JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 1075–1082
© 2003 The Authors
Received February 5, 2003
Accepted April 29, 2003
DOI 10.1211/0022357021413
ISSN 0022-3573

Prednisolone 21-sulfate sodium: a colon-specific pro-drug of prednisolone

Yun Jin Jung, Min Ju Doh, In Ho Kim, Hye Sik Kong, Jeoung Soo Lee and Young Mi Kim

Abstract

Prednisolone 21-sulfate sodium (PDS) was synthesized as a colon-specific pro-drug of prednisolone with the expectation that it would be stable and non-absorbable in the upper intestine and release prednisolone by the action of sulfatase once it was delivered to the colon. In-vitro/in-vivo properties were investigated using rats as test animals. PDS was chemically stable at pH 1.2, 4.5, 6.8 and 8.0, and the apparent partition coefficient was 0.11 in 1-octanol/pH 6.8 buffer solution at 37 °C. PDS was stable on incubation with the contents of the stomach or small intestine. When PDS (0.1 mg equiv. of prednisolone) was incubated with the caecal contents (0.05 g), prednisolone was produced to a maximum 54% of the dose in 6 h and decreased thereafter, which suggested that reduction of the A ring took place in addition to the hydrolysis by sulfatase. After oral administration of PDS, a small portion of prednisolone was recovered from the cecal contents but not from the small intestine. Neither PDS nor prednisolone was detected in the plasma, suggesting that absorption of PDS is limited. The data demonstrate that the sulfate ester can serve as a novel colon-specific pro-moiety by limiting the absorption of the pro-drug in the upper intestine and releasing the active compound by the action of microbial sulfatase in the colon.

Introduction

Glucocorticoids have been used most frequently for the treatment of inflammatory bowel disease, such as ulcerative colitis and Crohn's disease (Crotty & Jewel 1992; Friedman & Blumberg 2001). They are well absorbed in the upper intestine and only a limited fraction of the orally administered dose is delivered to the inflammatory site in the distal ileum or colon. Long-term administration causes serious side effects, such as osteoporosis, hypertension, oedema, diabetes or decreased immunity through systemic absorption of the drugs. For this reason, they are used for an attack therapy using a large dose and are not suitable for long-term maintenance therapy for the prevention of relapse. To reduce the serious side effects caused by systemic absorption, colon-specific delivery of corticosteroid is highly desirable. Development of a pro-drug is one of the methods used to deliver drugs specifically to the colon. A colon-specific pro-drug should be chemically and enzymatically stable and non-absorbable in the upper intestine so that it can be delivered to the colon as an intact form. Hydrophilic small molecules or polymers are used as the colon-specific pro-moiety to prevent absorption (Brown et al 1983; Larsen & Johansen 1985; Kopeckova & Kopecek 1990; Istran et al 1991; Ryde 1992; Jung et al 2000, 2001). Once delivered to the colon, the linkage between the drug and pro-moiety should dissociate to liberate the active drug in the colon. Generally, it is dissociated by enzymes originating from the microbes in the colon (Rubinstein 1990; Faigle 1993). McLeod et al (1993, 1994) synthesized dextran esters of methylprednisolone or dexamethasone by the use of a succinate linker and suggested them as potential colon-specific pro-drugs of glucocorticoids. Friend & Chang (1984, 1985) introduced steroid glycosides as colon-specific pro-drugs of corticosteroids. They reported that β-D-glycosides of prednisolone and dexamethasone were poorly absorbed from the small intestine after intragastric administration and that hydrolysis of the prodrug took place by β-glucosidase and faecal homogenates to

College of Pharmacy, Pusan National University, Pusan 609-735, Korea

Yun Jin Jung*, Min Ju Doh, In Ho Kim, Hye Sik Kong, Jeoung Soo Lee†, Young Mi Kim

Correspondence: Y. M. Kim, College of Pharmacy, Pusan National University, Pusan 609-735, Korea. E-mail: ymikim@pusan.ac.kr

Funding: This work was supported by grant no. RO4-2002-000-00019-0 from the Korea Science & Engineering Foundation.

Present address: *Department of Cell & Cancer Biology, Medicine Branch, NCI, 9610 Medical Center Dr., Suite 300, Rockville, MD 20850, USA; †CCCD. BPRB no. 205 University of Utah S.L.C., UT 84112, USA.

release free drug, which might be useful for the treatment of inflammatory bowel disease. Fedorak et al (1995) tested the efficacy of dexamethasone-β-D-glucuronide as a colon-specific pro-drug of dexamethasone using the acetic-acid-induced rat colitis model and reported that it should be useful in the treatment of ulcerative colitis and Crohn's disease without serious side effects caused by adrenal suppression.

