Oral Delivery of Sodium Cromolyn: Preliminary Studies In Vivo and In Vitro

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Purpose. Herein we report the discovery of a group of derivatized α-amino acids that increase the oral bioavailability of sodium cromolyn. Methods. We prepared three N-acetylated α-amino acids and used these compounds to demonstrate the oral delivery of cromolyn in an in vivo rat model. In vitro experiments, including permeation studies and near infrared spectroscopy, were also performed to initiate an understanding of the mechanism by which these compounds facilitate cromolyn oral delivery.

Results. Following oral administration to rats of solutions containing a combination of cromolyn and the delivery agent, significant systemic plasma concentrations of the drug were detected. In vitro studies suggest that absorption of the drug across the gastrointestinal membrane is a passive process.

Conclusion. The absolute oral bioavailability of sodium cromolyn in the rat model is estimated to be ~5%. Preliminary mechanistic studies suggest that a complex of the cromolyn/delivery agent facilitates permeation across/through the membrane.

KEY WORDS: cromolyn; oral drug delivery; acetylated α-amino acids; intestinal permeability; near infrared spectroscopy.

INTRODUCTION

Cromolyn sodium is an anti-inflammatory agent that has been used for the past 25 years in the prophylactic treatment of bronchial asthma (1). For this indication, the drug is administered locally several times daily by inhalation of either a solution or a dry powder. Although it is effective, this route of administration commonly causes irritation of the throat and trachea.

Cromolyn's mechanism of action remains poorly understood, however, it is believed to inhibit the degranulation of pulmonary mast cells (2). Its efficacy is highly dependent upon the inhalation technique of the patient. Even under these conditions, long-term cromolyn therapy is effective in maintaining decreased bronchoconstriction and improved lung capacity in most asthma patients (3).

Sodium cromolyn is also used to treat allergic rhinitis (4). In order to provide symptomatic relief of the nasal congestion, sneezing and postnasal drip associated with this condition, cromolyn must reach the nasal mucosa (2). Thus, it is administered locally as an intranasal solution which is effective, but causes irritation of the nasal mucosa. Less common indications for sodium cromolyn are in the treatment of allergic ocular disorders and Crohn's disease. In both cases, local administration is required.

To date, local administration of cromolyn has sufficed as a treatment for its clinical indications, in spite of the undesired and commonly reported irritations at the dosing site. Although an oral dosage form of the drug would be highly desirable, cromolyn is not effective when administered orally (5) because it is poorly absorbed from the gastrointestinal tract (5–7). The oral bioavailability of cromolyn (8) is less than 1% as opposed to 7.5% of an inhaled dose of cromolyn that is absorbed systematically (9). An oral formulation of sodium cromolyn would offer several advantages over the current dosage forms, including improved patient compliance and elimination of the respiratory tissue irritation associated with powder inhalation. By increasing the oral bioavailability of cromolyn, significant systemic concentrations of the drug could be obtained. This is of particular interest in light of the recent finding that mast cell stabilization is important in the prevention and treatment of atherosclerosis (10), suggesting that systemic cromolyn may prove to be useful in coronary artery disease prophylaxis.

Previously, the preparation and attempted oral delivery of various cromolyn prodrugs has been reported (11,12). These studies have met with limited success. Herein, we report the discovery of compounds that facilitate the oral delivery of sodium cromolyn in a rat model with an absolute bioavailability of ~5%.

MATERIALS AND METHODS

Materials

Sodium cromolyn was obtained from Silaviton s.p.a., Casaleno Lodigiano, Italy. Compound 3 and reagents and solvents for the syntheses of 1 and 2 were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin and were used without further purification. Thorazine and ketamine were obtained from Henry Schein, Port Washington, New York. Histopathological analyses were conducted by Cenvet Laboratories, Woodside, New York. NIR dye (2-[4'-chloro-7'-(3'-ethyl-2'"-benzoazolinylidene)-3',5'-1''''(3''''-propanediyl)-1', 3',5'-heptatriene-1'-yl]-3-ethylbenzoazolium bromide) was supplied by Dr. Raymond Ottenbrite at Virginia Commonwealth University. All other reagents and chemicals were purchased from Sigma, St. Louis, Missouri.

