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Tertiary amine mediated targeted therapy against metastatic lung cancer

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Tert-butyl 4-(chloromethyl) benzoate (PubChem CID: 18959012)
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Tetraethylammonium chloride (PubChem CID: 5946)
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ABSTRACT

In this work, two tertiary amine-derived 4′-demethylepipodophyllotoxin (DMEP) conjugates (DC and DP) have been designed and synthesized using *N,N,N′*-trimethyl-*N′*-(4-carboxyl benzyl)-1,3-propanediamine (CPDM) and 4-(4-methylpiperazinomethyl)benzoic acid (PBA) as the targeting ligands. Both DC and DP exhibited strong in vitro cytotoxicity against small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) cell lines. Cellular uptake efficiencies of DC and DP in human alveolar type II epithelial cells were significantly enhanced compared to DMEP and etoposide (VP-16), which were demonstrated to be concentration-, time- and energy-dependent. The active transport process of DC and DP might be mediated by organic cation transporters (OCTs). After systemic administration in mice, both DC and DP selectively accumulated in the lung, displaying the highest C_{max} and $AUC_{0\text{-}t}$ values of all tested tissues. Compared with DMEP and VP-16, DC and DP remarkably reduced the lung weight and the number of lung metastases of B16 melanoma in mice, and further prolonged the survival of tumor-bearing mice. Also, DC and DP exhibited comparable levels of cell cycle arrest and cell apoptosis. Furthermore, DC and DP demonstrated minimum toxicity towards vital organs and reduced gastrointestinal injury compared to DMEP and VP-16. Taken together, tertiary amine-derived moieties such as CPDM and PBA represent an efficient yet safe strategy to achieve lung-targeted drug delivery.

1. Introduction

Lung cancer remains one of the most commonly diagnosed cancers and the leading cause of cancer-related mortality in males worldwide [1,2]. Also, lung metastasis has been a common outcome for metastatic tumor progression [3,4]. Though surgery may effectively control tumor at the primary site, concurrent chemotherapy is necessary, especially for locally advanced lung cancer and lung metastasis cancer [5]. Despite improvements in chemotherapy over the years, 5-year survival after standard lung cancer treatment remains low with serious concomitant adverse effects, mainly due to off-target distributions [6–8]. Therefore, the development of targeted therapies to deliver chemotherapeutics specifically to the tumor site is highly demanded, which will likely result in enhanced therapeutic efficacy and reduced systemic toxicities.

Current targeted strategies to lung include size-driven delivery systems such as microparticles, nanoparticles and liposomes via systemic administration [9,10]. Although these micro- and nanoscale carriers show promising results, drawbacks such as complex structures, poor stability and toxicity issues remain unresolved [11]. Macromolecular carriers-based lung targeting strategies such as antibody- and peptide-drug conjugated systems have also been successfully developed [12–14], however, disadvantages including immunogenicity, insufficient potency, or linker stability issues have greatly limited their

Abbreviations: DMEP, 4′-demethylepipodophyllotoxin; VP-16, etoposide; CPDM, *N,N,N′*-trimethyl-*N′*-(4-carboxyl benzyl)-1,3-propanediamine; PBA, 4-(4-methylpiperazinomethyl)benzoic acid; DC, podophyllotoxin-CPDM conjugate; DP, podophyllotoxin-PBA conjugate; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; OCTs, organic cation transporters; pK_a, ionization constant; ESI, electrospray ionization; MRM, multiple reaction monitoring; logP, partition coefficient; PI, propidium iodide; TEA, tetraethylammonium; OCTN, novel organic cation transporter; PS, phosphatidylserine.

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application [15]. Thus, small molecule drug-ligand conjugates with well-defined structures, sufficient stability and high targeting efficiency may help overcome existing challenges.

Previously, our group reported that a phenolic propanediamine moiety modified rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) derivative acted as a potent anti-inflammatory compound, which significantly enhanced the pulmonary accumulation and showed a potent therapeutic effect against lung injury [16]. The propanediamine moiety is a linear moiety with two tertiary amines. Lung has been proven a site for accumulation and sequestration of numerous basic amines [17]. Thus, both small ligands with either a linear tertiary amine or a cyclic tertiary amine may benefit lung targeted drug delivery, which inspired us to explore the potential of tertiary amine-derived moieties as lung-targeted ligands for lung cancer and lung metastasis cancer therapy.

