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Dual pancreas- and lung-targeting therapy for local and systemic complications of acute pancreatitis mediated by a phenolic propanediamine moiety

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ABSTRACT

To inhibit both the local and systemic complications with acute pancreatitis, an effective therapy requires a drug delivery system that can efficiently overcome the blood–pancreas barrier while achieving lung-specific accumulation. Here, we report the first dual pancreas- and lung-targeting therapeutic strategy mediated by a phenolic propanediamine moiety for the treatment of acute pancreatitis. Using the proposed dual-targeting ligand, an anti-inflammatory compound Rhein has been tailored to preferentially accumulate in the pancreas and lungs with rapid distribution kinetics, excellent tissue-penetrating properties and minimum toxicity. Accordingly, the drug–ligand conjugate remarkably downregulated the proinflammatory cytokines in the target organs thus effectively inhibiting local pancreatic and systemic inflammation in rats. The dual-specific targeting therapeutic strategy may help pave the way for targeted drug delivery to treat complicated inflammatory diseases.

1. Introduction

Acute pancreatitis (AP) is a sudden inflammation of exocrine pancreas caused by the activation of tripsinogen within the acinar cells [1,2]. Following the local leukocyte sequestration in the pancreas, cytokines were rapidly released from damaged pancreatic acinar cells into the blood circulation and consequently other tissues, leading to severe life-threatening complications [3]. Lung injury, the most frequent systemic complication after onset of AP, accounts for the majority of AP-related deaths worldwide [4]. However, no specific cure is available to downregulate the local and systemic inflammatory responses involved in the progression of AP [3]. Protease inhibitors, such as somatostatin and octreotide, could only reduce the incidence of complications in the initial autodigestive phase of AP [5]. Antibiotics are demonstrated to inhibit the pancreatic infection when good pancreatic tissue penetration is provided [6]. Currently, supportive care and surgical operation remain the primary treatment options, causing unfavorable pain and high medical cost. The lack of specific therapy is mainly due to the extremely poor permeability of anti-inflammatory drugs across the blood-pancreas barrier (BPB), leading to insufficient accumulation in pancreas [7]. Meanwhile, anti-inflammatory drugs often fail to achieve therapeutically relevant concentrations against fatal systemic complications, such as lung injury, rendering AP very difficult to cure [8]. Therefore, it is crucial to develop a dual-specific targeting drug delivery system with high BPB penetration as well as lung targetability for effective AP therapy.

Herein, we proposed a dual-specific targeting strategy to the pancreas and lungs as a new therapeutic option to overcome the existing challenges in the treatment of AP. We aimed at engineering drug–ligand conjugates with excellent dual-targeting efficiency. Thus, the key is to develop small molecule ligands with high specificity as well as low toxicity that deliver anti-inflammatory agents specifically to both the pancreas and lungs. We were inspired by a phenolic propanediamine derivative, N,N,N′-trimethyl-N′-(2-hydroxyl-3-methyl-5-^{123}I iodobenzyl)-1,3-propanediamine (^{123}I-labeled HMPDM), which was a lung-imaging agent for lung-specific accumulation [9]. Of note, recent human studies have implicated its strong ability in crossing the BPB [10–12]. The interesting findings have driven us to explore the potential of ^{123}I-labeled HMPDM as a drug targeting ligand. However, its chemical structure is inappropriate for drug conjugation. Lipophilic basic molecules are reported to be stored in acidic organelles of tissues such as the pancreas and lungs [13]. Given this, we speculated that the phenolic propanedimine moiety on ^{123}I-labeled HMPDM may be responsible for

Abbreviations: AP, acute pancreatitis; HMPDM, N,N,N′-trimethyl-N′-(2-hydroxyl-3-methyl)-1,3-propanediamine; HPDM, N,N,N′-trimethyl-N′-(4-hydroxy-3-methyl-benzyl)-1,3-propane diamine; HPDM–Rhein, HPDM–Rhein conjugate; DTI, drug targeting efficiency index; OTCs, organic cation transporters.

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its dual pancreas- and lung-specific affinity. Motivated by this rationale, our group designed HMPDM analogs aimed at facilitating its conjugation to active therapeutics. Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid), a potent anti-inflammatory drug, [14,15] was proven ineffective against AP for the lack of specific accumulation in pancreas, [16] and therefore was selected as the model anti-inflammatory drug. The most promising ligand, N,N,N′-trimethyl-benzyl)-1,3-propanediamine (ligand 2, HPDM), was employed in the following studies for its easy conjugation to Rhein (HPDM–Rhein) and optimal plasma stability. The cytotoxicity, cellular uptake efficiency and mechanisms of HPDM–Rhein were investigated in cell-based studies. The in vivo stability and dual-targeting efficiency of HPDM–Rhein were also evaluated. Moreover, we assessed the anti-inflammatory therapeutic efficacy of HPDM–Rhein via systemic administration to an AP complicating lung injury model in rats. This is the first report of a dual-targeting strategy to achieve efficient AP therapy and the first use of a phenolic propanediamine derivative as a dual-specific targeting drug ligand.

2. Materials and methods

2.1. Materials

Rhein was purchased from Rongsheng Biological Co., Ltd (Xian, China) and vanillin was obtained from Kemiou Chemical Co., Ltd (Tianjin, China). 4-Hydroxybenzaldehyde, N,N,N′-trimethylpropane-1,3-diamine, taurocholic acid sodium salt hydrate (TAS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were commercially purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical or high performance liquid chromatography grade. TLC (silica gel GF254) was used to detect spots by UV radiation. Purification of intermediates and desired compound was achieved by column chromatography on silica gel. Chemical shifts were expressed in parts per million (ppm, δ units). Coupling constants were in units of hertz (Hz). MS spectroscopy was evaluated on Bruker microTOF-QII. ^{1}H NMR and ^{13}C NMR analysis were performed with an AMX-400 Bruker Spectrometer.

