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Research Paper

Dexamethasone 21-Sulfate Improves the Therapeutic Properties of Dexamethasone Against Experimental Rat Colitis by Specifically Delivering the Steroid to the Large Intestine

Inho Kim,[1] Hyesik Kong,[2] Younghyun Lee,[1] Sungchae Hong,[1] Jungoh Han,[1] Sunhwa Jung,[1] Yunjin Jung,[1,3] and Young Mi Kim[1,3]

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Purpose. We investigated *in vivo*-colon targetability and therapeutic properties of DS against experimental rat colitis.
Methods. The systemic absorption and colonic delivery of D after oral administration of DS was analyzed by examining the concentration of drugs in the GI tract, plasma, urine and feces. Therapeutic activity of DS was determined using a TNBS-induced rat colitis model. Adrenal suppression by DS administration was evaluated by monitoring the concentration of ACTH and corticosterone in the plasma.
Results. DS administered orally was delivered efficiently to the large intestine resulting in D accumulation at the target site. In addition, DS was not detectable in the plasma and was detected very low in the urine after DS administration. The fecal and urinary recovery of D (after DS administration) was much greater and less than that after D administration, suggesting that DS should exhibit enhanced therapeutic activity and reduced systemic side effects. Consistent with this notion, DS was more effective than D in healing rat colitis. Moreover, oral administration of either D or DS reduced the plasma corticosterone and ACTH levels from the normal levels, which is significantly greater for D.
Conclusion. DS is a promising colon specific prodrug that improves therapeutic properties of D

KEY WORDS: colon specific prodrug; dexamethasone; dexamethasone 21-sulfate; inflammatory bowel disease; systemic adverse effect.

INTRODUCTION

Inflammatory bowel disease (IBD), a form of autoimmune disease, is a general term for a group of chronic inflammatory disorders of unknown cause involving the gastrointestinal tract. Chronic IBD may be divided into two major groups, chronic nonspecific ulcerative colitis (UC) and Crohn's disease (CD). The incidence and prevalence of the two diseases differ slightly, UC being the more common. The major symptoms of UC are bloody diarrhea and abdominal pain, often with fever and weight loss in more severe cases. The basic pathologic features of CD are the same regardless of whether the disease involves the small bowel or the colon (1–3). Initial treatment of all forms of uncomplicated IBD is primarily medical, and the principles of medical therapy are similar for the two types of disease. The principal drugs used in the therapy of ulcerative colitis are the anti-inflammatory agents sulfasalazine and glucocorticoids (4,5).

Glucocorticoids including dexamethasone, which have been used most frequently for the treatment of inflammatory bowel diseases, are well absorbed in the upper intestine and only a limited fraction of the administered dose is delivered to the inflammatory site in the ileum or colon (4,6). Because of systemic side effects such as osteoporosis, hypertension, edema, diabetes, or decreased immunity, administration of glucocorticoids by the oral and intravenous routes is generally reserved for treatment of severe acute disease (6). Administration of glucocorticoids by the rectal route is often limited by poor patient compliance and the drug is largely confined to the distal region of the colon. To reduce the serious side effects caused by the systemic absorption, development of a colon-specific delivery system of corticosteroids is highly desirable (7–11). This site-specific delivery of the drugs would increase therapeutic concentration at the target site along with decreasing systemic absorption of the drugs, thereby enhancing therapeutic potency and reducing adverse effects.

Development of a prodrug where the drug is coupled to a carrier by way of covalent bond is an efficient way to deliver

[1] Laboratory of Biomedicinal/Medicinal Chemistry, College of Pharmacy, Pusan National University, Pusan, 609-735, Republic of Korea.
[2] Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892, USA.
[3] To whom correspondence should be addressed. (e-mail: jungy@pusan.ac.kr; ymikim@pusan.ac.kr)

ABBREVIATIONS: ACTH, Adrenocorticotropic hormone; CD, Crohn's disease; D, Dexamathasone; DS, Dexamathasone 21-sulfate or sulfate conjugated dexamathasone; DSI, Distal small intestine; IBD, Inflammatory bowel disease; PSI, Proximal small intestine; TNBS, 2,4,6-Trinitrobenzenesulfonic acid.

drugs specifically to a target site. Design of colon-specific delivery system requires accessibility to the colonic site after oral administration and activation in the gut lumen. Hydrophilic small molecules or polymers are used as colon-specific carriers to limit the absorption in the upper intestine (12). The linkage between the drug and promoiety should be chemically and enzymatically stable during the transit through the stomach and small intestine. After delivered to the colon, the prodrug is presumed to be activated by the enzymes originated from the microbes which are especially abundant in that portion of the alimentary canal (13). There are numerous reports on the development of colon specific drug delivery systems using polymeric or small molecular carriers (12).

