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Solid lipid nanoparticles: Reversal of tamoxifen resistance in breast cancer☆

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ABSTRACT

The objective of the present study was to investigate the effect of tamoxifen (Tam) loaded solid lipid nanoparticles (SLNs) on MCF7 Tam-resistant breast cancer cells (MCF7-TamR). Tam-SLNs were produced by the hot homogenization method. The characterization studies of Tam-SLNs demonstrated good physical stability with small particle size. The *in vitro* cytotoxicity results showed that Tam-SLNs enhanced the efficacy of Tam and reversed the acquired Tam resistance by inducing apoptosis, altering the expression levels of specific miRNA and the related apoptosis-associated target-genes in both MCF7 and MCF7-TamR cells without damaging the MCF10A control cells ($p < 0.05$). In conclusion, we demonstrated a molecular mechanism of the induction of apoptosis by Tam-SLNs in MCF7 and MCF7-TamR cells, and thus, we demonstrated that Tam-SLNs were more effective than Tam. The present study suggests that the use SLNs may be a potential therapeutic strategy to overcome Tam-resistance in breast cancer for clinical use.

1. Introduction

Tamoxifen (Tam) has been used to treat patients with estrogen receptor-positive (ER+) breast cancer. However, it has been reported that long-term Tam therapy leads to a higher incidence of endometrial and liver cancer, increased blood clotting, retinopathy, and corneal opacities and to the development of drug resistance. Furthermore, acquired Tam resistance is a major clinical problem for ER+ breast cancer patients due to cause of tumor recurrence and/or metastasis (Clarke et al., 2015; García-Becerra et al., 2012; Ring and Dowsett, 2004; Schafer et al., 2002).

Drug delivery systems have attracted considerable attention from cancer researchers for the optimization of drug features such as solubility, stability and safety (Dikmen et al., 2011; Güney et al., 2011; Hu and Zhang, 2012; Khan, 2010; Park, 2007; Shahin et al., 2012; Silva et al., 2014; Singh and W., 2009). In recent years, solid lipid nanoparticles (SLNs) have been extensively studied. SLNs offer attractive features, including small particle size, good stability, controlled drug release and elimination of the need for toxic organic solvents. Additionally, SLNs have been indicated to overcome multi-drug resistance (MDR) by passing drug efflux transporters while protecting incorporated drugs from the external biological environment and increasing the therapeutic efficacy of drugs (Cavaco et al., 2017; Guney and Kutlu, 2011; Guney Eskiler et al., 2015; Güney et al., 2011; Jawahar et al., 2012; Mäder and Mehnert, 2005; Müller et al., 1995; Müller et al., 2000; Nair et al., 2011; Souto and Müller, 2007; Svilenov and Tzachev, 2014; Wolfgang and Mäder, 2001).

For this purpose, Tam-loaded SLN formulations were produced to investigate whether SLNs could potentially overcome Tam resistance in the present study. First, Tam-loaded SLNs were produced by the hot homogenization technique and then characterized to evaluate physicochemical characteristics, including size, zeta potential and interactions. Additionally, we investigated the *in vitro* effects of these SLNs on cellular viability, drug retention and apoptosis of MCF7-TamR cells, a representative Tam-resistant breast cancer cell line, compared with the parent MCF7 breast cancer cells. The results of this study show that, the use of Tam-SLNs could be a potentially effective strategy for overcoming Tam resistance.

2. Material and methods

2.1. Preparation of Tam-loaded SLNs

Tam-loaded SLNs were prepared by the hot homogenization

Abbreviations: Tam, tamoxifen; SLNs, solid lipid nanoparticles; MCF7-TamR, MCF7 Tam-resistance breast cancer cell lines; ER+, estrogen receptor positive; DDS, drug delivery systems; MDR, multi-drug resistance; AO/EtBr, acridine orange/ethidium bromide

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Table 1
Particle size, PDI and zeta potential values of Tam, Tam-SLNs and free-SLNs at three different temperatures (4, 25 and 40 °C).

Formulations		Particle size (nm)		Polydispersity index (PDI)		Zeta potential (mV)		
		Average (n = 3)	SD	Average (n = 3)	SD	Average (n = 3)	RSD%	SE
Tam	4 °C	2283	1.88	0.207	0.04	3.67	0.98	0.17
	25 °C	2675	2.37	0.219	0.05	4.21	1.17	0.12
	40 °C	2756	2.10	0.198	0.03	3.98	1.06	0.23
Tam-SLNs	4 °C	277.4	1.26	0.298	0.05	−40.5	1.61	0.24
	25 °C	284.2	1.42	0.342	0.08	−38.7	1.98	0.32
	40 °C	298.6	0.55	0.367	0.01	−45.6	1.86	0.29
Free-SLNs	4 °C	268.7	1.33	0.247	0.02	−31.5	1.45	0.21
	25 °C	286.4	1.12	0.364	0.04	−35.7	1.82	0.28
	40 °C	264.7	1.76	0.388	0.07	−34.9	1.73	0.25

SD: standard deviation, RSD: relative standard deviation, SE: standard error.

