Design and Evaluation of Nanotherapeutic System(s) for Management of Hormone Receptor-Positive Breast Cancer

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SUMMARY

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Summary

In the field of drug delivery, nanocarriers have a number of benefits to offer, including the capacity to dissolve hydrophobic drugs and an improvement in retention. Nanocarriers are most commonly prepared with materials of synthetic and natural origin. The efficacy of nanocarriers as a drug delivery vehicle has increased due to the advantage of novel lipid-based natural biomaterials. These natural biomaterials are environmentally friendly since they are derived from renewable sources and have nontoxicity and biodegradability features. Lipids are GRAS (Generally Recognised As Safe), and they may be less opsonized by the RES (reticuloendothelial system) while passing across an aqueous barrier.

Breast cancer is a major cause of death in women throughout the world and contributes substantially to the escalating costs of health care. Age, family history, reproductive variables, and lifestyle are all risk factors for breast cancer. Higher estrogen levels in the body are one of the major risk factors for breast cancer, as they promote cell proliferation and worsen the condition. So, estrogen is a vital target for the prevention as well as treatment of breast cancer. Some breast cancers are caused by hormonal imbalances, such as estrogen and progesterone. These hormones play a function in directing the growth of breast cells. The hormone receptors in hormone receptor-positive breast cancer lead breast cells to proliferate out of control. In cancerous breast cells, estrogen is mainly responsible for the proliferation of the epithelial cells. Thus, the main enzyme which changes androgen to estrogen is the basic target for breast cancer treatment, i.e., the aromatase enzyme. Therefore, obstructing the aromatase enzyme declines the level of estrogens in the body, thus making it a selective and efficacious treatment for patients with HR+ breast cancer, mainly estrogen.

All phospholipids biomaterial possesses strong antioxidant activities, which are responsible for most of their biological effects. The antioxidant and free radical scavenging properties of

biomaterials make DSPC the material of choice for encapsulation any drug for the prevention of oxidative stress-associated diseases.

Exemestane (Exe) and Bosutinib (Bos) respectively were selected as drug candidates because of their anticancer activities. It was expected that the encapsulation and targeting capabilities would improve the biological characteristics of drugs put into lipid vesicles. These were taken as model drugs for the present study. Limitations like inadequate solubility, less absorption, low bioavailability, drug metabolism, side effects and patient non-compliance are noticed with these active moieties, which can be overcome by using lipid-based nanocarriers.

PART I

In part I, lipid nanocapsules were prepared by the modified phase inversion method. The exemestane-loaded lipid nanocapsules (Exe-LNCs) were characterized *in-vitro* for size, shape, charge, dispersity index, release profile, drug loading, entrapment efficiency, and surface morphology. Subsequently, cell cytotoxicity and cellular uptake investigation have been attempted on MCF-7 cell lines. Concomitantly, the *in-vivo* efficacy was monitored through various parameters viz., biochemical estimation, animal weight variation, carmine staining of whole-mount mammary glands, histopathological examinations, organ biodistribution, and pharmacokinetic studies.

The LNCs are the third generation of modifications that overcome the shortcomings of conventional lipid-based delivery systems such as SLNs, NLCs, nanoemulsions etc., which have a low drug loading efficiency and the added risk of the drug ending up as a non-homogeneous dispersion. The lipids are both biodegradable and biocompatible in nature and thus lipid-containing systems are preferred ascribing to the presence of natural lipids. These can be partially modified into semisynthetic lipids and can be prepared as nanoformulation-

based dosage forms. These also efficiently deliver the drugs and biologicals to the tumour target site without any disruption credited to the nanosized range that facilitates passive targeting.

The pharmacokinetic profile of Exe drug suspension Exe and Exe-LNCs were shown in table. Where Exe-LNCs showed higher plasma concentration as compared to Exe drug suspension Exe. The enhanced half-life could be due to the controlled release pattern of drugs from the Exe-LNCs. A controlled release formulation, from Exe-LNCs increases Exe bioavailability and longer circulation half-life in the body, allowing for dose reduction.

The histopathological analysis revealed normal cellular architecture and intact epithelium seen in the normal control group, whereas the positive control group (DMBA administered) showed loss of normal cellular architecture with a damaged epithelium layer. The Exe-LNCs treated group showed a marked restoration of tissue architecture owing to improved therapeutic efficacy which was able to treat mammary gland carcinoma more efficiently.