Previously, we reported that sulfation of D greatly reduces apparent partition coefficient of D and dexamethasone 21-sulfate (DS) is desulfated efficiently to dexamethasone (D) in the cecal contents of rats while stable in the contents of the upper intestine. These data suggested that DS would deliver D specifically to the large intestine without significant loss in the upper intestine (11). In this report, DS was evaluated as a colon specific prodrug of D *in vivo* along with investigation of therapeutic activity of DS against TNBS-induced colitis of rats. We also inspected whether DS could reduce adverse effects of D upon oral administration.

MATERIALS AND METHODS

Dexamethasone (D) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were purchased from Sigma Chemical Co. (St. Louis, Mo., USA) and used as received. Solvents for HPLC were obtained from Merck Inc. (Darmstadt, Germany). All other chemicals were reagent grade, commercially available products. An Orion 320 pH meter (Orion, Boston MA, USA) was used for pH measurements. A Polytron PT 3100 homogenizer was used for homogenization of the tissue of rats and an Effendorf Centrifuge 5415C (Effendorf, Hamburg, Germany) was used for centrifugation. A Taitec microincubator M-36 (Tokyo, Japan) was used for incubation. HPLC system consisted of Model 305, 306 pumps, a 117 variable UV detector, a Model 234 autoinjector, a Model 805 manometric module, a Model 811C dynamic mixer from Gilson. A μBondapak C_{18} (3.9×300 mm, Waters, Milford, MA, USA) column with a guard column (3.9×20 mm, Waters) was used. Male Sprague–Dawley rats (200~260 g, 6 to 7 weeks old) were purchased from Daehan Biotec Co. Ltd (Daegu, Korea) and housed in the animal care facility at Pusan National University, Pusan, Korea. Dexamethasone 21-sulfate (DS) was prepared as described previously (11). The chemical structures of D and DS are shown in Supplementary data. Buffer solutions were prepared as described in USP XXIII.

HPLC Analysis

The concentration of DS and D was measured by a reverse-phase HPLC. Standard or blank solution (1 mL) was mixed on a vortex mixer for 2 min, centrifuged at 14,000 rpm for 5 min and filtered through a membrane filter (0.45 μm). A portion of the filtrate (20 μL) was injected on a μBondapak C_{18} column and eluted with the mobile phase at a flow rate of 1.5 mL/min. The mobile phase consisted of acetonitrile/0.067 M, pH 4.5 phosphate buffer (40/60) solution, which was filtered through 0.45 μm membrane filter before use. The eluate was monitored by measuring the absorption at 254 nm at sensitivity of AUFS 0.01. The Gilson 712 HPLC software was employed for the data analysis. The retention time of D and DS was 5.27 and 3.54 min, respectively. Concentration of D and DS in the sample was calculated from the calibration curve.

Time–Concentration Profiles of D and DS in Various Segments of the GI Tract and Plasma after Oral Administration of Drugs to Rats

Male Sprague–Dawley rats (250–260 g) were maintained on a stock diet and water *ad libitum*. These animals were fasted overnight (16 h) prior to administration of D or DS. Water bottles were removed from the cages at least 30 min prior to drug administration to assure that stomach would be empty. D (1 mg) or DS (equivalent to 1 mg of D) in 0.3 mL of 25% ethanol solution was administered to rats by a gastric intubation. After an appropriate time interval, the animals were sacrificed by ether anesthesia. Blood was collected by intracardiac puncture through a heparinized syringe before death. The blood was then centrifuged for 5 min at 14,000 rpm. The serum was removed and stored frozen before being analyzed by HPLC. The contents of proximal small intestine (PSI), distal small intestine (DSI), cecum and colon were collected separately. To each intestinal contents, was added an equal volume of pH 6.8 phosphate buffer solution, and centrifuged at 14,000 rpm for 3 min. To a 100 μL portion of the supernatant, was added 900 μL of methanol, vortexed for 2 min, and centrifuged for 5 min at 14,000 rpm. The concentration of DS and/or D in a 20 μL portion of the supernatant was determined by HPLC.

