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Tamoxifen-encapsulated vesicular systems: cytotoxicity evaluation in human epidermal keratinocyte cell line

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Abstract

Aim: Tamoxifen is a nonsteroidal estrogen receptor modulator indicated in the treatment of breast cancer. Apoptosis has been reported to be a major mechanism for its antitumor effect. Tamoxifen has also shown significant potential in treating various dermatological disorders including psoriasis, characterized by hyperproliferation of epidermal keratinocytes. An endeavor was made in the current studies to investigate the potency of vesicle-encapsulated tamoxifen on human epidermal keratinocyte cell lines. *Methods*: Drug was encapsulated in the phospholipid-based vesicular systems, namely, conventional liposomes and flexible-membrane liposomes. In vitro cytotoxicity evaluation of the formulations was carried out employing MTT cell proliferation assay. *Results*: A composition-dependent strong inhibition in the viability of epidermal keratinocyte cells was observed. *Conclusion*: The encouraging findings of this work construe immense potential of the tamoxifen-encapsulated vesicular systems in the management of psoriasis.

Key words: *HEK001; liposome; MTT assay; phospholipid; skin; topical*

Introduction

Tamoxifen (TAM) is a nonsteroidal selective estrogen receptor modulator widely employed in the chemotherapy of breast cancer[1,2]. It provides effective treatment for the patients with metastatic breast cancer and reduces the risk of recurrence and death from breast cancer when administered as an adjuvant therapy. Use of TAM is specifically indicated in postmenopausal women suffering with breast cancer[3,4]. A case study reports the treatment of a breast cancer patient with TAM who was also suffering with chronic plaque psoriasis. Serendipitously, it was found during the study that her psoriasis responded quite favorably to TAM administration[5]. There are a few more such reports in support of TAM in the management of psoriasis[6–9].

TAM is currently available only as the oral dosage forms. Systemic absorption of TAM can result in the increased risk of endometrial cancer, deep vein thrombosis and pulmonary embolism, alteration in liver enzyme levels, ocular disturbances, etc., accountable to its wide distribution in the body[10–14].

Accordingly to surmount these problems, our research group developed liposomal systems of TAM for direct topical application to the target skin sites (i.e., breast and psoriatic lesions), which could serve the desired objectives more efficiently without affecting other naive body organs[15]. Development and characterization of the test formulations has already been reported by us earlier[16]. In this study, we aim to explore the in vitro cytotoxicity of these developed lipid vesicular systems employing human epidermal keratinocyte (HEK001) cell line.

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Materials and methods

Materials

TAM citrate and saturated phospholipid (soya phosphatidylcholine; 90H) were the generous gift samples from M/s. Biochem Pharmaceutical Industries (Mumbai, India) and Phospholipid GmbH (Nattermannallee, Germany), respectively. Dimethyl sulfoxide, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum, ethylenediamine tetraacetic acid, trypsin, cholesterol, and sorbitan monooleate were procured from M/s. Sigma Chemicals Co. (St. Louis, MO, USA). The HEK001 (ATCC-CRL-2404™) cell line and keratinocyte serum-free (KSF) medium were obtained from American Type Culture Collection (Manassas, VA, USA). All other chemicals used in the study were of analytical grade.

Methods

Cell culture and growth conditions

Epidermal keratinocyte cells were grown in the KSF medium containing 5 ng/mL of human recombinant epidermal growth factor and 2 mM L-glutamine (without bovine pituitary extract and without serum). The cells were grown in a CO_2 incubator (Thermo Electron Corporation, Milford, MA, USA) at a temperature of 37°C with 98% humidity and 5% CO_2 gas environment. The HEK cells were grown in a T-75 cell-culture flask. When the cells were growing in the logarithmic phase (i.e., about 80% confluent), the culture media was removed and cells were rinsed with the Ca^{2+}/Mg^{2+}-free phosphate buffer saline of pH 7.4. To this, 2.0 mL of 0.53 mM ethylenediamine tetraacetic acid solution containing 0.05% (w/v) of trypsin was added to detach the cell layers. Detached cells were dispersed gently by pipetting into the fresh KSF medium containing 10% fetal bovine serum. Thereafter, the cell suspension was transferred into the centrifuge tubes and spun for 5 minutes at $125 \times g$ at 4°C temperature. The supernatant obtained was discarded and the sedimented cells were resuspended in the fresh KSF medium. The required cell suspension (1×10^4/100 µL) was distributed into a 96-well plate and incubated in the CO_2 incubator.

