Role of Solid Lipid Nanoparticles in Augmentation of Carcinoma Therapy: An *in-vitro* Study

Original Research Article

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(Received: July, 2017) (Accepted: July, 2017)

ABSTRACT

Tamoxifen (TMX) is universally used drug for treatment of breast cancer. However, it has shown risk for development of colorectal malignancies. Hence, the present study focused on study of the effect of TMX and Solid lipid nanoparticles (SLN) loaded with TMX on Human colorectal cancer cell line (HT 29).

SLN were prepared using cholesterol and span 80 as drug delivery system for TMX using solvent injection method. They were characterized for their particle size, entrapment efficiency, shape, drug release studies and stability followed by *in vitro* cytotoxicity studies using HT 29 cancer cell lines. The SLN-TMX were round in shape and highly stable at 4˚C. Herein, the SLN showed sustained release of TMX under physiological and endosomal milieu. A significant inhibition in growth of HT 29 was noticed when treated with TMX and SLN-TMX with IC_{50} of 87.587.5µg and 52.5µg respectively under *in vitro* conditions. SLN-TMX is observed as highly stable and promising delivery system for sustained release of TMX to cancer cells HT 29 and may be suitable for use in clinical settings.

KEY WORDS: cancer cells, colorectal cancer, solid lipid nanoparticles (SLN), tamoxifen (TMX)

INTRODUCTION:

Colorectal cancer is the third most frequently diagnosed cancer and is one of the leading causes of cancer death. In recent years, cancer incidence rate has declined due to periodic screening, radiotherapy, chemotherapy, surgery and use of targeted anticancer drug delivery. Tamoxifen, a selective estrogen receptor modulator (SERM), is widely used regime in hormonal therapy of ER-positive cancer cases and certain skin specific disorders[1]. Although Tamoxifen is highly effective but its administration may cause short term and long term side effects including endometrial and uterine cancers[2].

SLN offer great possibility for site-specific cancer drug delivery via drug targeting. These nano-carriers retain drug efficiently and deliver it directly to tumor cells thus lowering the side effects caused by drugs[3]. Nano-carriers retain drug efficiently and deliver it directly to tumor cells thus lowering the side effects caused by drugs[3]. The ability of the engineered nanoparticles to interact with cells and tissues at a molecular level provides them with a distinct advantage over other polymeric or macromolecular substances[4]. Thus, the present study aimed to develop a vesicular delivery system for tamoxifen citrate to delivered drug at a lower concentration over a prolonged period of time and thereby reducing the potential dose related side effects.

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MATERIALS & METHODS:

Tamoxifen citrate (TMX) and Span 80 were procured from Sigma Aldrich Chemical Co. (St Louis, MO, USA), cholesterol and all the other reagent grade chemicals were procured from Himedia, India.

Preparation of solid lipid nanoparticle (SLN)

Solid lipid nanoparticles of testosterone were prepared by solvent injection method given by Goymann and Schubert[5], with slight modification. Glyceryl monostearate was melted and then TMX,

propylene glycol and chloroform were mixed and dissolved with slight heating. Separately, Brij 35 solution in an aqueous phase was prepared at the same temperature. Then, organic phase was quickly injected into aqueous phase at the same temperature with constant stirring using magnetic stirrer. Thereafter, chloroform was evaporated with continuous stirring. SLN were formed and subjected to centrifugation at 9000 rpm for 50 min. This semisolid dispersion of SLN-TMX was kept in nitrogen flushed vials for further use.

Particle size and percentage entrapment efficiency measurement: The size and size distribution of SLN was measured by dynamic light scattering technique (DLS) using a computerized Malvern instrument (Zetasizer 500962, DTS Ver. 4.10, U.K.). Appropriately diluted SLN suspension was filled in the zeta sizer cell and size was measured.

