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Metronomic chemotherapy using orally active carboplatin/deoxycholate complex to maintain drug concentration within a tolerable range for effective cancer management

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ABSTRACT

Metronomic chemotherapy has translated into favorable toxicity profile and capable of delaying tumor progression. Despite its promise, conventional injectable chemotherapeutics are not meaningful to use as metronomic due to the necessity of frequent administration for personalized therapy in long-term cancer treatments. This study aims to exploit the benefits of the oral application of carboplatin as metronomic therapy for non-small cell lung cancer (NSCLC). We developed an orally active carboplatin by physical complexation with a deoxycholic acid (DOCA). The X-ray diffraction (XRD) patterns showed the disappearance of crystalline peaks from carboplatin by forming the complex with DOCA. In vivo pharmacokinetic (PK) study confirmed the oral absorption of carboplatin/DOCA complex. The oral bioavailability of carboplatin/DOCA complex and native carboplatin were calculated as 24.33% and 1.16%, respectively, when a single 50 mg/kg oral dose was administered. Further findings of oral bioavailability during a low-dose daily administration of the complex (10 mg/kg) for 3 weeks were showed 19.17% at day-0, 30.27% at day-7, 26.77% at day-14, and 22.48% at day-21, demonstrating its potential for metronomic chemotherapy. The dose dependent antitumor effects of oral carboplatin were evaluated in SCC7 and A549 tumor xenograft mice. It was found that the oral carboplatin complex exhibited potent anti-tumor activity at 10 mg/kg (74.09% vs. control, $P < 0.01$) and 20 mg/kg dose (86.22% vs. control, $P < 0.01$) in A549 tumor. The number of TUNEL positive cells in the tumor sections was also significantly increased during oral therapy (3.95% in control, whereas 21.37% and 32.39% in 10 mg/kg and 20 mg/kg dose, respectively; $P < 0.001$). The enhanced anti-tumor efficacy of oral metronomic therapy was attributed with its antiangiogenic mechanism where new blood vessel formation was notably decreased. Finally, the safety of oral complex was confirmed by three weeks toxicity studies; there were no significant systemic or local abnormalities found in mice at 10 mg/kg daily oral dose. Our study thus describes an effective and safe oral formulation of carboplatin as a metronomic chemotherapy.

1. Introduction

Since its introduction, metronomic chemotherapy has gained interest in the major scientific community because of its non-toxic and inexpensive way of treatment that can target the tumor vasculature, cancer cells and immune system. Metronomic chemotherapy is characterized as the continuous administration of low amount of anticancer drugs, which is much lower than the usual maximum tolerated dose (MTD) based treatment [1,2]. The term, "metronomic therapy" was first introduced by Hanahan et al. and has been often considered as a low-dose antiangiogenic therapy [3]. Tumor vasculature could hence be another important target for cancer therapy, as first reported in 2000 by Judah Folkman and Robert Kerbel [4,5]. In general, the standard approach for cancer treatment relies on high payload of anticancer drugs usually with four to six cycles of therapy. Unfortunately, parenterally administered anticancer agents are often associated with amounts of drug that are high above the maximum tolerated concentration (MTC) in plasma immediately after its administration, thereby enhancing the drug induced adverse effects. Meanwhile, rapid elimination of anticancer

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drugs from the plasma leads to the poor prognosis in the treatment of various cancers [6]. On the other hand, orally administered anticancer drugs have become the most favorable towards metronomic therapy because it can reduce the side effects by maintaining the drug concentration below MTC level and may optimize the therapeutic efficacy by increasing the exposure time to cancer cells. Furthermore, oral metronomic chemotherapy can reduce the burden of having continuous infusion in clinics, thereby decreasing the overall treatment cost, and ultimately increasing the patients' quality of life [7].

Lung cancer has been documented as one of the most common types of malignancy that attributes to more than one million deaths per year worldwide [8]. Approximately, 85–90% of diagnosed lung cancers are reported as non-small cell lung cancer (NSCLC). A majority of lung cancer patients are often diagnosed with locally advanced or metastatic diseases (stage IIIB-IV), where surgical procedures are not operable. Tumor histology from NSCLC patients has revealed that heterogeneity in tumor mass is a crucial factor for determining tumor response to therapy. Since the discovery that genetic factors account for NSCLC, patients are routinely analyzed for phenotypic characteristics of this disease including epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) translocations [9,10]. Recently, Barlesi et al. reported that 50% of genetic alteration was present in the form of six different target genes during routine molecular analysis of patients with advanced NSCLC [11]. Thus, therapeutic efficacy and survival could be improved by targeting respective mutations. Another important treatment modality for NSCLC is the use of metronomic therapy. Oral vinorelbine, etoposide, and erlotinib have been tested for metronomic therapy in various clinical trials and found with superior efficacy as well as tolerability in the context of NSCLC [12–14]. Besides, low-dose metronomic chemotherapy has shown its promise as potent antiangiogenic and immunomodulatory activity. There is an evidence of higher survival rate in NSCLC patients during treatment with immune checkpoint inhibitors (such as anti-PD1 antibody). Thus, the concurrent administration of metronomic cytotoxic agents in combination with immune therapy may prevail the successful treatment against lung cancer [15,16].