In an effort to design a novel colon-specific pro-drug of glucocorticoids, we noticed that sulfate ester might serve as a potential colon-specific pro-moiety based on several reports stating that microbial sulfatase activity in the intestinal contents was high in the caecum, but very low in the small intestine (Huijghebaert et al 1984a, b; Van Eldere et al 1988). These reports imply that the sulfate linkage is stable in the small intestine and is hydrolysed by sulfatase originating from microbes in the colon. If the sulfate ester linkage is indeed chemically and enzymatically stable in the upper intestine and is hydrolysed by the enzymes originating from microbes in the colon, it could be a promising colon-specific pro-moiety. No studies have been reported that have used the sulfate ester as a pro-moiety for the development of colon-specific pro-drugs. Introduction of the sulfate group as a sodium salt will increase the hydrophilicity of the compound and consequently limit transcellular absorption by way of lipid-membrane permeation. We synthesized prednisolone 21-sulfate sodium (PDS) and examined its in-vitro/in-vivo properties as a colon-specific pro-drug of prednisolone using rats as test animals. We expect that if PDS is stable and non-absorbable in the upper intestine, a large fraction of the orally administered dose will be delivered to the colon in intact form. Hydrolysis of the sulfate ester by the sulfatase in the colon would release prednisolone, which should be available for the treatment of inflammatory bowel disease.

Materials and Methods

Materials

Prednisolone, sulfatrioxide triethylamine complex (STT) and sulfatrioxide pyridine complex (STP) were purchased from Sigma Chemical Co. (St Louis, MO) and were used as received. Solvents for NMR and HPLC were obtained from Merck Inc. (Darmstadt, Germany). All other chemicals were reagent-grade, commercially available products. IR spectra were recorded on a Bomem MB 100 FT-IR spectrophotometer (Bomem). ^{1}H NMR spectra were taken on a Varian AS 500 spectrometer and the chemical shifts were in ppm downfield from tetramethylsilane. Elemental analysis was carried out by an Elemental Analyzer System (Profile HV-3). An Orion 320 pH meter (Orion, Boston, MA) was used for pH measurements. Melting points were taken on a Mel Tem II (Laboratory Devices, Holliston, MA) and were uncorrected. A Polytron PT 3100 homogenizer was used for homogenization of the tissue of the gastrointestinal tracts of rats and an Eppendorf Centrifuge 5415C (Eppendorf, Hamburg, Germany) was used for centrifugation. A Taitec micro-incubator M-36 (Japan) was used for incubation. TLC was performed on Merck Keiselgel 60 F_{254} and RP_8 F_{254s} and the mobile phase consisted of chloroform–methanol (7:3). Open-column chromatography was performed on Merck silica gel (70–230 mesh) column and the mobile phase consisted of chloroform–methanol (7:3). The HPLC system consisted of Model 305 and 306 pumps, a 117 variable UV detector, a Model 234 auto-injector, a Model 805 manometric module, and a Model 811C dynamic mixer from Gilson (Middleton, WI).

Preparation of PDS

To the solution of prednisolone (3.60 g, 10.0 mmol) in 64 mL of anhydrous benzene and pyridine (1:1), STT (3.80 g, 17.0 mmol) was added in portions with stirring at 56~60 °C for 20 min. The reaction mixture was evaporated under reduced pressure to remove the solvent and prednisolone 21-sulfate triethylammonium (PDST) was obtained as oily residue. After purification by silica-gel open-column chromatography, PDST was dissolved in a minimum amount of water and suspended into a solution of 10% NaCl with mechanical stirring for 1 h. The resulting precipitate, PDS, was collected by suction filtration and recrystallized from absolute ethanol (yield 67%). mp: 120 °C (dec.); IR (nujol) ν_{max}(OH): 3450 cm^{-1}, (C=O): 1675 cm^{-1}, (C=C): 1640, 1600 cm^{-1}, (S=O): 1259, 1034 cm^{-1}; ^{1}H NMR (D_2O): δ 0.78 (s, 3H, C-18), 1.30 (s, 3H, C-19), 4.89 (AB q, 2H, C-21), 5.94 (s, 1H, C-4), 6.21 (d, 1H, C-1), 7.42 (d, 1H, C-2); EA for $C_{21}H_{27}SO_8Na$: Calculated (C; 54.55, H; 5.84, S; 6.93), Found (C; 54.24, H; 6.20, S; 6.72).

Analysis of prednisolone and PDS in various biological specimens

A male Sprague-Dawley rat was anaesthetized by diethyl ether and a midline incision was made and various segments of gastrointestinal tract were obtained. The tissue and contents of the stomach and small intestine, and the contents of the caecum and colon, were homogenized separately and diluted with isotonic phosphate buffer (pH 6.8) to the concentration of 10% (w/v). To a 100-μL portion of the above homogenates, was added 20, 40, 100 or 200 μL of the stock solution of prednisolone or PDS (50 μg mL^{-1} in methanol) and an appropriate volume of methanol to make the final volume of 1 mL, which provided the standard solutions of prednisolone or PDS in a concentration of 1, 2, 5 or 10 μg mL^{-1}, respectively, in various biological specimens. Standard or blank solution (1 mL) was mixed on a vortex mixer for 2 min, centrifuged at 10 000 rev min^{-1} for 5 min, filtered through a membrane filter (0.45 μm) and the filtrate was used for the HPLC analysis. For the standard solutions (5 mL) in concentration of 0.01, 0.05, 0.2 or 0.5 μg mL^{-1}, the filtrate (4 mL), which was obtained according to the same treatment, was evaporated to dryness, reconstituted in 200 μL of methanol and used for the HPLC analysis. A 20-μL

sample was injected on a Lichrospher 100 RP-18 column (250 × 4.6 mm, 5 μm) and eluted with the mobile phase at a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of 0.067 M acetonitrile – pH 4.5 phosphate buffer solution (3:7), which was filtered through a 0.45-μm membrane filter before use. The eluate was monitored by measuring the absorption at 248 nm at a sensitivity of AUFS 0.01. The Gilson 712 software was employed for data analysis. Calibration curves for prednisolone or PDS in the intestinal contents or plasma were constructed from the concentration versus the peak area on HPLC (n = 6 for each concentration). The retention time of prednisolone and PDS was 9.95 min and 6.12 min, respectively. The lower limit of detection at a signal-to-noise ratio of three was 0.02 μg mL^{-1}. The precision was 6.5~14.5% and the accuracy was 91–112% for each concentration.