The amount of unentrapped drug in the external aqueous solution was recovered after centrifugation at 150,000*g* for 90 min, (HEMLA laboratory, Germany) at 25^0C assayed in triplicate at 530nm by UV spectroscopy (Shimadzu UV1601, Kyoto, Japan UV1601, Kyoto, Japan UV1601, Kyoto, Japan). The entrapment efficiency was expressed as the percentage of TMX entrapped with respect to the theoretical value by (Te – Tun / Ti) x 100% (where Te is the total drug added in the formulation, and Tun is the unentrapped drug measured in the supernatant)[6]. The study was carried out in a set of six replica.

Transmission electron microscopy: The SLN shape was viewed using Transmission Electron Microscope (TEM) (Morgagni 268, FEI Electron Microscope, Netherland). Firstly, copper grid was kept with the suspension of TMX-SLN for one min, followed by negative staining with aqueous solution of phosphotungstic acid for 30 s. After drying the grid on Whatman filter paper. It was observed under microscope at 4.4KX magnification.

***In vitro* drug release studies at physiological and endosomal milieu:** The *in vitro* drug release from the formulations was studied using dialysis tube (MW cut-off point 3,500 Da) method[7]. The SLN formulation was centrifuged at 2,000 rpm, using a Sephadex G-50 mini-column to remove unentrapped TMX from SLN. Formulation (1 ml) free from any unentrapped drug was taken into a dialysis tube, suspended in a beaker containing 20 ml of PBS (pH 7.4 at 37 ± 0.5 °C or pH 4 at 37 ± 1 °C) and was continuously stirred through a magnetic stirrer. Samples were withdrawn (0.5 ml) periodically at time interval of 15 min, 30 min, 1 h, 2h, 6 h, 8 h, 12 h, 24 h and 48 h, thereafter replenished with the same volume of fresh PBS (pH 7.4 or 4) and amount of the drug was quantified (n = 3) using UV spectrophotometer at a wavelength of 274 nm. The study was carried out in a set of six replica.

Storage stability studies: Stability studies were conducted according to the ICH guidelines by storing formulations at 4±3°C and 25±2°C for 6 mo in nitrogen flushed sealed ampoules. The samples were withdrawn at 0, 30, 60, 90, 120, 150 and 180 days in 20% (vol/vol) methanol. The study was carried out in a set of six replica. The drug content was determined in the withdrawn samples using UV spectrophotometer (Picodrop spectrophotometer).

***In vitro* studies:** Human colorectal cancer cell line (HT 29), procured from National Centre of Cell Science, Pune (India), were cultured in complete growth medium DMEM containing 5% (v/v) fetal bovine serum and 100 μl/ml Streptomycin. The cell line was maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Subculture was developed with cells from sub-confluent cultures treated with TPEG (0.25% Trypsin, 0.02 g EDTA and 0.05 g glucose in PBS).

Cell line growth and sub culturing: Colorectal cancer cell line HT 29, ($5 X 10^3$) were seeded evenly into 96-well flat-bottomed tissue culture plate supplemented with 10% heat inactivated FBS (Sigma, USA) and 1% Penicillin-Streptomycin (Sigma, USA) at 37.5°C with in a humidified incubator in an atmosphere of 95% air and 5% CO_2. These cells were sub-cultured every 48 h and harvested from subconfluent cultures (60-70%) using 0.05% trypsin-EDTA.

Cell viability study: Trypan blue assay was performed under aseptic conditions, equal volume of trypan blue dye and cell suspension (10^7 cells/ml) were mixed and incubated for 1 min in CO_2 incubator[8]. The study was carried out in a set of six replica. Approximately, 10 μl of this cell-dye suspension was loaded in Neubauer chamber and observed under low (100X) and high (400X) power of an inverted microscope. Number of viable (bright cells) and non-viable cells (stained blue) were counted. Concentration and percentage of viable cells was calculated as follows:

(a) Viable Cell Count:

$$= \frac{\text{Number of live cells counted}}{\text{Number of large corner squares counted}} \text{ X dilution factor X } 10^4$$

(b) Non-viable cell count:

$$= \frac{\text{Number of dead cells counted}}{\text{Number of large large corner squares counted}} \text{ X dilution factor X } 10^4$$

Determination of Inhibitory Concentration (Ic50): The viability of HT 29 cancer cell lines in presence of TMX and TMX-loaded SLN was assessed by SRB assay. The HT 29 cell density 105cells/ml was seeded into a 96-well plate and incubated for 24 hours under pre stated conditions. The cells were treated with TMX and TMX-loaded SLN at concentrations ranging from 20 to 120 µg for 24, 48 and 72 hour. The control wells received PBS as the vehicle. Experiment was carried out in set of six wells per treatment condition. The quantification assessment for cell viability and IC50 versus free TMX and TMX-loaded SLN concentrations was exercised.

