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Temoporfin-loaded 1-tetradecanol-based thermoresponsive solid lipid nanoparticles for photodynamic therapy

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ABSTRACT

We developed fully biodegradable/metabolizable nanosystem based on polymer surfactant-stabilized thermoresponsive solid lipid nanoparticles with non-covalently bound photosensitizer temoporfin (T-SLNP) with particle size below 50 nm. The efficacy of T-SLNP was compared with commercial temoporfin formulation in terms of *in vitro* phototoxicity in 4T1 (murine mammary carcinoma) and MDA-MB-231(human breast adenocarcinoma) cells and of *in vivo* anticancer effect in Nu/Nu mice bearing MDA-MB-231 tumors. *In vitro* study demonstrated faster accumulation kinetics in the cells for our formulation design resulting in higher phototoxicity against the tumor cells. *In vivo* anticancer efficacy was markedly improved by T-SLNP compared with commercial temoporfin formulation. Owing to controlled and sustained release properties, subcellular size, biocompatibility with tissue and cells, the T-SLNP nanodispersion prepared in this study represents promising drug delivery system applicable in cancer treatment.

1. Introduction

Photodynamic therapy (PDT) is widely studied and used for the treatment of cancer and consists in an application of photosensitizer followed by laser irradiation of tumor lesions [1–6]. Photosensitizer after irradiation with light activates the oxygen present in tissues to singlet oxygen, which is highly destructive due to damage of important biomolecules such as proteins, lipids, DNA and nearby cell membranes. Temoporfin (Foscan®) is a hydrophobic porphyrin derivative approved as the photosensitizer for photodynamic therapy [7] of squamous cell carcinoma of the head and neck. Temoporfin is transported in the blood, adsorbed onto blood proteins and subsequently is translocated into lipoproteins [8].

The efficacy of photodynamic therapy is often limited by insufficient accumulation of photosensitizers in tumor tissue and unfavorable retention in the skin leading to whole body surface photosensitization of the patients [9]. Therefore, delivery of photosensitizers specific just for tumor tissue is still a challenging and desirable goal.

Liposomes were among the first nanocarriers proposed for drug formulation in medicine [10]. In order to improve temoporfin bioavailability and efficacy, and to reduce side effects, two liposomal forms of temoporfin were introduced: conventional liposomes (Foslip®; Biolitec Research GmbH), and poly(ethylene oxide)-coated liposomes (Fospeg®; Biolitec Research GmbH). Grafting of poly(ethylene oxide) to the surface of liposomes serves to inhibit their recognition and uptake by the reticuloendothelial system (RES), which is a major disadvantage of conventional liposomes. The temoporfin liposomal formulation Fospeg® exhibits higher photodynamic efficacy then Foslip® in tumor-grafted mice due to the enhanced EPR-based accumulation in

Abbreviations: ^{1}H NMR, ^{1}H-nuclear magnetic resonance spectroscopy; ATCC, American Type Culture Collection; b.p., boiling point; D_H, hydrodynamic diameter (nm); DL, drug loading (%); DLS, dynamic light scattering; DSC,, differential scanning calorimeter; EDTA, ethylenediaminetetraacetic acid; EE, entrapment efficiency (%); FCS, fetal calf serum; GPC, gel permeation chromatography; IC_{50}, half maximal inhibitory concentration; LDL, low density lipoproteins; m.p., melting point; MPEO, poly (ethylene oxide) monomethyl ether; NP, nanoparticle; PBS, phosphate buffer saline; PCL, ε-caprolactone; PDI, polydispersity index; PDT, photodynamic therapy; PEO, poly(ethylene oxide); PEO_X-*b*-PCL_Y, copolymer poly(ethylene oxide)-*block*-poly(ε-caprolactone); SD, standard deviation; SLNP, solid lipid nanoparticles; SLNP A, solid lipid nanoparticles with surfactant A (PEO_{45}-*b*-PCL_{34}); SLNP B, solid lipid nanoparticles with surfactant B (PEO_{45}-*b*-PCL_{17}); SLNP C, solid lipid nanoparticles with surfactant C (PEO_{45}-*b*-PCL_7); surfactant A, PEO_{45}-*b*-PCL_{34}; surfactant B, PEO_{45}-*b*-PCL_{17}; surfactant C, PEO_{45}-*b*-PCL_7; TEM, transmission electron microscopy; TGI, tumor growth inhibition; T-orig., original Foscan® formulation containing 4-mg/mL temoporfin with ethanol (solvent) and propane-1,2-diol (co-solvent); T-SLNP, temoporfin-loaded solid lipid nanoparticles; T-SLNP A, temoporfin-loaded solid lipid nanoparticles with surfactant A (PEO_{45}-*b*-PCL_{34}); T-SLNP B, temoporfin-loaded solid lipid nanoparticles with surfactant B (PEO_{45}-*b*-PCL_{17}); T-SLNP C, temoporfin-loaded solid lipid nanoparticles with surfactant C (PEO_{45}-*b*-PCL_7); UV-VIS, ultraviolet-visible spectroscopy; ZP, zeta potential.