2.2. Animals and cell cultures

Male Sprague-Dawley rats (body weight: 200 ± 20 g) were supplied by the West China Experimental Animal Center of Sichuan University (Chengdu, China). Rats were maintained in a germ-free environment and allowed free access to food and water. All animal experiments were approved by the Institutional Animal Care and Ethic Committee of Sichuan University, according to the requirements of the National Act on the use of experimental animals. (People's Republic of China).

AR42J cells (a rat pancreatic acinar cell line), A549 cells (a human pulmonary alveolar epithelial cell line), HepG2 cells (human liver cells) and Hela cells (human carcinoma cervical cells) were obtained from ATCC and cultured in DMEM with high glucose (GIBCO, USA) supplemented with 20% fetal bovine serum, 1% penicillin/streptomycin. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2, and the cell medium was changed every other day.

2.3. Synthesis, characterization and in vitro stability assay of ligand–Rhein conjugates

Ligands 1–3 and corresponding drug–ligand conjugates were synthesized and presented in Fig. 1A (see Scheme S1–6 for detailed information). The structure of the ligands and drug–ligand conjugates were confirmed and characterized by electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR).

To assess the stability of ligand–Rhein conjugates, the conjugates were incubated with serum from SD rats at 37 °C. To stimulate the stability of HPDM–Rhein in in vivo tissues, HPDM–Rhein were incubated with lung homogenates and pancreas homogenates from SD rats at 37 °C, respectively. The stability of HPDM–Rhein in PBS with varying pH values (pH = 2, 4, 6.8, 7.4, 9) at 37 °C were also investigated. At predetermined time intervals, samples were immediately diluted by equal amounts of methanol to precipitate protein, vortexed for 5 min and centrifuged at 13,225 *g* for 10 min. The supernatants were then subjected to LC–MS/MS.

2.4. Sample preparation and LC–MS/MS analysis

Cell digestion, tissue homogenates and plasma samples were mixed with suitable volume of methanol to precipitate protein. The mixtures were vortexed for 5 min and centrifuged at 13,225 *g* for 10 min. Then the supernatants were analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

To accurately evaluate the dual-targeting efficiency of the conjugate, we developed a rapid and sensitive LC–MS/MS to directly quantify free Rhein and HPDM–Rhein conjugate in the following studies. The analysis was performed on an Agilent 1200 series RRLC system equipped with an SL autosampler, degasser and SL binary pump as well as an Agilent triple-quadrupole MS. Separations were carried out using a Diamonsil ODS column (50 mm × 4.6 mm, 1.8 μm) with the corresponding guard column (ODS, 5 μm). For the determination of Rhein, the mobile phase consisted of acetonitrile and 0.1% formic acid (85:15, v/v). For HPDM–Rhein, the mobile phase was adjusted to acetonitrile and 0.7% formic acid (80:20, v/v). Flow rate was 0.4 mL/min. Detection was operated on a mass spectrometer and quantification was performed using multiple reaction monitoring (MRM). Negative and positive electrospray source ion modes were used to monitor Rhein and HPDM–Rhein, respectively. MRM of m/z 284 → 267 and 518 → 267 were adopted to qualify Rhein and HPDM–Rhein, respectively. The responding optimized collision-induced dissociation voltages of the analysis were 159 eV and 32 eV for the fragmentor and collision energy, respectively. Instrumental parameters were as follows: gas temperature of 350 °C, gas flow of 8 mL/min, nebulizer of 30 psi, capillary of 4000 v.

2.5. Cell-based studies

2.5.1. Cytotoxicity assay

Cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) method (n = 5). Briefly, AR42J cells or A549 cells were seeded in 96-well culture plates at a density of 1×10^4 cells/well and incubated for 24 h at 37 °C. Then the cells were treated with different concentrations of Rhein solution, HPDM–Rhein solution or HPDM solution (dispersed in serum-free culture medium) for 4 h. 20 μL MTT PBS solution (5 mg/mL) were added into each well and incubated at 37 °C for another 4 h. Thereafter, the medium was removed, and the cells were mixed with 150 μL DMSO. Cell viability was assessed by measuring the absorbance at 570 nm with a microplate reader (Thermo, Varioskan Flash). Cells not exposed to samples were used as control with 100% viability.

2.5.2. Cellular uptake studies

The indicated cell lines were seeded in 6-well culture plates (5×10^5 cells/plate) 2 d before the drug treatments. To evaluate the cellular uptake efficiency of HPDM–Rhein, the cells were incubated with Rhein or HPDM–Rhein solution at increasing concentrations (2.17, 4.34, 8.69, 17.57 and 35.15 μM, respectively, equivalent to Rhein) at 37 °C for 1 h. Rhein and HPDM–Rhein were dissolved in serum free culture medium.

2.5.3. Mechanistic studies on intracellular delivery of HPDM

To explore the cell delivery mechanism of HPDM, the cells were pre-incubated with indicated inhibitors for 1 h and then incubated with HPDM–Rhein (17.57 μM) for another 1 h. For energy depletion, the cellular uptake was performed at 4 °C or in the presence of NaN_3 (1 mg/mL) for 1 h. L-Arginine and lysine were inhibitors of basic