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In vitro investigations on the toxicity induced by tamoxifen and tamoxifen-loaded solid lipid nanoparticles on two breast cancer cell types

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ABSTRACT

Objectives: Colloidal drug delivery system, solid lipid nanoparticles (SLNs), helps to increase the solubility of the drug and its oral bioavailability.

Methods: Tamoxifen (TAM) as a nonsteroidal antiestrogen drug was formulated in SLN and an *in vitro* study was conducted to determine the cytotoxicity effect of TAM-loaded SLNs on human breast cancer cell lines MCF-7 (estrogen receptor-positive) and MDA-MB231 (estrogen receptor-negative) cells. The cytotoxicity was measured by (3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay).

Results: The results showed that tamoxifen-loaded SLNs has an equally efficient cytotoxic activity against MCF-7 and MDA-MB231 cells, compared with free tamoxifen, and the half maximal inhibitory concentration (IC_{50}) of TAM-loaded SLNs was generally lower than that of free TAM.

Conclusion: This finding indicates that tamoxifen's cytotoxicity may result from improved drug internalization through encapsulation into the SLN matrix and endocytosis. Therefore, when TAM is incorporated into the SLN carrier system, its antitumoral activity is still preserved, suggesting that SLN is a good carrier for the drug insoluble in water.

Keywords: *Breast Neoplasms; Nanoparticle; Tamoxifen; Toxicity*

Introduction

Breast cancer is one the most important health concerns of the modern society [1]. The main options for breast cancer treatment include surgery, radiation therapy and chemotherapy [2]. The cytotoxic drugs treat cancers by causing cell death or growth arrest. Although tamoxifen (TAM) was primarily used as a drug against hormone-dependent breast cancers [3], it has also been used in the treatment of hormone-insensitive estrogen receptor-negative breast cancers [4]. In addition to anticarcinogenic and antioxidant effects, TAM also has toxic side-effects [5-6].

Depending on the administration dose and target tissues, the function of TAM can be estrogenic or anti-estrogenic. For example, while TAM is anti-estrogenic to the breast, it is estrogenic to the uterus. One of the most adverse and serious side-effects of postmenopausal TAM therapy seems to be its proliferated effect on the endometrial tissue. Endometrial disease such as hyperplasia, polyps, carcinoma and sarcoma has been recog-

nized in approximately 36% of postmenopausal patients with breast cancer and in the cases under TAM treatment. The dose-dependent side-effects of TAM also include liver cancer, increased blood clotting and ocular adverse effects such as retinopathy and corneal opacities. These findings suggest that small doses given through colloidal delivery systems would be useful for long-term therapy of breast cancers [7].

It has been shown that tamoxifen, a nonsteroidalantiestrogen drug, which was recently encapsulated in lipid based nanoparticles -solid lipid nanoparticle (SLN)- shows similar effects as free tamoxifen by promoting apoptosis in the mammary tumor gland in Sprague-Dawley rats [8]. However, today there is increasing acceptation for *in vitro* tests as the method for determining cytotoxicity and viability of chemotherapeutic drugs. In the current study, the objective was to determine the *in vitro* chemotherapeutic effects of TAM-loaded SLN on breast cancer cell lines (MCF-7 and MDA-MB231). The responses of breast cancer cell lines were determined by (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay).

Materials and Methods

Cells and chemicals

Softisan® 154 (S154) or hydrogenated palm oil, was a gift from CONDEA (Witten, Germany). Lipoid S100 (soy lecithin) was a gift from Lipoid KG (Ludwigshafen, Germany). Thimerosal, sorbitol, tamoxifen and tetrazolium salt were purchased from Sigma (Kuala Lampur, Malaysia)

Preparation of SLN and TAM-loaded SLN

SLN was prepared using the high pressure homogenization (HPH) technique [11[. Briefly, 70 g palm oil and 30 g soy lecithin were weighed, mixed and ground in a ceramic crucible and then heated up to 65 - 70°C until a clear yellowish solution was obtained. A solution consisting of 1mL oleyl alcohol, 0.005 g thimerosal, 4.75 g sorbitol and 89.25 mL re-distilled water was added to each of the lipid matrices. The mixtures were stirred on a magnetic stirrer using a teflon coated magnet, for 30 min at room temperature. The lipophilic drug model, TAM, with concentration of 10 mg was dissolved in 1 ml oleyl alcohol and mixed with 50 mg SLN using an Ultra Turrax® (Ika, Staufen Germany) at 13000 rpm for 10 min. The mixture of TAM-SLN was then incubated at 50 - 60°C while stirring overnight with a teflon coated magnet at 500 rpm and then exposed to air until solidification.

