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*-acylated 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; acylated 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 admininstration 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 (4) 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 systemically (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 Lodigrano, 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″-benzothaizolinylidene)-3′,5′-(1‴,3‴-propanediyl)-1′, 3′,5′-heptatriene-1′-yl]-3-ethylbenzothiazolium bromide) was supplied by Dr. Raymond Ottenbrite at Virginia Commomwealth University. All other reagents and chemicals were purchased from Sigma, St. Louis, Missouri.

Analytical Methods

NMR spectra were recorded at 300 MHz in DMSO-d_6. 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 chromotography on a Vydac 250 × 4.6 mm, 5μm Protein and Peptide C_{18} 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/H_2O) in solution A (0.01% TFA in H_2O) 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.

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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 ^{1}H NMR (300 MHz, d_6-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. (C_{13} H_{22} NO_3) C, H, N.

Analytical data for **2**: mp 109–111°C; ^{1}H NMR (300 MHz, d_6-DMSO) δ 13.0 (br, 1H), 12.0 (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. ($C_{16}H_{15}NO_4$) 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 thorazine, 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 $MgCl_2$, 1.25 mM $CaCl_2$, 114 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.65 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, pH 7.4). The intestine was slit open along the mesenteric border and segments about 4 cm in length were mounted in 1.78 cm^2 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% O_2/5% CO_2 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 monitered 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.

Hydrophobicity 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/

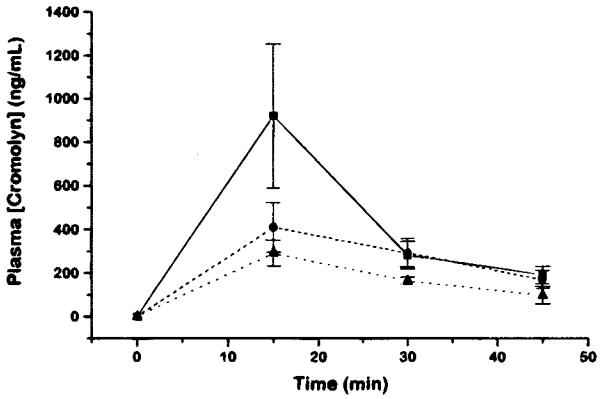

Fig. 1. Oral delivery of cromolyn in rats. The squares represent the response following PO dosing of an aqueous solution of **1** and cromolyn. The circles represent the response following PO dosing of an aqueous solution of **2** and cromolyn. The triangles represent the response following PO dosing of an aqueous solution of **3** and cromolyn. Delivery agents **1** and **2** were dosed at 200 mg/kg and cromolyn was dosed at 25 mg/kg. Delivery agent **3** was dosed at 400 mg/kg and cromolyn was dosed at 50 mg/kg.

kg cromolyn for **1** and **2** and 400 mg/kg delivery agent and 50 mg/kg cromolyn for **3**. Control groups received cromolyn alone or delivery agent alone. Blood samples were collected serially from the tail artery at 15, 30 and 45 minutes post-dosing, the plasma samples were prepared according to the method of Kobayashi and Machida (15) and concentrations of cromolyn were measured according to the method of Yoshimi *et al* (12) (Figure 1). A peak plasma concentration of 920 ± 332 ng/mL was obtained from a single PO dose of a cromolyn/**1** solution. Peak plasma concentrations of 408.1 ± 113.8 ng/mL and 290.4 ± 59.4 ng/mL were obtained from a single oral dose of cromolyn/**2** and cromolyn/**3**, respectively. In a separate experiment, a control group receiving solutions of drug alone (50 mg/kg) at twice the dose previously administered, did not produce any significant plasma drug concentrations (Figure 2). However, the peak plasma concentration of cromolyn doubled following PO dosing at twice the dose (400 mg/kg **1** and 50 mg/kg cromolyn).