Apparent partition coefficient

To a solution (10 mL) of prednisolone or PDS (100 μg mL^{-1}) in pH 6.8 isotonic phosphate buffer pre-saturated with 1-octanol, 10 mL of 1-octanol pre-saturated with pH 6.8 isotonic phosphate buffer was added. The mixture was shaken for 10 h and left for 4 h at 37 °C. The concentration of prednisolone or PDS in the aqueous phase was analysed by HPLC as described in the preceding section. A calibration curve was constructed from the standard solutions (0.01~10 μg mL^{-1}) of prednisolone or PDS in pH 6.8 isotonic phosphate buffer. The apparent partition coefficients were calculated by using the equation $(C_o - C_w)/ C_w$, where C_o and C_w represent the initial and equilibrium concentration of the drug in aqueous phase, respectively.

Solubility

PDS (50 mg) was placed in a microtube containing 1 mL of pH 6.8 isotonic phosphate buffer solution and agitated for 24 h at 25 °C. After centrifugation, a 20-μL portion of the supernatant was analysed by HPLC as described previously.

Chemical stability

A solution of PDS (100 μg mL^{-1}) in pH 1.2 hydrochloric acid buffer, pH 4.5 acetate buffer and pH 6.8 and pH 8.0 phosphate buffer was incubated at 37 °C for 10 h. At a predetermined time interval, a 20-μL portion of the solution was removed, and the concentration of PDS was analysed by HPLC.

Incubation of PDS with rat gastrointestinal-tract segment contents

Collection of the contents of various segments of gastrointestinal tract was undertaken in a glove box, which was previously displaced by nitrogen. Tissue and contents of the stomach or small intestine were homogenized and diluted to half concentration with pH 4.5 isotonic acetate buffer for stomach and with pH 6.8 isotonic phosphate buffer for small intestine. To a 0.2-g portion of each homogenate, 0.8 mL of PDS solution in pH 6.8 isotonic phosphate buffer (100 μg equivalent of prednisolone) was added and the mixture was incubated under nitrogen at 37 °C. At appropriate time intervals, the sample was centrifuged at 5000 rev min^{-1} for 3 min. To a 0.1-mL portion of the supernatant, methanol (0.9 mL) was added to precipitate protein in the sample, vortexed for 2 min and centrifuged for 5 min at 10 000 rev min^{-1}. The concentration of PDS or prednisolone in a 20-μL portion of the supernatant was determined by HPLC. We adopted the pH values for stomach and small intestine as 4.5 and 6.8, respectively, which were the reported pH values normally found in the rat gastrointestinal tract (William-Smith 1965; McLeod et al 1993).

For incubation with 10% caecal contents, a 0.1-g portion of the caecal contents and 0.9 mL of PDS solution in pH 6.8 isotonic phosphate buffer (100 μg equivalent of prednisolone) were mixed and incubated at 37 °C. For incubation with 5% and 3% caecal contents, the relative amounts of the caecal contents and PDS solution were adjusted accordingly. At appropriate time intervals, the amount of PDS or prednisolone in the incubation medium was determined by HPLC as described previously.

Recovery of prednisolone and PDS from the plasma and rat gastrointestinal-tract segment contents after oral administration of prednisolone and PDS

Male Sprague-Dawley rats weighing 200~250 g were maintained with free access to a stock diet and water. These rats were fasted overnight (16 h) before administration of prednisolone or PDS. Water bottles were removed from the cages at least 30 min before drug administration to assure that the stomach would be empty. Prednisolone or PDS (equivalent to 5 mg of prednisolone) dissolved in 0.3 mL of ethanol–water (1:3) was administered by an oral zonde. At an appropriate time interval, the rat was anaesthetized by ethyl ether and blood was collected by intracardiac puncture through a heparinized syringe. After blood collection, the rat was sacrificed and the tissue and contents of various segments of gastrointestinal tract were obtained. Blood samples were centrifuged for 5 min at 14 000 rev min^{-1}, the plasma was removed and stored frozen until analysis by HPLC. The amount of prednisolone or PDS in each sample was analysed by the same protocol as described previously.

Statistical analysis

The results are expressed as mean ± s.e. At least 6 replicate studies were carried out for in-vitro hydrolysis studies and 3~5 replicate studies were executed for in-vivo studies. The non-paired Student's t-test was used to assess the statistical significance ($P < 0.05$) of results for all measurements.

Results

Preparation of PDS

Prednisolone was reacted either with STP or STT using anhydrous benzene and pyridine as solvent. Sulfation did not take place by STP, but it proceeded readily by STT (Figure 1). The overall yield was 67%. The ratio of the reactant and optimization of the reaction time was very critical to the suppression of by-product formation. Use of 1.5∼1.7 molar excess of STT provided optimum result. The reaction proceeded rapidly and we terminated the reaction when a trace of by-product was monitored on TLC. No impurities were detected from HPLC after executing column chromatography once. PDS was obtained by the treatment of PDST with 10∼20% salt solution. IR and ^{1}H NMR spectra, as well as results of elemental analysis, confirmed the structure of PDS. Compared with prednisolone, the most characteristic change in the ^{1}H NMR spectrum of PDS was the downfield shift of the two protons on C-21 to 0.4 ppm by the introduction of the sulfate group.