Determination of DS and/or D in Feces and Urine after Oral Administration of the Drugs to Rats

Male Sprague–Dawley rats (250–260 g) were placed in a metabolic cage and starved for 24 h prior to use for the experiments but had free access to water. D or DS (1 mg equivalent of D/day) was orally administered. The fecal and urinary samples were collected separately at 2 h intervals for 24 h and stored immediately in a freezer before being analyzed by the following procedure. The fecal or urinary sample was diluted with isotonic phosphate buffer solution (pH 6.8) to 20-fold or ten-fold, respectively. The sample was vortexed and centrifuged at 5,000 rpm for 3 min. To a 0.1 mL portion of the supernatant, was added 0.9 mL of methanol, vortexed for 2 min, and centrifuged for 5 min at 14,000 rpm. The concentration of D and/or DS in a 20 μL portion of the supernatant was analyzed by HPLC.

Induction of Inflammation

The rats were lightly anesthetized with ether. A rubber catheter (OD, 2 mm) was inserted rectally into the colon such that the tip was 8 cm proximal to the anus, approximately at the splenic flexture. 2,4,6-Trinotrobenzenesulfonic acid (TNBS) dissolved in 50% (*v*/*v*) aqueous ethanol was instilled

into the colon via the rubber cannula (30 mg/0.5 mL/rat). The instillation procedure required about 5 s to complete.

Evaluation of TNBS-Induced Colitis

A gross colonic damage score was calculated according to the criteria set forth previously (14,15). The modified scoring system is: 0, normal appearance; 1, localized hyperemia but no ulcer; 2, linear ulcers without significant inflammation; 3, 2–4 cm site of inflammation and ulceration without scab; 4, serosal adhesion to other organs, 2–4 cm site of inflammation and ulceration with scab; 5, stricture, serosal adhesion involving several bowel loops, >4 cm site of inflammation and ulceration with scab. Four independent observers blinded to the treatment did the assessment of colonic damage score. Using the distal colon (5 cm), myeloperoxidase (MPO) activity was measured as described previously (16). One unit of MPO activity is defined as that degrading 1 μmol of peroxide per minute at 25°C.

Measurement of Plasma Corticosterone and Adrenocorticotropic Hormone Levels

To measure plasma corticosterone and adrenocorticotropic hormone (ACTH) levels after oral administration of D or DS (0.22 and 0.44 μmol/kg day) to rats once a day for 14 days, blood samples (2 mL) were collected in heparinized or EDTA tubes from normal or TNBS-induced colitis rats by intracardiac puncture between 8 AM and 10 AM on the day of sacrifice. The blood was then centrifuged at 4,000 rpm for 10 min at 4°C. The plasma was immediately removed and stored at −80°C until analysis. Corticosterone and ACTH concentrations in the plasma were measured using commercial enzyme immunoassay kits (Cayman chemical company, Michigan, USA and Calbiotech. Inc., CA, USA). Determination of corticosterone and ACTH concentrations was achieved according to the protocol described in the kits.

Dose Regimen

D or DS was administered to rats 24 h after induction of colitis by an oral zonde at a dose ranging from 0.0137 to 0.44 μmol/kg day. The daily dose of D, 0.22 μmol/kg (86.24 μg/kg day), is approximately equivalent to that of prednisolone (0.6 μmol/kg day) prescribed for the treatment of human idiopathic ulcerative colitis. After medication for 6 days, the rats were sacrificed, and used for measurement of MPO activity and colonic damage score.

Statistical Analysis

The results are expressed as mean±SE. Three to five replicate studies were executed for *in vivo* studies. The nonpaired Student's *t* test was used to assess the statistical significance ($P<0.05$) of results for all measurements.

RESULTS

Time–Concentration Profiles of D and DS in Various Segments of GI Tract and Plasma after Oral Administration of DS

In a previous report, we demonstrated that sulfation of D greatly reduces the apparent partition coefficient of D and DS is cleaved to liberate D in the cecal contents while stable in the contents of the upper intestine, suggesting that DS administered orally is specifically delivered to and converted to D in the large intestine without significant loss in the upper intestine (11). We examined whether DS behaved as suggested by the *in vitro* results after oral administration. DS (equivalent to 1 mg of D) was administered to rats by a gastric intubation, sacrificed the rat according to the predetermined time schedule, and the concentration of D and DS from various segments of the gastrointestinal tract was determined. For comparison, the same experiment was done

