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ORIGINAL ARTICLE

5-[(3-Carboxy-4-hydroxyphenyl)diazenyl] nicotinic acid, an azo-linked mesalazine-nicotinic acid conjugate, is a colon-targeted mutual prodrug against dextran sulfate sodium-induced colitis in mice

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Abstract

Purpose We aimed to develop a 5-aminosalicylic acid (5-ASA, mesalazine)-based anti-colitic drug with higher efficacy than sulfasalazine (SSZ), a colon-targeted prodrug of 5-ASA, for the treatment of inflammatory bowel disease (IBD). To this end, we synthesized a colon-targeted mutual prodrug (ASA-azo-NA) consisting of 5-ASA and the GPR109A agonist nicotinic acid, 5-[(3-carboxy-4-hydroxyphenyl)diazenyl] nicotinic acid.

Methods In our previous study, oral gavage of ASA-azo-NA delivered 5-ASA and 5-aminonicotinic acid specifically to the large intestine in a 2,4-dinitrobenzene sulfonic acid (DNBS)-induced rat colitis model and ameliorated colonic damage and inflammation more effectively than oral SSZ. To increase the therapeutic convincibility of ASA-azo-NA for the treatment of IBD with multifactorial pathologies, the colon targetability and therapeutic activity of ASA-azo-NA were examined using a dextran sulfate sodium (DSS)-induced colitis mouse model with a different pathogenesis from that of DNBS-induced colitis in rats.

Results ASA-azo-NA liberated 5-ASA in the cecal contents of mice while remaining stable in the small intestinal contents, with a cecal conversion rate and extent comparable to those of SSZ. Oral ASA-azo-NA and SSZ accumulated similar concentrations of 5-ASA in the cecum, indicating that ASA-azo-NA was delivered to and activated in the large intestine as efficiently as SSZ. In mice with DSS-induced colitis, oral ASA-azo-NA mitigated colonic damage and inflammation, as assessed using macroscopic and molecular indices, and was therapeutically superior to SSZ.

Conclusion ASA-azo-NA acted as a colon-targeted mutual prodrug against DSS-induced mouse colitis. Thus, ASA-azo-NA may be therapeutically applicable to patients with IBD who are resistant to SSZ treatment.

Keywords 5-Aminosalicylic acid · Nicotinic acid · GPR109A · Colon-targeted drug delivery · Mutual prodrug · Inflammatory bowel disease · DSS-induced mouse colitis

Introduction

Inflammatory bowel disease (IBD), represented by ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic and recurrent episodes of inflammation in the gastrointestinal tract (GIT), particularly the distal part (Podolsky 2002; Xavier and Podolsky 2007). Although the etiology of IBD remains obscure, perturbation of the interaction between the host and microflora, leading to a dysregulated immune response, has been implicated in the pathogenesis of this inflammatory condition (Neurath 2014).

Currently, there is no curative medicine for IBD; thus, the therapeutic aim of anti-IBD drugs is prolonged maintenance of remission (Lofberg 2003). Anti-IBD drugs include aminosalicylates (AS), glucocorticoid (GC), and immunosuppressant (IS) agents, which have treatment disadvantages such as low efficacy with AS drugs and the serious side effects of long-term therapy with GC and IS agents (Lofberg 2003; Taylor and Irving 2011). Recently, biologics such as anti-tumor necrosis factor (TNF)-α agents (ATAs) have been introduced as anti-IBD pharmacotherapy and have shown effectiveness in patients who are resistant to conventional drugs.

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However, biopharmaceuticals are not free of side effects, and therapeutic tolerance and resistance can occur largely due to the formation of antibodies against the agents. In addition, high medication cost and poor patient compliance due to parenteral administration are major drawbacks, particularly with the required lifelong use of biologics (Crowe et al. 2018). Therefore, there is still an unmet medical need to develop small-molecule anti-IBD drugs with improved therapeutic and toxicological properties over those of conventional drugs.

The drug 5-aminosalicylic acid (5-ASA), a first-line treatment for mild to moderate IBD (Lofberg 2003; Taylor and Irving 2011), has advantages over other therapeutic options in safety (GC, IS, and ATA), cost (ATA), and patient compliance (ATA). However, 5-ASA is limited therapeutically and is ineffective against severe IBD (Berends et al. 2019). In our previous study (Jeong et al. 2020), we developed a 5-ASA-based anti-colitic drug (ASA-azo-NA) with enhanced therapeutic activity and reduced risk of side effects by coupling 5-ASA with the GPR109A agonist. This design, which produced a colon-targeted mutual prodrug against colitis, adopted pharmaceutical concepts of a codrug and colon-targeted drug delivery.

GPR109A agonists, including butyric acid and nicotinic acid, produce metabolites by the action of colonic microflora that suppress colonic inflammation via GPR109A (Segain et al. 2000; Singh et al. 2014; Graff et al. 2016; Salem and Wadie 2017). The codrug concept, also known as a mutual prodrug, is widely used to improve the therapeutic properties of one or both drugs combined in the formulation (Lau et al. 2008; Das et al. 2010), and the design involves chemically linking two synergistic drugs. In addition, colon-targeted drug delivery is a well-established pharmaceutical strategy used to enhance therapeutic activity and reduce systemic side effects of anti-IBD drugs (Jung and Kim 2010; Lautenschlager et al. 2014).