Preparation of lipid vesicular systems

Drug-loaded liposomes (TAM-Lipo) for topical application were prepared by thin-film hydration technique, as described previously[16]. Briefly, a dry film of lipid (phospholipid and cholesterol; 2:1 weight ratio) was hydrated in water. The flexible-membrane liposomes (TAM-FMLipo), on the other hand, were composed of phospholipid and sorbitan monooleate in a weight ratio of 4:1. Analogously, the placebo formulations were prepared employing the identical composition and methods of preparation, but without the addition of drug.

Cytotoxicity test

Cell proliferation was studied using modified MTT assay[17,18]. The HEK cells (1×10^4 cells) were grown in a 96-well tissue culture plate in 100 µL of complete KFS medium. The cells were treated with pure drug and vesicular suspensions (incorporated in 100 µL KSF medium) at different drug concentrations, whereas the control cells received the placebo formulations only. The cells were incubated in a CO_2 incubator at 37°C for 48 hours. Cells were then washed twice with phosphate buffer saline. Thereafter, an aliquot of 20 µL of sterile MTT solution (2.5 mg/mL in phosphate buffer saline) was added to each well. Culture plates were gently stirred on plate-shaker (Titertek™; Flow Laboratories, Meckenheim, Germany) and incubated in CO_2 incubator at 37°C for 2 hours. The plates were centrifuged at $1200 \times g$ for 15 minutes. The supernatant was discarded, whereas MTT-formazon crystals were dissolved in 100 µL of dimethyl sulfoxide. The plates were further stirred for 20 minutes and the optical density (OD) was measured at 570 nm taking 620 nm as the reference wavelength using an ELISA plate reader (Thermo Electron Corporation). The tetrazolium compound (i.e., MTT) was bioreduced by cells into colored formazan product that absorbs light at 570 nm. The viability of cells was computed from the OD of the MTT-formazon violet color crystal, as the OD is known to be directly proportional to the number of viable cells present[17]. The IC_{50} values of the different tested formulations were calculated by plotting the graphs of cell viability at different graded concentrations of drug in the formulations[18]. The photomicrographs of the treated cells were taken with the help of DP-12 digital camera coupled to a 1X-70 microscope (Olympus, Tokyo, Japan).

Statistical analysis

Two-way analysis of variance was performed, employing SigmaStat software version 2.0 (Jandel Scientific, San Rafael, CA, USA), on the raw data of cell viability obtained with three tested formulations at different dose levels.

Results and discussion

Microphotographs of the HEK001 cell samples following various treatments are depicted in Figure 1. The photographs vividly indicate significant reduction in the cell viability with all the three treatments investigated, namely TAM, TAM-Lipo, and TAM-FMLipo. Close supervision of the microphotographs reveal the unambiguous superiority of vesicularly entrapped drug (i.e.,

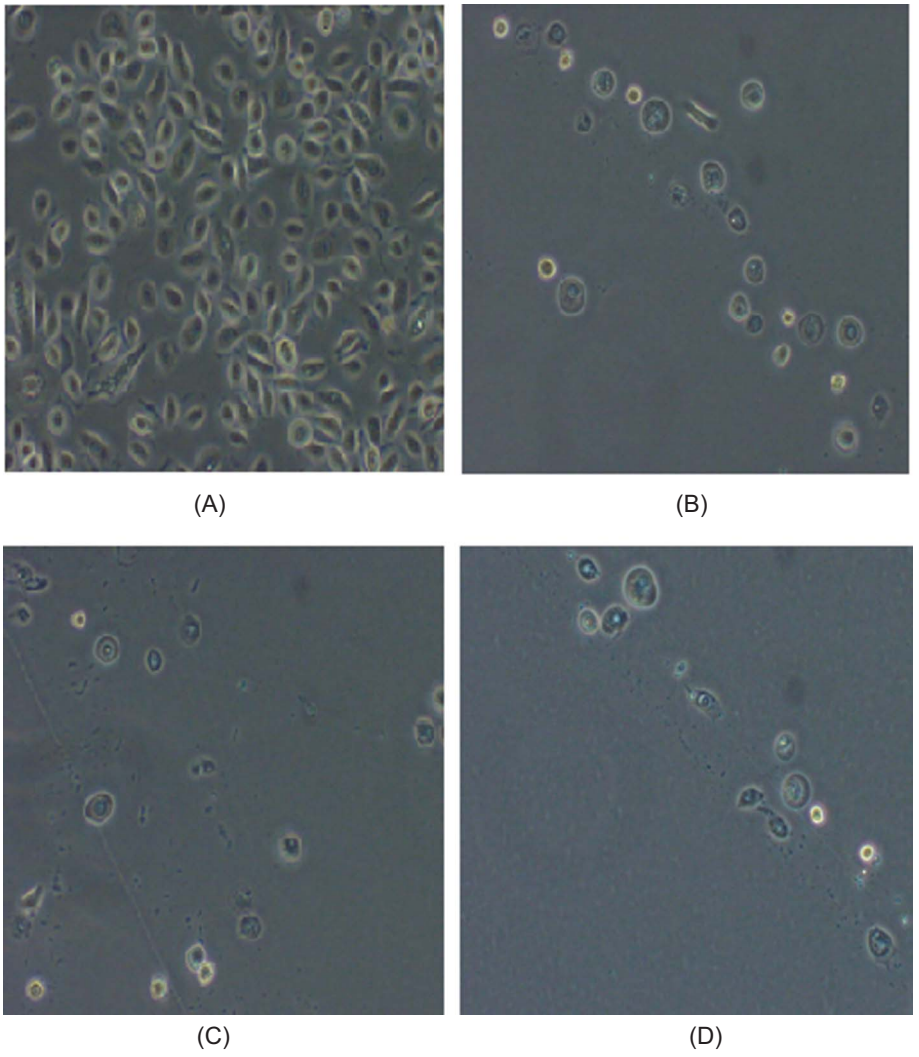

Figure 1. Effect of various treatments of TAM formulations (equivalent to 30 μg/mL of tamoxifen) on the survival of MCF-7 cells, namely (A) placebo; (B) free drug; (C) TAM-Lipo; and (D) TAM-FMLipo.

TAM-FMLipo and TAM-Lipo formulation) in reducing the cell growth vis-à-vis the free drug. Two-way analysis of variance on the cell viability data indicates statistically significant high values of Fisher's criterion [$F(2,12) = 358.42$, $P < 0.001$] for different formulation treatments as well as for their different dose levels [$F(6,12) = 3881.35$, $P < 0.001$], vouching considerable influence of both studied treatments and drug dose levels.

Figure 2 portrays quantitative measure of cell viability of the aforesaid three treatments at various drug concentrations. As indicated in the figure, all the tested formulations were found to induce cytotoxicity in human epidermal keratinocyte cells following 48 hours of incubation. Significantly higher efficacy (≈twice) was observed in the case of vesicularly entrapped drug vis-à-vis free drug. The inset of the Figure 2 vouches for the same. This enhanced efficacy may be ascribed to the lipo-solublized state of the drug, owing to its entrapment within multiple lipoidal domains of vesicles. Furthermore, the phospholipid lamella in the periphery of the vesicles may integrate with cell membrane facilitating internalization of the vesicular contents. Several studies[19–23] reported in the literature vouch for such internalization of vesicular content in cell. Lower cellular uptake may be attributable to the absence of such facilitated transport in case of free drug.

