Camptothecin delivery systems: enhanced efficacy and tumor accumulation of camptothecin following its conjugation to polyethylene glycol via a glycine linker

Abstract

Purpose: This study was designed to assess the circulatory retention, antitumor activity and tissue biodistribution of polyethylene glycol (PEG)-conjugated camptothecin-20-O-glycinate, PEG-β-camptothecin (PEG-β-CPT). PEG-β-CPT is a novel water-soluble transport form (macromolecular prodrug) of the naturally derived antitumor drug, 20-(S)-camptothecin (CPT).

Methods: Circulatory retention studies were performed in nontumor-bearing mice injected intravenously (i.v.) with 875 mg/kg of PEG-β-CPT. Antitumor activity was evaluated both intraperitoneally (i.p.) and i.v. in nude mouse xenograft models. Biodistribution studies were performed in nude mice bearing colorectal carcinoma xenografts with tritium-labelled PEG-β-CPT and CPT injected i.v.

Results: PEG-β-CPT had a blood t1/2a of approximately 6 min and a t1/2b of 10.2 h. Significant antitumor activity was seen in all treated xenograft models. Biodistribution studies demonstrated that PEG-β-CPT in saline provided more available labelled CPT in the circulation than unconjugated CPT dissolved in intralipid. In addition, it appeared that more labelled CPT accumulated in solid tumors when delivered in the PEG-β-CPT form, with greater preference for tumor tissue than normal tissue.

Conclusion: This soluble transport form of CPT and its underlying technology may have clinical application especially for the treatment of solid tumors.

Key words: Xenograft · Polyethylene glycol · Camptothecin · Prodrug · Biodistribution

Introduction

Camptothecin (CPT), an extract from the Chinese tree Camptotheca acuminata, has shown significant antitumor activity in nude mice bearing human lung, ovarian, breast, pancreas and stomach cancers [12]. Mechanistically, CPT stabilizes a topoisomerase I-induced single-strand break in the phosphodiester backbone of DNA, preventing subsequent religation of the broken single strand. During replication, when the advancing fork encounters the covalent complex it results in a double-strand DNA break, which if left unrepaired, results in apoptosis. CPT, however, is extremely insoluble which has severely restricted its clinical application and has led many investigators to pursue water-soluble analogs [8, 20, 27]. However, a great deal of interest in pursuing water-insoluble CPT congeners still remains, because of their reported superior antitumor activity against in vitro human cancers and in vivo animal xenografts [24].

These two divergent issues can be resolved by employing a macromolecular prodrug strategy or, perhaps more accurately, a transport form [3, 13] of CPT. Prodrugs are temporary chemical modifications of the parent drug which are devised to enhance its aqueous solubility and biodistribution, while keeping its inherent pharmacological properties intact [23]. These transport forms are designed to be cleaved in vivo, in a predictable fashion, to the active drug by either an enzymatic mechanism or simple hydrolysis initiated under physiological pH conditions [31].

It has previously been reported that CPT can be solubilized as a nonionic α-alkoxyester conjugated to nonimmunogenic polyethylene glycol (PEG)40kDa [15]. Fortuitously, it has been found that modifying CPT at the 20 position as a PEG ester stabilizes the active lactone ring (essential for activity) under physiological conditions [15]. This 20-camptothecin PEG40kDa ester (PEG-α-CPT) has been shown to hydrolyze in the blood gradually releasing the active ingredient CPT [15]. The solubility in water of CPT in the PEG-α-CPT transport
form of minimally 2 mg/ml is dramatically greater than that of CPT (0.025 mg/ml) [20]. PEG-α-CPT exhibits a blood t₁/₂b of approximately 4 min and a t₁/₂a of 3.5 h with significant antitumor activity [5]. However, PEG-α-CPT is a heterogeneous mixture of mono- and disubstituted ester prodrugs and therefore not clinically suitable. A new homogeneous form of disubstituted CPT has been achieved by employing a bifunctional spacer group (glycine) in the PEG prodrug strategy that yields a watersoluble nonionic α-amidoester prodrug, PEG-β-CPT (Fig. 1). In vitro P388/0 cell toxicity for PEG-β-CPT (IC₅₀ 12 nM) is in the expected range for a prodrug that releases CPT (IC₅₀ 7 nM) [16]. The in vitro half-life of hydrolysis of PEG-β-CPT to CPT at 37 °C is 40 h in pH 7.4 phosphate buffer and 6 h in rat plasma [16]. The objective of the current study was to assess in vivo circulatory retention, antitumor activity and tissue biodistribution of this new PEG-conjugated camptothecin-20-O-glycinate, PEG-β-CPT.