Analytical Methods

NMR spectra were recorded at 300 MHz in DMSO-d6. Combustion analyses performed by Microlit Laboratories, Madison, New Jersey, were within acceptable limits (C, H, N ± 0.4%). Reactions were monitored by high pressure liquid chromatography on a Vydac 250 × 4.6 mm, 5μm Protein and Peptide C18 column using a gradient of 0–50% acetonitrile in water with 0.1% trifluoroacetic acid (TFA) as follows. The concentration of solution B (0.1% TFA in 50% acetonitrile/ H2O) in solution A (0.01% TFA in H2O) was increased from 0–100% over 20 minutes at a flow rate of 1 mL/min. Ultraviolet detection at 220 nm was employed. Melting points, performed using a Mel-Temp II from Laboratory Devices, are uncorrected and are in agreement with the literature values.
Oral Delivery of Sodium Cromlyn

General Synthetic Methods

The following procedure was used to prepare 1 and 2. The preparation of N-cyclohexanoyl-(L)-leucine (1) is given as a representative example. (L)-leucine (43.5 g, 331 mmol) was dissolved with stirring in aqueous sodium hydroxide (360 mL, 2N) in an open flask. The resulting solution was cooled to about 10–15°C in an ice/water bath and cyclohexanecarbonyl chloride (38.5 mL, 331 mmol) was added dropwise maintaining the reaction temperature at about 10–15°C. After the addition was complete, the reaction solution was stirred for 2.5 hours at room temperature. The pH of the reaction mixture was adjusted to 9.5 with aqueous hydrochloric acid (37%), the unreacted leucine separated as a white solid and was removed by filtration. The pH of the filtrate was then further lowered to 4.5 and crude 1 precipitated from solution. This solid was removed by filtration and recrystallized from methanol to give N-cyclohexanoyl-(L)-leucine (1, 43 g, 54%) as a white crystalline solid. mp 153–155°C. 1H NMR (300 MHz, d6-DMSO) δ 4.2 (m, 1H), 2.3 (m, 1H), 1.8 (br m, 4H), 1.6 (br m, 4H), 1.3 (br m, 5H), 0.8 (dd, 6H). Anal. (C13H22NO3) C, H, N.

Analytical data for 2: mp 109–111°C; 1H NMR (300 MHz, d6-DMSO) δ 13.0 (br, 1H), 1.20 (br, 1H), 9.0 (d, 1H), 7.9 (d, 1H), 7.4 (t, 1H), 7.2 (m, 5H), 6.8 (dd, 2H), 4.7 (m, 1H), 3.2 (m, 2H). Anal. (C16H21NO4) C, H, N.

Oral Gavage Studies

All animal experiments and protocols were reviewed and approved by the Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Taconic Farms, Germantown, New York) weighing 250–325 g were used after a 5-day acclimation period. The rats were housed in the animal unit at Emisphere Technologies under a 12 h light/dark cycle. Rats were fasted for 12 h prior to dosing. On the morning of dosing the animals were anesthetized with 44 mg/kg ketamine and 0.5 mg/kg thora- mine, and the test articles were administered by oral gavage using a Nelaton catheter (8 fr).

The test articles were prepared by dissolving 100 mg/ml of delivery compound in 0.85 N citric acid and then adding 12.5 mg/ml sodium cromolyn powder to the solution. The pH of the test articles was 3.3 to 4.4. For each test article, six to seven rats were given 2 mL/kg. The total dose of cromolyn was 25 mg/kg and the total dose of delivery compound was 200 mg/kg. Control solutions of cromolyn were similarly prepared in aqueous citric acid. Blood samples were collected serially from the tail artery prior to oral gavage and at 15, 30 and 45 minutes after dosing. Heparinized plasma was isolated from these samples, extracted with ethyl acetate and cromolyn concentrations were measured by HPLC as described by Yoshimi et al (12).

