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Preparation and Evaluation of Co-Delivery of Tamoxifen and Curcumin Solid Lipid Nanoparticles (Slns) on Breast Cancer Cells MCF-7

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Abstract

Breast cancer is caused by the growth of a malignant tumor mass. Luminal subtypes mainly contain estrogen and progesterone receptors. Three classes of drugs can be noted for the treatment of breast cancer, one of which is the selective estrogen receptor modulator (SERMs) such as TMX. The oral bioavailability of TMX is increased in the presence of CUR. CUR can also influence the expression of oncogenic proteins involved in the breast cancer progression. SLNs have been developed as leading nanocarriers in the treatment of cancer. SLNs containing TMX and CUR were prepared by microemulsion techniques. The average size of nanoparticles was 19.46 nm. Around 99.97% of CUR and 98.7% of TMX had loaded on nanoparticles, about 78% of TMX and 80% of CUR were released. An in vitro study of antiproliferative activity, carried out on MCF-7 cell line, demonstrated that TMX-CUR SLNs showed an antitumoral activity. The results of characterization assessments and in vitro antiproliferative activity intensely support the potential application of TMX-CUR SLNs as a carrier system at extended-release useful for breast cancer treatment. TMX-CUR SLNs had a suitable size and high loading capacity and are controlled release. They have a higher efficacy than free drugs in vitro situations.

Keywords: Breast neoplasm; Curcumin; Solid Lipid Nanoparticles; Tamoxifen, MCF-7.

Abbreviations: SLN: Solid Lipid Nanoparticles; TMX: Tamoxifen, CUR: Curcumin; TMX SLN: Tamoxifen loaded Solid Lipid Nanoparticles; CUR SLN: Curcumin loaded Solid Lipid Nanoparticles; MX-CUR SLN: Tamoxifen and Curcumin combination loaded Solid Lipid Nanoparticles; DLS: Dynamic Light Scattering, PdI: polydispersity index; Zp: zeta potential; Transmission Electron Microscopy (TEM); P-XRD: powder X-ray diffraction, EE: Entrapment efficiency, DL: Drug Loading, DMDM: Dulbecco's Modified Eagle's medium, MTT: Methylthiaozol Tetrazolium, MCF-7: Michigan Cancer Foundation-7, IC50: inhibitory concentration.

Introduction

Breast cancer is highly prevalent globally and is the fifth most common cancer type. It is commonly detected in women and ranks second as a cause of death amongst females. In women, breast cancer is the second principal reason of death after lung cancer. Breast cancer is more common in women between the ages of 20 and 59[1] In the USA, 12.5 percent of women are at risk to develop breast cancer [2] Meanwhile, In Asia, there is a growth in the occurrence of breast cancer due to lifestyle alterations. Mortality rates are also increasing due to the absence of screening plans and therapeutic services for breast cancer in developing countries [3]. TMX is the first selective estrogen receptor modulator (SERM) [4] Oral TMX is considered as the standard adjuvant endocrine therapy for postmenopausal female with hormone receptor-positive localized breast cancer for 5 years. It decreases the risk of developing the progressive types of breast cancer in women who have had ductal carcinoma in situ. TMX binds to the estrogen receptors. It performs as an anti-estrogen agent and has lower toxicity than other chemotherapeutics [5]. CUR is a popular natural drug, traditionally used for the treatment of a wide range of diseases derived from Curcuma longa [6,7]. Various laboratories and clinical investigations have shown the role of CUR in the prevention and treatment of several types of cancer [8] In studies various mechanisms of CUR as an antitumor agent such as apoptosis [9] and cell cycle arrest [10] have been approved.