For example, oral ASA-azo-NA showed specific activity on the colon and did not exhibit skin toxicity, which is a typical systemic side effect of GPR109A agonists. Moreover, oral ASA-azo-NA effectively ameliorated 2,4-dinitrobenzene sulfonic acid (DNBS)-induced rat colitis with therapeutic superiority to sulfasalazine (SSZ), a colon-targeted 5-ASA prodrug currently used as an anti-IBD drug, with benefits ascribed to the mutual action of 5-ASA and the GPR109A agonist (Jeong et al. 2020).

Intrarectal administration of the haptenating agents, 2,4,6-trinitrobenzene sulfonic acid (TNBS) or DNBS, induces colitis by haptenizing colonic proteins. This effect involves the initiation of a mucosal immune response to the immunogenic colonic proteins, resulting in transmural colitis driven by a T_H1-mediated immune response. The clinical and immunological features of the resultant condition resemble those of CD (Kiesler et al. 2015; Kolios 2016). The pathology of dextran sulfate sodium (DSS)-induced colitis is quite different from that of haptenating agent-induced colitis (Randhawa et al. 2014). DSS is directly toxic to the colonic epithelium, causing epithelial cell injury and consequently disrupting the intestinal epithelial barrier. This effect facilitates the entry of luminal bacteria or bacterial antigens into the mucosa, thereby inducing immune responses to the excess influx of luminal antigens.

Thus, administration of DSS to mice in drinking water for a short period induces a very reproducible, acute inflammation limited to the colon, where innate immunity plays a major role (Randhawa et al. 2014; Kiesler et al. 2015). Examining the anti-colitic activity of a drug using two colitis models with different inflammatory pathologies may enhance the success of translating results of the animal models to outcomes in clinical IBD trials (Valatas et al. 2015). Therefore, it is worth investigating whether ASA-azo-NA is effective against DSS-induced colitis in mice. In this study, we examined whether ASA-azo-NA was converted to 5-ASA and GPR109A agonist in the cecal contents of mice and if they were delivered specifically to the large intestine following oral administration. Moreover, we examined whether oral ASA-azo-NA was effective against DSS-induced colitis in mice.

Materials and methods

Materials

5-Aminonicotinic acid (5-ANA), 5-ASA, salicylic acid, sodium nitrite ($NaNO_2$), and sulfamic acid were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Nicotinic acid (NA), SSZ, and tetrabutylammonium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents were obtained from Junsei Chemical Co. (Tokyo, Japan). Enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Systems (Minneapolis, MN, USA). All other chemicals were reagent-grade, commercially available products. ASA-azo-NA was prepared as described previously (Jeong et al. 2020).

High-performance liquid chromatography (HPLC) analysis

The HPLC system consisted of a model 306 pump, a 151 variable UV detector, and a model 234 autoinjector obtained from Gilson (Middleton, WI, USA). A Symmetry R18 column (Waters, Milford, MA, USA; 250 × 4.6 mm, 5 µm) with a guard column (Waters, 20 × 4.6 mm) was used. Samples prepared from each experiment were passed through a membrane filter (0.45 µm). HPLC analysis was conducted at a flow rate of 1 mL/min using a mobile phase comprising

acetonitrile and 1.0 mM phosphate buffer (pH 7.4) with 0.5 mM tetrabutylammonium chloride (1.5:8.5, v/v) for 5-ASA and 5-ANA. The eluate was monitored at 330 nm (for 5-ASA) and 265 nm (for 5-ANA) by a UV detector measuring the absorption with a sensitivity of AUFS 0.01. The retention times of 5-ASA and 5-ANA were 10.5 min and 3 min, respectively.

Animals

Male C57BL/6 mice (6–7-week-old) were purchased from Samtako Co. Ltd. (Osan, Korea) and acclimatized to the animal care facility for at least 7 days before the experiments. Animals were housed in an air-conditioned atmosphere under a 12-h light/dark cycle and given free access to standard rodent chow (Samtako) and water. The animal study protocols used in this study were approved by the Institutional Animal Care and Use Committee of Pusan National University (Approval No.: PNU-2017-1525).

Conversion of ASA-azo-NA to 5-ASA in the contents of the GI tract of mice

Male C57BL/6 mice were euthanized by CO_2 asphyxiation and a midline incision was made. The contents of the small intestine and the cecum were collected separately and suspended in pH 7.4 phosphate buffered saline (PBS) to prepare a 10 (w/v)% suspension. Either ASA-azo-NA or SSZ (2 mg/mL) in PBS was added to the same volume of the 10% intestinal suspension and the mixture was incubated at 37 °C. Incubation of drugs in the cecal contents was conducted in a nitrogen gas bag (AtmosBag, Sigma-Aldrich). At the appropriate time intervals, the samples were centrifuged at 10,000×*g* for 5 min. Methanol (0.45 mL) was added to 0.05 mL of the supernatants, vortexed, centrifuged at 10,000×*g* at 4 °C for 10 min, and then passed through a membrane filter (0.45 µm). The concentrations of 5-ASA in the filtrate were determined using HPLC.

To compare the ability of the mouse cecal contents to activate ASA-azo-NA with that of the rat cecel contents, male Sprague-Dawley rats (Samtako) were euthanized by CO_2 asphyxiation and a midline incision was made. The contents of the cecum were collected and suspended in PBS to prepare a 10 (w/v)% suspension. Either ASA-azo-NA (2 mg/mL) in PBS was added to the same volume of the 10% cecal content suspension and the mixture was incubated at 37 °C under nitrogen. At the appropriate time intervals, the samples were centrifuged at 10,000×*g* for 5 min. Methanol (0.9 mL) was added to 0.1 mL of the supernatants, vortexed, centrifuged at 10,000×*g* at 4 °C for 10 min, and then passed through a membrane filter (0.45 µm). The concentration of 5-ASA in the filtrate was determined using HPLC.

