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Research paper

A new nanostructured carrier design including oil to enhance the pharmaceutical properties of retinoid therapy and its therapeutic effects on chemo-resistant ovarian cancer

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ABSTRACT

All-trans retinoic acid (ATRA) is an appealing alternative drug for the cancers that have failed the conventional chemotherapy and become chemo-resistant and more tumorigenic. In this study, we specifically addressed two issues commonly associated with ATRA nanotherapeutics: (1) insufficient, unstable entrapment and uncontrolled release of the highly lipophilic ATRA and (2) lack of studies in therapeutically relevant chemo-resistant cancer cell models. A polymer-oil nanostructured carrier (PONC) composed of oil and PLGA was designed and studied in an ovarian cancer cell subline SKOV-3_{PR} that could withstand up to 300 nM paclitaxel and expressed high levels of multidrug resistance transporter ABCB1 and tumorigenic marker CD133. Differential scanning calorimetry of PONC revealed superior polymer amorphosity and dispersion of the entrapped ATRA in a manner comparable to nanostructured lipid carriers. With this design, the ATRA encapsulation efficiency was increased up to 8.5-fold and a 5-day controlled release profile was obtained. ATRA-PONC was able to induce extensive apoptotic cell death and exert substantially higher long-term anti-tumorigenic effects (IC_{50} of ATRA-PONC: 2 μg/ml *versus* free ATRA: 17.5 μg/ml; $p < 0.05$) in SKOV-3_{PR} cells. Mechanistic studies indicated that these enhanced anticancer effects were likely attributable to higher cell permeation by the well-dispersed drug/oil steadily released from PONC. To conclude, a nanostructured, oil-in-polymer hybrid carrier design has been developed for efficient ATRA delivery and treatment of the chemo-exposed, chemo-resistant sub-population of ovarian cancer, exemplifying a convenient strategy to vastly improve the pharmaceutical and therapeutic properties of tough-to-deliver lipophilic, poorly water-soluble anticancer compounds.

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1. Introduction

Solid malignant diseases such as ovarian cancer frequently acquire chemo-resistance after exposing to the conventional cytotoxic chemotherapy. 30% Ovarian cancer treated with the standard taxane/platinum-based chemotherapy failed to respond, and the majority recurred over time and became refractory to further chemo-treatment [1,2]. In view of these issues, researchers in recent years have begun to focus on the less cytotoxic drugs as alternative therapies for chemo-resistant cancers. All-*trans*-retinoic acid (ATRA) is one of these alternatives [3,4]. Instead of relying heavily on inducing cytotoxicity in the cycling cancer cells, ATRA controls cancer progression with its unique cell-differentiating, anti-proliferative and apoptosis-inducing activities mediated by the retinoic acid receptors and retinoid X receptors [5]. This drug is therefore officially approved for the treatment of acute promylocytic leukemia, a subtype of blood cancer [5,6]. However, repurposing ATRA for non-blood, solid malignancies remains a daunting task. Systemic delivery of ATRA to these cancers is inefficient which leads to fre-

Abbreviations: ABC, ATP-binding cassette; ATRA, all-trans-retinoic acid; BSA, bovine serum albumin; DSC, differential scanning calorimetry; EE, encapsulation efficiency; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic) acid; PLGA-np, poly(lactic-co-glycolic) acid nanoparticles; PONC, polymer-oil nanostructured carriers; PVA, polyvinyl alcohol; TEM, transmission electron microscope.

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quent side effects [5,7–9]. There is clearly an unmet clinical need for improved delivery of this valuable alternative anticancer drug.

Delivery of ATRA is mainly limited by its poor physico-chemical properties (e.g. high lipophilicity: log$P$ = 6.3 and low aqueous solubility: 29 μg/ml) [7,8,10] and unfavorable pharmacokinetic behaviors (e.g. non-specific binding: serum binding > 95%, V_D > 100 L; short half-life: $t_{1/2}$ = 0.5–2 h) [11]. To improve these aspects, nanosystems of ATRA such as liposomes, micro/nano-emulsion and solid lipid nanoparticles were developed with most of them demonstrating promising anticancer activities [7,11–13]. However, two key issues still need to be addressed. First, there remain challenges in designing a nanosystem to optimally deal with the issues related to the high lipophilicity of ATRA molecules. For instance, loading of ATRA in nanosystems is often inadequate and unstable. Many nanosystems reported had ATRA payloads at around 1–2% by weight or below. Moreover, we noticed in our previous study that even after apparently successful loading, the poorly-soluble ATRA molecules tended to deposit on or near the surface of polymeric nanocarriers instead of being actually entrapped, and this led to significant burst releases of ATRA once in aqueous environment [14]. If not adequately solved, these issues will compromise the effectiveness, efficiency and safety of ATRA nanotherapeutics.

Second, considering the unique anticancer mechanisms of ATRA, it is foreseeable that ATRA nanoformulations will eventually be used as a second-line treatment or an adjunct for the patients who have failed the first-line chemotherapy. The chemo-exposed cancers in these patients have generally developed various chemo-resistance mechanisms [2,15,16]. For ovarian cancer, while the mechanisms of acquired chemoresistance are not completely known, many findings indicated that the ATP-binding cassette (ABC) drug efflux transporters such as ABCB1 (also known as P-glycoprotein or MDR1), ABCC2 (known as MRP2) and ABCG2 (known as BCRP) play a crucial role [2,15]. The expression of ABCB1 is particularly responsive to paclitaxel exposure [2,17,18]. However, in previous studies, ATRA nanosystems were mostly evaluated in normal cancer cell lines that are still chemo-sensitive. In other words, the true therapeutic value of ATRA nanotherapeutics has not been fully validated. The use of a chemo-exposed, chemo-resistant cell model is thus highly warranted.