Hence, co-administration of TMX with CUR can lead to improved/superior treatment programs. The combination of active ingredients that sensitize cancer cells to chemotherapeutics has been well known as an efficient method of improving the efficiency of the drugs [11] Natural compounds from medicinal plants are mainly safe and low toxicity [12]. For the last several decades, the pharmaceutical industry has had various problems in developing poorly soluble active ingredients into dosage forms. Numerous approaches including solid dispersion in carriers, particle size reduction, salt formation and prodrug, polymorphism, complexation, and solubilization have been used to increase the solubility of poorly water-soluble active ingredients [13] TMX and CUR are cases of such molecules that are highly permeable but poorly soluble. Among these methods, for making better solubility, preparation solid dispersions over hot melt mixing, solvent methods, and melt extrusion has achieved acceptance. SLNs were first introduced by Muller. They are a colloidal system, composed of biological lipid and emulsifiers that are solid at room condition [14]. SLNs are a substitute vehicle system for liposomes and polymer nanoparticles. Another benefit of SLNs is that they improve biodegradability of drugs. They consist of biocompatible and physiological lipids, which are suitable for the incorporation of hydrophilic, lipophilic, and poorly water-soluble active ingredients. Their ability to decrease the particle size offers promising prospects in making them vehicles for effective, sustained release, and extended stability of the drug [15]. The main aim in this study was to improve the dissolution and bioavailability of TMX when used in combination with CUR in the treatment of breast cancer on the MCF-7 cell lines.

Materials and Methods

Materials

TMX and Sodium bicarbonate were purchased from Sigma Aldrich (Canada). CUR and Polysorbate 80 (tween 80) were bought from Merck (Germany). Cetyl palmitate was obtained from Oleon (Italy). DMEM high glucose medium, Gentamycin, and FBS were provided from Gibco (Maryland, United States). MCF-7 cells were attained from the Pasteur Institute (Tehran, Iran).

Fabrication of TMX-CUR SLNs

TMX-CUR SLNs were prepared using the microemulsion cooling method. In brief, to prepare TMX-CUR SLNs formulation, lipid phase (cetyl palmitate) was heated to 65°C, then tween 80 as a surfactant was added and after the mixing was completed, TMX powder was added to lipid phase and stirring continued to dissolve TMX entirely. Afterwards, the aqueous phase (containing CUR, tween 80, and distilled water) was heated up to 65°C and was added to the lipid phase and was mixed at 1400 rpm. Following fabrication of Nano emulsion, the mixture was sonicated using a probe ultrasonic (Hielscher up 400s, Germany) with 0.6 cycle and 200-watt power for 3 minutes. Ultimately, the prepared nano emulsion was diluted gradually by deionized water on the magnetic stirrer at 4-8°C in the ratio of 1:1 to fabricate the TMX-CUR SLNs.

Characterization of TMX-CUR SLNs

Dynamic Light Scattering (DLS) assessments

The TMX-CUR SLNs were well-diluted in distilled water with the ratio of 1:10 and analyzed for average Zp, particle size, and polydispersity index (PdI) using zetasizer (Nano flex, Particle meter, UK). For analyzing Zp, at first optical refractive index was measured by Abbe refractometer (AR2008, A.Kruss optronic, Germany). All the results resemble the average ± SD of three different tests at 25±2°C.

Transmission Electron Microscopy (TEM)

TMX-CUR SLNs was specified in terms of their size, structure, and surface morphology by TEM, utilizing an electron microscope (Zeiss EM10C, Germany). Nanoparticles were coated by a thin film of uranyl acetate 2% and then electron photography was performed by the electron microscope.

Powder X-Ray Diffraction (P-XRD)

P-XRD measurment was performed to prove the formation of a new solid-state. P-XRD analysis was conducted for SLNs without the drug (placebo) and TMX-CUR SLNs, using an X-ray diffractometer X'Pert PRO MPD (PANalytical, Holland). Cu Kα radiation in the scanning range of 2θ = 5–80° was used in room temperature and pressure.

