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Long chain lipid based tamoxifen NLC. Part I: Preformulation studies, formulation development and physicochemical characterization

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ABSTRACT

Tamoxifen citrate (Tmx) was formulated in nanostructured lipid carrier system (NLC) using long chain solid lipids (LCSL) and oils (LCO) with the aim to target lymphatic system to improve its bioavailability in plasma and lymphnode (initial sites for metastasis) and reduce its drug associated toxicity. Tamoxifen loaded NLC (Tmx-NLC) was formulated using solvent diffusion technique. Preformulation studies comprised evaluation of drug–excipients compatibility. Solubility of Tmx was screened in LCSL and LCO, surfactants and co-surfactants to identify NLC components. Surfactant co-surfactant combinations were studied for their ability to stabilize the system. Tmx-NLC was physicochemically characterized by TEM, DSC, XRD, and FTIR studies. Drug–excipients chemical compatibility study facilitated anticipation of excipients induced oxidative degradation of Tmx. Suitable storage condition below 30 °C could stabilize Tmx. Tmx-NLC with >90% entrapment efficiency and 215.60 ± 7.98 nm particle size were prepared and freeze dried. Freeze dried Tmx-NLC could withstand various gastrointestinal tract (GI) media (pH 1.2, pH 3.5, pH 4.5, pH 6.8, pH 7.4). Dissolution profile of Tmx-NLC in various media showed sustained release pattern irrespective of pH of medium. No significant change in characteristics of Tmx-NLC was observed after 3 months of accelerated stability studies.

1. Introduction

Breast carcinoma is identified to be the second most dreadful cancer afflicting adult women. Annually, more than one million women are diagnosed with breast cancer and more than five lakhs die from the disease (Jemal et al., 2008). Tmx, a non-steroidal anti-oestrogen agent is being used as first line treatment for breast cancer since several decades. It is administered as tablets or solutions orally with the dose of 10 mg twice a day. Despite being a promising molecule, Tmx exhibits numerous biopharmaceutical and toxicological issues. High susceptibility to liver metabolism and precipitation as free base in acidic environment of stomach are important factors contributing to low Tmx bioavailability (20–30%) (Shin and Choi, 2009). This necessitates high dose administration which leads to dose dependent side effects and large inter-individual variation (Hard et al., 1993; McVie et al., 1986; Tukker et al., 1986). Further, prolonged treatment and dose accumulation render patients highly vulnerable to oxidative stress mediated hepatotoxicity (Parvez et al., 2006) and endometrial cancer (Shin et al., 2006).

Prime reason of mortality in breast cancer patients is metastasis which predominantly occurs *via* regional lymph nodes (Chua et al., 2001; Cunnick et al., 2002). Lymphangiogenesis aggravates the migration of tumour cells into lymph node which further leads to systemic spread thus reducing patient survival (He et al., 2004; Salven et al., 2003; Skobe et al., 2001).

This scenario demands an effective lymphatic targeting therapeutic strategy which could help overcome drug allied biopharmaceutical and toxicological issues and additionally confer anti-metastatic property.

Targeting intestinal lymphatic system (ILS) presents numerous advantages like increased bioavailability, reduced hepatotoxicity, improved systemic toxicity profile (Trevaskis et al., 2008) and significant drug concentration in lymphatic system helps in prevention of metastasis (Arya et al., 2006; Cense et al., 2006; Trevaskis et al., 2008). Co-administration of drug with lipid is the most studied and successful approach to target drug to ILS. Long chain lipid (LCL) based formulation unlike short and medium chain lipids generally enhance drug bioavailability *via* ILS (Caliph et al., 2000; Noguchi et al., 1985; O'Driscoll, 2002; Porter et al., 2007). LCL based nanoformulations on oral administration are dissolved and assimilated into non polar core of enterocytes generated chylomicrons and promotes drug uptake into ILS (Porter and Charman, 2001). This mechanism is called transcellular uptake which constitutes a major pathway for lipid uptake in ILS. Other minor pathways like Gut asso-

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ciated lymphoid tissue (GALT) (Muranishi et al., 1997; Yoshikawa et al., 1981) and paracellular mechanism further contribute augmentation of lipid pool in ILS.

Few attempts have been reported to enhance lymphatic uptake of Tmx by nanoformulation *viz.* solid lipid nanoparticles (SLNs) (ALHaj et al., 2008; Reddy et al., 2006), polymeric (Jain et al., 2011), alginate–chitosan (Coppi and Iannuccelli, 2009), and cyclodextrin (Memisoglu-Bilensoya et al., 2005) for oral delivery. However, the SLNs remarkably displayed low drug loading and drug expulsion during storage whereas polymeric nanoparticles were claimed to target Peyer's patches which constitute 1–2% of intestinal surface and thus confer a minor pathway for lymphatic uptake. Further, lipids are non-toxic and cost effective than polymers (Humberstone and Charman, 1997). NLC bears improvised properties like higher drug loading, modulated release profile and storage stability compared to other nanodelivery systems (Muller et al., 2002a). These merits are attributed to their less ordered inner structures formed by the blend of solid lipids and oils (Mehnert and Mader, 2001).

Considering these therapeutic benefits and the fact that the drug has physicochemical characteristics suitable for lymphatic transport, it was decided to formulate LCL based Tmx-NLC using solvent diffusion technique. A detailed preformulation study was undertaken in order to select appropriate excipients and to stabilize the drug molecule. Developed NLC was physicochemically characterized and evaluated for *in vitro* release performance and stability.