RESULTS:

Treatment of cancers using drugs is one of the most widely used therapeutic procedures. However, lesser bioavailability of the drug has been a clinical challenge. In addition, the prolonged use of these drugs pose serious and long lasting side effects on health and immune system of patient. Drugs with high specificity are required to be developed that selectively kills the cancerous cell. TMX, being one of the most accepted and widely used drug for treatment of cancer especially breast cancer alongwith its effectiveness in treating metastatic cancer, where it blocks estrogen's effect in effected tissues[2].

SLN containing TMX and without TMX were of particle size of 183 ± 4 nm and 140 ± 3 nm, respectively with TMX entrapment efficiency of 57 ± 3.3 %. The morphological details of SLN containing TMX studied using TEM shown round shape of particles (Figure 1).

Percentage release of TMX at physiological milieu was fast in case of pure TMX and almost complete drug release occurred in 12 h, while the release of TMX from SLN had prolonged to 48 h (Figure 2). The amount of drug entrapped in SLN vs TMX concentration is described in Table 1.

Storage of formulation at 25±2°C have shown very less loss in stability of SLN-TMX (Figure 3) and no loss in stability occurred with SLN-TMX on storage at 4±3°C upto 180 d. Our study revealed that

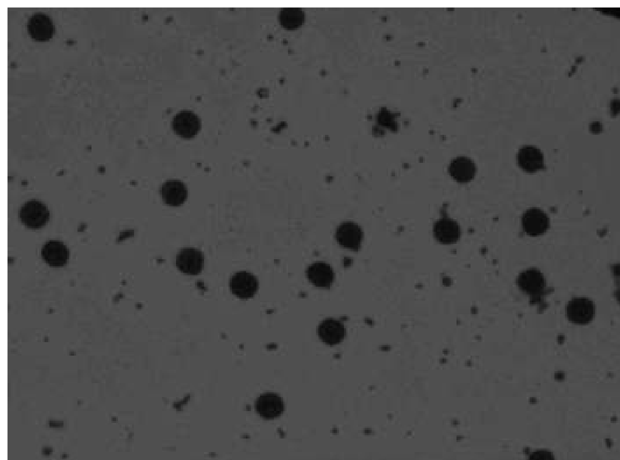

Figure 1: TEM photograph of TMX entrapped SLN at 400X magnification.

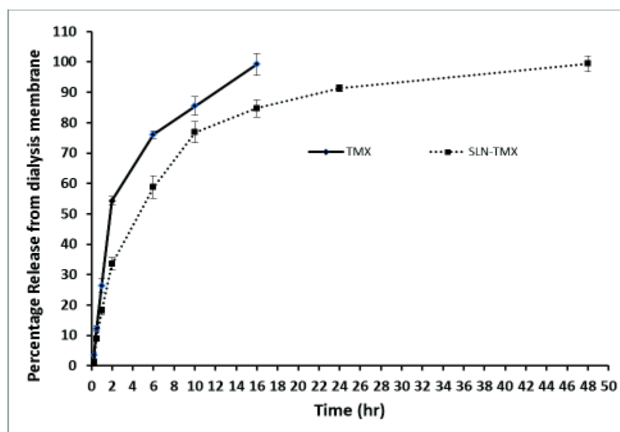

Figure 2: Release of TMX from dialysis membrane at physological milieu under in vitro conditions.

the SLN prepared from higher percentage of cholesterol were highly stable[9]. Most of the formulations containing cholesterol as additive have been reported to be highly stable since it stabilizes twin layers thereby preventing loss of drugs[10]. It also hinders gel to lipid phase transition that further improves its effectively[11,12].