Treatment of the cells

Breast cancer cell lines (MCF-7 and MDA-MB231 – ATCC) were maintained in RPMI culture medium supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/mL streptomycin and 100 IU/mL penicillin at 37°C in a humidified incubator containing 5% CO_2 / 95% air. The cells were allowed to grow in 25 cm^2 cell culture flask until confluent. The old media was removed and the confluent cells washed with PBS and detached with trypsin. A haemocytometer was used to estimate the count. Then, 100 µL of cell suspentions (10^5 cell/mL) were seeded into a 96-well plate using culture medium and allowed to reach confluency. The confluent cells were treated with TAM dissolved in Dimethyl sulfoxide (DMSO) and TAM-loaded SLN dispersed in DMSO at concentration ranging 37.5, 75, 150, and 300µg/mL for 24, 48 and 72 h. The control wells received 0.1% DMSO as the vehicle. The viability of the breast cancer cells versus free TAM and TAM-loaded SLN concentration was assessed by the MTT assay.

MTT Assay

After the treatment of the cells for defined time (24, 48 and 72h), 10 µL of (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5mg/mL in PBS) was added to each well and the plates incubated at 37°C for 2 additional hours. Fifty microliters of DMSO was added to each well and shaken for 15 min. The optical density of the cell suspension was read on an ELISA reader (Tecan, Magellan, Austria) at 570 nm after background correction at 690 nm. Average cell viability of treated cells

was expressed as percentage of the absorbance of control-treated cells.

Statistical analysis

The data obtained were subjected to statistical analysis. The differences in means among the groups were expressed as mean ± standard deviation. All the data were subjected to one-way analysis of variance (ANOVA) followed by Post Hoc multiple comparison and Duncan test after verification of the normal distribution of the data. The Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS 2006) was used to perform all statistical tests and p-value less than 0.05 were considered significant.

Results

In the MTT assay viable cells are able to reduce the water-soluble yellow tetrazolium salt into a water-insoluble purple formazan compound [9]. The color reaction determined spectrophotometically is used as a measure of cell viability and proliferation [10]. The cytotoxicity effect of TAM and TAM-loaded SLN on MCF-7 and MDA-MB231 cells are presented in Fig. 1 and Fig. 2.

The results suggest that the TAM-loaded SLN has an equally efficient cytotoxic activity as free tamoxifen. Therefore TAM-loaded SLN still preserves the antitumoral activity of the free drug. The cytotoxicity of TAM-loaded SLN maintains an antitumoral activity on MCF-7 and MDA-MB231 cells that was comparable to that of the free drug (Fig. 1 and Fig. 2). Therefore the biological activity of tamoxifen is not negatively affected when incorporated into SLN.

The percent cell viability versus free TAM and TAM-loaded SLN concentration graph was constructed using Microsoft Excel and the IC_{50} value was calculated by linear interpolation principle. Table 1 and Table 2 show the 50% inhibitory concentration (IC_{50}) of TAM and TAM-loaded SLN on MCF-7 and MDA-MB231 respectively. The results showed that the IC_{50} of TAM-loaded SLN on the breast cancer cell lines were generally lower than those for free TAM. The IC_{50} of TAM and TAM-loaded SLN for MDA-MB231 cells (ER-negative or ER-independent) was higher than for MCF-7 cells (ER-positive or ER-dependent).