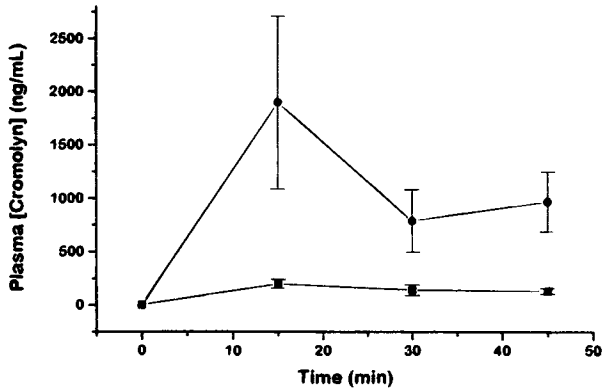

Fig. 2. Oral delivery of cromolyn in rats. The circles represent the response following PO dosing of an aqueous solution of 400 mg/kg **1** and 50 mg/kg cromolyn. The squares represent the response following PO dosing of an aqueous solution of cromolyn (50 mg/kg).

By comparing dose-corrected areas under the plasma concentration-time curves (16) for an intravenous dose of 10 mg/kg cromolyn in saline, the absolute bioavailability of an oral dose of a cromolyn/**1** solution was estimated to be 4.6%. Cromolyn alone, dosed orally, had an absolute bioavailability of 0.5% in these experiments. Acute toxicology studies (not presented here) indicated that the LD_{50} of **1** in mice was greater than 5 g/kg when administered orally as an aqueous solution. Additionally, histopathological examinations of rats dosed orally with **1** were conducted. Autopsy focusing on the gastrointestinal tracts of the animals, followed by histological examination, indicated no pathology. These studies suggest that drug transit across the intestinal membrane is not the result of mucosal damage.

Based on this preliminary data, we focused our attention on delivery agent **1** because it appeared to be the most active of the materials tested. To begin our understanding of the mechanism by which **1** increases the oral delivery of cromolyn, an *in vitro* permeation system was developed. In these studies, rat proximal small intestine was mounted in Ussing-type diffusion chambers where permeation and electrophysiological parameters could be measured simultaneoulsy (17,18). To ensure tissue viability, the electrophysiological response to prostaglandin (PGE_2) added to the serosal side of the tissue was measured (19). At both 60 and 120 minutes following initiation of a permeation experiment, the intestinal tissue showed an increase of both the potential difference (PD) and short-circuit current (I_{SC}) in response to PGE_2. Two hours was therefore chosen as the time length of a typical permeation experiment. Cromolyn concentrations in these samples were quantitated by high pressure liquid chromotography (HPLC) as cited previously (12).

The permeation of cromolyn through rat small intestine *in vitro* was measured in the absence and presence of delivery agents **1** and **3** to compare with the results obtained *in vivo*. The apparent permeability coefficient of cromolyn measured *in vitro* increased to a similar extent as the bioavailability increased *in vivo*. The average *in vitro* permeability coefficient of the drug in the presence of delivery agent **1** was 7-fold higher than that of cromolyn alone (Table 1). This is in agreement with the *in vivo* data which showed that the absolute bioavailability of orally-dosed cromolyn increased from 0.5% without **1** to 4.6% with **1**.

In an attempt to identify a convenient model compound for the transport of small polar drugs like cromolyn in Ussing-type diffusion chambers, the permeation of Lucifer Yellow (LY) was measured. This compound was chosen because it is chemi-

Table 1. *In Vivo* Cromolyn Absorption and *In Vitro* Cromolyn and Lucifer Yellow Permeability

Delivery Agent	Peak Plasma [Cromolyn] (ng/mL)*	Cromolyn Permeation ($\times 10^{-6}$ cm/sec)	Lucifer Yellow Permeation ($\times 10^{-6}$ cm/sec)
none	155 ± 20	1.30 ± 0.55	2.18 ± 2.03
1	920 ± 332	9.13 ± 3.21	11.08 ± 3.84
3	290 ± 59	3.19 ± 1.00	4.12 ± 1.99

* Compounds **1** and **3** were dosed at 200 mg/kg and cromolyn was dosed at 25 mg/kg.

