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LDI–glycerol polyurethane implants exhibit controlled release of DB-67 and anti-tumor activity in vitro against malignant gliomas

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Abstract

The purpose of the present study was to develop a biodegradable and biocompatible polyurethane drug delivery system based on lysine diisocyanate (LDI) and glycerol for the controlled release of 7-*tert*-butyldimethylsilyl-10-hydroxy-camptothecin (DB-67). DB-67 has yet to be implemented in any clinical therapies due to the inability to delivered it in sufficient quantities to impact tumor growth and disease progression. To remedy this, DB-67 was covalently incorporated into our delivery system by way of an organometallic urethane catalyst and was found to be dispersed evenly throughout the LDI–glycerol polyurethane discs. Scanning electron micrographs indicate that the LDI–glycerol discs are uniform and possess a pore distribution typical of the non-solvent casting technique used to prepare them. The release rates of DB-67 from the LDI–glycerol discs were found to vary with both time and temperature and were shown capable of delivering therapeutic concentrations of DB-67 in vitro. Cellular proliferation assays demonstrate that empty LDI–glycerol discs alone do not significantly alter the growth of malignant human glioma cell lines (U87, T98G, LN229 and SG388). DB-67-loaded LDI–glycerol polyurethane discs were found to inhibit cellular proliferation by 50% on average in all the malignant glioma cell lines tested. These results clearly demonstrate the long-term, slow release of DB-67 from LDI–glycerol polyurethane discs and their potential for postoperative intracranial chemotherapy of cancers.

Keywords: Drug delivery; Polyurethane; Camptothecin; Glioma; Lysine diisocyanate

1. Introduction

Camptothecin (CPT) and its numerous synthetic analogs comprise a special class of anticancer agents active in lung, ovarian, breast, pancreas and stomach cancers previously reported resistant to chemotherapy [1–4]. CPT is a naturally occurring alkaloid that was initially isolated from the Chinese tree *Camptotheca acuminata* (Nyssaceae) by Wall and co-workers [5]. Unsuccessful results in three early Phase I studies lessened the interest in the drug for a number of years [6–8]. It was later shown that CPT inhibited the enzyme topoisomerase I, a nuclear protein essential for DNA repair during replication, and interest in the drug and its analogs was reborn [9,10]. Currently, two CPT analogs, topotecan and irinotecan, have been approved by the FDA, and at least 10 additional CPT derivatives are in various stages of clinical trials – including 9-amino-CPT in advanced clinical trials [3,4].

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The pharmaceutical development and clinical utility of the camptothecins are limited by the distinctive dynamics of these agents in the bloodstream. All of the camptothecins now in clinical development contain a α-hydroxy-δ-lactone moiety, and they exist in two distinct forms at the physiological pH of 7.0 and above. The biologically active "lactone-closed" form reacts with water to form a biologically inactive "lactone-opened" (carboxylate) form [11,12]. This simple chemical hydrolysis dynamically inactivates the parent drug. Furthering compounding the problem, the predominant human blood serum protein, albumin (HSA), preferentially binds the carboxylate form – shifting the equilibrium towards the inactive moiety [13–15]. Because camptothecins are S-phase specific drugs [16], optimal topoisomerase (topo) I inhibitory activity is only obtained when the tumors of a patient are continuously exposed to the drug. Accordingly, establishing conditions where a therapeutically relevant concentration of the lactone-closed form of a camptothecin is present over a suitable period for tumor cells to cycle through the S-phase is a major challenge.