Apparent partition coefficient, solubility and pH stability of PDS

The solubility of PDS increased greatly (46.2 mg mL^{-1} in pH 6.8 isotonic phosphate buffer solution) in comparison with prednisolone. The apparent partition coefficient of prednisolone and PDS in the 1-octanol–phosphate buffer solution (pH 6.8) system at 37 °C was 21.8 and 0.11, respectively. Incubation of PDS for 10 h in pH 1.2, pH 4.5, pH 6.7 and pH 8.0 buffer solution produced neither prednisolone nor any change in the concentration of PDS.

Incubation of PDS with the tissue and contents of rat gastrointestinal tract segments

PDS was stable when it was incubated with the homogenate of the tissue and contents of the stomach or small intestine; there was no change in the concentration of PDS during 24 h incubation period at 37 °C. When PDS was incubated with the caecal contents, the amount of PDS decreased progressively, producing prednisolone. The rate of PDS conversion on incubation with varied concentration of caecal contents was compared. Figure 2 shows the results when PDS (1 mg) was incubated in 10 mL of 10%, 5% or 3% caecal contents in pH 6.8 isotonic phosphate buffer solution. When PDS (1 mg) was incubated in 10 mL of 5% caecal contents, the amount of PDS decreased to 79, 52, 30 and 18% of the initial dose at 2, 4, 6 and 8 h, respectively. The level of prednisolone increased to give a maximum 54% of the dose at 6 h and decreased thereafter. The amount of prednisolone produced was less than the amount by which PDS decreased - the greater the gap, the longer the incubation period. As the concentration of caecal contents increased, the rate of PDS conversion increased (left section on Figure 2). The amount of prednisolone in the medium (right section on Figure 2) increased as the concentration of caecal contents increased in the early part of the incubation period. As the incubation period extended, the level of prednisolone began to decrease and it was no longer in parallel order with the concentration of caecal contents. The maximum level of prednisolone amounted to 12, 54 and 39% of the initial dose when the concentration of caecal contents was 3, 5 and 10%, respectively. When prednisolone was incubated with caecal contents under the same conditions, the amount of prednisolone decreased progressively as shown in Figure 3. The rate of decrease was greater as the concentration of caecal contents increased.

Recovery of prednisolone and PDS from the plasma and contents of rat gastrointestinal tract segments after oral administration of prednisolone or PDS

After oral administration of PDS (equivalent to 5 mg prednisolone) to rats, the amount of prednisolone and PDS in the contents of various segments of the gastrointestinal tract and plasma was determined at predetermined time intervals. As shown in Figure 4, the level of PDS in the proximal small intestine (PSI) amounted to 691 μg mL^{-1} at 0.5 h and decreased rapidly. The level of PDS in the distal small intestine (DSI) reached a maximum (1399 μg mL^{-1}) around 2 h and decreased drastically to become undetectable after 8 h. The level of prednisolone was below the limit of detection throughout the whole section of the small intestine. Recovery of prednisolone and PDS from the caecal contents is shown in Figure 5. The concentration of PDS increased rapidly after 2 h, reached a maximum (340 μg mL^{-1}) around 4 h and decreased slowly. Prednisolone appeared after 3 h, giving

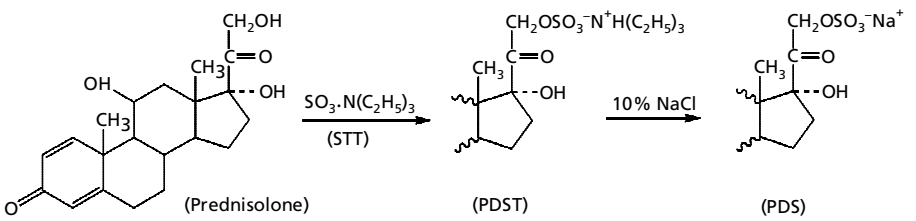

Figure 1 Preparation of prednisolone 21-sulfate sodium.

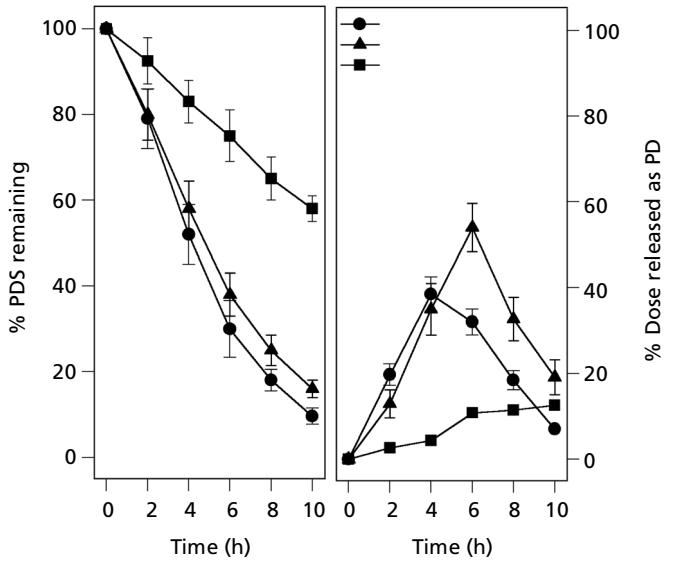

Figure 2 Hydrolysis of PDS (left) and release profile of prednisolone (PD) (right) during incubation of PDS (equivalent to 1 mg PD) in 10 mL of varied dilution of caecal contents of rats in pH 6.8 isotonic phosphate buffer at 37 °C. Data are mean ± s.e. (n = 6).