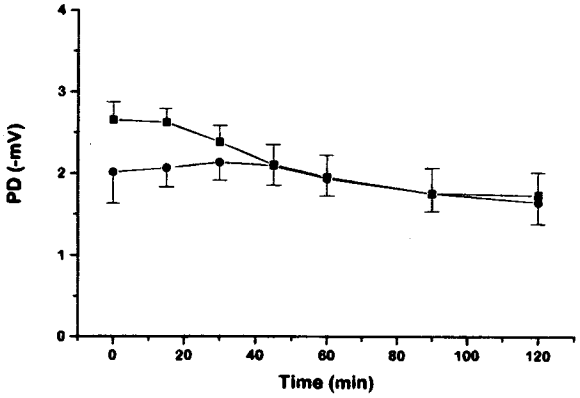

Fig. 3. Effect of delivery agent **1** on the potential difference (PD) of the small intestine *in vitro*. The squares represent the PD measured when LY alone was added to the mucosal side of the tissue, and the circles represent the PD with LY and delivery agent **1**. Data are presented as mean ± SEM, n = 6–8.

cally similar to cromolyn and can be readily quantified by a straight-foward fluorometric assay. The data obtained showed that the permeation of LY through rat intestinal tissue was comparable to that seen for cromolyn in both the presence and absence of delivery agents **1** and **3** (Table 1). The delivery agents had no effect on the PD or I_{SC} during the duration of these experiments indicating that they did not damage the tissue (Figure 3). Also, the serosal to mucosal flux of LY was measured in the presence of delivery agent **1** on the mucosal side of the tissue, and was not significantly different from the flux measured in the absence of **1** (79 ± 26% of control, n = 6).

In order to evaluate the possible involvement of active transport processes in the enhanced permeation of lucifer yellow observed with delivery agent **1**, a series of *in vitro* experiments were performed using LY/**1** in the presence of various metabolic inhibitors. Azide was chosen as an inhibitor of ATP synthesis, and ouabain, amiloride and dimethylamiloride were used to block various sodium transport processes (20). Figure 4 shows that none of these compounds were able to inhibit the enhanced permeation of LY in the presence of **1**, suggesting that the delivery agent-mediated transport of LY is not an active process.

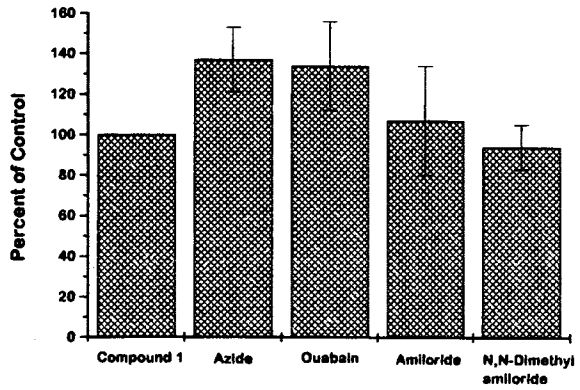

Fig. 4. Effect of metabolic inhibitors on LY permeation in the presence of **1**. The enhanced permeation of the LY/**1** combination is given as 100% and the LY/**1**/inhibitor combinations are reported relative to that 100%.

The possibility that a passive transport mechanism could facilitate the permeation of the highly hydrophilic cromolyn molecule through a lipophilic membrane prompted a preliminary study of the nature of the drug/delivery agent combination. Near infrared spectroscopy (NIR) using 2-[4′-chloro-7′-(3″-ethyl-2″-benzothaizolinylidene)-3′,5′-(1‴,3‴-propanediyl)-1′,3′,5′-heptatriene-1′-yl]-3-ethylbenzothiazolium bromide (dye) whose relative absorbance varies with the hydrophilicity of its environment (13), was employed to estimate the relative lipophilicity of the cromolyn/**1** mixture.