Materials and methods

Materials

PEG-β-CPT (camptothecin-di-20-O-ester of PEG₄₀kDa glycine) was produced as described previously [16]. Radiolabelled PEG-β-[³H]CPT was prepared using ¹²⁵I-labelled CPT (Moravec Biochem. Brea, Calif.) and PEG₄₀kDa glycine acid synthesized according to a previously published procedure for PEG₅kDa glycine acid [14]. ³H-CPT itself is expected to be relatively stable in biological systems [18]. Topotecan was synthesized according to published procedures [20]. For in vivo administration, 20-(S)-CPT was dispersed in intralipid (Liposyn III 10%, Abbott Laboratories, North Chicago, Ill.) by sonication. CPT was found to be stable in the intralipid vehicle as verified by high-performance liquid chromatography. 5-Fluorouracil (5-FU; Aldrich Chem. Co., Milwaukee, Wis.), formulated as previously prescribed [11], was used as a clinically relevant control for the xenograft experiments. All PEG-β-CPT dosages were dissolved in sterile water for injection (WFI) or physiological saline (0.9% NaCl) prior to in vivo drug treatments. With the exception of the circulatory retention study, all PEG-β-CPT dosages were given as their CPT equivalents (absolute amount of CPT given).

Circulatory retention

PEG-β-CPT circulatory retention studies were performed in 25 g nontumor-bearing CD1 female mice (Charles River Laboratories, Stone Ridge, N.Y.). Mice received an intravenous (i.v.) bolus of 875 mg/kg of PEG-β-CPT (14 mg/kg CPT equivalents) in saline via the tail vein and were exsanguinated over a 24-h period (0.05, 0.25, 2, 4, 6, 8 and 24 h) with three mice per time-point. Exsanguination was conducted in animals rendered unconscious in 100% CO₂ via orbital bleeding into a sterile tube to remove a minimum of 1.0 ml whole blood. Blood samples were processed and assayed as previously described [5]. The pharmacokinetics of injected PEG-β-CPT and released CPT were calculated using a two-compartment i.v. bolus first-order elimination model (WinNonlin, Scientific Consulting, Apex, N.C.).

Cell lines

Antitumor activity

Antitumor activity studies of PEG-β-CPT, given i.p. to HT-29 tumor-bearing nude mice, were conducted as previously reported [5]. Briefly, when tumors reached an average volume of 150 mm³, the mice were divided into their experimental groups. Groups (n = 10) consisted of a control (untreated) group and groups treated with 5-FU, CPT, topotecan and a range of PEG-β-CPT dosages in WFI (1–4 mg/kg per day). Mice received CPT derivative treatments i.p. five times a week, Monday through Friday, for 5 weeks with 500 µl of test solution per treatment. Mice in the 5-FU treatment group were dosed (80 mg/kg) twice a week for 5 weeks. Mouse weight and tumor size were measured weekly over a 10-week period which spanned from tumor inoculation through post-treatment regrowth. The overall growth of tumors (%T/B) was calculated as the mean tumor volume at the end of the treatment minus the mean initial (pretreatment) tumor volume, divided by the initial tumor volume. Thus, any tumor group which did not respond to treatment and grew over the course of the experiment would display a positive percent change and treatment groups in which tumors regressed would exhibit a negative percent change.

Intravenous treatment with PEG-β-CPT against HT-29, SKOV3, A549 and LS174T were carried out in nude mice (seven or eight per group) bearing initial tumor volumes of approximately 110, 65, 100 and 65 mm³, respectively. PEG-β-CPT in saline was given as a single dose (15 mg/kg CPT equivalent) or three doses (5 mg/kg CPT equivalent on each of days 1, 5 and 9) and tumor volume and body weight were measured weekly for 5 weeks. Tumor growth inhibition (%T/C) was used to determine antitumor effectiveness [6]. Treatment and control groups were measured when the control group’s median tumor volume reached approximately 800–1100 mm³ (exponential growth phase). The differences between treatment groups were assessed by one-way ANOVA. Multiple comparisons, when significant differences existed, were determined by least significant differences techniques. Statistical significance was defined as P < 0.01 to reject a null hypothesis. Statistical analysis was conducted using the StatView software program (Abacus Concepts, Berkeley, Calif.).