Lipid Nanocarriers for Treatment of Certain Types of Cancer

A Thesis Submitted for Partial Fulfillment of the Requirements for the Master Degree of Pharmaceutical Sciences (Pharmaceutics)

By

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List of Abbreviations

Antibodies	Ab
Atorvastatin	ATV
ATP-binding cassette	ABC
Confocal laser scanning microscopy	CLSM
D- Optimal mixture design	DOMD
Differential scanning calorimetry	DSC
Distilled water	DW
Drug loading	DL
Drug to lipid	D/L
Dynamic light scattering	DLS
Encapsulation efficiency	EE
Enhanced permeability and retention	EPR
Farnesyl pyrophosphate	FPP
Fluvastatin	FV
Generally regarded as safe	GRAS
Geranyl geranyl pyrophosphate	GGPP
High performance liquid chromatography	HPLC
High resolution transmission electron microscopy	HR-TEM
HMG-Co A reductase	HMGCR
Hydrophile lipophile balance	HLB
Hydroxy-methyl glutaryl	HMG
Hypoxia-inducible factor 1 α	HIF-1 α
Kilogray	KGy
Labrafac lipophile® WL 1349	LL
Lipid nanocapsules	LNCs
Lipid nanoparticles	LNPs
Long circulating liposomes	LCL
Lovastatin	LV
Low density lipoproteins	LDL
millipascal-second	mPa.s
Molecular weight	Mw
Mononuclear phagocytic system	MPS
Multi-drug resistance	MDR
Nanoparticles	NPs
Nanostructured lipid carriers	NLCs

Nitric oxide	NO
Paclitaxel	PTX
Particle size	PS
Percent drug released after 48 hours	%Q48h
P-glycoproteins	P-gp
Phase-inversion temperature	PIT
Phase-inversion zone	PIZ
Phosphate buffer saline	PBS
Phosphatidylcholine	PC
Poly (glycolic acid)	PGA
Poly (lactic acid)	PLA
Poly (lactic-co-glycolic acid)	PLGA
Poly ethylene glycol	PEG
Polydispersity index	PDI
Pravastatin	PRV
Reticuloendothelial systems	RES
Simvastatin	SV
Solid lipid nanoparticles	SLNs
Solid lipid to oil	S/O
Surfactant	SAA
Tumor associated macrophages	TAMs
Zetapotential	ZP

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Abstract

Simvastatin (SV) belongs to the lipophilic class of statins. It is one of the most pharmacologically potent inhibitor of HMG-CoA reductase leading to inhibition of mevalonate synthesis and hence cholesterol biosynthesis. It has also been reported that high levels of mevalonate have been associated with breast malignancies. Recently, SV demonstrated anti-proliferative effect on wide varieties of cancer cell lines as it induces cell cycle arrest at G1phase.

Hence, the purpose of this study was to formulate and to *in vitro* evaluate SV-loaded lipid nanocarriers .i.e. lipid nanocapsules (LNCs) and nanostructured lipid carriers (NLC) followed by an assessment of their cytotoxicity effect on breast cancer cells aiming to passively target cancer cells when administered *via* the intravenous route.

SV-loaded LNCs were successfully prepared based on the phase inversion method. They consisted of Solutol® as the main ingredient having PEG moieties influencing both LNC formation and its stealth properties. Labrafac® was employed as the oily phase which was additionally stabilized by lecithin (Epikuron®). Moreover, the aqueous phase including sodium chloride (NaCl) greatly affected LNCs formation. The LNCs were optimized using the D-Optimal mixture statistical design where the influence of the percentages of the three independent components (variables) were studied, namely; Labrafac ®, Solutol® and water. The observed dependent variables were the mean particle size (PS), polydispersity index (PDI) and percentages of SV released after 48 hours (%Q48h). The selected SV-LNCs formulation was characterized by HR-TEM and DSC studies and subjected to sterilization by gamma irradiation. Moreover, the cytotoxicity of SV-loaded LNCs was evaluated on MCF-7 breast cancer cell lines using crystal violet assay.