2. Materials and methods

2.1. Materials

Tmx was kindly provided as a gift sample from Khandelwal Pharmaceuticals Pvt. Ltd., Mumbai, India. Glycerylmonosterate (GMS), Compritol 888 ATO, stearic acid, Ovucire WL 2944, isopropyl myristate, ethyl oleate, Precirol ATO 5, olive oil, Gelucire 50/30, Gelucire 44/14, Gelucire 39/01 were obtained as a gift from (Gattefosse, St-Priest, Germany). Dynasan 114, Dynasan 116, Dynasan 118, Softisan 154, Softisan 142, Softisan 100, Imwitor 380 (Sasol GmbH, Hamburg, Germany) and Capmul MCM (Abitec Corporation, Janesville, USA), were obtained as a gift samples. Oleic acid, Corn oil, Arachis oil, sunflower oil, cottonseed oil, soybean oil, Tween 20, Tween 80, α-tocopheryl polyethylene glycol 1000 succinate (TPGS), Transcutol, Oleique CC, and Lauroglycol – FCC, HPLC grade acetonitrile, tetrahydrofuran (THF) were procured from (S.D. Fine Chemicals, Mumbai, India). Labrafil WL 1349, Labrafil WL 2609 BS, Labrafil M 1944 CS, Labrafil M2125 CS, Labrasol, Maisine 35-1 (Gattefosse, St-Priest, France) Cremophor RH 40, Cremophor EL, Poloxamer 188, Poloxamer 407, Solutol HS 15 (BASF India Ltd., Mumbai, India) were obtained as gift samples. Freshly prepared double distilled water and buffers were filtered through 0.22 μm membrane filter (Pall India Pvt. Ltd., Ahmedabad, India) and used whenever required.

2.2. HPLC quantification of Tmx

A reversed phase HPLC method was developed for analysis of Tmx. The parameters were Jasco UV 2075 Intelligent UV/VIS detector (Jasco, Japan), Rheodyne 7725 injector (Rheodyne, USA), Jasco Borwin Chromatography Software (Version 1.50) integrator software, Supelcosil^{TM} LC-8, 5 μm HPLC column (4.6 mm × 250 mm) (Supelco Analytical, Bellefonte, USA), mobile phase: acetonitrile: potassium dihydrogen orthophosphate, pH 2.0 (84:16) at flow rate 1.5 ml min^{-1}, detection at 275 nm with retention time 8.00 ± 0.36 min The method was validated according to the International Conference on Harmonization (ICH) guidelines, Q2(R1) (2005). Assay was linear (r^2 = 0.9997) in the concentration range of 1–30 μg/ml. For identification of potential degradation products, an array of forced degradation tests were performed on bulk drug in accordance with the guidelines presented by ICH Q3B(R2) (2006).

2.3. Preformulation studies

2.3.1. Solubility studies

The solubility of Tmx was determined in LCSL and LCO by test tube method. Lipids and oils (1 g) were transferred to a test tube maintained at temperature 5 °C above the melting point of lipids. The drug was added in increments of 1 mg until Tmx was completely dissolved and amount of lipid required to solubilize Tmx was determined. Drug solubility in various 1% surfactants solutions was determined similarly at 25 ± 2 °C. Experiments were conducted in triplicates (Patel et al., 2012).

2.3.2. Drug–excipients chemical compatibility

Accurately weighed amounts of Tmx (5 mg) and each of selected excipients (300 mg) were placed in 5 ml amber coloured glass vials and mixed thoroughly. Closed vials containing blends were stored in stability chambers at 30 °C/65% RH, 40 °C and 60 °C/75% RH for 14 days (Borhade et al., 2012). A pure Tmx sample alone was also kept under similar conditions. Duplicate samples of drug–excipients blends were analysed after 14 days by validated HPLC method.

2.3.3. Tmx-NLC development

Tmx-NLC was prepared by solvent diffusion technique using GMS as the solid lipid and Labrafil WL 2609 BS as oil. These were dissolved in THF along with Tmx at room temperature. Organic phase was then added slowly to aqueous phase containing stabilizers under stirring. The resulting o/w emulsion was probe sonicated on ice for 10 min (on and off cycle of 3 s at 60% amplitude) (Sonics VC-750-220 Ultrasonic processor, Vibra cell^{TM}) (Newtown, CT, USA). The resulting microemulsion was kept for constant stirring to diffuse and finally evaporate the organic solvent to effect nanoprecipitation of Tmx-NLC.

2.4. Optimization of process variables

2.4.1. Screening of surfactants: assessment of dispersion properties

Tmx-NLC was formulated using selected surfactants *viz.* Tween 20, Tween 80, Cremophor EL and Cremophor RH 40 at different concentrations by aforementioned procedure. The best surfactant was identified based on the optimum particle size and polydispersity index (PI).

2.4.1.1. Screening for optimum concentration of surfactant and co-surfactant. The best suitable surfactant identified as above was then screened for the optimum concentration ratio with co-surfactant for the preparation of Tmx-NLC. Poloxamer 188 at various concentrations was screened as co-surfactant. The optimum concentrations of surfactant and co-surfactant were determined on the basis of particle size, PI and entrapment efficiency.

2.5. Tmx-NLC evaluation

2.5.1. Dynamic light scattering (DLS) and zeta potential measurements

The average particle size and distribution (PI) of Tmx-NLC were measured in triplicates at 25 °C by DLS using Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Zeta potential was estimated on the basis of electrophoretic mobility under an electric field, as an average of 30 measurements using Zeta Sizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK).