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In situ thermosensitive Tamoxifen citrate loaded hydrogels: An effective tool in breast cancer loco-regional therapy

Dalia S. Shaker [a], Mohamed A. Shaker [b, *], Anke Klingner [c], Mahmoud S. Hanafy [b]

[a] Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, FUE, Cairo, Egypt
[b] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Helwan University, Cairo, Egypt
[c] Department of Physics, Faculty of Engineering, German University, Cairo, Egypt

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ABSTRACT

One of the main challenges for using Tamoxifen citrate (TMC) in breast cancer therapy is achieving proper target and efficient delivery of adequate concentration to the adenocarcinoma without harming healthy glandular and soft fatty tissue. Herein, TMC niosomal thermosensitive hydrogels were proposed as a tool to resolve this challenge. Niosomes were prepared by film hydration technique and incorporated into Pluronics thermosensitive gels prepared using cold method. The prepared hydrogels were evaluated for gelation temperature, rheological behavior and *in vitro* drug release. Moreover, *in vivo* anti-tumor activity was examined in Ehrlich carcinoma mice model through reporting solid tumor volume regression and tissue distribution of TMC. Type and ratio of used poloxamers were manipulated to provide the optimal gelation temperature (34–37 °C). Rheological analysis showed low viscosity and elasticity values at low and room temperature while these values significantly increased at the physiological temperature. A prolonged diffusion-driven release of TMC was detected. *In vivo* data showed, evidently, that anticancer activity was improved with significant retention of the drug at the tumor site. These encouraging results confined that this *in situ* hydrogel depot offers an attractive approach for controlled delivery of TMC and clinically expected to be useful delivery system in loco-regional therapy for breast cancer.

1. Introduction

Nowadays, the world faces an awful battle with the cancer in consequence with the incredibly increasing number of people who die from this fatal disease [1]. Among the different types of diagnosed cancer today, breast cancer is considered the second leading cause of cancer related death in women [2]. There are several types of breast cancer; however, estrogen receptor positive breast cancer is deemed the most common breast cancer among women. TMC which is estrogen receptor modulator in the breast tissue is commonly indicated to treat that type of breast tumor which requires high estrogen level to grow. TMC has been approved for more than 40 years and is currently prescribed as a hormonal chemotherapy in treating early, advanced and metastatic breast cancer in both pre- and post-menopausal women, as well as, a hormone therapy to treat male breast cancer [3]. Despite the fact that different administration routes and delivery systems for TMC have been extensively investigated and commonly used, it still possesses several limitations and properties that restrict its full clinical relevance [3]. Oral TMC therapy which is the main route of administration has always been associated with poor bioavailability due to poor aqueous solubility, liability to intestinal metabolism and rapid first pass metabolism. At the same time TMC is not a single treatment, the course required one or two oral doses daily for 10 years (according to the latest guidelines) which eventually leads to failure of therapeutic efficacy as much as poor patient compliance [4,5]. This long-term systemic therapy is usually associated with life-threatening toxicities such as oxidative stress mediated liver toxicity, ototoxicity, retinopathy, cataract, thromboembolic events as well as increased risk of endometrial and liver cancer [6,7].

With the purpose of finding solutions to the aforementioned complications, several attempts alternating modes and strategies for delivering this drug were made, trying to achieve two major goals. The first goal is to deliver and target TMC specifically and

* Corresponding author. Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Helwan University, P.O. Box: 11477, Cairo, Egypt.
E-mail addresses: dalia.samuel@fue.edu.eg (D.S. Shaker), mshaker@mun.ca (M.A. Shaker), anke.klingner@guc.edu.eg (A. Klingner), pharm.mahmoud@gmail.com (M.S. Hanafy).

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safely to its site of action, through localized drug delivery (intra-/peri-tumor) to maximize its therapeutic efficacy while minimizing its toxicity, based on the fact that the anticancer activity can be reinforced by increasing its concentration at the target site (tumor) [8–12]. The second is to prolong the duration of tumor exposure to TMC by sustaining its release from the formulated pharmaceutical carrier, to help maintaining its therapeutic effect after administration to the body [13]. As administration of free TMC into tumor site leads to rapid diffusion of TMC out of the tumor after intra-tumor injection [12], work in this area is ongoing since therapists are still in need for a delivery system to slow down TMC diffusion (increase the retention of TMC in the tumor). To address this need, we proposed "package within a package" *in situ* drug delivery system. A depot drug delivery system composed of nano-size vesicles embedded within biodegradable thermosensitive hydrogels. Once locally injected, it forms a drug depot at the site of injection that can reduce the total number of injection throughout the TMC therapy course. Niosomes are spherical lamellar structures obtained upon self-assembly of non-ionic surfactant with or without membrane stabilizer e.g. cholesterol during hydration in buffer, i.e. surfactant molecules forming a closed concentric bilayer structure in which the lipophilic tails are sandwiched between the hydrophilic heads. The high drug entrapment, slow drug release, improved cellular uptake, biodegradable and biocompatible features as well as the economic considerations distinguish niosomes from other lipid nano-carriers [12,14–16].