Permeation Studies

Proximal small intestine was obtained from male Sprague- Dawley rats weighing 350 to 400 g which were anesthetized with ketamine, and the tissue was gently flushed with cold Kreb's bicarbonate buffer (1.1 mM MgCl2, 1.25 mM CaCl2, 114 mM NaCl, 5 mM KCl, 25 mM NaHCO3, 1.65 mM Na2HP04, 0.3 mM NaH2PO4, pH 7.4). The intestine was slit open along the mesenteric border and segments about 4 cm in length were mounted in 1.78 cm² lucite diffusion chambers (Precision Instrument Design, Tahoe City, CA). Kreb's-bicarbonate buffer was added to each side of the tissue, the temperature was maintained at 37°C with a water jacket and the buffer was circulated by gas lift with 95% O2/5% CO2 which maintained the pH at 7.3. Glucose (10 mM) was added to the serosal side of the tissue to aid with viability, and 10 mM mannitol was added to the mucosal side to balance the osmotic pressure. The tissue was equilibrated for 30 minutes before beginning permeation experiments. The potential difference and short-circuit current across the tissues were monitored throughout the experiments using four electrodes and a six channel voltage-current clamp (Physiologic Instruments, San Diego, CA). Fluxes were measured with the tissue kept in an unclamped state except for brief periods during the experiment to measure the electrophysiological parameters.

Solutions of 500 µg/mL cromolyn or 100 µg/mL Lucifer Yellow CH, dipotassium salt (LY) with or without 20 mg/mL 1 in Kreb's-bicarbonate buffer were placed in the mucosal chamber, and the serosal side was sampled serially for 2 hours. LY was quantified by fluorometric assay and cromolyn by HPLC as described by Yoshimi et al (12). The total amount of the compound, cromolyn or LY, which crossed the tissue was plotted versus time and the flux calculated by linear regression. The apparent permeability coefficient (cm/sec) was obtained by dividing the flux by the initial concentration of compound on the mucosal side of the tissue. The effects of active transport processes on permeation were examined by adding 10 mM azide to the mucosal chamber, 10 mM ouabain to the serosal chamber, 1 µM amiloride to the mucosal chamber, or 1 µM dimethylamiloride to the mucosal chamber for the 2 h duration of the experiments.

Hydropobicity Measurements

Absorption measurements were performed using a Hitachi U-2000 UV-vis spectrophotometer. All solutions were prepared and handled as described in Patonay et al (13) and exemplified for 1 as follows.

A stock NIR dye solution (5 × 10⁻⁴ M) was prepared in spectrophotometric grade methanol. Stock solutions of cromolyn and 1 were prepared in 50 mM phosphate buffer, pH 7. The study solutions were prepared from the stock solutions directly into 1 mL plastic cuvettes such that the concentration of 1 was 100 mg/mL, the concentration of cromolyn was 12.5 mg/mL and the concentration of the dye was 5 × 10⁻⁶ M. The absorbance of the dye was measured at 700 and 800 nm. The relative absorbance of the dye was calculated as a ratio of the 700 nm absorbance to the sum of the absorbance measurements at 700 and 800 nm.

RESULTS AND DISCUSSION

Previously we reported the use of N-acylated-α-amino acids as delivery agents for protein drugs (14). During the course of this work, N-cyclohexanoyl-(L)-leucine (1), N-salicyloyl-(L)-phenylalanine (2), and 3-cyclohexanepropionic acid (3) were tested for their ability to promote the oral delivery of cromolyn in rats.

An aqueous solution of cromolyn and either compound 1, 2, or 3, was dosed by oral gavage to rats (n = 7). Each animal received a total dose of 200 mg/kg delivery agent and 25 mg/