Conclusion

The encouraging results of this study indicate tremendous promise of vesicular systems in ameliorating the efficacy of TAM through topical route of administration for the management of psoriasis. Accordingly, it can be

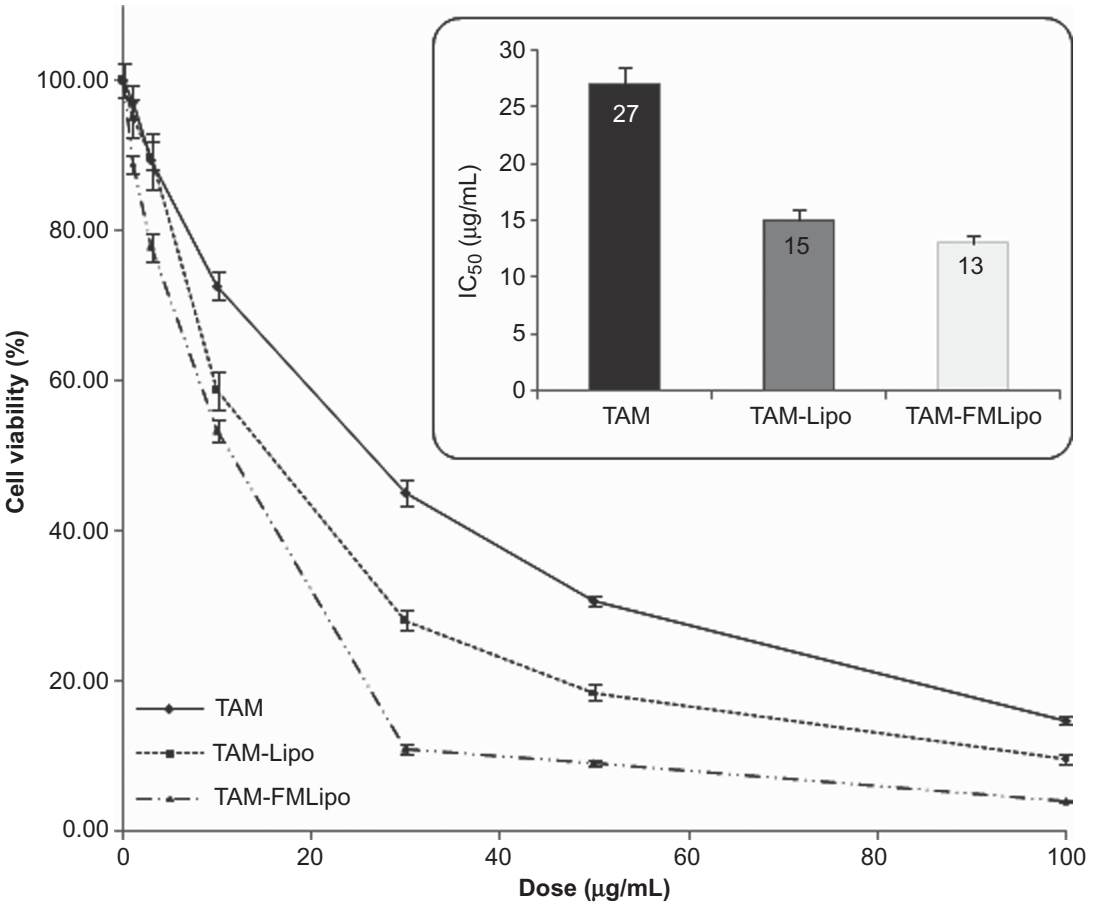

Figure 2. Cytotoxicity of TAM and its liposomal formulations in human epidermal keratinocyte cell line. Each data point represents the mean ± SD of eight wells, carried out in triplicate under analogous conditions. The inset figure depicts the bar diagram of the IC_{50} values of the different investigated TAM formulations.