Recently we have reported on the TMC loaded niosomes which are able to bind *in vitro* to breast cancer cells (MCF-7) with more cytotoxic activity compared to free drug. As an injectable delivery system for localized breast cancer therapy, we also demonstrate an improvement in the *in vivo* anti-tumor efficacy of TMC [12]. Nonetheless, the low viscosity of such a colloidal suspension of niosomes does not provide sufficient retention time for the injected dose at the tumor site. As such TMC loaded niosomes can be dispersed into high viscous thermosensitive polymeric hydrogels such as poloxamers. Poloxamers are bio-inert synthetic tri-block copolymers, poly(ethylene oxide)-poly(propylene oxide)-poly(-ethylene oxide) (PEO-PPO-PEO), with thermosensitive behavior in aqueous solutions and are widely used as a biocompatible vehicle in various drug delivery systems [17]. Their thermal sensitivity can be easily controlled throughout adjusting composition for the reversible gelation to occur *in situ*, i.e. spontaneous transition from sol to gel when injected at the tumor site [18]. Herein, we have focused to develop a smart delivery of TMC loaded niosomes by incorporation into *in situ* thermosensitive hydrogels using different poloxamers to localizes TMC action and activity into the vicinity of tumor, hence improving therapeutic outcome with much less side effects or toxicity on other organs. With the aspect of bench-to-bedside translation, the *in vitro* characterization of their gelation temperature, rheological behaviors as well as drug release profiles were examined. The *in vivo* antitumor efficacy was also investigated using Ehrlich carcinoma mice model through reporting solid tumor volume regression and drug tissue distribution in comparison to the free drug.

2. Material and methods

2.1. Material

Tamoxifen citrate (purity 98.9%) was kindly gifted by Medical Union Pharmaceuticals, Egypt. Span 60 was procured from Sigma Aldrich, USA. Pluronic F127® (poloxamer 407) and Pluronic F68® (poloxamer 188) were procured from Sigma Aldrich, Germany. HPLC grade methanol and acetonitrile were purchased from Sigma Aldrich, Germany, whereas chloroform was purchased from United Company for medical and chemical preparations, Egypt. Other chemicals used were reagent grade and all of the chemicals were used as received without any further purification.

2.2. Preparation of Tamoxifen citrate niosomes

TMC niosomes were prepared as previously described, using the thin lipid film-hydration technique [19]. Briefly, 30 mg of TMC along with 300 mg of Span 60 and cholesterol in 1:1 Molar ratio were dissolved in chloroform: methanol (2:1) mixture in a round bottom pear like shaped round flask. The formed mixture was then evaporated under reduced pressure using IKA rotary evaporator (HBIO basic, RV10B S99, Germany) at 40 °C, until a thin lipid film was formed on the inner wall of flask and kept overnight drying at room temperature. The dried thin film was then hydrated with 30 ml phosphate buffer (PB) of pH 7.4 at 60 °C temperature (just above the glass transition temperature of the Span 60). The resultant niosomal dispersion was sonicated using ultra-probe sonicator (UP50H, Teltow, Germany) for 3 min, followed by centrifugation using bench top high speed refrigerated centrifuge (Bio Lion, XC-HR20, Manassas, USA) at speed 20,000 rcf at temperature 4 °C for 1 h to separate the un-entrapped from entrapped drug. The collected niosomal pellets were washed three times with PB to ensure removal of the un-entrapped TMC and finally re-suspended again in the buffer to form niosomal suspension containing only the entrapped TMC.

2.3. Preparation of Tamoxifen citrate niosomal thermosensitive gels

Different TMC niosomal thermosensitive gel bases were prepared by simple cold method technique as reported previously [20], using various concentrations and ratios of poloxamer 407 (P407) and/or poloxamer 188 (P188) as demonstrated in Table 1. In brief, the calculated amount of poloxamer(s) was/were added to 30 ml of the niosomal suspension in PB (equivalent to 30 mg of TMC) with continuous agitation at room temperature. The preparations were kept for overnight in refrigerator at 4°C for complete dissolution/hydration of poloxamer(s).

2.4. In vitro characterization of Tamoxifen citrate niosomal thermosensitive gels

2.4.1. Measurement of gelation temperature ($T_{sol\text{-}gel}$ transition temperature)

Few milliliters of the formula were placed in a small transparent beaker containing a small magnetic bar and a digital thermometer was immersed in the poloxamer(s) solution. The beaker was placed in controlled thermostat water bath at low temperature (4 °C) and then heated gradually at slow heating rate with a continuous stirring at 30 rpm. Once the magnetic bar stopped moving, the temperature displayed on digital thermometer was considered as gelation temperature. Three independent measurements were taken for each sample and results are represented as mean ± standard deviation [21].

2.4.2. Rheological behavior using piezoelectric axial vibrator (PAV)

Dynamic visco-elastic parameters of the prepared hydrogels were determined using Piezoelectric Axial Vibrator (Institute for Dynamic-mechanical Material Testing IdM at Ulm University, Germany). Samples were carefully applied between two stainless steel plates of 10 mm radius; axial vibration driven lower plate and temperature driven upper plate [22]. Vibration are driven by piezoelectric actuator material such as Ceramics or Quartz crystals which convert the very high electric voltage into vibration with a vibration distance between two plates ranging from 9 up to 89 μm.