a maximum around 5~6 h (59 μg mL^{-1}) and became undetectable after 8 h. Figure 6 shows the concentration profile of prednisolone in the contents of PSI, DSI and caecum after oral administration of prednisolone (5 mg) to rats. The concentration of prednisolone in the contents of PSI reached a maximum (21 μg mL^{-1}) around 0.7 h and decreased to be undetectable at 1.5 h. The concentration of prednisolone in the contents of DSI reached a maximum (22 μg mL^{-1}) around 1.5 h and decreased slowly. The level of prednisolone in the caecal contents was below the limit of detection for 6 h. Concentration profiles of prednisolone in the plasma after oral administration of prednisolone or PDS (equivalent to 5 mg prednisolone) are shown in Figure 7. The plasma level of prednisolone after oral administration of prednisolone increased to give a maximum around 1.5 h (1.6 μg mL^{-1}) and decreased rapidly. The level of prednisolone or PDS in the plasma was below the limit of detection after oral administration of PDS.

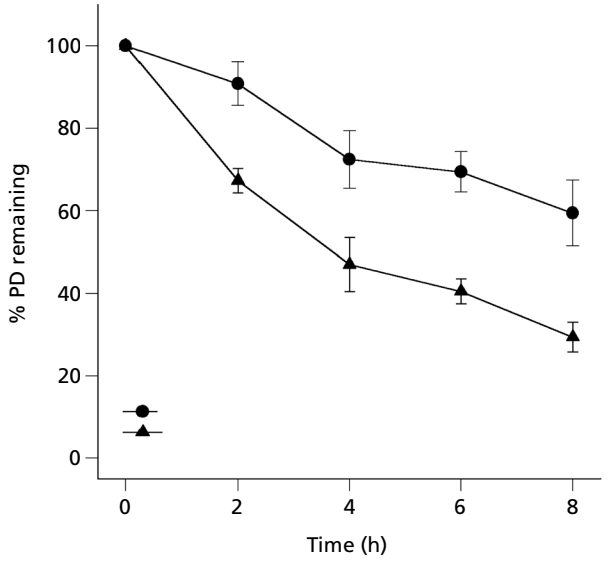

Figure 3 Incubation of prednisolone (PD) (1 mg) in 10 mL of varied dilution of caecal contents of rats in pH 6.8 isotonic phosphate buffer at 37 °C. Data are mean ± s.e. (n = 4).

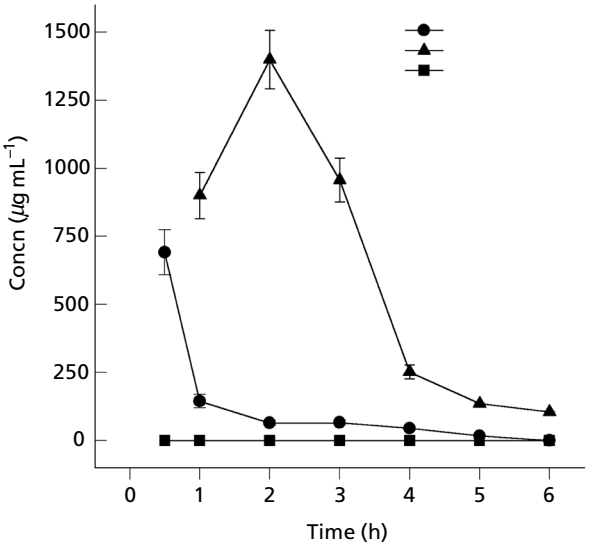

Figure 4 Concentration profile of prednisolone (PD) and PDS in the contents of the proximal and distal small intestine (PSI and DSI, respectively) after oral administration of PDS (5 mg) to rats. Each data point represents mean ± s.e. (n = 3~5).

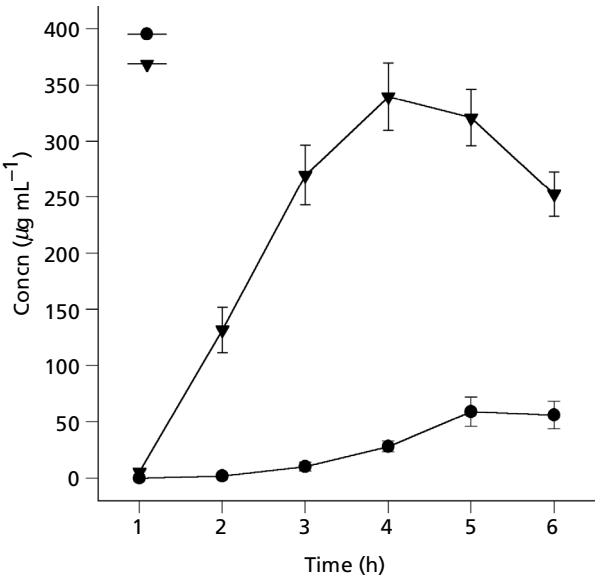

Figure 5 Concentration profile of prednisolone (PD) and PDS in the contents of the large intestine of rats after oral administration of PDS (5 mg) to rats. Each data point represents mean ± s.e. (n = 3 ~ 5).