Table 1: Proportion of TMX entrapped in SLN at varying concentrations.

Concentration (µM)	TMX	Blank SLN	TMX entrapped SLN
20	81.22	99.24	69.56
40	77.33	99.24	57.23
60	62.35	99.24	47.11
80	53.02	99.24	34.53
100	44.71	99.24	22.92
120	42.31	99.24	21.15

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DISCUSSION:

The delivery of drugs at a controlled rate, targeting the affected area has gained much attention in past few decades. Solid Lipid Nanoparticles are bilayer delivery system formed by self-association[13]. The prepared SLN-TMX formulation was found to be highly stable at 4˚C making it suitable, stable drug delivery system for clinical usage. These nano-drug delivery systems have been reported to possess long shelf life with greater stability enabling targeted delivery of drug in a controlled manner[14]. SLN carriers, well known for their potential in topical drug delivery, have been chosen to help transport tamoxifen molecules in the skin layers[15]. Among ongoing scientific researches, new carriers of anticancer drugs developed till date include: liposomes, polymeric microspheres, SLN etc[16,17].

The effect of TMX and SLN-TMX on cell viability of HT 29, quantified using Trypan blue assay under in vitro conditions is mentioned in Figure 4. Inhibitory Concentration (IC50) for the pure TMX was found to be 87.5μg and 52.5μg with the SLN-TMX. The effectivity of SLN-TMX over TMX was further confirmed by the SRB assay. The IC50 value of

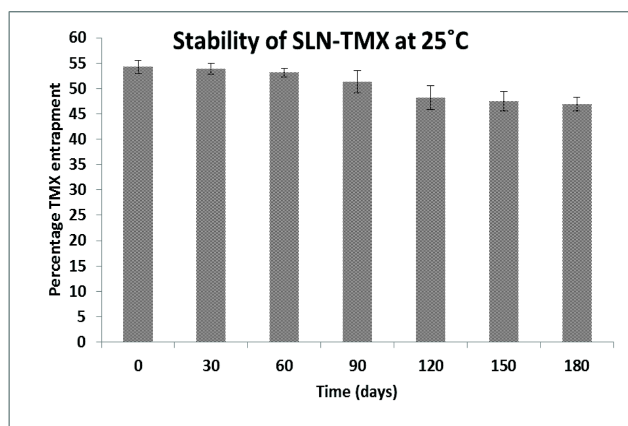

Figure 3: Stability of SLN-TMX on storage at 25˚C.

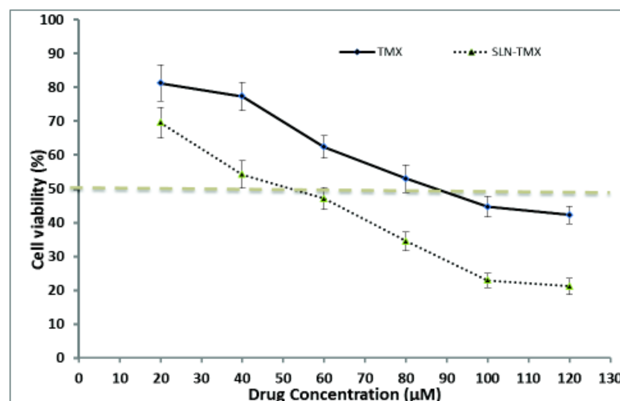

Figure 4: Cell viability studies of SLN-TMX and TMX.

TMX and SLN-TMX were observed as 100 μg. and 65.3 μg respectively. Shaker et al[18] prepared nanoparticles of Tamoxifen citrate, for localized cancer therapy by film hydration technique and reported that cellular uptake of optimized SLN formulation was 2.8-fold of tamoxifen and exerted significantly greater cytotoxic activity on breast cancer cell line (MCF-7).

CONCLUSION:

The present study revealed both TMX and SLN-TMX inhibited growth of human colorectal cancer cell lines HT 29 under in vitro conditions. The SLN offered greater advantage over other drug delivery systems due to their high biocompatibility along with ease in preparation. The SLN- TMX is highly stable formulation for sustained release of TMX to cancer cells. This delivery system may also be conscientious for targeting other adenocarcinomas offering wide dimensions to its applicable potential for clinical applications.