The present study focuses on tackling the above two issues. Here we report the use of a new class of hybrid nanocarriers known as polymer-oil nanostructured carriers (PONC) that are designed for systemic delivery of ATRA. Fig. 1 presents the proposed oil-in-polymer design of PONC, in which the oil-soluble ATRA is well-dispersed together with the oil component within the polymeric matrix. We hypothesize that (1) the oil-in-polymer design of PONC will facilitate more efficient, stable entrapment and controlled release of ATRA when compared with the standard "solid core" polymeric nanoparticles and (2) being in a highly dispersed state in the presence of oil, the ATRA molecules in PONC can be easily utilized by the ovarian cancer cells that have been exposed and became resistant to paclitaxel treatment. Our overall goal is to study this original ATRA nanotherapeutic design with a more therapeutically relevant cell model, and understand the mechanistic basis of its enhanced anticancer effects.

2. Materials and methods

2.1. Materials

Poly(lactic-co-glycolic) acid (PLGA) (50:50, viscosity 0.4 dl/g, molecular weight 44 kDa, ester end cap) and Captex 200 (i.e. propylene glycol dicaprylate/dicaprate) were kindly donated from Purac (Gorinchem, Netherlands) and Abitec (Janesville, WI, USA),

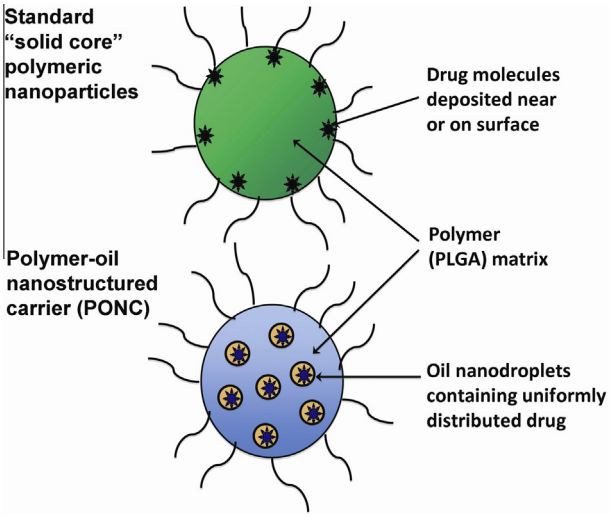

Fig. 1. Schemes comparing the designs of the standard polymeric nanoparticles (top) with polymer-oil nanostructured carriers (PONC, bottom). Green/blue color refers to polymeric component, yellow color refers to oil component, and the carrier surfaces are coated with polyethylene glycol or targeting moieties. It is hypothesized in the scheme that the oil-in-polymer design of PONC will increase the amorphosity of the polymer so the drug molecules can be entrapped in a more efficient and uniform manner. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

respectively. Polyethylene glycol–PLGA (PEG–PLGA, PEG (2000)–PLGA(4110)) was purchased from Polyscitech (West Lafayette, IN, USA). MK-571 was purchased from Santa Cruz Biotechnology (Dallas, TX, USA), CD133 antibody and blocking reagent from Miltenyi Biotec (Auburn, CA, USA), Spectra/Por dialysis membrane from Spectrum Labs (Gardena, CA, USA), bovine serum albumin (BSA, Fraction V) and polyvinyl alcohol (PVA, molecular weight 30–70 kDa, 87–90% hydrolyzed) from Fisher Scientific (Pittsburgh, PA, USA). ATRA, verapamil, Ko-143, amantadine, cytochalasin-D and other chemicals were purchased from Sigma Aldrich, Inc. (St Louis, MO, USA).

2.2. Cell culture

Human ovarian carcinoma SKOV-3 cells were purchased from American Type Culture Collection (Manassas, VA). The cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 50,000 units penicillin G, and 50,000 μg streptomycin at standard cell culture conditions (37 °C, humidified atmosphere of 5% CO_2). Cells were passaged every 4–6 days.

2.3. Preparation of PONC and control nanosystems

PONC were prepared using standard emulsification–solvent evaporation technique. In brief, ATRA and oil (Captex 200) were mixed in 0.3, 1.5 or 3.0 to 6.0 w/w ratio. In a typical preparation, the drug–oil phase containing 6 mg oil was diluted with 1 ml dichloromethane, and to this organic phase a mixture of 21.6 mg PLGA and 2.4 mg PEG–PLGA was dispersed. The final organic phase was added dropwise to 5 ml aqueous solution of 1.5%w/v PVA over a period of 2 min under constant stirring. The mixture was vortexed for 10–15 s and subjected to sonication for 6 min on ice (40 kHz, 120 V, Bransonic 3510, Danbury, CT). The emulsion formed was stirred overnight for solvent evaporation. The nanocarriers formed were separated from the aqueous phase by centrifugation at 15,000 rpm for 25–30 min. Unbound PVA and unencapsulated drug were washed away with 50 ml cold double-

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