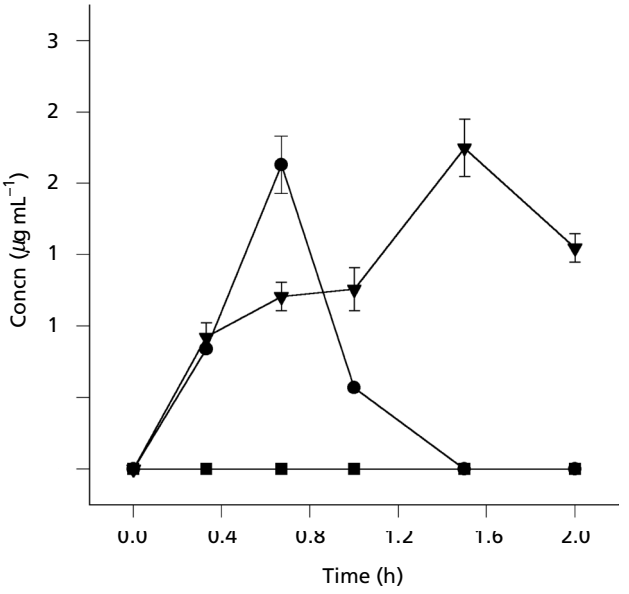

Figure 6 Concentration profile of prednisolone in the contents of proximal small intestine (●), distal small intestine (▼) and caecum (■) of rats after oral administration of prednisolone (5 mg) to rats. Each data point represents mean ± s.e. (n = 3 ~ 5).

Discussion

Delivery of orally administered drugs specifically to the colon is highly desirable for the efficient treatment of diseases developed locally at the colon, such as ulcerative colitis, Crohn's disease, or colorectal cancer. Being delivered

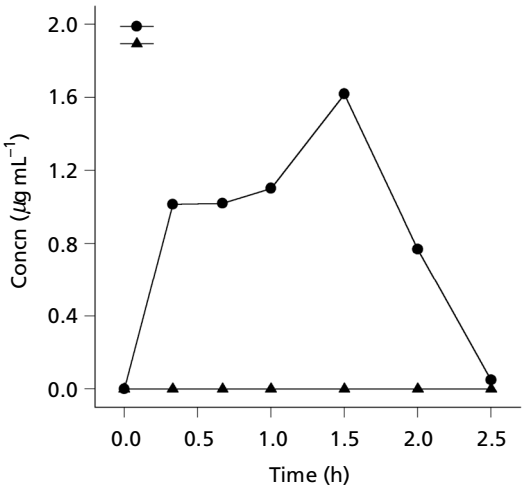

Figure 7 Concentration profile of prednisolone in the plasma after oral administration of prednisolone (PD) or PDS (equivalent to 5 mg PD) to rats. Each data point represents mean ± s.e. (n = 3 ~ 5).

specifically to the site of action with limited systemic absorption, only a small dose is needed, which subsequently results in reduced side effects. Glucocorticoids with a limited systemic effect are desirable for the efficient treatment of inflammatory bowel disease.

Budesonide is considered to be one such drug due to its high first-pass bio-inactivation. It has been evaluated for use in patients with inflammatory bowel disease either as an enema or as a controlled ileal-release formulation (Robinson 1998). Colon-specific pro-drugs aiming at the delivery of glucocorticoid to the colon have been introduced utilizing polymers (McLeod et al 1994) or highly hydrophilic small molecules (Friend & Chang 1984, 1985; Fedorak et al 1995) as carriers.

It is reported that sulfatase activity is high in the caecum but very low in the small intestine of rats (Huijghebaert et al 1984a, b). Van Eldere et al (1988) reported on the intestinal steroid-desulfating bacteria from rats and man. Huijghebaert et al (1984b) reported that 3-α-sulfates of bile acids were desulfated on incubation with faecal flora of man, rat and mouse. Even though there might exist some interspecies differences between rat and man, these studies suggested that sulfate ester would selectively be hydrolysed by the microbes in the large intestine of rats and man. Based on this information, we prepared PDS as a colon-specific pro-drug of prednisolone, expecting that sulfate ester could serve as a promising colon-specific pro-moiety.