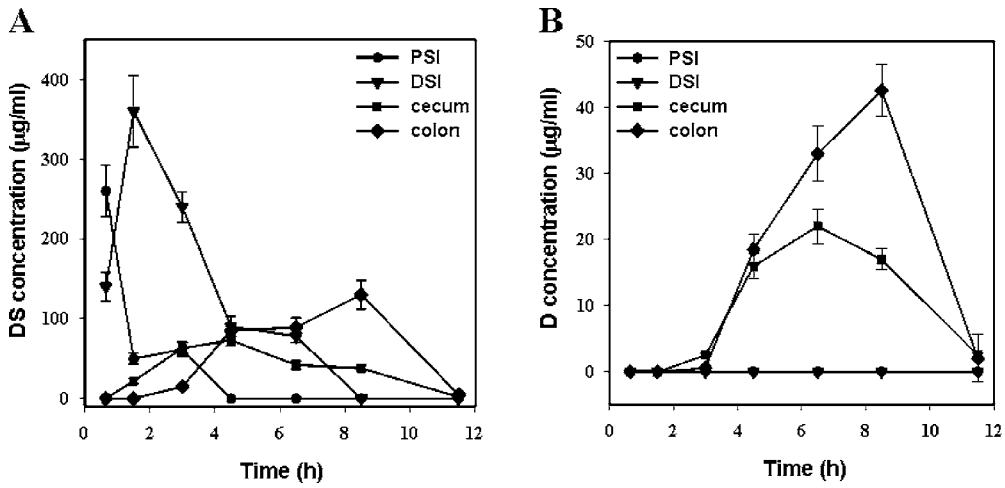

Fig. 1. Concentration profiles of D and DS recovered from the contents of various segments of the GI tract after oral administration of DS to rats. **A** DS (equivalent to 1 mg of D) in 0.3 mL of 25% ethanol solution was administered to rats (250–260 g) by a gastric intubation. After an appropriate time interval, the animals were sacrificed by ether anesthesia. The contents of proximal small intestine (*PSI*), distal small intestine (*DSI*), cecum and colon were collected separately. The concentration of DS in the contents was determined by HPLC. **B** The same experiment was done to monitor the distribution of D. The data **A** and **B** are mean± SE ($n=4$).

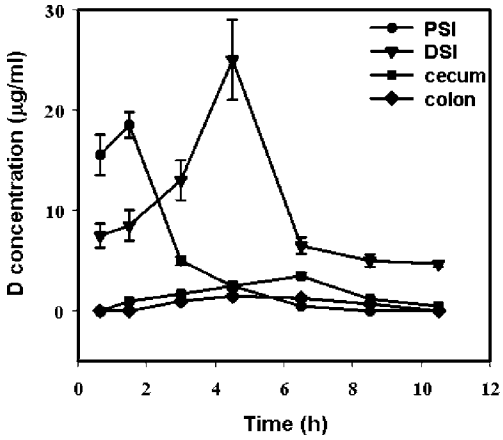

Fig. 2. Concentration profiles of D recovered from the contents of various segments of the GI tract after oral administration of D to rats. D (1 mg) in 0.3 mL of 25% ethanol solution was administered to rats (250–260 g) by a gastric intubation. After an appropriate time interval, the animals were sacrificed by ether anesthesia. The contents of proximal small intestine (*PSI*), distal small intestine (*DSI*), cecum and colon were collected separately. The concentration of D in the contents was determined by HPLC. The data are mean±SE (n=4).

with D. As shown in Fig. 1A, the C_{max} (t_{max}) of DS for the PSI, DSI, cecum and colon was 262 μg/mL (0.5 h), 360 μg/mL (1.5 h), 74 μg/mL (4.5 h) and 132 μg/mL (8.5 h), respectively. After 4.5 h, the concentration level of DS in the large intestine began to exceed that in the small intestine. Fig. 1B shows the time–concentration profiles of D from various segments of the gastrointestinal tract after oral administration of DS. While D was not detected from the entire region of the small intestine, the C_{max} (t_{max}) of D for the cecum and colon was 22 μg/mL (6.3 h) and 42 μg/mL (8.5 h), respectively. On the other hand, as shown in Fig. 2, after oral administration of D, the C_{max} (t_{max}) for the PSI and DSI was 18 μg/mL (1.5 h), and 26 μg/mL (4.5 h), respectively. The concentration of D from the cecum and colon was very low during the entire experimental period (10.5 h). To show the different disposition after oral administration of D or DS more clearly, the above data were presented as cumulative amount of D in the small and large intestine after oral administration of D or DS. Fig. 3A and B showed that while substantial amount of D appeared in the small intestine, very low level of D was monitored in the large intestine following oral administration of D. In contrast to this, D was accumulated significantly in the large intestine but not in the upper intestine following DS administration. These results indicate while D administered orally hardly reached the large intestine, DS was delivered to the large intestine without conversion to D in the upper intestine and was deconjugated to liberate D. The disposition data and low partition coefficient suggest poor systemic absorption of DS. To verify that the systemic absorption of DS was limited, DS was administered orally and the levels of DS in the blood and urine collected for 24 h were monitored. For comparison, D was subjected to the same experiment. As shown in Fig. 4, the C_{max} was 2.3 μg/mL 3 h after D administration and D in the plasma was observed until 6.5 h. The urinary recovery was about 26% of the dose (The urinary recovery was presented as amount in Fig. 5B). On the contrary, DS was not detected in the plasma during the entire experimental period (24 h) and the urinary recovery of DS was about 5% of the dose (data not shown).