Entrapment efficiency (EE)

EE was analyzed as the ratio of the trapped drug in the carrier system over the total drug used in the preparation of SLN. LC was explicated as a ratio of drug-loaded in carriers to the lipid of carriers. To calculate EE with a spectrophotometer, the first calibration curve of different concentrations of each drug in solvent (deionized water and tween 80) was carried out. Then, 10 ml of TMX-CUR SLNs suspension was precisely weighed. The sample was centrifuged at 4000 rpm for 10 min by centrifuge (TDZ5-WS, Selecta Lab). The absorbance of the supernatant was measured using the Fluorescence spectrophotometer instrument (cary eclipse, Agilent) and UV-VIS spectrophotometer instrument (Cary 60, Agilent) at 360 nm and 361 nm for TMX and CUR, respectively. Finally, the mass of each active ingredient was analyzed with the standard calibration curve. The EE and drug loading of the fabrication were measured by the following equations for each active ingredient:

where "M initial drug" is the mass of the initial drug, "M free drug" is the mass of the free drug present in the supernatant. "WDL" is the weight of drug-loaded in nanoparticles and "WNP" is the weight of nanoparticles (lipids).

Drug Release Study through the dialysis membrane

The in vitro drug release studies were carried out using the dialysis bag technique. 5 ml of TMX-CUR SLNs suspension was put into the dialysis bag (10 KD cut-off, Merck) which was then

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placed inside a screw cap glass beaker filled with release media (deionized water, tween 80 at the ratio of 33:1) with pH =7, agitated at 100 rpm and 37 °C in a shaking water bath. 3 ml of samples were withdrawn and analyzed spectrophotometrically. The percentages of the drug release from nanoparticles were plotted against time intervals 0, 0.5, 1, 2, 4, 8, 24, and 48 h for each drug separately. The following formula was used to measure the drug release percentage:

Assessment of TMX-CUR SLN cytotoxicity on MCF-7 cell line

Breast cancer cell line MCF-7 was obtained from the National Center for Genetic and Biological Reserves in Iran and cultured in DMEM medium supplemented with 10% FBS. The cells were maintained in an incubator in 37 °C, 5% CO2 concentration and 95% relative humidity (Memmert GmbH, Germany). The medium was altered every 2-3 days. The cell lines were subcultured when they were around 80–90% confluent with 0.25% trypsin. The cells were classified into eight groups as follows:

- Free TMX: treated with 40 µmol/mL
- Free CUR: treated with 40 µmol/mL
- Free TMX-CUR: treated with 40-40 µmol/mL
- TMX SLN: treated with 40 µmol/mL
- CUR SLN: treated with 40 µmol/mL
- TMX-CUR SLN: treated with 40-40 µmol/mL
- SLN: treated with unloaded SLN
- Control: without any treatment

MTT assay was used to compare the Cytotoxic effect of study groups on cell viability. In brief, MCF-7 cells (1 × 104 cells/well) were cultured in 96-well plates. After treatment and further incubation for 72 hours, the MTT solution at a concentration of 0.5 mg/mL was added to each well and kept at 37 °C for 3 h. After removing the supernatants, 150 µL of DMSO was added to each well. Using a microplate reader (Epoch) absorbance at 570 nm was determined. To measure the toxic effect of groups on the MCF-7 cells, IC50 values were analyzed by MTT assay. The IC50 value of free TMX was calculated using GraphPad Prism (version 5, USA) software with a dose-response curve after 72 hours. The viable cells percentage was calculated using the below equation:

Percentage of viable cells= (ODTest / ODcontrol) × 100

Statistical analysis

The data achieved was subjected to statistical analysis. The alterations in mean values among the groups were expressed as mean ± standard deviation. Data analysis was examined by using Paired sample t-test and equivalency and noninferiority test. The entirety of statistical calculations is taken using Statgraphics18 (version 12, USA) software. Moreover, a P-value of ≤0.05 is considered statistically significant.

Results and Discussion

Developments in drug delivery systems including nanoparticle-based vesicular drug delivery systems have improved various factors such as solubility, bioavailability, stability, release properties, and improve the therapeutic effects of already-efficient molecules. Principally, SLNs have developed as promising nanocarriers in cancer therapy. SLNs accelerate the cellular uptake of the incorporated drugs through the modulation of active, passive, and cotransport mechanisms [16]. Drugs are not specific against tumor cells; therefore, they accumulate not only in tumor cells but also in healthy tissues. New approaches that can deliver active ingredients more specifically and make less toxicity are required [17].

