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Tumor accumulation, degradation and pharmacokinetics of elastin-like polypeptides in nude mice

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Abstract

ELPs are genetically engineered, thermally responsive polypeptides that preferentially accumulate in solid tumors subjected to focused, mild hyperthermia. In this paper, we report the biodegradation, pharmacokinetics, tumor localization, and tumor spatial distribution of ^{14}C-labeled ELPs that were radiolabeled during their biosynthesis in *Escheriehia coli*. The in vitro degradation rate of a thermally responsive ^{14}C-labeled ELP1 ([^{14}C] ELP1) with a molecular weight of 59.4 kDa, upon exposure to murine serum, was 2.49 wt.%/day. The apparent in vivo degradation rate of ELP1 after intravenous injection of nude mice was 2.46 wt.%/day and its terminal half-life was 8.7 h. The tumor accumulation and spatial distribution of intravenously administered ELP1 and a control ELP that was designed to remain soluble in heated tumors (ELP2) were examined in both heated (41.5 °C) and unheated tumors. ELP1 accumulated at a significantly higher concentration in heated tumors than ELP1 in unheated tumors and ELP2 in heated tumors. Quantitative autoradiography of tumor sections provided similar tumor accumulation results as the whole tumor analysis but, in addition, showed that ELP1 had a more homogeneous distribution in heated tumors and a greater concentration in the tumor center than either control treatment.

Keywords: Drug delivery; Elastin-like polypeptide; Macromolecular drug carriers; Degradation; Pharmacokinetics; Tumor localization; Carbon-14

1. Introduction

The full potential of anticancer agents for the treatment of solid tumors has not yet been realized due to the difficulty of delivering therapeutic concentrations of these agents to the target site. Current anticancer therapies, such as radiotherapy and chemotherapy, are a double-edge sword, as they kill both tumor cells and normal cells. In order to limit normal tissue toxicity and maximize anti-tumor efficacy, it is essential to maximize delivery of therapeutically active drug to a tumor and at the same time, limit systemic exposure.

Macromolecular drug carriers [1–7] are a promising drug delivery technology that have been developed to accomplish this goal. Macromolecular drug carriers consist of high molecular weight (MW) molecules that are typically linked to a therapeutic agent through a stable or labile bond. Macromolecular carriers can target solid tumors either "passively," through a mechanism known as enhanced permeability and retention (EPR) effect [8–10], or "actively," due to the incorporation of a specific affinity or stimulus that targets the macromolecule-drug conjugate to the tumor [11–13]. In addition to the EPR effect, macromolecular drug carriers are attractive for drug delivery because they have longer plasma half-lives, reduced normal tissue toxicity, activity against multiple drug-resistant cell lines and the ability to increase the solubility of poorly soluble drugs [7,11]. These attributes often result in a higher anticancer efficacy for macromolecular therapeutics as compared to low molecular weight drugs [7,11].

Mild hyperthermia treatments may also improve tumor therapy and drug delivery. For example, hyperthermia has been shown to synergistically enhance tumor cytotoxicity when combined with

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chemo- or radio-therapy in clinical studies [14–16]. Furthermore, hyperthermia preferentially increases the permeability of tumor vasculature compared to normal vasculature [17–19] and enhances perfusion, which can further improve the delivery of drugs to a tumor [20–24]. In previous studies, we have developed a new thermal targeting approach using a thermally responsive elastin-like polypeptide (ELP) drug carrier that takes advantage of three components to enhance the accumulation of cancer drugs in solid tumors: (1) passive tumor targeting of tumors by macromolecules; (2) the enhanced vascular permeability and perfusion afforded by hyperthermia; and (3) the thermally triggered soluble-insoluble phase transition of a genetically engineered ELP [25].

ELPs belong to a unique class of biopolymers that undergo an inverse temperature phase transition (also called a lower critical solution temperature [LCST] transition); they are soluble at temperatures below their transition temperature but become insoluble and aggregate at temperatures above their T_t [26–28]. ELPs are composed of a Val-Pro-Gly-Xaa-Gly pentapeptide repeat (where the "guest residue" Xaa is any amino acid except Pro) derived from a structural motif found in mammalian elastin [29,30]. The inverse temperature transition is fully reversible, such that the aggregated ELP becomes soluble when the temperature is decreased below its T_t. T_t can be tuned by adjusting the guest residue identity, MW and ELP concentration [31,32].

The ELP drug delivery strategy that we have previously investigated was to heat the tumor to ~42 °C [25] with externally focused hyperthermia followed by intravenous administration of the ELP conjugated to an anticancer drug. We designed the ELP to have a T_t of ~40 °C by selecting the appropriate guest residue composition, MW and target plasma concentration [25]. Therefore, the ELP was designed to be highly soluble in systemic circulation as its T_t is greater than normal body temperature (T_n=37 °C) but upon entering the hyperthermic tumor vasculature, the ELP was designed to undergo its phase transition ($T_n < T_t < T_h$) and accumulate in the tumor. We have previously shown that a thermally responsive ELP exhibited a 2-fold greater accumulation in heated tumors [25] compared to the same ELP in unheated tumors.