Reduction in cancer growth in female rats also certified the significantly enhanced therapeutic efficacy of the Exe-LNCs in comparison to the Exe drug suspension drug. Further, the biodistribution studies strengthen the targeting effect of Exe on estrogen receptors when formulated as LNCs.

The main objective of the present research was to synthesize and characterize lipid-based nanocarrier system(s) for delivery of exemestane and bosutinib. In the first study, it was envisaged to use of DSPC as the lipid phase, olive oil for the oily core, and Pluronic® F-68 (PF-68) as a surfactant to develop LNCs for the successful delivery of Exe to the tumor cells. Due to the stealth vesicles of lipids and surfactant, they carry the drug to the target site (receptor) and offer passive targeting facilitated EPR (Enhanced permeation and retention) into the tumor vicinity.

PART II

In part II, we designed and formulated DSPC-based liposomes for the successful delivery of Bosutinib against estrogen-positive cancer. Physicochemical evaluations demonstrated that optimization aided in the successful development of LPs. Biotin modification increased the apoptotic potential of Bos in cancer cells and demonstrated high antioxidant properties in normal cells. The attachment of biotin to LPs was successfully affirmed by characteristic FTIR peaks and NMR spectrum of modified stearic acid and HABA/avidin assay. HABA is a dye that shows UV maxima (λmax) at 350 nm. It can bind to avidin to form HABA avidin complex, in the same binding site that is used by biotin to form an avidin-biotin complex. HABA after the formation of a complex with avidin gives maximum UV absorption maxima at 500 nm. The affinity of HABA is much weaker (Kd, 10-6 M)) as compared to the affinity of biotin to avidin (Kd, 10-15 M). Therefore, the addition of biotinylated LPs to the solution of avidin/HABA complex, HABA is competitively and quantitatively replaced by biotin from avidin/HABA complex, and this replacement was quantitatively observed by a decrease in absorbance maxima at 500 nm.

The positive outcomes observed through in vitro studies provided strong e to further explore the delivery system for in vivo anticancer efficacy of biotin decorated LPs systems in DMBA induced breast tumor in the rat animal model. A reduction (54%) in mean animal weights was observed in the case of the toxic group. In the case of other groups, Bos treated group showed a 38.65% reduction, the Bos-LPs treated group showed 12.34% and the b-Bos-LPs treated group showed no reduction of weight from 0-day reduction. In line with above data, the highest number of animals survived after the completion of the study in case of b-Bos-LPs treated groups. The lowest percentage of survival was observed in

case of the toxic group. Further, total tumor burden demonstrated the induction and progression of mammary gland cancer in DMBA-treated animal groups was found to be highest in the case of toxic group. The Exe-LNCs treated group demonstrated lowest toxicity. Aerobic cells constantly produce reactive oxygen species which are counter balanced by antioxidant enzymatic biochemicals like GSH, SOD and catalase. However, under the stress conditions like tumor, these enzymatic biochemicals are suppressed.

Angiogenesis and cellular proliferation are distinctive characteristics of the cancer growth and progression represented through Alveolar buds/terminal end buds (AB/TEB) count and cellular architecture of mammary gland tissue. The DMBA administration resulted in a significant increase in the number of AB/TEB and significant changes in ducts, adipocytes, Loose connective tissue, Dense connective tissue and tissue architecture in mammary gland tissues as depicted by carmine staining and H&E stain. Bos-LPs and b-Bos-LPs were more efficient in diminishing the count of AB/TEBs as compared to the standard groups. Similarly, b-Bos-LPs treatment resulted in the restoration of cellular architecture and lowering in the AB/TEBs count.

The formulation's apoptotic and antiproliferative properties were validated in MCF-7 cell lines. The biotin modified-Bos-LPs were found to be cytotoxic to malignant cells. The regulation of apoptotic proteins by biotin modified-Bos-LPs was discovered using immunoblotting. In vivo findings in a carcinogen-induced rat model revealed that biotin-modified LPs were more effective and safer than simple formulations. The preferential concentration of the drug in the tumor was confirmed by biodistribution. In conclusion, the encapsulation of Bos within LPs can be utilized as a better alternative to standard formulations in the treatment of estrogen-

positive cancer. Also, after subsequent clinical testing, the nanoformulation's therapeutic effectiveness can be translated come bench-to-bedside.