The use of TMX-CUR SLNs to improve the physical, pharmacokinetic characteristics and antitumor efficacy is one such approach. TMX and CUR are efficient and multitarget ingredients for the prevention and treatment of numerous cancers, such as breast cancer [18]. In this study, the microemulsion technique was chosen because of its accessibility and appropriate particle characteristics. TMX and CUR were successfully incorporated into SLNs. The average size of TMX-CUR SLNs was (found at REMOVE) 19.46±1.45 nm with a PdI of 2.70±0.04 nm. The surfaces of TMX-CUR SLNs carried a negative charge due to the presence of cetyl palmitate. The Zp was -21.9±0.07 mV. Particle size is a major characteristic property to be examined in a nanometric system to ensure the efficiency of drug delivery. Abbasalipour Kabir et al. [35] fabricated SLN of TMX and reported that the average size of preparation was around 251.6 nm [19]. Senthil Kumar et al prepared SLN of CUR with the particle size, polydispersity index, zeta potential of 214.60± 3.55nm, 0.49±0.03, -29.63±0.50 mV, respectively [20]. Our results were concordant with the findings of this paper.

The PdI measures the width of size distribution and homogeneity of droplet size, which is usually about 0.5 or less [21]. The Zp was stated as a function of surface properties of particles and can be used to forecast their long-term stability [22]. An upper value of Zp has shown the stability of the dispersion [23] Righeschi et al. [23] and Marangoni et al. showed that Zp of CUR nanoparticles were -33 mV and -29.6 mV, respectively [24,25]. Furthermore, Al Haj et al. revealed Zp of TMX nanoparticles of -15.4 mV [26].

TEM results revealed that the TMX-CUR SLN had spherical shapes with smooth round edges. The results also demonstrated their nanoscale size range [Figure 1]. The EE depends on the physicochemical properties of formulation, the reaction between drug and vehicle, and drug and membrane. TMX-CUR SLN showed EE of 97.48% and 99.41% for TMX and CUR, respectively.

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Moreover, TMX-CUR SLN showed DL of 9.77% and 9.76% for TMX and CUR, respectively. Our results established that the almost complete EE showed high efficiency of SLN in taking up the drugs. The considerably higher EE might be recognized for its lipophilic nature and is indicative of the formulation and method of fabrication.

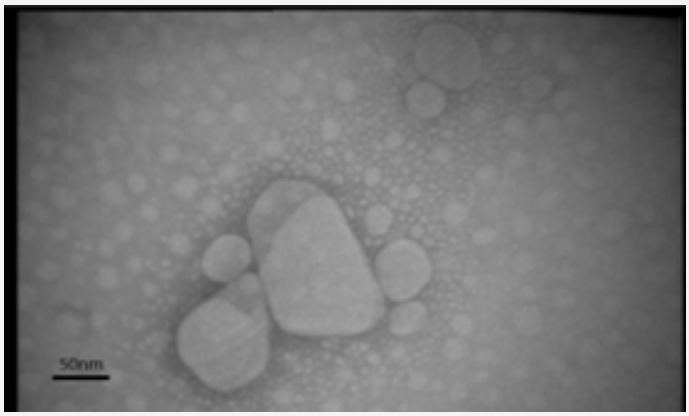

Figure 1: TEM topography of TMX-CUR SLNs.

As P-XRD results shown TMX, CUR powder, and cetyl palmitate showed a series of sharp peaks verifying its crystalline nature. However, in TMX-CUR SLN and placebo SLN, these peaks disappeared, indicating the amorphous nature of the particles [Figure 2]. Comparison of area under the P-XRD peaks of TMX-CUR SLN and placebo SLN showed the area under the peak of TMX-CUR SLN is larger than placebo SLN which demonstrates high EE of TMX-CUR SLN and changing crystalline phase.