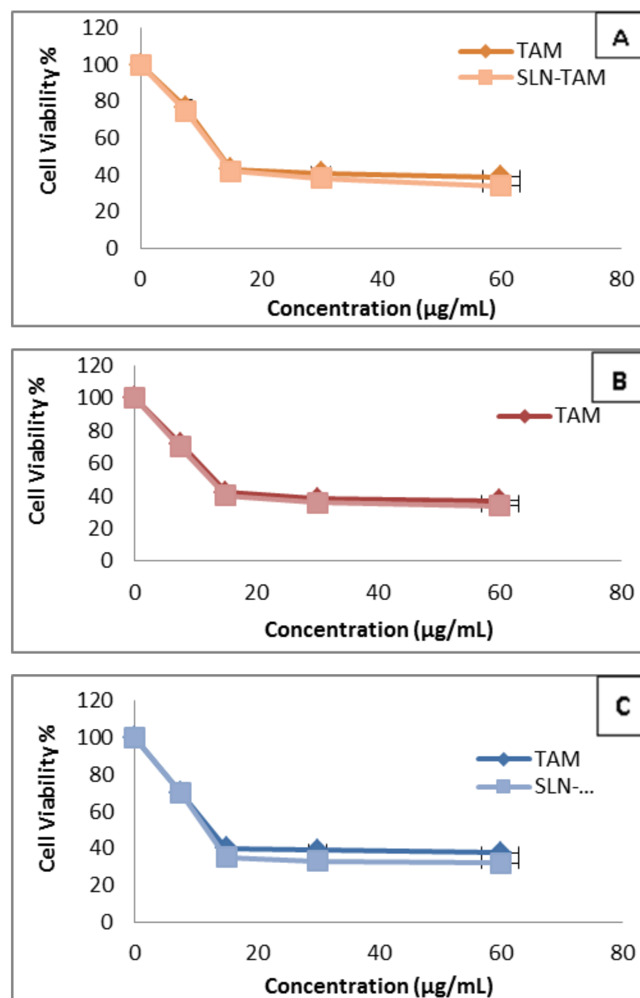

Figure 1. MCF-7 cell viability after incubation with TAM and TAM-Loaded SLN formulation. (A) after 24 h, (B) after 48 h and (C) after 72 h. (The percentage of cell viability was expressed as a ratio of treated cells to the untreated control cells. Each point represents the mean ± SD of 5 wells).

Table 1. The IC_{50} of TAM and TAM-loaded SLN formulations on MCF-7 cells after 24, 48 and 72h (n=5)

Treatment	IC_{50} (µg/mL)		
	24h	48h	72h
TAM	13.45 ± 0.46	13.00 ± 0.98	12.50 ± 0.91
TAM-SLN	13.18^{a} ± 0.66	12.50^{a} ± 1.5	11.78^{b} ± 0.18

All value represent the mean ± S. [a,b]means in each row with different superscripts are significantly different ; *P*<0.05. TAM, Tamoxifen; TAM-SLN, Tamoxifen-loaded solid lipid nanoparticles**.**