Patients with advanced NSCLC are usually treated with multimodality therapy including combination chemotherapy, targeted agents, radiation, and or surgery [17]. Among various anticancer agents, platinum-based therapy is the most commonly used as the first-line treatment option for the treatment of NSCLC. To date, cisplatin is considered most widely used cytotoxic drugs, however, the dose limiting toxicity and higher case of resistance to tumors restrict its clinical applications. In terms of nephrotoxicity, gastrointestinal, and neurotoxicity, carboplatin is associated with less adverse effects compare to first generation cisplatin [18,19]. But the major disadvantages of carboplatin therapy are required 4–20 times higher concentration for achieving same effects compare to cisplatin, and thus makes it more toxic due to elevation of drug concentration above MTC level in blood, and followed by rapid elimination which decrease the chance of adduct formation on DNA in tumor site [20,21]. Consequently, low dose with frequent administration could be the ideal option for carboplatin because it may sustain the plasma drug concentration in desired level over longer periods of time and lowers the possibility of unwanted adverse effects. Under this circumstance, there is an unmet need to develop a patient friendly and customized treatment strategy, that is, oral therapy for carboplatin as low-dose metronomic chemotherapy for patients.

In this study, we aimed to formulate bile acid based orally active anticancer drug, particularly using physical complex between deoxycholic acid (DOCA) and carboplatin. DOCA is an endogenous molecule in human and produced from cholic acid via bacterial metabolism in the small intestine [22]. Several publications have previously reported the effectiveness of deoxycholic acid as an oral permeation agent based on the fact that it may increase the hydrophobicity of active molecule and also transport across the basolateral membrane in the small intestines via apical sodium bile acid transporter (ASBT). We previously demonstrated that DOCA conjugated low molecular weight heparin successfully interacted with ASBT and absorbed in the small intestine [23,24]. Furthermore, lysine conjugated DOCA could increase the permeation of negatively charged macromolecules in the small intestine such as insulin as well as small therapeutics [25–27]. Recently, Sun et al. showed the insulin delivery through oral route by making hydrophobic complex with sodium deoxycholate [28]. On the basis of favorable physicochemical properties for the oral delivery of carboplatin, DOCA was selected as a bile acid enhancer. We speculated that carboplatin physically associated with DOCA had an increased hydrophobic property, thereby increasing the permeability across enterocytes, and also enhancing the uptake into the cells through ASBT. Finally, the feasibility of oral carboplatin therapy was evaluated as the metronomic chemotherapy for lung cancer. Our aim was to explore a new possibility for the effective cancer management by means of the better efficacy, well tolerability, and adherence to chemotherapy associated with orally active carboplatin therapy.

2. Materials and methods

2.1. Materials

Carboplatin and sodium deoxycholate (DOCA) were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents used in this study were of analytical grade. The physical complex between carboplatin and DOCA was formed by their hydrophobic interactions in water. We had selected 1:2 molar ratio between carboplatin and DOCA as a physical complex and used in all in vivo experiments.

2.2. Powder X-ray diffraction (XRD)

The formation of complex was confirmed by powder XRD (D8 advance with DAVINCI; Bruker, Germany) equipped with LYNXEYE XE detector. The analysis was recorded at 40 kV voltage and 40 mA current. The scan speed and step size were set at 1°/min and 0.02°, respectively, over 5–70° in 2θ range.

2.3. Differential scanning calorimetry (DSC)

DSC thermogram was performed with a DSC-Q1000 instrument (TA Instruments, New Castle, DE). The complex was prepared in different mole ratios between carboplatin and DOCA in water, followed by lyophilization prior to analysis. Afterwards, 1 mg of powder samples was subjected to analysis at heating rate of 10 °C/min and gradually increased the temperature up to 400 °C.

2.4. Pharmacokinetic study

Animal studies were carried out in compliance with the Regulations for the Care of Animals established by the guidelines from the institutional Animal Ethics Committee. Male Sprague-Dawley (SD) rats were equilibrated in optimum room temperature and relative humidity with free access of normal diet and water. Prior to experiment, the animals were fasted for 12 h and neutralizing the gastric pH using magnesium hydroxide solution (3% v/v). The test sample was administered either intravenous (IV) injection of carboplatin at a dose of 5 mg/kg in rat via the tail vain or orally administered carboplatin/DOCA complex at a dose of 50 mg/kg. The rats were lightly anesthetized under diethyl ether and the blood samples were taken from the retro-orbital plexus using heparinized capillary tubes (Kimble Chase, Rockwood, TN) at different time intervals. After centrifuging at 4000g for 15 min, the plasma samples were isolated from the blood and stored at −70 °C until analysis. For quantification, the sample was prepared by digesting with 70% HNO_3 (Sigma-Aldrich) for 4 h at 200 °C in glass beaker equipped with watch glass and continued the heating for another 4 h with deionized water. The platinum content of the plasma samples from each time point was analyzed by ICP-MS (Elan 6100, Perkin Elmer, Waltham, MA).