The CPT analog, 7-*tert*-butyldimethylsilyl-10-hydroxycamptothecin (DB-67), was synthesized in an attempt to further enhance the therapeutic performance of this class of drugs [17–19]. DB-67 displays superior lactone stability in plasma relative to other camptothecin agents, likely due to its lipophilicity and reduced interactions with the carboxylate-binding site on serum albumin. The analog also possesses a high intrinsic potency against the topo I target enzyme with a unique DNA cleavage profile. Ultimately, the combination of its potency and stability profiles suggests that it may be more efficacious than the currently used FDA-approved CPT-based therapies. However, the compound is largely water-insoluble, making it difficult to deliver into the body through the conventional routes such as oral, intravenous or intramuscular injection [15]. As such, DB-67 has yet to be implemented into any clinical therapies due to an inability to be delivered in adequate quantities to impact tumor growth and disease progression.

Surgically implantable polymer matrices loaded with chemotherapeutic agents are a proven approach to localized drug delivery [20–22]. The matrix is loaded with the desired agent and then implanted within the tumor site, following as complete a surgical resection as clinically possible. The matrix releases its drug load over a period predetermined by the characteristics of the encapsulating polymer, delivering drug to neoplastic cells existing in the peritumoral region that otherwise would give rise to recurring tumor. Typically, these types of drug delivery systems have been created by dissolving drug in a polymer and processing it into the desired morphology. The drug remains physically entrapped in the polymer, but it is not anchored through chemical bonds – and a burst release of drug subsequently follows. CPTs have previously been delivered in this manner [22–25]. Because the degree of survival improvement with previous polymer-based local delivery approaches, although statistically significant, has been modest at best, there is a rationale to build on this strategy in order to further enhance therapeutic efficacy. Our goal was therefore to develop a synthetic, biodegradable matrix incorporating DB-67 through labile chemical bonds, resulting in controlled long-term delivery of the agent to tumor cells.

Our laboratory has developed a new generation of biocompatible polyurethanes composed of lysine diisocyanate (LDI) and glycerol that degrade into the non-toxic components – lysine, glycerol and CO_2 [26,27]. Only a handful of studies have reported the successful use of polyurethanes in drug delivery applications, and their favorable results support further examination of this approach [28–33]. Our peptide-based urethane possesses the versatility of commercial polyurethane systems, but lacks the toxicity associated with commercial urethane degradation products. The goal of this study was to develop a biodegradable polyurethane disc incorporating DB-67 through reactive isocyanate groups capable of controlled, long-term drug delivery to tumor cells through hydrolysis of urethane linkages. We hypothesized that it is possible to incorporate DB-67 into the backbone of such a polymer and impact cellular growth in vitro – the ultimate goal being long-term delivery of active compound over a period of months in vivo.

2. Materials and methods

2.1. Materials

Lysine diisocyanate methyl-ester (LDI) was purchased from Chemical Division, Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan). 7-*Tert*-butyldimethylsilyl-10-hydroxycamptothecin (DB-67) was obtained from Dr. Dennis Curran at the University of Pittsburgh Chemistry Department (Pittsburgh, PA). The human malignant glioma cell lines U87 and T98G were obtained from the American Type Tissue Culture Collection. LN229 was kindly provided by Dr. Nicolas de Tribolet at the University of Lausanne (Lausanne, Switzerland). The SG388 cell line was established at Children's Hospital of Pittsburgh from a tumor specimen identified by a neuropathologist. MTS reagents were obtained from Promega. Other chemicals were obtained from Sigma–Aldrich Chemical Co. (Milwaukee, WI) and were of reagent grade unless otherwise specified.

2.2. Reaction of DB-67 with LDI

Lysine methyl-ester diisocyanate (M_W 212, 4.43 mg, 0.021 mmol) was added to DB-67 (M_W 479, 10.0 mg, 0.021 mmol) in a 1:1 molar ratio and dissolved in 1 ml of tetrahydrofuran (THF) in a dry flask. Two samples were prepared and 10 μl of Sn(II)-2-ethylhexanoate (M_W 405, 1.251 mg, 0.031 mmol) was added to one sample. Samples were sealed and stirred in the dark at room temperature for 48 h. THF was then removed at 50 °C in a vacuum oven, and the resulting solid was incorporated into a potassium bromide window. Fourier transform infrared (FT IR)

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