A limitation of currently used preclinical models of colitis is that disease and treatment assessment methods differ from clinically used methods. Thus, a modified Mayo score and an endoscopic index (EI) were developed for use in cynomolgus macaques with 0.25% dextran sulfate sodium (DSS)-induced ulcerative colitis. Macaques were treated with water with DSS for two weeks followed by water without DSS for two weeks. Disease activity was classified according to a modified Mayo score: stool consistency, rectal bleeding, colonoscopy examination and global assessment. Findings on colonoscopy were further graded according the Rachmilewitz EI. To demonstrate the sensitivity of the modified Mayo score and EI to therapeutic intervention, macaques were treated with the anti-inflammatory steroid prednisolone followed eight weeks later by the integrin antibody vedolizumab. Before DSS treatment, normal stool consistency and no rectal bleeding were observed. Colonoscopy demonstrated no mucosal abnormalities. Following the first DSS treatment, Mayo score and EI indicated signs of mild colitis. Following subsequent DSS treatments, mild to moderate colitis emerged with each DSS treatment and reduced signs of colitis were observed 2 weeks after DSS treatment termination. Prednisolone treatment during DSS treatment suppressed the emergence of colitis. Vedolizumab reduced signs of colitis during DSS treatment and further reduced signs of colitis that persisted after termination of DSS treatment. The current study demonstrated the potential of utilizing clinical outcome measures to assess experimentally-induced colitis in the macaque. Furthermore, signs of colitis, as assessed with the current methods, were reduced following therapeutic treatment. The current findings suggest that clinically relevant outcome measures in the macaque model of ulcerative colitis could be used to test novel treatments.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammation of the gut characterized by patches of ulcerations of the intestinal mucosa. Over time, these ulcers may progress to involve the entire thickness of the intestine, which may necessitate surgical intervention [1]. Signs and symptoms are intermittent, including loose stools or diarrhea and abdominal pain. Understanding of the pathophysiology of IBD has greatly increased in recent years and potential biomarkers have been identified that could be used to aid in stratification of disease severity in patients, differentiate IBD from other gastrointestinal disorders that present with similar signs and symptoms and serve as possible objective indicators of treatment efficacy [2]. Nonetheless, efficacious therapeutics, in which mucosal healing and disease remission are primary treatment objectives, are lacking. In addition, currently available treatments, including immunosuppressants and corticosteroids, have adverse effects on immunologic functioning when taken over an extended period of time.

Genetically-modified and chemically-induced rodent models are the primary preclinical models used to explore the pathophysiology of IBD and to test novel therapeutics, with the understanding that mechanisms observed in rodents are clinically relevant. A limitation of rodent models is that the key outcome measure is “semi-quantitative histopathology”, which is obtained following animal euthanasia [3, 4, 5]. By contrast, the US Food and Drug Administration (FDA) suggests the use of an assessment scale which captures signs and symptoms of disease activity with “endoscopic” assessment, to track therapeutic efficacy in clinical trials in each patient over time [6]. The FDA also suggests the need for the use of
two outcome measures to ensure that observed improvements in signs and symptoms are related to the underlying disease state (as well as the converse, that the underlying disease in fact mediates the signs observed by clinicians and symptoms reported by patients) [6]. One such outcome measure is the Mayo score, a combination of semi-quantitative observations of clinical signs, endoscopic examination and clinician’s general assessment of disease activity [7].

Recently, a rhesus macaque (Macaca mulatta) model of dextran sulfate sodium (DSS)-induced chronic colitis was developed [8]. Macaques freely consumed a suspension of DSS in water over a two-week period, followed by a two-week period of water without DSS—a cycle of three treatments of water with DSS and water without DSS was used to induce a cycle of disease and remission, as observed clinically. With the final treatment of DSS, these macaques were euthanized and histopathological examination of the colon demonstrated robust infiltration of polymorphonuclear neutrophils (PMN) into the mucosa, lamina propria and submucosa, similar to that seen in clinical IBD [8, 9]. While colonoscopic findings were not reported for these macaques, Hao et al. noted the presence of “few-to-no overt clinical signs” [8]. In the same study, African green monkeys (Chlorocebus sabaeus) with longstanding simian immunodeficiency virus infection treated with DSS for 10 days demonstrated a colonic inflammatory and immune response, characterized by “multifocal mucosal thickening, redness and ulcerations” as observed with colonoscopy. Whether repeated treatment of DSS in either rhesus macaques or African green monkeys induces a cycle of disease onset and remission that can be visualized with colonoscopy has yet to be reported. Furthermore, it is not known if cyclic induction of colitis as described by Hao et al. is responsiveness to standard therapeutic treatment [8].

To further enhance utilization of the macaque model of DSS-induced colitis, the current study sought to determine if clinically used outcome measures, colonoscopy and Mayo scoring, could be developed using this model in order to quantify disease activity and determine if these outcome measures are sensitive to therapeutic intervention.

2. Materials and methods

2.1. Subjects

A total of eight male cynomolgus macaques (captivity-bred Macaca fascicularis from Vietnam obtained from EBS Co.; 3–4 years old, 4–5 kg body weight) were used in the current study.