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tumor cells resulting in improved stability in the blood circulation and photosensitizer release properties [11].

In 2013, V. Reshetov et al. [12] presented a comparison of conventional (Foslip®) and poly(ethylene oxide)-modified liposomal (Fospeg®) formulations of temoporfin in tumor-grafted mice. They showed the correlation between a set of parameters within one model – drug pharmacokinetics, drug release properties, liposome stability, uptake into tumor intratumoral distribution and the efficacy of PDT treatment at different drug–light intervals (DLIs) of Foslip® and Fospeg®. They used female $NMRI^{nu/nu}$ mice with exponentially growing HT29 human colon adenocarcinoma cells. Foslip® or Fospeg® was administered intravenously at a dose of 0.15 mg/kg of temoporfin. If we compare the efficacy of PDT treatment, tumor response to Foslip®–PDT was maximal at DLIs of 15 h and 24 h, significantly higher than at DLIs 3 h and 6 h, respectively. The PDT 3 h was ineffective, with no statistical difference observed from the control group. Therefore, efficient EPR-based accumulation cannot be achieved with Foslip®. In case of Fospeg®, the PDT was more effective than Foslip® treatment at all other DLIs. However, the treatment at DLI of 3 h showed the efficacy similar to that of the treatment with Foslip® at 15 h. Surfactant-stabilized lipid nanoparticles (SLNP) represent emerging drug delivery concept with noncovalently bound drugs [13]. With appropriate selection of the surfactant and core material, it is possible to create a fully biodegradable/metabolizable formulation with low side effects and longer shelf-life than, *e.g.* liposomes. The SLNP may also be transported into tissues from bloodstream due to adhesion of apolipoproteins after which they mimic transport low - density lipoproteins (LDL) [14,15]. The receptors for LDL are overexpressed on the surface of numerous tumor cells [16,17] leading to tumor targeting. In the same time, when nanoparticles of suitable size (less than *ca.* 200 nm) are used, it is possible to exploit the Enhanced Permeation and Retention (EPR) effect [18], *i.e.* the solid tumor accumulation of macromolecular objects caused by enhanced permeability of the tumor neovasculature for macromolecules and nanoparticles and also by the poor or missing lymphatic drainage in tumor tissue. Surprisingly, only one model lipid-based system was proposed for temoporfin so far. Authors prepared lipid nanoparticles composed of a lipid core (soybean oil and SuppocireNB (as a waxy ingredient)), stabilized by phospholipids (lipoid s75) and pegylated by surfactants (Myrj s40) in an aqueous phase (sterile phosphate buffer saline) [19].

In this study, we describe the SLNP-based temoporfin delivery system for photodynamic therapy with a core composed of lipid – fatty alcohol 1-tetradecanol. 1-Tetradecanol was newly used due to its melting point (35–39 °C) and also because it is more polar than, *e.g.* triacylglycerols, so it better solubilizes temoporfin. In addition, it is fully metabolizable *via* tetradecanoic acid by β-oxidation to acetyl-CoA and Krebs cycle to CO_2 and H_2O [20,21]. Nontoxic polymeric surfactant copolymer poly(ethylene oxide-*block*-ε-caprolactone) (PEO_X-*b*-PCL_Y) was utilized to stabilize the nanoparticles and provide them PEO coating to avoid entrapment into reticuloendothelial system. The surfactant itself is also degraded to 6-hydroxyhexanoic acid and poly(ethylene oxide), which are both eliminable by kidneys due to relatively low molecular weight below renal threshold [22]. The SLNP are solid and stable against aggregation at the storage temperature of 4 °C. The core melting temperature is set to 38–40 °C to release temoporfin in a controlled way in tumor tissue, which is, due to intensive metabolism, hyperthermic (analogy with thermoresponsive liposomes), and melts the SLNP to liquid surfactant-stabilized droplets. We have shown that our system has remarkable advantages compared to the published delivery systems for temoporfin.

