liver, 11.5±0.98 µg/g in heart, 4.9±1.67 µg/g in kidney, 2.6±2.01 µg/g in breast, 1.4±1.23 µg/g in uterus and 0.95±0.87 µg/g in brain. Both the liposomal formulations showed change in the biodistribution profile. Targeted formulation accumulates more in breast and uterine tissues. The concentration of estrone appended liposomal doxorubicin and plain liposomal doxorubicin in breast tissues after 1 hr tail *i.v.* injections were 9.66±2.07 µg/g and 6.12±1.96 µg/g. Similarly in uterine tissue were 2.9±1.67 µg/g and 1.63±1.59 µg/g respectively. The lower concentration of plain and estrone appended liposomal-DXR in the heart, kidney tissues as compared to free DXR may provide platform for lower toxicity to these tissues. Biodistribution study on breast, uterus and brain reveled that greater accumulation of estrone appended liposomal-DXR as compared to plain liposomal-DXR and free DXR. This changed biodistribution of liposomes might be due to differential expression of receptors for the attached ligand over the liposomes. In a human tumor xenograft nude mouse model, estrone-targeted liposomes significantly reduced the tumor volume compared to non-targeted liposomes or free doxorubicin.

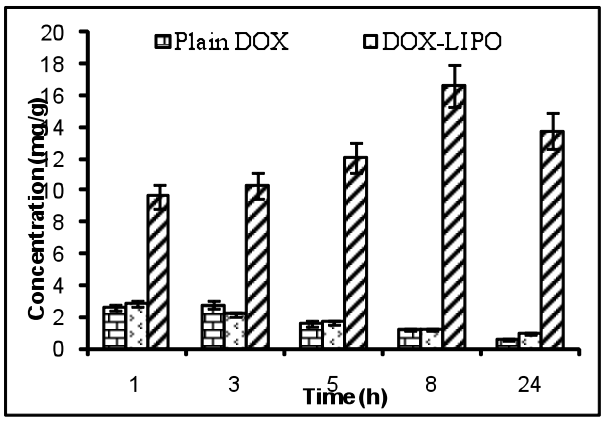

Figure 5: Liposome encapsulated DOX accumulation in breast tissues

CONCLUSION

The use of targeting ligand for over-expressed tumor cell receptors is widely accepted to selectively deliver therapeutic agents to these cells while sparing non-target healthy cells. A novel ER-targeted liposomal doxorubicin formulation was developed with good drug loading properties. The formulation exhibited excellent colloidal stability and the stability in bio-fluid. The formulation also retained their specificity towards the estrogen receptors. Thus it can be concluded that ER-targeted formulation of antineoplastic agents could be potentially useful for treatment of ER positive tumors such as breast, uterus and opens the new possibility for non-immunogenic, site-specific delivery of bioactive(s).

REFERENCES

- Rai S et al. (2007) *Estrogen(s) and analogs as a non-immunogenic endogenous ligand in targeted drug/DNA*. Current Medicinal Chemistry (14) 2095-2109.
- Zalipsky S et al. (1983) *Attachment of drugs to polyethylene glycols*. Eur Polym J (19) 1177-1183.
- Rose LM et al. (1988) *High-performance liquid chromatographic determination of doxorubicin and its metabolites in plasma and tissue*. J Chromatogr Biomed appl (425) 419-423.
- Lasic DD et al. (1992) *Gelation of liposome interior: a novel method for drug encapsulation*. FEBS Letter (312) 255-258.
- Speth PA et al. (1988) *Clinical pharmacokinetics of doxorubicin*. Clin Pharmacokinet (15) 15-31.