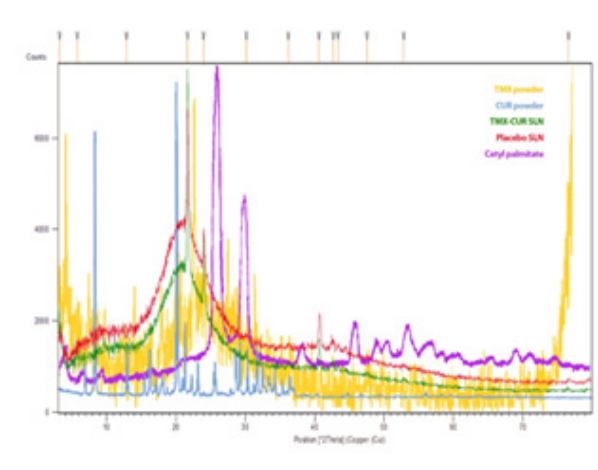

Figure 2: P-XRD curve of TMX powder, CUR powder, TMX-CUR SLN, Placebo SLN, and Cetyl palmitate.

Drug release kinetics involve the application of mathematical models to predict the drug release profile. The correlation coefficient (R2) of various kinetic models was calculated for TMX-CUR SLNs. First-order and Weibull kinetic models obtained the best model with the linearity of R2=0.936 for CUR-SLN and R2≥0.9853 for TMX SLN, respectively. First-order kinetics refers to drug release that is concentration-dependent and the Weibull model the logarithm of the released drug and logarithm of the time would have a linear relationship. The Weibull equation mostly fits all kind of dissolution curves and builds up the fraction of the drug (m) when applied the equation to release of drugs [27]. As shown in the results, after 48 hours of in vitro releasing 78% of TMX and 80% of CUR were released [Figure 3]. These two release patterns showed the slow release of TMX and CUR from preparation. Furthermore, the release of CUR was slower in the beginning compared to TMX. These results confirmed that the extended and completed release pattern of TMX-CUR SLNs fabrication was attained. SLNs were dispersed in a liquid lipid medium which was enclosed by a solid lipid shell. This structure consequently offered the extended-release profile of the drug from nanostructure. This

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controlled release pattern can decrease the adverse reaction of drugs. In another in vitro release study, fabricated TMX loaded SLNs demonstrated a similar release [28]. Cell survival and apoptosis are regularly applied to assess the efficacy of anti-cancer drugs. Anticancer active ingredients commonly kill dividing cells by stimulation of the apoptosis process [29]. In this study, we have revealed that TMX-CUR SLN is effective in decreasing the population/number of MCF-7 cells due to growth suppression and stimulating cell death. After treatment cells by 40 µm of TMX SLN, CUR SLN, Free TMX-CUR, and TMX-CUR SLN the percentage of cell viability were 53.12 ± 1.55, 39.18 ± 2.04, 52.50 ± 0.49, and 42.54 ± 8.95, respectively [Figure 4].

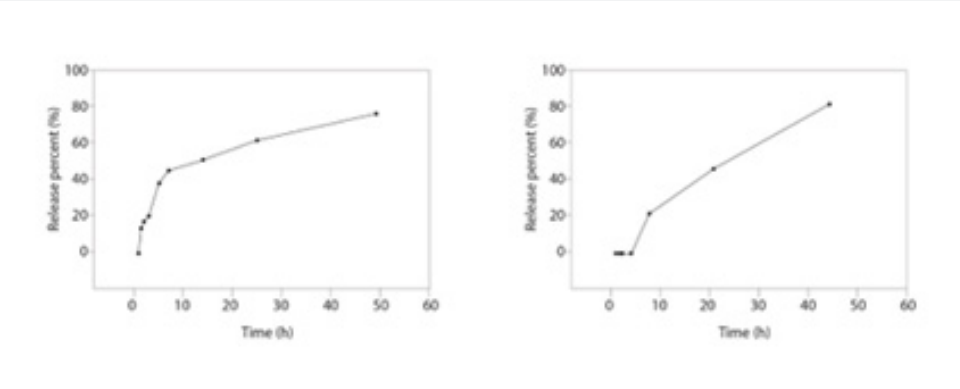

Figure 3: Percentage of drug release from A) SLN and, B) CUR-SLN.