2. Materials and methods

2.1. Materials

1-Tetradecanol (PubChem CID: 8209); dichloromethane (PubChem CID: 6344);methanol (PubChem CID: 887); phosphate buffered saline (PubChem CID: 24978514);phosphate buffered saline tablet; trypsin-EDTA (ethylendiamino-tetraacetic acid) - (1 × 0.05% trypsin, 0.02% EDTA without Phenol Red);RPMI 1640 medium (supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 units/mL), streptomycin (0.1 mg/mL), human serum (from human male AB plasma) and L-glutamine (2 mM) were obtained from Sigma-Aldrich Ltd. (Czech Republic). Cell lines 4T1 (murine mammary carcinoma) and MDA-MB-231 (human breast adenocarcinoma) were purchased from American Tissue Culture Collection (ATCC®, Manassas, Virginia). Foscan® (5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin) was purchased from Advanced Technology Industrial Co., Ltd. (Hong Kong). Temoporfin has been synthesized according to ref. [23,24], purity of the synthesized temoporfin was >98% (HPLC), ^{1}H NMR (600 MHz, DMSO-d_6) $\delta = -1.64$ (s, 2H), 4.14 (s, 4H), 7.08 (m, 2H), 7.15 (m, 2H), 7.26 (m, 2H), 7.29 (m, 2H), 7.45 (m, 2H), 7.50 (m, 6H), 8.25 (d, 2H), 8.40 (s, 2H), 8.65 (d, 2H), 9.79 (s, 2H), 9.84 (s, 2H); MS-ESI$^+$ 681 M + H$^+$.

2.2. Synthesis copolymer poly (ethylene oxide)-block-poly (ε-caprolactone)

Synthesis of copolymer poly(ethylene oxide)-*block*-poly(ε-caprolactone) copolymers(PEO_X-*b*-PCL_Y) based on catalyst-free ring-opening polymerization of ε-caprolactone was initiated by poly(ethylene oxide) monomethyl ether(MPEO, degree of polymerization = 45) [25]. The^{1}H-nuclear magnetic resonance spectroscopy(^{1}H NMR) based ratio of monomer units in PEO_X-*b*-PCL_Y copolymers, prepared by variation of amount of PCL monomer in the reaction mixture, was calculated according to Eq. (1), where DP(PCL) is a degree of polymerization of the poly(ε-caprolactone) block, DP(PEO) is a degree of polymerization of the poly(ethylene oxide) block and *a* and *c* are integrated signals of the corresponding protons (see Fig. 1). Details of the obtained products are summarized in the Table 1.

The general procedure for PEO_{45}-*b*-PCL_{34} synthesis is fully described below, the others PEO_{45}-*b*-PCL_{17} and PEO_{45}-*b*-PCL_7have been prepared in analogy. In a typical procedure 3 g (1.49 mM, 1 eq) of MPEO was placed into 50 mL flask equipped with magnetic stirrer. The system was degassed with 5 vacuum/argon cycles. Under argon, 3.4 g (30 mM, 20 eq) of PCL was added. The mixture was cooled with dry ice/ethanol bath, evacuated and heated to 185 °C. Ring-opening polymerization was carried out for 33 h. After polymerization, the system was let cool down to the room temperature. The solid in the flask was dissolved in 10 mL of dichloromethane and precipitated in 350 mL of diethylether. After drying, the polymer was suspended in water and dialyzed (MWCO 2 kDa, Spectra/Por® membrane) against water and freeze-dried. Lyophilized product (2.99 g; 47% yield) was characterized by gel permeation chromatography (GPC) with results: $M_w = 12{,}100$; $M_n = 8600$; and PDI = 1.41. ^{1}H NMR based composition: PEO_{45}-*b*-PCL_{34}, $M_n = 5900$.

$$DP(PCL) = DP(PEO) * \frac{c}{a} \quad (1)$$

2.3. Preparation of solid lipid nanoparticles

Temoporfin-loaded solid lipid nanoparticles (T-SLNP) were prepared according to procedures describing preparation of other types of solid lipid nanoparticles [26–29] with some modifications by the following high-performance hot homogenization and ultrasonication method. Briefly, 1-tetradecanol (250 or 50 mg, respectively) was transferred to a glass vial and melted by heating at 45 °C. Temoporfin (50 or 100 mg, respectively) was dissolved in dichloromethane (1 mL) and was added to 1-tetradecanol to obtain a drug-lipid mixture after evaporation of dichloromethane. The aqueous phase was obtained by dissolving the stabilizer PEO_X-*b*-PCL_Y (50 or 250 mg, respectively) in dichloromethane