We have also previously developed a method for incorporating ^{14}C into the backbone of ELPs during their expression from a plasmid-borne gene in *Escheriehia coli* [33]. A primary motivation for developing this radiolabeling method was that the degradation of the ELP itself can be quantified after in vivo administration, unlike polypeptides that are labeled by conjugation to a radiolabeled prosthetic group. Biodegradation is a primary consideration in the development of drug delivery systems, because in vivo degradation of a drug carrier should be sufficiently slow to allow it to accumulate in the target site, while being rapid enough to be ultimately cleared from systemic circulation and improve its biocompatibility [34,35]. Furthermore, similar to the use of radiolabeled conjugates of polymer drug carriers, this intrinsic labeling method also permits the biodistribution to be quantified using beta-counting and allows visualization of the intratumoral spatial distribution by autoradiography.

In this study, we report the degradation, pharmacokinetics, tumor localization, and tumor spatial distribution of a thermally responsive ^{14}C-labeled ELP1 ([^{14}C] ELP1) and the tumor accumulation and spatial distribution of a control ELP (ELP2) that is designed to remain soluble in tumors that are heated to 42 °C. These data provide a complete picture of the behavior of a thermally responsive biopolymer in vivo, and thereby provide a rational strategy for exploiting this class of macromolecular carriers for the delivery of cancer therapeutics to solid tumors.

2. Materials and methods

2.1. Synthesis of [^{14}C] ELP

The ELPs were homogeneously labeled with ^{14}C by inducing ELP expression from *E. coli* bearing a plasmid-borne ELP gene in modified M9 medium spiked with [U-^{14}C]-D-glucose (264 mCi/mmol; Moravek Biochemicals, Brea, CA) and unlabeled glucose as the sole carbon source [33]. Two different ELPs were used in these studies and the T_t of each ELP was controlled by the identity of the guest residue [36]. ELP1 was designed to be the thermally sensitive carrier with a T_t of ~40 °C by incorporation of Val, Ala and Gly residues in a 5:2:3 ratio at the fourth, guest residue position. ELP2 was designed to have a T_t significantly greater than the hyperthermic tumor temperature of 41.5 °C (T_t=~56 °C) by incorporation of Val, Ala, and Gly in a 1:8:7 ratio at the guest residue position. Briefly, the *E. coli* strain BLR(DE3) (Novagen) harboring a gene encoding ELP1 (MW=59.4 kDa) and ELP2 (MW=61.1 kDa) in a modified pET-25b expression plasmid (Novagen, Madison, WI, USA) were grown in 125 ml M9 medium in 500-ml Erlenmeyer flasks at 37 °C with aeration by shaking at 300 rpm. Bacterial growth was determined by monitoring the optical density (OD) at 600 nm [37]. M9 media consisted of 6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 1 g/L NH_4Cl, 0.5 g/L NaCl, 4g/L glucose and 100 mg/L ampicilin and supplemented with 1 mg/L thiamine, 1 mM $MgSO_4$, 0.3 mM $CaCl_2$, and trace elements (TE) consisting of 0.016 mg/L $MnCl_2$, 8.3 mg/L $FeCl_3$, 0.84 mg/L $ZnCl_2$, 0.13 mg/L $CuCl_2$, 0.1 mg/L H_3BO_3 and 0.1 mg/L $CoSO_4$. In preliminary experiments, we found that a specific ELP radioactivity of 0.2 μCi/mg ELP was sufficient to produce a signal above the detection limit of the beta counter (1214 Rackbeta, LKB/Wallac, Gaithersburg, MD) while mitigating safety concerns about the in vivo experiments. Because the specific radioactivity of [^{14}C] ELP was dependent on the ratio of [^{14}C] glucose to unlabeled glucose in M9 medium during ELP expression, the percentage of [^{14}C] glucose in M9 medium was adjusted to yield the desired specific ELP radioactivity [33]. When the OD_{600} of the *E. coli* culture reached 2.0 (typically about 5 h after inoculation), expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM. The ELPs were purified from other *E. coli* proteins in the soluble fraction of cell lysate by inverse transition cycling (ITC) [38,39]. Typically, three rounds of ITC (warm centrifugation, pellet resuspension, and subsequent cold centrifugation) were sequentially performed to purify the ELP.

2.2. Characterization of [^{14}C] ELP

The thermal properties of ELPs were measured by heating a 25-μM ELP solution (in PBS supplemented with 1 mM BSA as