Determination of 5-ASA concentration in the cecum

ASA-azo-NA (36.0 mg/kg, equivalent to 50 mg/kg of SSZ) or SSZ (50.0 mg/kg) was orally administered to male C57BL/6 mice and the mice were euthanized by CO_2 asphyxiation to obtain the cecal contents in the cecum 2, 4, 6 h after the administration of the drugs. The cecal contents was suspended in PBS at 10%, which was centrifuged at 10,000×*g* for 5 min. Methanol (0.45 mL) was added to 0.05 mL of the supernatants, vortexed, centrifuged at 10,000×*g* at 4 °C for 10 min, and then passed through a membrane filter (0.45 µm). The concentrations of 5-ASA in the filtrate were determined using HPLC.

DSS-induced mouse colitis and drug treatment

For induction of colitis, male C57BL/6 mice were given 3% (w/v) DSS (MP Biomedicals, Irvine, CA) in drinking water for 7 days. Then, mice were treated daily with drugs by oral gavage while being given 3% (w/v) DSS (MP Biomedicals) in drinking water for another 7 days. The drugs were dissolved or suspended in PBS (50 µL) for oral gavage. Mice were randomly grouped as follows: normal group: oral gavage of PBS; colitis control group: oral gavage of PBS; ASA-azo-NA-treated group: oral gavage of ASA-azo-NA (36.0 mg/kg); SSZ-treated group: oral gavage of SSZ (50 mg/kg). Each group consisted of 12 mice.

Evaluation of anti-colitic effects

Macroscopic assessment

During treatments, the mice were monitored daily for mortality and clinical symptoms. The degree of symptoms was graded on a scale of 0–4 and was presented as disease activity index (DAI). DAI was measured by stool consistency and rectal bleeding. Briefly, stool consistency was scored in a four-point scale: well-formed pellet stool was assigned 0, semiformed stool was assigned a score of 2, and liquid stool was assigned a score of 4 points. For rectal bleeding, no blood was assigned a score of 0, positive finding was assigned a score of 2, and gross bleeding was assigned a score of 4 (Siegmund et al. 2002). Four independent trained observers blinded to the treatment information carried out the DAI assessment. DAI was presented by the summation of the daily scores divided by 2. At the same time, the loss in body weight was estimated for each mouse. After receiving medication for 7 days, all mice were euthanized by CO_2 asphyxiation after 24 h of the last drug treatment and the colons were carefully removed. The resected colons were rinsed with cold PBS and colon length was measured.

MPO activity and inflammatory cytokines

Myeloperoxidase (MPO) activity and levels of inflammatory cytokines in the colon were measured as described previously (Kim et al. 2012). Briefly, the colon specimens (50 mg) were homogenized in 1 mL of pre-chilled potassium phosphate buffer (50 mM K_2HPO_4 and 50 mM KH_2PO_4, pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) followed by sonication for 10 s, three freeze–thaw cycles, and centrifugation at 14,000 rpm at 4 °C for 3 min. The clarified supernatants (0.1 mL) were added to 2.9 mL of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/mL *o* -dianisidine dihydrochloride and 0.0005% hydrogen peroxide, and the change in absorbance at 460 nm was measured using a UV spectrophotometer (Shimadzu) for 5 min at 25 °C. Concentrations of the tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in the colon tissue of the untreated and treated mice were determined by an enzyme-linked immunosorbent assay (ELISA) using kits (R & D systems) according to the manufacturer's instructions.

Data analysis

Results are represented as mean ± standard deviation (SD). Difference in survival was shown by Kaplan–Meier plot. The log-rank test was used to compare significant survival difference. Group data were compared by one-way analysis of variance followed by a Newman–Keuls post-hoc test to assess differences between groups. The nonparametric Mann–Whitney U test was used to compare DAI and body weight difference. Differences with α or $P < 0.05$ were considered significant.

Results

Oral ASA-azo-NA acts as colon-specific prodrug in mice

In our previous study (Jeong et al. 2020), we demonstrated the colon targetability of oral ASA-azo-NA in rats, and, in the present study, we determined the reproducibility of those findings in mice. First, the cecal contents of mice were incubated with ASA-azo-NA, and the release of 5-ASA was monitored, and a similar experiment was performed with the cecal contents of rats for comparison. As shown in Fig 1a, 5-ASA corresponding to 86% of the initial dose of ASA-azo-NA was released during a 24-h incubation in the cecal contents of mice, which was greater than 5-ASA release (about 68 %) in the cecal contents of rats. 5-ASA was not detected during incubation of ASA-azo-NA with the small intestinal contents.

As shown in Fig. 1b, a comparison of 5-ASA release from the pro-drugs ASA-azo-NA and SSZ in the mouse cecal contents demonstrated similar release profiles. We next examined the colon targetability of ASA-azo-NA in vivo. The colonic delivery efficiency of ASA-azo-NA was compared with that of SSZ, and the results showed that 5-ASA was detected in the cecum 2, 4, and 6 h after oral gavage of ASA-azo-NA and SSZ. As shown in Fig 1c, concentrations of 5-ASA accumulated in the cecum were similar at each time point after oral gavage of ASA-azo-NA and SSZ, indicating that the prodrug ASA-azo-NA exhibited colon specificity and a comparable colonic delivery efficiency to that of SSZ.