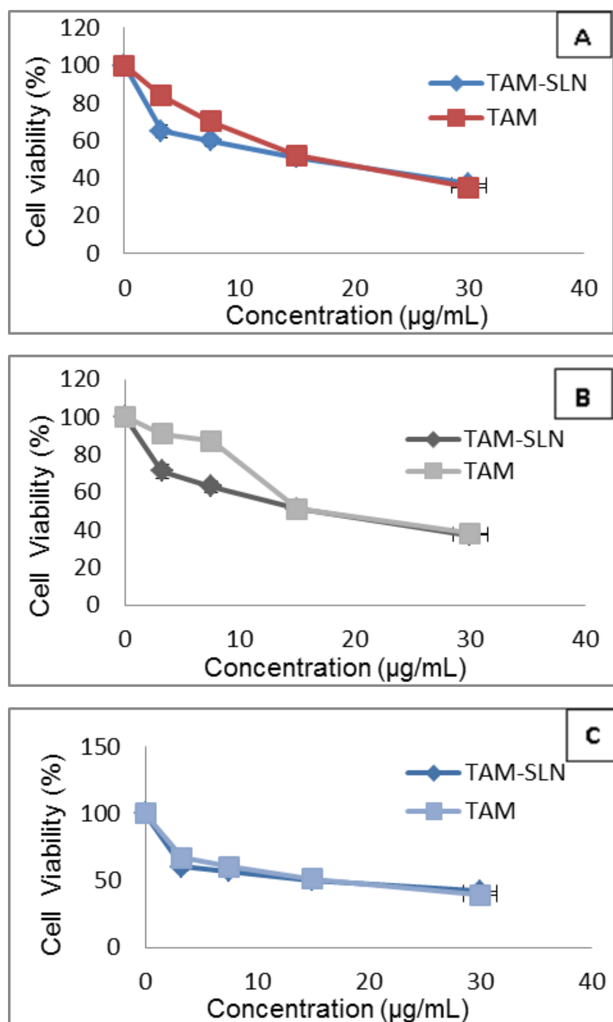

Figure 2. MDA-MB231 cell viability after incubation with TAM and TAM-Loaded SLN formulation. (A) after 24 h, (B) after 48 h and (C) after 72 h. (The percentage of cell viability was expressed as a ratio of treated cells to the untreated control cells. Each point represents the mean ± SD of 5 wells).

Table 2. The IC_{50} of TAM and TAM-loaded SLN formulations on MDA-MB231 cells after 24, 48 and 72h (n=5)

Treatment	IC_{50} (µg/mL)		
	24h	48h	72h
TAM	17.21 ± 1.44	16.87 ± 1.97	15.97 ± 0.86
TAM-SLN	16.93 ± 0.82	16.00 ± 0.10	15.80 ± 0.69

All value represent the mean ± SD. TAM, Tamoxifen; TAM-SLN, Tamoxifen-loaded solid lipid nanoparticle

Discussion

The results showed that when TAM is incorporated into the SLN carrier system, its antitumoral activity is still maintained, suggesting that SLN is a good carrier for the drug. Although the TAM-loaded SLN was dispersed in DMSO, the final concentration of DMSO in each well was maintained at 0.1%. At this concentration, there is no possibility of cytotoxicity on the cancer cells. The SLN formulation will help to increase the solubility of the drug and facilitate the entrapment of high amounts of drug in the nanoparticles.

The mechanisms of ER-independent, TAM-induced apoptosis may be through the inhibition of protein kinase C. The IC_{50} value of tamoxifen for protein kinase C inhibition is 4 to 10 times the concentration for ER inhibition in ER-positive cells. Therefore, the dose of tamoxifen for treatment of patients with ER-positive breast cancer would have to be increased over the usual 20 mg per day used. High dose of tamoxifen might decrease the therapeutic index by increasing toxicity [12].

In our study the IC_{50} of TAM-loaded SLN on the breast cancer cell lines were generally lower than those for free TAM. This indicates that TAM cytotoxicity may be the result of improved drug internalization through encapsulation into SLN matrix and endocytosis [13]. A previous study showed a similar finding, where there was reduced MCF-7 cell viability in the presence of TAM-loaded SLN [14]. It seems that improved cytotoxicity of incorporated drug is not dependent of the composition on the SLN. In fact it was reported that the IC_{50} value of drug-loaded SLN composed of different materials were lower than that of free drug solution [15]. There are at least two mechanisms that have been associated with the cytotoxicity of drug-loaded SLN. Using Doxorubicin (DOX)-loaded SLN, it was suggested that the first mechanism involves the release of DOX from DOX-SLN outside the cells, and the cytotoxicity of DOX is increased by the nanoparticles. The second mechanism suggested was, release of the drug inside the cell and thus produces greater cytotoxicity [16].

Since unloaded SLN is nontoxic [17], formulating TAM by incorporating into SLN will potentially enhance the solubility of the drug through inclusion into the lipid phase and facilitating the entrapment of greater amounts of the drug in the SLN.

Conclusion

Tamoxifen-loaded SLN like free TAM displayed antitumoral activity against human breast cancer cells. The biological availability of drug is not affected when incorporated into SLN. Therefore SLN could be applied as a drug delivery system for cancer treatments. In conclusion, the TAM-loaded SLN, because of its small size, could not be easily phagocytosed by macrophages and therefore the nanoparticles could be potentially used in long-term circulating carrier system for breast cancer therapy.

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Conflict of interest

The authors report no conflict of interest.

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