Recovery of D in the Feces and Urine after Oral Administration of DS

Since fecal and urinary recovery of an active agent for a colon specific prodrug administered orally is relevant to the therapeutic activity and systemic side effects, we compared concentration of D in the feces and urine after oral administration of either D or DS. As shown in Fig. 5A and B, from the specimen collected for the 24 h, while D was not detected in the feces after D administration, 11.7 μg of D was

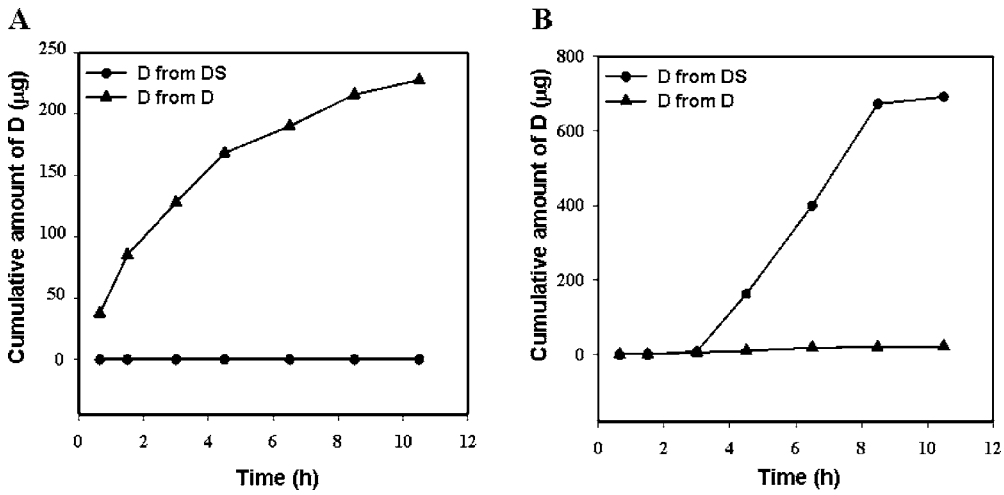

Fig. 3. Cumulative amount of D recovered from the contents of the GI tract after oral administration of D or DS to rats. **A** D or DS (equivalent to 1 mg of D) in 0.3 mL of 25% ethanol solution was administered to rats (250–260 g) by a gastric intubation. After an appropriate time interval, the animals were sacrificed by ether anesthesia. The contents of proximal small intestine (*PSI*) and distal small intestine (*DSI*) was collected. The concentration of D in the contents was determined by HPLC and cumulative amount of D was calculated. **B** The same experiment was done except that the concentration of D in the contents of the large intestine was determined by HPLC.

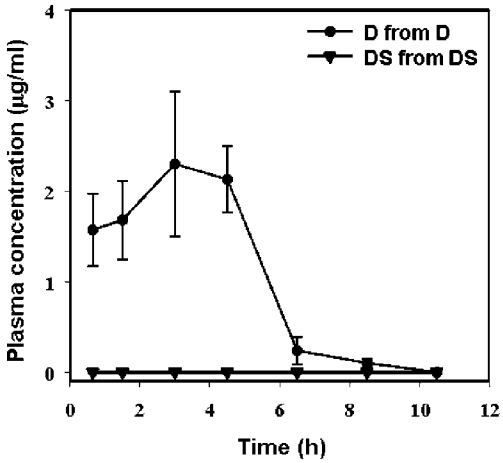

Fig. 4. Plasma concentration of DS after oral administration of DS. DS (equivalent to 1 mg of dexamethasone) in 0.3 mL of 25% ethanol solution was administered to rats (250–260 g) by a gastric intubation. After an appropriate time interval, the animals were sacrificed by ether anesthesia. Blood was collected by intracardiac puncture through a heparinized syringe before death. The concentration of DS in the plasma was determined by HPLC. For comparison, the same experiment was done with D (1 mg). The data are mean±SE (n=4).

recovered from the feces after DS administration. Moreover, the urinary recovery of D was much greater after administration of D than that of DS. These data suggest that DS should exhibit improved therapeutic activity and reduced systemic side effects compared with D. On the other hand, DS was not detected from the feces, which suggests that DS is completely deconjugated by the time of defecation.