ASA-azo-NA is more effective than SSZ against DSS-induced colitis in mice

In our previous study (Jeong et al. 2020), ASA-azo-NA was found to be more effective than SSZ against DNBS-induced colitis in rats; therefore, we examined whether this therapeutic superiority would also be evident in a DSS-induced colitis mouse model. The mice were randomly grouped as follows: normal group, colitis control group (colitis group with no medication), ASA-azo-NA-treated, and SSZ-treated groups. DSS was administered to mice in the drinking water to induce colitis. Medication started 7 days after the colitis induction by DSS. The drugs were orally administered once daily for 7 days with feeding DSS in the drinking water to mice. During the treatments, the severity of colitis was assessed using the disease activity index (DAI) score as previously reported (Siegmund et al. 2002), body weight, and survival rates.

As shown in Fig 2a, the DAI score of mice orally treated with ASA-azo-NA for 7 days was lower (at normal level) than that of the colitis control; however, treatment with SSZ for 7 days did not improve the score. Colitis-induced reduction of body weight (Fig 2b) was significantly prevented by treatment with ASA-azo-NA for 7 days but not upon treatment with SSZ. Consistent with these results, colitis-mediated mortality was significantly reduced by treatment with ASA-azo-NA, but SSZ treatment did not make a difference in colitis-mediated mortality (Fig 2c). Assessment of the anti-colitic effects using DAI, loss in body weight, and mortality showed that ASA-azo-NA was significantly superior to SSZ.

To further examine the anti-colitic activity of the drugs against DSS-induced mouse colitis, the mice were euthanized 7 days after the treatments; colon length, myeloperoxidase (MPO) activity, and inflammatory cytokine levels were determined in the inflamed colons of mice. As shown in Fig 3a, DSS induction shortened the length of