Podophyllotoxin, a well-known naturally occurring aryltetralin lignin, is a bioactive component extracted from the roots of *Podophyllum* species, which shows potent antitumor effects against various cancer cell lines by disrupting microtubule organization and inhibiting the formation of mitotic spindles [18]. However, the initial attempts to use podophyllotoxin in clinic failed due to its severe adverse reactions, including gastrointestinal toxicity and damages to normal tissues [19,20]. Nevertheless, podophyllotoxin has remained an important lead compound and numerous semisynthetic derivatives have been synthesized, which showed enhanced cytotoxicity against various tumor cells and reduced toxicity to vital organs [18,20,21]. Among them, etoposide (VP-16, Fig. 1B) is a C-4β-glucoside substituted derivative of 4′-demethylepipodophyllotoxin (DMEP, Fig. 1A), which act as an inhibitor of topoisomerase II, has been extensively used as a potent chemotherapeutic agent clinically [22], and shows a high response rate in patients with small cell lung carcinoma (SCLC) [23,24]. Despite significant clinical success against SCLC, VP-16 displays only minimal activity against non-small cell lung carcinoma (NSCLC) accounting for about 80% of lung cancers [25], possibly due to an insufficient distribution in the lung tissue [26,27]. Moreover, the lack of selectivity often leads to severe adverse effects such as myelosuppression and gastrointestinal toxicity [28]. Thus, the development of novel podophyllotoxin derivatives with improved lung-specificity is highly desirable to provide alternative treatment options with improved therapeutic efficacy and reduced systemic toxicity.

In this work, we selected DMEP as the model compound and VP-16 as the positive control for the following study. Structure-activity relationship demonstrates the critical role of N-linkage at C-4β site in the antitumor activity of podophyllotoxin derivatives [29–31]. Thus, we replaced the C-4β hydroxyl group of DMEP with an amino group. Next, we selected *N*,*N*,*N*′-trimethyl-*N*′-(4-carboxyl benzyl)-1,3-propanediamine (CPDM) and 4-(4-methylpiperazinomethyl)benzoic acid (PBA) moieties which possess two tertiary amines and a carboxyl group that could be easily coupled to the C-4β amino site of DMEP through an amide bond. DMEP-CPDM (DC, Fig. 1C) and DMEP-PBA (DP, Fig. 1D) conjugates were synthesized, and the stability and pK_a of DC and DP were characterized, respectively. Next, cytotoxicity of DC and DP against both SCLC and NSCLC cell lines were evaluated in vitro, and the tissue distribution study was performed in mice. Additionally, we evaluated the cellular uptake efficiency and the internalization pathways of DC and DP in human alveolar type II epithelial cells. A B16 melanoma lung metastasis mice model has been established to evaluate the antitumor effect of DC and DP in vivo.

2. Materials and methods

2.1. Materials

4′-Demethylepipodophyllotoxin was purchased from J&K Scientific Ltd. (Beijing, China). Etoposide and tert-butyl 4-(chloromethyl)

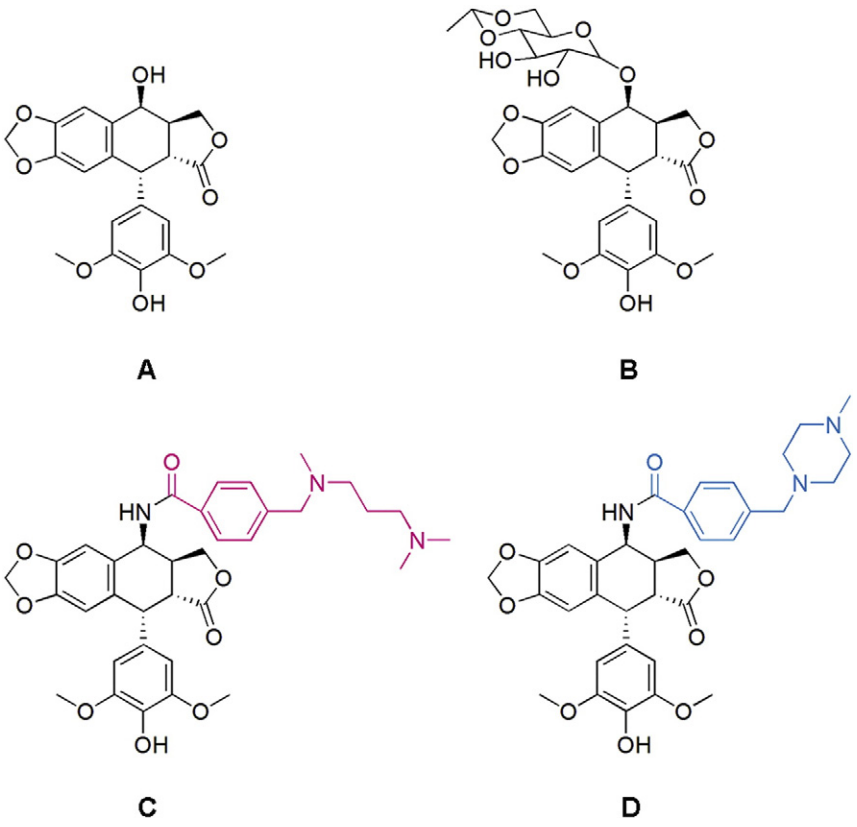

Fig. 1. Chemical structures of (A) DMEP, (B) VP-16, (C) DC and (D) DP.