The NIR spectrum of the dye was measured alone and in solutions of **1**, **2**, and **3**. In a hydrophilic environment, the NIR relative absorbance of the dye is greater than its absorbance in a hydrophobic environment (7). In dye solutions containing 100 mg/mL of added delivery agent, the relative absorbances were 0.675 (**1**), 0.162 (**2**), and 0.437 (**3**). This data indicates that **1** provides a significantly more hydrophilic environment for the dye than any of the other delivery agents tested.

Based on these observations, **1** and **3** were chosen for further study because they represent extremes of both hydrophobicity and *in vivo* activity. Thus, at a concentration of 12.5 mg/mL the relative absorbance of the dye/cromolyn solution was 0.275. The same spectra were recorded in solutions containing **1** or **3** (100 mg/mL) and cromolyn (12.5 mg/mL). The relative absorbances were 0.175 for **1** and 0.451 for **3**. This data indicates that the combination of cromolyn and **1** is more hydrophobic than either of the two compounds separately, suggesting the formation of a non-covalent drug/delivery agent complex. The hydrophobicity of the cromolyn/**3** mixture is unchanged from that of **3** alone suggesting no significant interaction between the two compounds. The increased lipophilicity of the cromolyn/**1** complex would appear to render it more readily absorbed across the gastrointestinal membrane.

CONCLUSION

We have demonstrated the use of *N*-cycloalkanoyl-α-amino acids as delivery agents for the oral delivery of sodium cromolyn in an *in vivo* rat model. Three compounds were tested and evaluated. The absolute bioavailability of cromolyn dosed orally in solution with the lead compound (**1**) was estimated to be 4.6% relative to intravenous injection. Preliminary mechanistic studies were carried out using Ussing-type diffusion chambers and near infrared spectroscopy. The data obtained suggest that **1** forms a non-covalent complex with cromolyn. The lipophilic nature of this complex may facilitate the passive transport of cromolyn through the gastrointestinal membrane. Recent studies with new delivery agents (not presented here), producing bioavailabilites of 7–8%, will be the subject of additional communications. These new delivery agents will be used to gain a more detailed understanding of the exact mechanism of action of this novel class of drug delivery agents.

REFERENCES

1. G. G. Shapiro and P. Konig. Cromolyn sodium: a review, *Pharmacotherapy* **5**:156–170 (1985).
2. T. W. Rall. Sodium cromolyn. In A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor (eds), *The Pharmacological Basis of Therapeutics*, 8th ed., Macmillan, New York, 1990, pp 630–635.
3. A. M. Edwards. Sodium cromoglycate (Intal®) as an anti-inflammatory agent for the treatment of chronic asthma, *Clin. Exptl. Allergy* **24**:612–623 (1994).