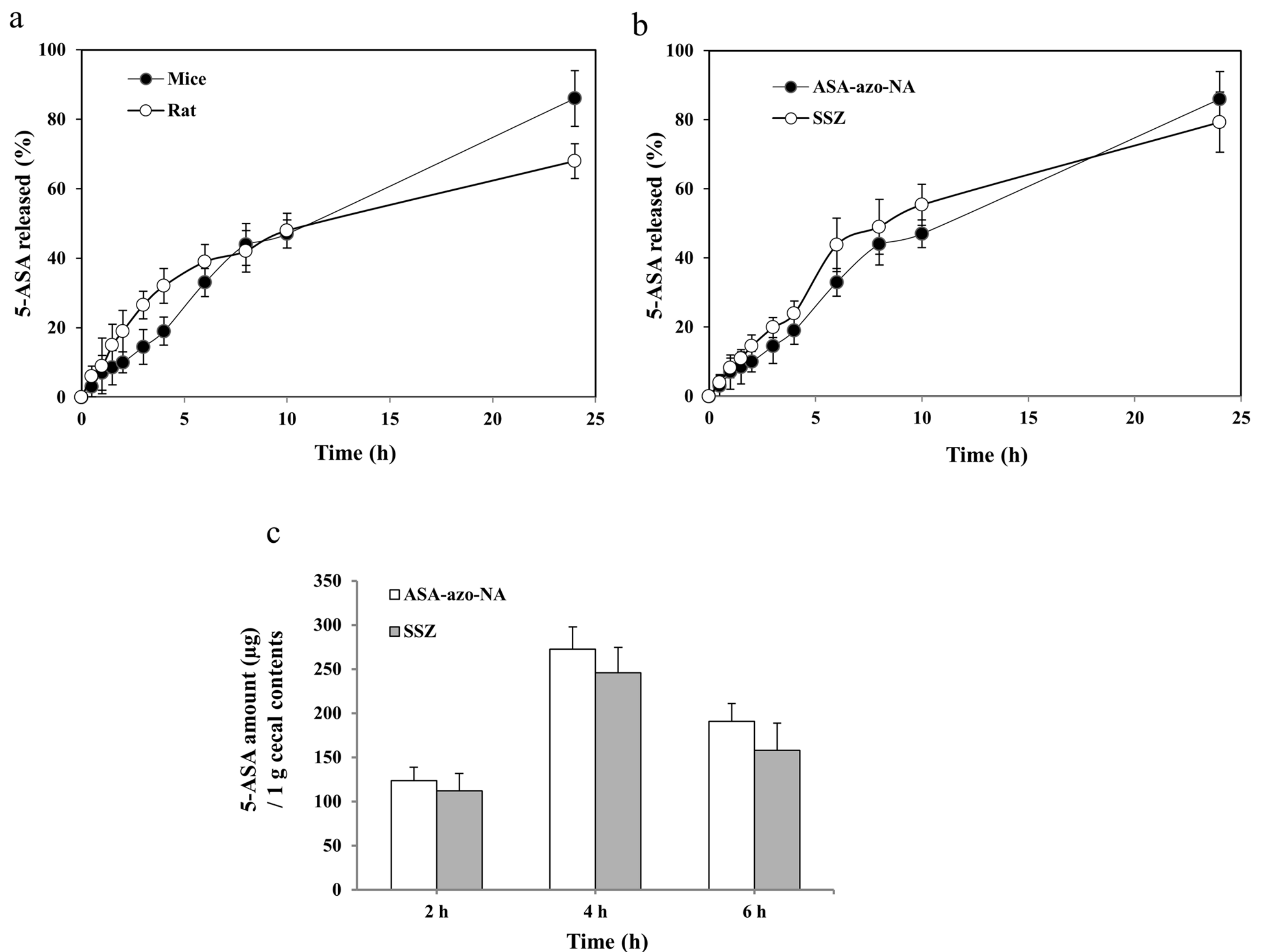

Fig. 1 ASA-azo-NA acts as a colon-specific prodrug in mice. **a** The cecal contents were obtained from rats and mice. ASA-azo-NA (1.0 mg/mL) was incubated with the cecal contents suspended in PBS (5%). The concentration of 5-ASA liberated from ASA-azo-NA was analyzed by HPLC at the indicated time points. **b** ASA-azo-NA or sulfasalazine (SSZ, 1.0 mg/mL) was incubated with the cecal contents of mice suspended in PBS (5%). The concentration of 5-ASA was analyzed by HPLC at the indicated time points. **c** Mice were fasted for 24 h, except for tap water. ASA-azo-NA (36.0 mg/kg, equivalent to 50.0 mg/kg of SSZ) or SSZ (50.0 mg/kg) suspended in PBS was administered to mice by oral gavage. The mice were sacrificed and concentrations of 5-ASA in the cecum were determined by HPLC at the appropriate time points. The data in **a**–**c** represent the mean ± SD (n = 5). $*P < 0.05$

the large intestine, whereas ASA-azo-NA and SSZ prevented the longitudinal shortening of the large intestine, although the effect was not statistically significant. MPO activity in the inflamed colons was substantially reduced following treatment with both prodrugs for 7 days, but ASA-azo-NA was more effective than SSZ (Fig. 3b). We also examined whether ASA-azo-NA suppressed the levels of the inflammatory cytokines, interleukin (IL)-6 and TNF-α, in the inflamed colon. As shown in Fig. 3c and d, the levels of inflammatory cytokines were decreased in the ASA-azo-NA- and SSZ-treated groups, and consistent with the above results, ASA-azo-NA was more effective in decreasing the levels of inflammatory cytokines than SSZ.

Discussion

Although biologics such as anti-TNF-α antibodies have been added to the list of current therapeutic options, 5-ASA remains the mainstay for the treatment of IBD (Crotty and Jewell 1992; Lofberg 2003). Despite its long clinical use, the use of 5-ASA is limited to the treatment of mild to moderate IBD because of its low anti-inflammatory efficacy (Hanauer 1996). In our previous study (Jeong et al. 2020), we sought to widen the therapeutic spectrum for IBD treatment with 5-ASA by enhancing its anticolitic efficacy. To that end, ASA-azo-NA was designed as a colon-targeted mutual prodrug and its efficacy was