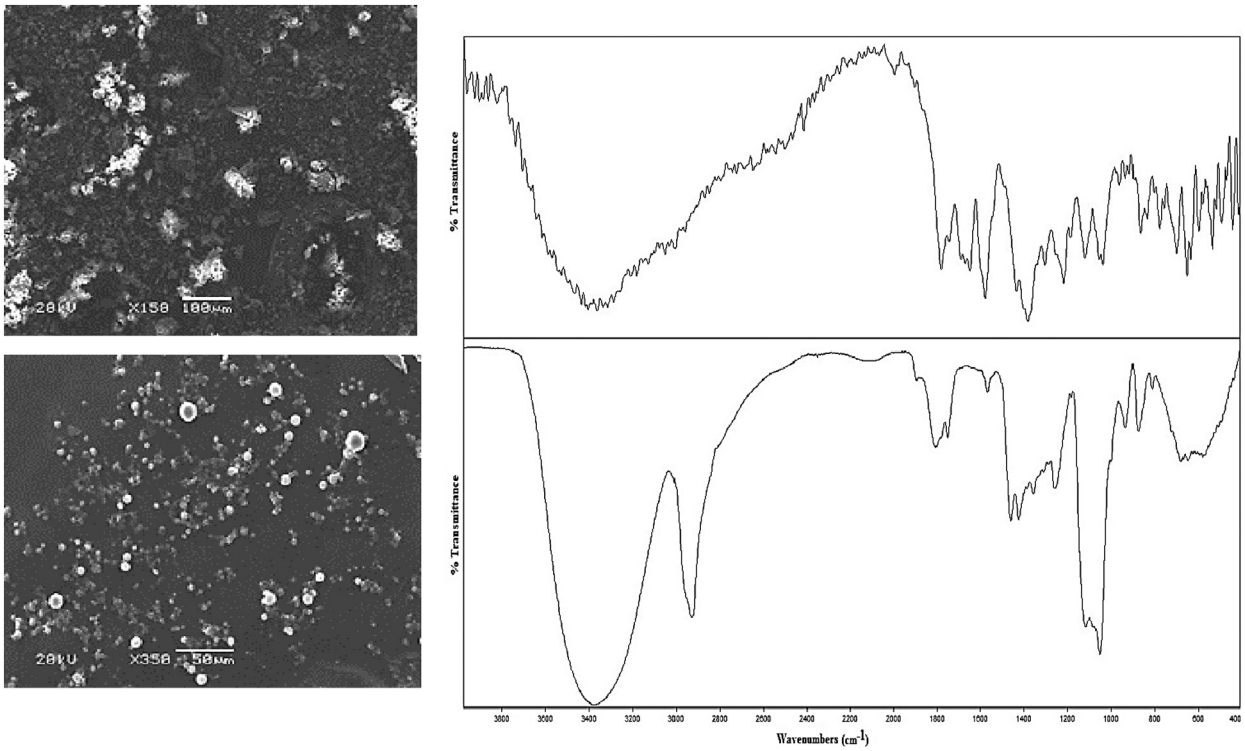

Fig. 1. SEM images and FTIR spectra of Tam (upper) and the Tam-SLN formulation.

technique. We have previously produced Tam-loaded SLNs by this technique using 2.5% stearic acid, 5% Tam and 2.5% Tween 80 (Eskiler et al., 2016; Eskiler Guney et al., 2017). However, stearic acid was used at 5% in the present study to improve the physical properties of the SLNs. Briefly, stearic acid was melted at 80 °C and Tam (2.5 mg/ml), was added to the melted lipid. The organic solvent (Tween 80 (200 mg)) was heated at the same temperature and then slowly added to the melted lipid-drug mixture. The mixture was homogenized (at 25,000 rpm) for 10 min using a homogenizer (T18 Turax, IKA, Germany). The SLN formulations were lyophilized and stored at 4 °C until further measurements. Additionally, drug-free SLNs were produced in parallel by using the same procedure.

2.2. Particle size, polydispersity index and zeta potential

The particle size, polydispersity index and zeta potential of Tam, the free SLNs and Tam-SLNs were measured using the a Zetasizer instrument (Malvern Instruments, UK) at three different temperature (4 °C, 25 °C and 40 °C) and duration times (30, 60 and 90 days) at a 90° scattering angle.

2.3. Surface morphology

The surface morphologies of Tam and Tam-SLNs were monitored using an SEM microscope at 20 kV. A drop of the Tam-SLN or free-SLN dispersions was spread on a small clean slide coverslip and dried overnight at room temperature. After one day, the samples were gold coated under an argon atmosphere, and then studied using SEM.

2.4. Fourier-transform infrared spectroscopy (FT-IR)

The interaction between the lipids (stearic acid) and Tam was determined by the Fourier-transform infrared (FT-IR) studies. The FTIR spectra of Tam and Tam-SLNs were recorded on a Bruker Optics IFS66v/s FT-IR spectrometer using the KBr disc technique in the range of 4000–400 cm^{-1}.