concluded that the phospholipid-based vesicular systems help in generating and retaining the most favorable physicochemical state of the drug to improve its cellular interactions.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Jordan VC, Brodie AM. (2007). Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. Steroids, 72:7–25.
2. Robertson JF. (2002). Current role of endocrine therapy in the management of breast cancer. Breast Cancer, 9:276–81.
3. Punglia RS, Kuntz KM, Winer EP, Weeks JC, Burstein HJ. (2005). Optimizing adjuvant endocrine therapy in postmenopausal women with early-stage breast cancer: A decision analysis. J Clin Oncol, 23:5178–87.
4. Gradishar WJ. (2003). Adjuvant systemic therapy of early stage breast cancer. Curr Treat Options Oncol, 4:141–50.
5. Boyd AS, King LE Jr. (1999). Tamoxifen-induced remission of psoriasis. J Am Acad Dermatol, 41:887–9.
6. Stevens HP, Ostlere LS, Black CM, Jacobs HS, Rustin MHA. (1993). Cyclical psoriatic arthritis responding to anti-oestrogen therapy. Br J Dermatol, 129:458–60.
7. Ferrari VD, Jirillo A. (1996). Psoriasis and tamoxifen therapy: A case report. Tumori, 82:262–3.
8. Hu D, Hughes MA, Cherry GW. (1998). Topical tamoxifen—a potential therapeutic regime in treating excessive dermal scarring? Br J Plast Surg, 51:462–9.
9. Kanda N, Watanabe S. (2003). 17-beta-estradiol inhibits the production of interferon-induced protein of 10 kDa by human keratinocytes. J Invest Dermatol, 120:411–9.

10. Strickland C. (2002). Benefits of tamoxifen for breast cancer prevention do not always outweigh overall risks. J Fam Pract, 51:1016.
11. Varras M, Akrivis Ch. (2003). Large endometrial polyp with sarcomatous stromal components following long-term tamoxifen treatment for breast cancer: A case report and review of the literature. Eur J Gynaecol Oncol, 24:565–8.
12. Bergh J. (2003). Breast-cancer prevention: Is the risk-benefit ratio in favour of tamoxifen? Lancet, 9379:183–4.
13. Bjarnason NH, Hitz M, Jorgensen NR, Vestergaard P. (2008). Adverse bone effects during pharmacological breast cancer therapy. Acta Oncol, 47:747–54.
14. Bernstein PS, DellaCroce JT. (2007). Diagnostic and therapeutic challenges. Tamoxifen toxicity. Retina, 27:982–8.
15. Katare OP, Sinha VR, Bhatia A, Kumar R, Gupta S. (2006). Synergistic liposomal tamoxifen composition for topical application and method of preparing thereof. International patent publication PCT No.: WO 2006/001035 A2.
16. Bhatia A, Kumar R, Katare OP. (2004). Tamoxifen entrapped topical liposomes: Development, characterization and in-vitro evaluation. J Pharm Pharm Sci, 7:252–9.
17. van de Loosdrecht AA, Beelen RH, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MM. (1994). A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. J Immunol Methods, 174:311–20.
18. Bhushan S, Kumar A, Malik F, Andotra SS, Sethi VK, Kaur IP, et al. (2007). A triterpenediol from Boswellia serrata induces apoptosis through both the intrinsic and extrinsic apoptotic pathways in human leukemia HL-60 cells. Apoptosis, 12:1911–26.
19. Batzri S, Korn ED. (1975). Interaction of phospholipid vesicles with cells. J Cell Biol, 66:621–34.
20. Ishimoto Y, Ohashi K, Mizuno K, Nakano T. (2000). Promotion of the uptake of PS liposomes and apoptotic cells by a product of growth arrest-specific gene, *gas6*. J Biochem, 127:411–7.
21. Peek LJ, Middaugh CR, Berkland C. (2008). Nanotechnology in vaccine delivery. Adv Drug Deliv Rev, 60:915–28.
22. Manconi M, Isola R, Falchi AM, Sinico C, Fadda AM. (2007). Intracellular distribution of fluorescent probes delivered by vesicles of different lipidic composition. Colloids Surf B Biointerfaces, 57:143–51.
23. Karanth H, Murthy RS. (2007). pH-sensitive liposomes—principle and application in cancer therapy. J Pharm Pharmacol, 59:469–83.