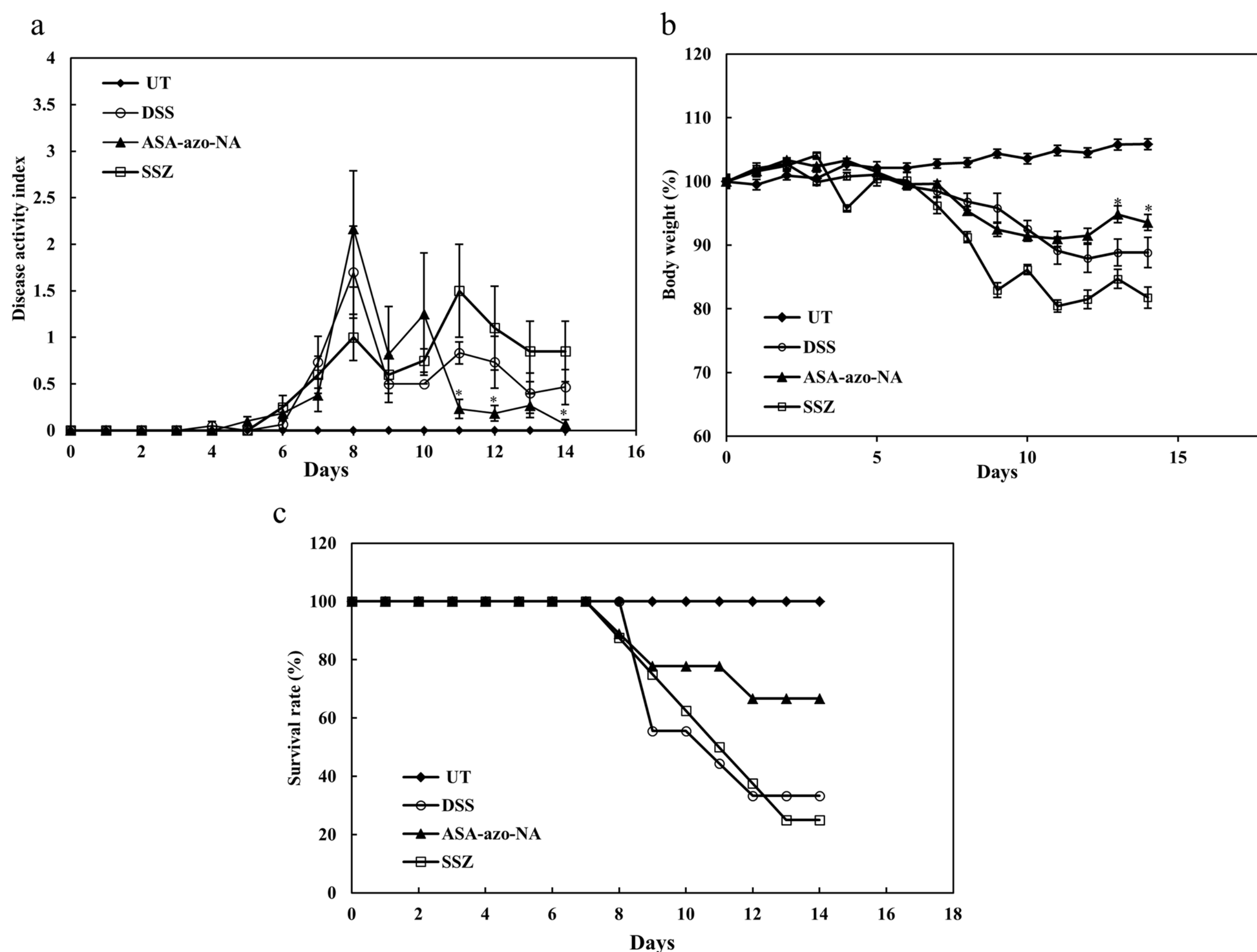

Fig. 2 ASA-azo-NA is more effective at ameliorating DSS-induced mouse colitis than SSZ. After 7-day induction of colitis by 3% dextran sulfate sodium (DSS) in the drinking water, sulfasalazine (SSZ, 50.0 mg/kg), or ASA-azo-NA (36.0 mg/kg, equivalent to 50.0 mg/kg of SSZ) suspended in PBS (50 μL) was orally administered to mice once daily. Mice were given 3% DSS in the drinking water during the treatments. **a** The disease activity index (DAI) of mice was determined daily after each treatment. *α < 0.05 vs DSS, and vs SSZ. **b** Body weight of mice was measured daily after each treatment. *α < 0.05 vs DSS, and vs SSZ. **c** Mortality of mice was monitored and indicated as survival rate. **d** The mice were sacrificed on the 7th day of treatment. Midline incision was made to obtain the large intestines of mice. Upper panel: Representative images of colons of mice. Lower panel: Colon lengths of mice were measured. **e** The mice were sacrificed on the 7th day of treatment. Midline incision was made to obtain the large intestines of mice. Myeloperoxidase (MPO) activities were measured using the inflamed colon. $^{*}P < 0.05$ vs DSS, $^{\#}P < 0.05$. The data in **a**, **b**, **d**, and **e** represent the mean ± SD (n = 3–12). *UT* normal group, *DSS* colitis control group, *ASA-azo-NA* ASA-azo-NA-treated group, *SSZ* SSZ-treated group

evaluated using a DNBS-induced rat colitis model, in which adaptive immunity plays a major role in inducing colitis. the previous study demonstrated that while ASA-azo-NA was colon-specific, similar to SSZ in the colitis rat model, it was significantly more effective than SSZ (Jeong et al. 2020).

DSS-induced mouse colitis has a considerably different pathogenesis from that of DNBS-induced colitis (Randhawa et al. 2014; Kiesler et al. 2015). Thus, it is worth investigating whether ASA-azo-NA also acts as a colon-targeted mutual prodrug in the DSS-induced mouse colitis model. This study increases the therapeutic convincibility of ASA-azo-NA as a drug for the treatment of IBD with a multifactorial nature of inflammatory and immune pathologies (Schmidt and Stallmach 2005; Xavier and Podolsky 2007).

ASA-azo-NA exhibited colon specificity in mice, similar to that in rats, as demonstrated by the data showing that (1) ASA-azo-NA liberated 5-ASA in the cecal contents while remaining stable in the small intestinal contents and (2) oral ASA-azo-NA delivered as much 5-ASA to the cecum as oral SSZ did. Considering that ASA-azo-NA and SSZ liberated 5-ASA at similar rates and extent in the cecal contents of mice, these in vivo results were largely attributable to the colonic delivery efficiency of the two prodrugs.