Therapeutic Effect of DS Against TNBS-Induced Colitis Rats

The data of the fecal recovery of D suggest that DS possesses a greater therapeutic activity than D at a same dose. To verify the notion, DS (0.0137~0.44 μmol/kg day) was administered orally to TNBS-induced colitis rats once a day starting from 1 day after induction of inflammation. Colonic damage score and myeloperoxidase activity, an indicator of neutrophil infiltration, were determined after medication for 6 days. The same experiment was done with D (0.0137~0.44 μmol/kg day) for comparison. As shown in Fig. 6A in which colonic damage scores represent the extent of colonic injury by TNBS-induced inflammation, the normal colon showed no damage but the control colon, the inflamed colon without medication, was severely damaged showing scab by the hemorrhagic necrosis of the mucosa, stricture and extensive serosal adhesion to other organs. Oral administration of DS or D significantly healed the damaged colon in a dose dependent manner. Moreover, DS was more effective than D in healing the colonic injury at the same doses. Fig. 6B showed that the level of MPO activity in the distal colon markedly increased by induction of inflammation and oral administration of DS or D lowered the level of MPO activity in a dose dependent manner. In accordance with the recovery of the colonic injury, the effectiveness of DS was greater than that of D at the same doses.

ACTH and Corticosterone Levels in the Blood After Oral Administration of DS

Our data comparing the urinary recovery of D suggest that DS should reduce the systemic side effects of D. We examined whether DS, indeed, has such a beneficial effect. Since adrenal suppression is a typical systemic adverse effect of glucocorticoids including D, it was tested whether DS could attenuate the adrenal suppressive effect caused by D. DS or D at the doses where the colonic injury was recovered significantly was administered orally to colitic rats once a day for 14 days and corticosterone and ACTH levels in the plasma were measured using Elisa kits. As shown in Fig. 7A and B, compared with the plasma levels of the hormones in the normal rats, a little increase in ACTH and corticosterone levels was observed in the inflamed rats. Although oral administration of either DS or D reduced the levels of the hormones from the normal levels, the adrenal suppression by D was much greater than that by DS.

DISCUSSION

In this study, we demonstrate that DS administered orally was delivered specifically to the large intestine without significant

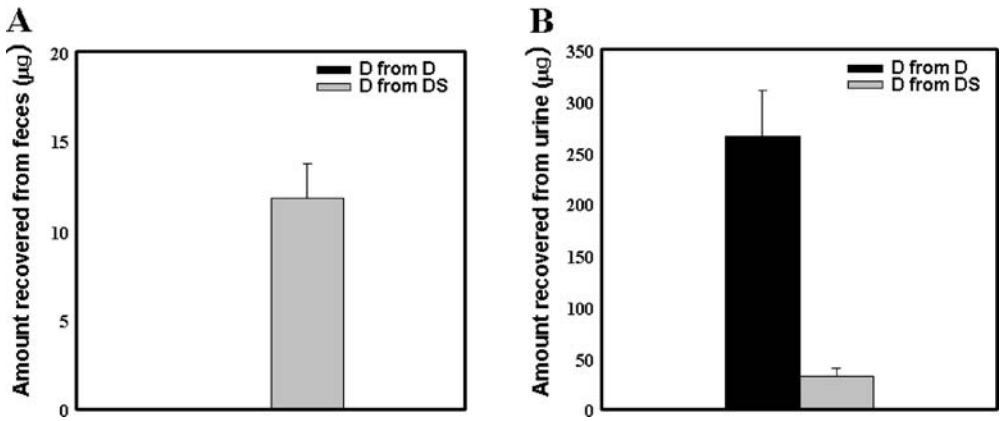

Fig. 5. The fecal and urinary recovery of D after oral administration of DS. **A** DS (equivalent to 1 mg of D) in 0.3 mL of 25% ethanol solution was administered to rats (250–260 g) by a gastric intubation. The fecal samples were collected separately at 2 h interval for 24 h. The concentration of D in the samples was analyzed by HPLC. For comparison, the same experiment was done with D. **B** The same experiment as in **A** was done except monitoring D in the urinary samples. The data in **A** and **B** are mean±SE (n=4).