The results demonstrated that the physical characterization of LNCs were greatly affected by the ratio of surfactant to oil. Increasing the amount of Solutol reduced the interfacial tension of the oily core of LNCs resulting in smaller and homogenously distributed particles. LNCs showed sustained release of SV owing to the hindrance property imparted by Solutol. The obtained statistical models were significant at a level of $p<0.05$ and highly fitting with $r^2 > 0.7$. High correlation between the experimental and the predicted runs together with high adequacy of the models were attained. The generated models achieved high prediction power scoring percentages bias less than 7%.

SV-LNCs were spherical with smooth surfaces and the drug was molecularly dispersed in the lipidic matrix as being represented by TEM and DSC, respectively. The selected formula was successfully sterilized at 25 KGy dose of radiation and was found to boost the cytotoxicity of SV.

SV-loaded NLCs were effectively prepared by the hot homogenization technique incorporating a mixture of solid and liquid lipids. The solid lipid comprised Compritol E ATO, while the oil consisted of Labrafac Lipophile WL 1349 as medium chain triglycerides without PEG moiety. Labrasol® and Labrafil® M 1944 CS, as examples of PEG containing lipids with different HLB, were employed replacing Labrafac® to produce PEGylated nanocarriers without physical or chemical synthesis. All the prepared formulations were stabilized by Polysorbate 80. Applying one-factor-at-a-time (OFAT) technique, a preliminary study was conducted studying several variables at different levels. The prepared formulae were characterized in terms of PS, PDI, ZP and EE%. Lyophilization of the selected formula was optimized using different types and concentrations of cryoprotectants. Selected NLC formulations were subjected to *in vitro* release studies, HR-TEM imaging, DSC and sterilization. The cytotoxicity of selected SV-NLCs was also evaluated on MCF-7 breast cancer

cell lines using crystal violet assay and further confirmed by confocal laser scanning microscope (CLSM) and quantitative analysis of intracellular drug concentrations.

The preliminary study revealed that prolonging the homogenization time, reducing Polysorbate 80 concentration and raising solid lipid to oil ratio led to significant increase in PS and PDI. All the prepared NLC carried negative charges having EE% greater than 97. Hence, the optimized formulation comprised 2% Tween 80, 85:15 solid lipid (Compritol): oil (Labrafac), 0.25:1 drug: total lipids and 1% total lipids exhibiting a small mean PS of 52.3 nm, a homogenous size distribution with a PDI value of 0.177, a negatively charged surface with a ZP at -13.2 mV and an entrapment efficiency of 98.5%.

By replacing Labrafac with either Labrasol or Labrafil, NLC acquired greater PS and PDI and decreased ZP. As all prepared SV-NLC suffer from instability upon storage, they were then subjected to lyophilization efficiently using either 10% (w/w) sucrose or trehalose.

HR-TEM images of SV-NLC showed dense spherical patches having smooth surfaces and DSC studies showed complete drug entrapment in lipidic NPs. The release profiles of PEGylated formulations were found sustained compared with that of PEG-free NLC. The lyophilized formulae exhibited faster SV release pattern than their corresponding fresh ones except for Labrafac®-based NLC. Labrasol®-NLC, fresh and lyophilized formulae, followed diffusion release mechanism while Labrafac®-NLC exhibited first order release mechanism. The results of sterilization showed that the lowest dose (5 KGy) of gamma radiation imparted sterilization with minimal changes in PS, PDI and ZP.

NLC incorporating Labrafac®, Labrasol® and Labrafil® showed variable cytotoxicities against MCF-7 cell lines. NLC including Labrasol® exhibited the highest cytotoxicity with IC50 scored 35.2±3 µg/ml. The optical CLSM sections expounded the boosted cytotoxicity of Labrasol®-based NLC where more dense fluorescence was defined. Furthermore, Labrasol®-NLC attained the highest intra-cellular SV accumulation at different drug concentrations owing to its effect on the integrity of plasma membrane impairing the tight junctions together along with its inhibitory action on P-gp. Therefore, we deduced that Labrasol®-NLC could be a promising candidate for enhancing SV cytotoxicity.

To this end, the investigated lipid nanocarriers has showed promising results as successful nanocarriers for simvastatin and have demonstrated high cytotoxicity effects on breast cancer cell lines.

Keywords: *Simvastatin; Lipid nanocapsules; D-Optimal Mixture design; Nanostructured lipid carriers; Medium chain triglycerides; PEGylated glycerides; Human breast adenocarcinoma cell lines; Cellular uptake.*