REFERENCES:

1. Petinari L, Kohn LK, De Carvalho JE, Genari SC. Cytotoxicity of tamoxifen in normal and tumoral cell lines and its ability to induce cellular transformation in vitro. Cell Biol Int 2004; 28: 531–539.
2. Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta analysis of Vascular and Neoplastic Events Associated with Tamoxifen. J Gen Intern Med 2003; 18(11):937-947.
3. Seleci DA, Seleci M, Walter JG, Stahl F, Scheper T. Niosomes as Nanoparticular Drug Carriers: Fundamentals and Recent Applications. J Nanomater . 2016; 1-13.
4. Bhaskar S, Tian F, Stoeger T, Kreyling W, Fuente JM, Grazu V et al. Multifunctional Nanocarriers for

diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. Part Fiber Toxicol 2010; 7:3.
5. Goymann M, Schubert M A. Solvent injection as a new approach for manufacturing lipid nanoparticles- evaluation of the method and process parameters. Eur J Pharm Biopharm 2003; 55: 125-131.
6. Jain A, Jain A, Gulbake A, Shilpi S, Hurkat P, Jain SK. Peptide and protein delivery using new drug delivery systems. Crit Rev Ther Drug Carrier Syst 2013; 30(4): 293-329.
7. Gupta Y, Jain A, Jain SK. Transferrin-conjugated solidpid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. J Pharm Pharmacol 2007; 59(7): 935-940.
8. Strober W. Trypan blue exclusion test of cell viability. Curr Protoc Immunol 2015; 111 A3-B:1-3.
9. Padhye SG, Nagarsenker MS. Simvastatin Solid lipid nanoparticles for oral delivery: formulation development and in vivo evaluation. Indian J Pharm Sci 2013; 75(5):591-598.
10. Rogerson A, Cummings J, Florence AT. Adriamycin-loaded niosomes-drug entrapment, stability and release. J Microencap. 1987; 4: 321–328.
11. Emami J, Mohiti H, Hamishehkar H, Varshosaz J. Formulation and optimization of solid lipid nanoparticle formulation for pulmonary delivery of budesonide using Taguchi and Box-Behnken design. Res Pharm Sci 2015; 10(1): 17-33.
12. Cable C. An examination of the effects of surface modifications on the physicochemical and biological properties of non-ionic surfactant vesicles. PhD Thesis; 1989, University of Strathclyde, Glasgow, UK.
13. Muller RH, Mader K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm 2000; 50(1): 161-177.
14. Mahale NB, Thakkar PD, Mali RG, Walunj DR, Chaudhari SR. Niosomes: novel sustained release nonionic stable vesicular systems—an overview. Adv Colloid Interfac 2012; 183: 46–54.
15. Bhatia A, Kumar R, Katare OP. Tamoxifen in topical liposomes: development, characterization and in-vitro evaluation. J Pharm Pharm Sci 2004; 7: 252-259.
16. Fontana G, Maniscalco L, Schillaci D, Cavallaro G, Giammona G. Solid lipid nanoparticles containing tamoxifen characterization and in vitro antitumoral activity. Drug Deliv 2005; 12(6): 385-392.
17. Reddy LH, Vivek K, Bakshi N, Murthy RS. Tamoxifen citrate loaded solid lipid nanoparticles (SLN): preparation, characterization, in vitro drug release, and pharmacokinetic evaluation. Pharm Dev Technol 2006; 11(2):167-177.
18. Shaker DS, Shaker MA, Hanafy M S. Cellular uptake, cytotoxicity and in-vivo evaluation of Tamoxifen citrate loaded niosomes. Int J Pharmaceut 2015; 493(1): 285–294.

Cite this article as: Jain R, Thakur KK, Gumashta R, Jain A:Role of Solid Lipid Nanoparticles in Augmentation of Carcinoma Therapy: An in-vitro Study. PJSR ;2017:10(2):
Source of Support : Nil, **Conflict of Interest**: None declared.