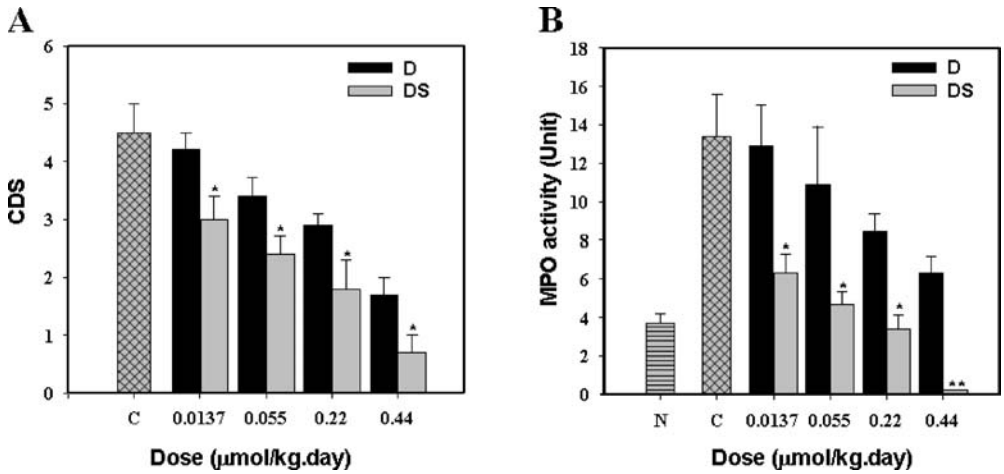

Fig. 6. Therapeutic effect of DS against TNBS-induced rat colitis. **A** DS was administered to rats 24 h after induction of colitis by an oral zonde at a dose ranging from 0.0137 to 0.44 μmol/kg day. After medication for 6 days, the rats were sacrificed, and colonic damage score (*CDS*) was calculated as described under "MATERIALS AND METHODS" For comparison, the same experiment was done with D *$P<0.05$ *vs.* D-treated mice at the same doses **B** DS was administered as described as in **A**. MPO activity in the distal colon (5 cm) was measured as described under "MATERIALS AND METHODS" For comparison, the same experiment was done with D. *$P<0.05$ *vs.* D-treated mice at the same doses, **$P<0.001$ *vs.* D-treated mice at the same dose The data in **A** and **B** are mean±SE ($n=4$).

absorption and (bio)chemical loss in the upper intestine, increasing colonic concentration and reducing systemic absorption of D. Consistent with the *in vivo* disposition of DS, DS exhibited a greater therapeutic activity against TNBS-induced experimental colitis and a lower adrenal suppression than D.

In our previous report, sulfation of D greatly reduces apparent partition coefficient of D, which probably renders DS to be poorly absorbable in the upper intestine (11). Consistent with this, we found that the systemic absorption of DS was limited after oral administration. This is demonstrated by the data showing that (1) DS was not detectable in the plasma and the urinary recovery of DS was very low following oral administration of DS and (2) DS (after DS administration) was accumulated in the small intestine at much greater amount than D (after D administration). This poor systemic absorption seems to be linked to efficient delivery of DS to the large intestine. This argument is supported by the observation that DS administered orally afforded much greater level of D in the large intestine and in the feces than did D. This observation that DS delivered to the large intestine generated D is in agreement with the previous *in vitro* result of conversion of DS to D in the cecal contents. Efficient colonic delivery of an active agent from its prodrug is relevant to improve therapeutic activity (17). In parallel