Sulfation of prednisolone proceeded readily and efficiently to yield PDS by using 1.7 molar excess of STT and adjusting the reaction time by TLC monitoring. PDS showed increased water solubility, low partition coefficient and chemical stability at wide ranges of pH. PDS was stable on incubation with the contents of small intestine. These properties indicated that PDS might be stable and non-absorbable during the transit through the upper intestine and delivered to the colon in intact form after

oral administration. On incubation with caecal contents, PDS released prednisolone, but the amount of prednisolone did not coincide with the decreased amount of PDS. When PDS (1 mg) was incubated in 10 mL of 5% caecal contents in pH 6.8 isotonic phosphate buffer solution, the amount of PDS decreased to 79, 52, 30 and 18% of the initial dose at 2, 4, 6 and 8 h, respectively (left side on Figure 2). If prednisolone is the only product of PDS, and if prednisolone is stable under the condition of incubation, the amount of prednisolone would be expected to be 21, 48, 70, and 82% of the initial dose at 2, 4, 6 and 8 h, respectively. It was less than that of the decreased PDS, and the greater the gap, the longer the incubation period. We incubated prednisolone under the same condition and found that the amount of prednisolone decreased progressively (Figure 3). Considering that reduction is one of the common metabolic reactions of the microbes in the large intestine, it seemed that reduction of the A ring of prednisolone had taken place because the A ring is the only chromophore in the structure of prednisolone and the reduced forms were not detectable by the UV detector of the HPLC system. The rate of PDS conversion and the level of prednisolone in the medium was affected by the concentration of the caecal contents. The rate of PDS conversion increased as the concentration of caecal contents increased (left side on Figure 2). However, we noticed that the level of prednisolone was not in parallel order with the concentration of caecal contents as the incubation period extended (right side on Figure 2). The maximum level of prednisolone was obtained in the order of 5% > 10% > 3% caecal contents. The amount of prednisolone in the medium was related to the relative rates of sulfate hydrolysis of PDS and reduction of prednisolone, which might be affected by the concentration of the caecal contents. Our results suggest that hydrolysis precedes the reduction process, which is slower than hydrolysis. It seems that hydrolysis dominates in the earlier period of incubation, to produce and accumulate prednisolone. As the incubation period extends, the amount of PDS, the rate of hydrolysis and consequently the production of prednisolone decreases, which lowers the level of prednisolone as the reduction proceeds dominantly. When PDS was incubated with a lower concentration of caecal contents (3%), the level of prednisolone was low and nearly constant for a considerable period, which implies that the production of prednisolone was comparable with the loss of prednisolone by reduction process. The anti-inflammatory action of glucocorticoids is known to diminish if the A ring of the steroid is reduced (Schimmer & Parker 2001). Therefore, the potential of PDS as a colon-specific pro-drug of prednisolone will be dependent on the relative rate of hydrolysis (bio-activation) and reduction of the A ring of the steroid (bio-inactivation) by the microbes in the large intestine (Figure 8).

After oral administration of prednisolone or PDS in an equivalent dose, the concentration of prednisolone or PDS from the plasma and contents of various segments of the gastrointestinal tract was determined. After oral administration of PDS, the concentration of PDS was very high (C_{max}: ~1399 ppm) in the contents of the small intestine, but the level of prednisolone was below the limit of detection (Figure 4). This indicated that PDS was stable and was hardly absorbed in the small intestine, which are the prerequisite characteristics of a colon-specific pro-drug. In this case, a large fraction of the pro-drug would be delivered to the large intestine in intact form. As the pro-drug reaches the large intestine, the level of PDS will increase until the amount of bioconversion, and the amount moving out to the next section of the gastrointestinal tract, surpasses that of the incoming amount, and then decrease. The level of prednisolone produced from PDS will increase until the amount of removal by reduction and absorption exceeds that of formation. This is illustrated in Figure 5, where T_{max} of prednisolone falls behind that of PDS. After oral administration of prednisolone (Figure 6), the concentration of prednisolone determined from the contents of the small intestine was very low (C_{max}: ~21 ppm) and that in the large intestine was below the limit of detection. This implied that a large fraction of the dose was rapidly absorbed from the small intestine as expected, which would afford only a small fraction of the dose for the colon delivery. In both cases, the level of prednisolone in the large intestine was much lower than one would expect. Prednisolone in the large intestine, whether it is delivered from the upper intestine after oral administration of prednisolone or produced from PDS by the microbial enzymes in the large intestine after oral administration of PDS, will undergo reductive metabolism as we stated in the in-vitro experiments and a portion of it might be absorbed. This might explain the low level of prednisolone in the large intestine in this study. The concentration of prednisolone in the plasma after oral administration of prednisolone, which originates mostly from upper intestinal absorption, approached a maximum

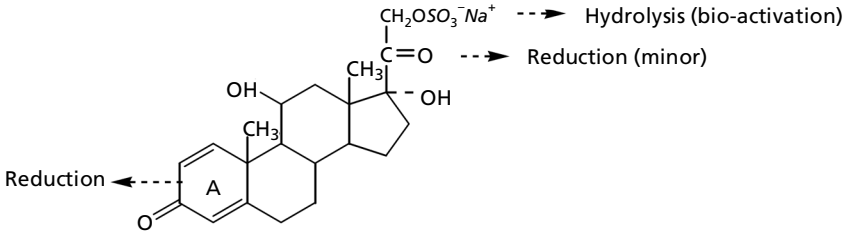

Figure 8 Proposed metabolic pathways of prednisolone 21-sulfate sodium in the large intestine.

at around 1.5 h (1.6 μg mL^{-1}), decreased rapidly and became below the limit of detection (0.02 μg mL^{-1}) after 2.5 h. After oral administration of PDS, the concentration of prednisolone or PDS in the plasma was below the limit of detection. PDS would not be detected in the plasma if the transcellular absorption of PDS were limited due to the low partition coefficient. The level of prednisolone in the blood would depend on the relative rate and extent of absorption and elimination. Prednisolone is produced from the pro-drug in the large intestine in a controlled manner and a portion of the released prednisolone undergoes reduction. These facts might keep the concentration of prednisolone at low level in the large intestine for an extended period of time (Figure 5) and restrict absorption of prednisolone, which are desirable characteristics for a colon-specific pro-drug of prednisolone.