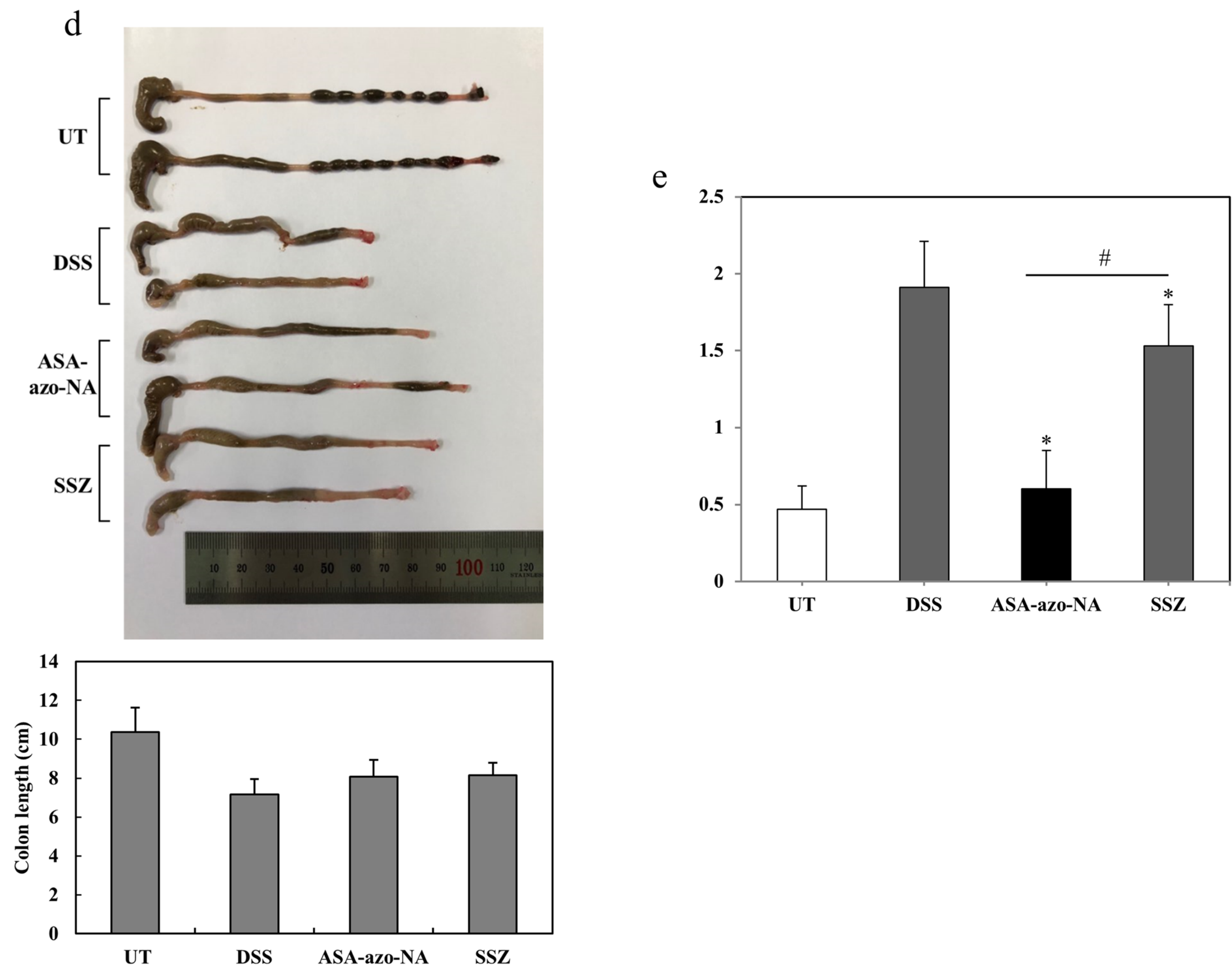

Fig. 2 (continued)

Our results in the DSS-induced mouse colitis model indicate that ASA-azo-NA also acted as a colon-targeted mutual anti-colitic prodrug. This was supported by the data showing that oral ASA-azo-NA alleviated DSS-induced colitis, assessed using the DAI score, survival rate, colon length, and body weight. Moreover, oral ASA-azo-NA reduced MPO activity, which is a biochemical inflammatory indicator, and suppressed the levels of the inflammatory cytokines TNF-α and IL-6 in the inflamed colon. Except for the effects on colon length, all anti-colitic activities of ASA-azo-NA were significantly superior to those of SSZ. As reported (Jeong et al. 2020), the enhanced anti-colitic activity of ASA-azo-NA is likely ascribable to the mutual anti-colitic action of 5-ASA and the GPR109A agonist 5-ANA released from ASA-azo-NA by colonic activation.

Conclusion

Oral ASA-azo-NA was delivered specifically to the large intestine, where it was converted to active 5-ASA and the GPR109A agonist, 5-ANA. Oral ASA-azo-NA was therapeutically superior to oral SSZ, a colon-targeted prodrug of 5-ASA, in ameliorating DSS-induced colitis in mice, indicating that ASA-azo-NA acted as a colon-targeted, anti-colitic mutual prodrug. Therefore, ASA-azo-NA may be applicable to the treatment of patients with SSZ-resistant IBD.