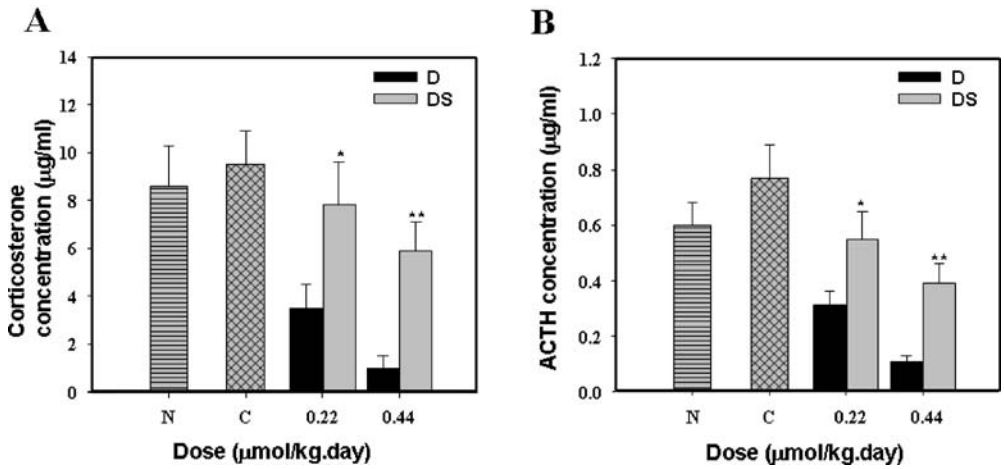

Fig. 7. Corticosterone and ACTH levels in the plasma after oral administration of DS. **A** DS (0.22 and 0.44 μmol/kg day) was administered orally to colitic rats once a day for 14 days. Blood samples (2 mL) were collected in heparinized or EDTA tubes from the rats by intracardiac puncture between 8 AM and 10 AM on the day of sacrifice. The corticosterone concentrations in the plasma were measured using commercial enzyme immunoassay kits. *$P<0.05$ *vs.* D-treated mice at the same doses, **$P<0.01$ *vs.* D-treated mice at the same dose. **B** The same experiment as in **A** was done except monitoring adrenocorticotropic hormone (*ACTH*) level. *$P<0.05$ *vs.* D-treated mice at the same doses, **$P<0.01$ *vs.* D-treated mice at the same dose The data in **A** and **B** are mean±SE ($n=5$).

with this notion, our data showed that DS was more effective than D at the same doses in healing colonic injury and lowering MPO activity in the inflamed colonic tissue, indicating that DS is more potent than D.

For an efficient therapeutic action against inflammatory bowel disease, a colon specific prodrug is required not only to deliver an active agent to the proximal part but also to the distal part of the large intestine where inflammatory lesion of target diseases such as ulcerative colitis and Crohn's disease frequently occurs (18). In this respect, the colonic metabolic susceptibility of an active agent and its prodrug should be considered upon design of a colon specific prodrug. If the prodrug is very susceptible to colonic metabolism(s), almost all of it would be converted to the active agent at the proximal part of the large intestine and would not reach the distal large intestine. In this case, it might depend on the colonic metabolic stability of the active agent whether the active agent could reach the distal large intestine. If the prodrug has an appropriate half-life for the metabolic conversion, it would arrive at and be converted to the active agent in the distal large intestine thus probably affording the active agent regardless of the metabolic stability of the active agent. Of course, the more stable the active agent is, the more it would be accumulated at the distal large intestine. Therefore, it is thought to be important to select an active agent and a colon-specific promoiety that have a proper metabolic property for design of an efficient colon specific prodrug. Since, in addition to our data showing that a significant amount of DS not only was detected in the cecum but also in the colon of rats, D is relatively resistant to colonic reductive metabolism(s) that can nullify or weaken therapeutic activity of the drug (10,11), it is very likely that a fractional dose of DS administered orally reaches the distal part of the large intestine, where DS produces D. Moreover, it is also possible that D produced from DS at the proximal part (cecum) moves down to the distal large intestine without the metabolic inactivation. However, DS seems to be completely deconjugated to liberate D during the transit of the large intestine as DS was not detectable in the feces collected for 24 h after oral administration of DS.

While a significant amount of D was shown in the plasma after D administration, D was hardly detected in the plasma after DS administration. In accordance with this observation, the urinary recovery of D (after DS administration) was much lower than that after D administration. These results strongly suggest that DS reduced the systemic absorption of D. Generally, the systemic absorption of a drug (even a lipophilic drug) from large intestine is lower than that from small intestine (19,20). Therefore, the systemic absorption of the active agent from its colon specific prodrug would be low compared with that from a conventional dosage form, even in the case that all of an administered prodrug is delivered to large intestine and is converted completely to an active agent. In agreement with this argument, D was detected very low in the plasma and urine although a substantial amount of D was accumulated in the large intestine after DS administration. In parallel with the low systemic absorption of D upon DS administration, DS did not affect the adrenal function in contrast to D that substantially induced adrenal suppression, a systemic side effect of D, which was analyzed by measuring the plasma levels of corticosterone and ACTH after oral administration of DS or D at the same dose. Taken together, our data suggest that DS is a potential colon specific prodrug of D that improves therapeutic and toxicological properties.

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