Conclusion

Our data demonstrate that the sulfate ester can serve as a novel colon-specific pro-moiety by limiting the absorption of the pro-drug in the upper intestine and releasing the active compound by the action of microbial sulfatase in the colon. Selection of a glucocorticoid that is resistant to the bio-inactivation process (reduction) in the colon might be crucial for the development of a potential colon-specific pro-drug.

References

Brown, J. P., McGarraugh, G. V., Parkinson, T. M., Wingard, R. E., Onderdonk, A. B. (1983) A polymeric drug for treatment of inflammatory bowel disease. *J. Med. Chem.* **26**: 1300–1307

Crotty, B, Jewel, J. P. (1992) Drug therapy of ulcerative colitis. *Br. J. Clin. Pharmacol.* **34**: 189–198

Faigle, J. W. (1993) Drug metabolism in the colon wall and lumen: In: Bieck, P. R. (ed.) *Colonic drug absorption and metabolism*. Marcel Dekker Inc., New York, NY, pp 29–54

Fedorak, R. N., Haeberlin, B., Empey, L. R., Cui, N., Nolen, H., Jewell, L. D., Friend, D. R. (1995) Colonic delivery of dexamethasone from a prodrug accelerates healing of colitis in rats without adrenal suppression. *Gastroenterology* **108**: 1688–1699

Friedman, S., Blumberg, R. S. (2001) Inflammatory bowel disease. In: *Harrison's principles of internal medicine*. 15th edn, CD-ROM, McGraw-Hill, New York

Friend, D. R., Chang, G. W. (1984) A colon-specific drug-delivery system based on drug glycosides and the glycosidases of colonic bacteria. *J. Med. Chem.* **27**: 261–266

Friend, D. R., Chang, G. W. (1985) Drug glycosides: potential prodrugs for colon-specific drug delivery. *J. Med. Chem.* **28**: 51–57

Huijghebaert, S., Sim, S. M., Back, D. J., Eyssen, H. J. (1984a) Distribution of estrone sulfatase activity in the intestine of germ-free and conventional rats. *J. Steroid Biochem.* **20**: 1175–1179

Huijghebaert, S., Parmentier, G., Eyssen, H. J. (1984b) Specificity of bile salt sulfatase activity in man, mouse and rat intestinal microflora. *J. Steroid Biochem.* **20**: 907–912

Istran, C., Gabor, S., Ferene, S. (1991) Glycosides of 5-aminosalicylic acid. *Magy. Kem. FOLY* **97**: 143–147

Jung, Y. J., Lee, J. S., Kim, Y. M. (2000) Synthesis and in vitro/in vivo evaluation of 5-aminosalicyl-glycine as a colon-specific prodrug of 5-aminosalicylic acid. *J. Pharm. Sci.* **89**: 594–602

Jung, Y. J., Lee, J. S., Kim, Y. M. (2001) Colon-specific prodrug of 5-aminosalicylic acid: synthesis and in vitro/in vivo properties of acidic amino acid derivatives of 5-aminosalicylic acid. *J. Pharm. Sci.* **90**: 1767–1775

Kopeckova, P., Kopecek, J. (1990) Release of 5-aminosalicylic acid from bioadhesive N-(2-hydroxypropyl) methacrylamide copolymers by azoreductases *in vitro*. *Makromol. Chem.* **191**: 2037–2045

Larsen, C., Johansen, M. (1985) Dextrans as carriers for drug compounds – realized and potential applications. *Arch. Pharm. Chem.* **92**: 809–830

McLeod, A. D., Friend, D. R., Tozer, T. N. (1993) Synthesis and chemical stability of glucocorticoid-dextran ester: potential prodrugs for colon-specific delivery. *Int. J. Pharm.* **92**: 105–114

McLeod, A. D., Friend, D. R., Tozer, T. N. (1994) Glucocorticoid-dextran conjugate as potential prodrugs for colon-specific delivery: hydrolysis in rat gastrointestinal tract contents. *J. Pharm. Sci.* **83**: 1284–1288

Robinson, M. (1998) Medical therapy of inflammatory bowel disease for the 21st century. *Eur. J. Surg. Suppl.* **582**: 90–98

Rubinstein, A. (1990) Microbially controlled drug delivery to the colon. *Biopharm. Drug Dispos.* **11**: 465–475

Ryde, E. M. (1992) Low-molecular-weight azo compounds. In: Friend, D. R. (ed.) *Oral colon specific drug delivery*. CRC press, Boca Raton, FL, pp 143–152

Schimmer, B. P., Parker, K. L. (2001) Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones. In: Hardman, J. G., Limbird, L. E. (Editors-in-chief) *Goodman and Gilman's the pharmacological basis of therapeutics*. 10th edn, McGraw-Hill, New York, NY, pp 1649–1677

Van Eldere, J., Robben, J., De Pauw, G., Merckx, R., Eyssen, H. (1988) Isolation and identification of intestinal steroid-desulfating bacteria from rats and humans. *Appl. Eviron. Microbiol.* **54**: 2112–2117

William-Smith, H. (1965) Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J. Pathol. Bacteriol.* **89**: 95–122