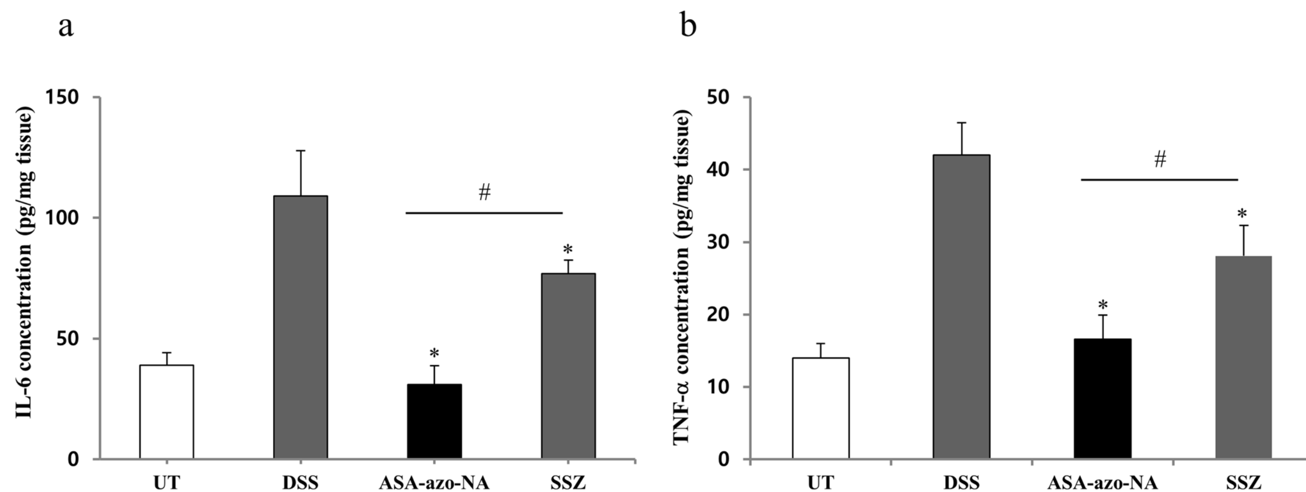

Fig. 3 ASA-azo-NA is more effective at suppressing inflammatory cytokines in DSS-induced mouse colitis than SSZ. After 7-day-induction of colitis by 3% dextran sulfate sodium (DSS) in the drinking water, sulfasalazine (SSZ, 50.0 mg/kg), or ASA-azo-NA (36.0 mg/kg, equivalent to 50.0 mg/kg of SSZ) suspended in PBS (50 µL) was orally administered to mice once daily. Mice were given 3% DSS in the drinking water during the treatments. The mice were sacrificed on the 7th day of treatment. Midline incision was made to obtain the large intestines of mice. Inflammatory indices interleukin-6 (IL-6) (**a**) and tumor necrosis factor (TNF)-α (**b**) were assessed in the inflamed colons. The data represent the mean ± standard deviation (n = 3–12). $^{*}P < 0.05$ vs DSS control, $^{\#}P < 0.05$. *UT* normal group, *DSS* colitis control group, *ASA-azo-NA* ASA-azo-NA-treated group, *SSZ* SSZ-treated group

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human and animal rights All institutional and national guidelines for the care and use of laboratory animals were followed. The animal study protocols used in this study were approved by the Institutional Animal Care and Use Committee of Pusan National University (Approval No: PNU-2017-1525).

References

Berends SE, Strik AS, Lowenberg M, D'haens GR, Mathot RA (2019) Clinical pharmacokinetic and pharmacodynamic considerations in the treatment of ulcerative colitis. Clin Pharmacokinet 58:15–37

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

Crowe JS, Roberts KJ, Carlton TM, Maggiore L, Cubitt MF et al (2018) Preclinical development of a novel, orally-administered anti-tumour necrosis factor domain antibody for the treatment of inflammatory bowel disease. Sci Rep 8:4941

Das N, Dhanawat M, Dash B, Nagarwal RC, Shrivastava SK (2010) Codrug: an efficient approach for drug optimization. Eur J Pharm Sci 41:571–588

Graff EC, Fang H, Wanders D, Judd RL (2016) Anti-inflammatory effects of the hydroxycarboxylic acid receptor 2. Metabolism 65:102–113

Hanauer SB (1996) Inflammatory bowel disease. N Engl J Med 334:841–848

Jeong S, Lee H, Kim S, Ju S, Kim W et al (2020) 5-Aminosalicylic acid azo-coupled with a GPR109A agonist is a colon-targeted anticolitic codrug with a reduced risk of skin toxicity. Mol Pharm 17:167–179

Jung Y, Kim YM (2010) What should be considered on design of a colon-specific prodrug? Expert Opin Drug Deliv 7:245–258

Kiesler P, Fuss IJ, Strober W (2015) Experimental models of inflammatory bowel diseases. Cell Mol Gastroenterol Hepatol 1:154–170

Kim JJ, Shajib MS, Manocha MM, Khan WI (2012) Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp. https://doi.org/10.3791/3678

Kolios G (2016) Animal models of inflammatory bowel disease: how useful are they really? Curr Opin Gastroenterol 32:251–257

Lau WM, White AW, Gallagher SJ, Donaldson M, Mcnaughton G et al (2008) Scope and limitations of the co-drug approach to topical drug delivery. Curr Pharm Des 14:794–802

Lautenschlager C, Schmidt C, Fischer D, Stallmach A (2014) Drug delivery strategies in the therapy of inflammatory bowel disease. Adv Drug Deliv Rev 71:58–76

Lofberg R (2003) Review article: medical treatment of mild to moderately active Crohn's disease. Aliment Pharmacol Ther 17(Suppl 2):18–22

Neurath MF (2014) Cytokines in inflammatory bowel disease. Nat Rev Immunol 14:329–342

Podolsky DK (2002) Inflammatory bowel disease. N Engl J Med 347:417–429

Randhawa PK, Singh K, Singh N, Jaggi AS (2014) A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol 18:279–288

Salem HA, Wadie W (2017) Effect of niacin on inflammation and angiogenesis in a murine model of ulcerative colitis. Sci Rep 7:7139

Schmidt C, Stallmach A (2005) Etiology and pathogenesis of inflammatory bowel disease. Minerva Gastroenterol Dietol 51:127–145

Segain JP, Raingeard De La Bletiere D, Bourreille A, Leray V et al (2000) Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut 47:397–403

Siegmund B, Lehr HA, Fantuzzi G (2002) Leptin: a pivotal mediator of intestinal inflammation in mice. Gastroenterology 122:2011–2025
Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R et al (2014) Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity 40:128–139
Taylor KM, Irving PM (2011) Optimization of conventional therapy in patients with IBD. Nat Rev Gastroenterol Hepatol 8:646–656
Valatas V, Bamias G, Kolios G (2015) Experimental colitis models: Insights into the pathogenesis of inflammatory bowel disease and translational issues. Eur J Pharmacol 759:253–264
Xavier RJ, Podolsky DK (2007) Unravelling the pathogenesis of inflammatory bowel disease. Nature 448:427–434

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