4. A. Yoshimi, H. Hashizume, M. Kitagawa, K. Nishimura, and N. Kakeya. Absorption mechanism of 1,3-bis-(2-ethoxycarbonylchromon-5-yloxy)-2-((S)-lysyloxy)propane dihydrochloride (N-556), a prodrug for the oral delivery of disodium cromoglycate, *Biol. Pharm. Bull.* **16**:375–378 (1993) references cited therein.
5. L. S. Schanker, D. J. Tocco, B. B. Brodie, and C. A. M. Hogben. Absorption of drugs from the rat samll intestine, *J. Pharmacol. Exp. Ther.* **123**:81–88 (1958).
6. C. A. M. Hogben, D. J. Tocco, B. B. Brodie, and L. S. Schanker. On the mechanism of intestinal absorption of drugs, *J. Pharmacol. Exp. Ther.* **125**:275–282 (1959).
7. G. F. Moss, K. M. Jones, J. T. Ritchie, and J. S. G. Cox. Distribution and metabolism of disodium cromoglycate in rats, *Toxicol. Appl. Pharmacol.* **17**:691–698 (1970).
8. S. K. Gupta, S. Kumar, S. Bolton, C. R. Behl, and A. W. Malick. Optimization of iontophoretic transdermal delivery of a peptide and non-peptide drug, *J. Cont. Rel.* **30**:253–261 (1994).
9. G. F. Moss, K. M. Jones, J. T. Ritchie, and J. S. G. Cox. Plasma levels and urinary excretion of disodium cromoglycate after inhalation by human volunteers, *Tox. Appl. Pharm.* **20**:147–156 (1971).
10. M. Kaartinen, A. Pentilla, and P. T. Kovanen. Mast cells of two types differing in neutral protease composition in the human arotic intima, *Arterioscler. Thromb.* **14**:966–972 (1994).
11. T. Mori, K. Nishimura, S. Tamaki, S. Nakamura, H. Tsuda, and N. Kakaya. Prodrugs for the oral delivery of disodium cromoglycate, *Chem. Pharm. Bull.* **36**:338–344 (1988).
12. A. Yoshimi, H. Hashizume, M. Kitagawa, K. Nishimura, and N. Kakeya. Characteristics of 1,3-bis-(2-ethoxycarbonylchromon-5-yloxy)-2-((S)-lysyloxy)propane dihydrochloride (N-556), a prodrug for the oral delivery of disodium cromoglycate in absorption and excretion in rats and rabbits, *J. Pharmacobio-Dyn.* **15**:681–686 (1992).
13. G. Patonay, M. D. Antoine, D. Srinivasan, and L. Strekowski. Near-infrared probe for determination of solvent hydrophobicity, *Applied Spectroscopy* **45**:457–460 (1991).
14. (a) A. Leone-Bay, C. Mcinnes, N. Wang, F. DeMorin, D. Achan, C. Lercara, D. Sarubbi, S. Haas, J. Press, E. Barantsevich, B. O'Broin, S. Milstein and D. R. Paton. Microsphere formation in a series of derivatized α-amino acids: properties, moledular modeling, and oral delivery of salmon calcitonin, *J. Med. Chem.* **38**:4257–4261 (1995).
14. (b) A. Leone-Bay, N. Santiago, D. Achan, K. Chaudhary, F. DeMorin, L. Falzarano, S. Haas, S. Kalbag, D. Kaplan, H. Leipold, C. Lercara, D. O'Toole, T. Rivera, C. Rosado, D. Sarubbi, E. Vuocolo, N. Wang, S. Milstein, and R. A. Baughman. *N*-Acylated α-amino acids as novel oral delivery agents for proteins, *J. Med. Chem.* **38**:4262–4269 (1995).
15. J. Kobayashi and Y. Machida. Studies on absorption, excretion and tolerance of an oral form of disodium cromoglycate (DSCG) in healthy volunteers, *Jpn. Pharmacol. Ther.* **8**:80–91 (1980).
16. M. Gibaldi, and D. Perrier. *Pharmacokinetics*, 2nd ed.; Marcel Dekker: New York, 1982; pp 169–180.
17. H. H. Ussing, and K. Zerahn. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin, *Acta Phys. Scand.* **23**:110–133 (1951).
18. S. C. Sutton, A. E. Forbes, R. Cargill, J. H. Hochman, and E. L. LeCluyse. Simultaneous *in vitro* measurement of intestinal tissue permeability and transepithelial electrical resistance (TEER) using Sweetana-Grass diffusion cells, *Pharm. Res.* **9**:316–319 (1992).
19. B. Yao, D. L. Hogan, K. Bukhave, M. A. Koss, and J. I. Isenberg. Bicarbonate transport by rabbit duodenum in vitro: effect of vasoactive intestinal polypeptide, prostaglandin E_2, and cyclic adenosine monophosphate, *Gastroenterology* **104**:732–740 (1993).
20. T. R. Kleyman, E, J. Cragoe. Amiloride and its analogs as tools in the study of ion transport, *J. Membrane Biol.* **105**:1–21 (1988).