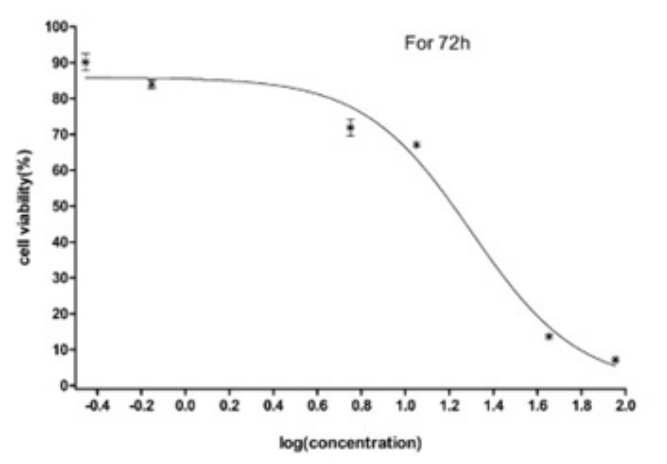

Figure 4: Response curve of free TMX.

The IC50 of TMX-CUR SLNs was normally lower than that of free TMX-CUR. In the presence of SLNs, the viability of cells reduces, and the cancer cells lose their normal morphological characteristics, detach, aggregates, and later develop apoptotic bodies [Figure 5].

The results showed that TMX-CUR SLNs have an equally efficient cytotoxic activity against MCF-7, compared to CUR SLNs. CUR-SLNs had the maximum toxicity effect on the cell line. Furthermore, the results showed that drug-loaded SLNs have more effective cytotoxic activity against MCF-7 than the free drug. Ho WY et al. [28] and Chowdhuty et al. [29] in two different studies showed that IC50 of TMX after 72 hr were 27.46 µM and after 48 hr was 30.11 µM, respectively [30,31]. The improved preventive effect of TMX-CUR SLNs on the growth of MCF-7 In comparison with Free TMX-CUR group cells may be related to the lipophilic characteristic of the carrier, which stimulates the intracellular uptake [32]. Fontana et al. [34] showed characterization assessments and antiproliferative activity intensely support the possible application of tamoxifen-loaded SLNs as a carrier system at sustaned-release useful for treatment of breast cancer. Furthermore, other studies revealed that TMX loaded SLNs have a better effect than free drug [33-36]. The major limitation of this research was hardly providing chemical materials. Lack of short time and long-term stability study is another limitation that would possibly affect the reliability of the data.

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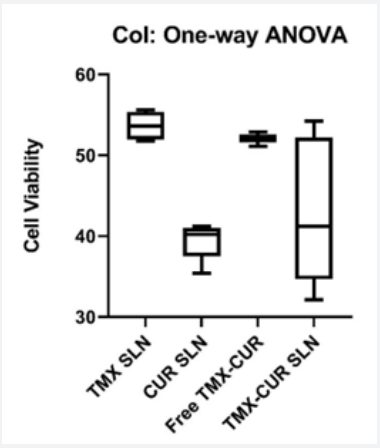

Figure 5: Percentage of cell viability after treatment by TMX SLN, CUR SLN, Free TMX-CUR, and TMX-CUR SLN.

Conclusions

In this study, we proposed a new pharmaceutical formulation of SLN for TMX and CUR in combination with appropriate characteristics results. This study was conducted to attain larger amounts of TXM and CUR loaded into SLNs with extended drug release profile. The cytotoxicity study revealed that the formulation was as toxic as free drugs on MCF-7 cell lines.

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