Procedures involving macaques were reviewed and approved by the Hamamatsu Pharma Research Animal Care and Use Committee. Environmental management and housing conditions were according to the Guide for the Care and Use of Laboratory Animals, Eighth Ed. (National Research Council, 2011). The HPR Primate Center is fully accredited by AAALAC International. Room temperature and humidity were continuously monitored. Upon arrival at HPR, the macaques underwent a two-week quarantine period, in which feeding, general behavior and stool consistency were examined daily. Macaques used in the current study showed no signs of ill health. Macaques were individually housed in order to individually monitor fluid intake and to monitor individual health parameters. Individual macaques were provided with manipulate and retained auditory, visual and olfactory contact with conspecifics. Throughout treatment, macaques were fed a standard nonhuman primate diet (Oriental Yeast Co., Ltd., Chiba, Japan). In addition, macaques were hand-fed by either study staff or animal care staff fresh fruit or vegetables at least once per week.

In case macaques showed a sudden, acute loss of greater than 25% of body weight, diminished feeding, lethargy and unresponsiveness to sensory stimuli, they were to be removed from the study and treated. Pentobarbital overdose was to be used if euthanasia was necessary. In the current study, none of the macaques demonstrated signs warranting euthanasia. Macaques at the end of the current study were taken off DSS and allowed to return to group housing following health evaluation by a veterinarian.

2.2. DSS treatment

The method based on Hao et al. was used to induce an acute and chronic colitis-like state in the cynomolgus macaque [8]. Dextran sulfate sodium (DSS; 0.25% (w/v); M.W. 36,000–50,000; MP Biomedicals, OH, US) was dissolved in distilled water mixed with non-colored drink powder (Kool-Aid® Invisible Drink Mix; Kraft Heinz Co., Chicago, IL), to add flavor and to encourage macaques to drink. Macaques were allowed free access to DSS-water. Daily total intake of DDS-water was 100 mL/kg. One treatment cycle consisted of 14 days of DSS-water followed by 14 days of tap water without DSS (DW). Macaques were treated for a total of five treatment cycles.

An outline of the DSS treatment cycles and periods of therapeutic interventions are shown in Figure 1.

2.3. Semi-quantitative measures of disease activity

Before administration of DSS-water and at regular intervals following DSS-water administration, disease activity was assessed using a modified Mayo score which consisted of: stool consistency (modified Bristol stool scale [10]), rectal bleeding, colonoscopy examination [11] and “global assessment,” or assessment of general signs of illness (Table 1). Subscores ranged from 0–3, with 3 being severe. The worst stool consistency and rectal bleeding from within three days of...
examination was used [6]. Representative colonoscopic images from untreated and DSS-treated macaques are shown in Figure 2. Stool consistency, rectal bleeding, and global assessment, except for body weight, were assessed daily and body weight was assessed weekly. Colonoscopies were performed once every two weeks using a small animal flexible video endoscope (Olympus VQ Type 5112B, Olympus, Tokyo, Japan) while under ketamine (10 mg/kg, intramuscular) and propofol (0.5–30 mg/kg/h, intravenous) anesthesia.

| Categories                  | Score                                      |
|-----------------------------|--------------------------------------------|
|                             | 0                                          | 1                                           | 2                                           | 3                                           |
| Stool consistency           | Normal (Bristol 3, 4)                      | Soft stools (Bristol 5)                      | Loose stools (Bristol 6)                     | Diarrhea (Bristol 7)                        |
| Rectal bleeding             | No blood seen                              | Positive occult blood test                   | Visible blood in stool                       | Rectal bleeding                            |
| Colonoscopy examination     | Normal                                     | Mild                                        | Moderate                                    | Severe                                      |
|                             | No friability or granularity               | Erythema                                    | Marked erythema                             | Marked erythema                            |
|                             | Intact vascularity pattern                 | Decreased vascularity pattern               | Absent vascularity pattern                  | Absent vascularity pattern                  |
|                             | Mild friability                            | Friability                                  | Granularity                                 | Granularity                                 |
| Global assessment           | Change in body weight                      | No change or Increase                       | ≤5%                                         | ≤10%                                        | >10%                                        |
|                             | Activity                                   | Normal                                      | reduced, none                               | –                                           | –                                           |
|                             | Appetite                                   | Normal                                      | Reduced, none                               | –                                           | –                                           |
|                             | Drinking volume                            | Normal                                      | None                                        | –                                           | –                                           |
|                             | Body temperature                           | Normal                                      | Either reduced or elevated                  | –                                           | –                                           |

A total score was calculated based on four sub-scores (stool consistency, rectal bleeding, colonoscopy examination, global assessment). A total score can range from 0-12, with 12 being the most severe. Change in body weight includes either an increase or decrease. Stool consistency subscore drived from “modified Bristol stool scale”, Degan, Phillips, Gut, 1996. Colonoscopy examination scoring based on Pineton de Chambrun et al., Nat. Rev. Gastro. Hepatol., 2010.

Figure 2. Representative colonoscopic images from macaques demonstrating various Mayo colonoscopy subscores. See Table 1 for Mayo colonoscopy scoring criteria. (a) Untreated macaque prior to DSS treatment. Mayo colonoscopy subscore = 0. (b) After one two-week DSS treatment, before water without DSS treatment. Mild, Mayo colonoscopy subscore = 1. (c) After one two-week DSS treatment, before water without DSS treatment. Moderate, Mayo colonoscopy subscore = 2. (d) After three two-week DSS treatments, before the third water without DSS treatment. (This macaque was also treated twice daily with placebo control. See section 2.4. Pharmacological interventions.) Severe, Mayo colonoscopy subscore = 3.
Components in global assessment included activity, appetite, drinking volume, body temperature and body weight and scores range from 0-7, with 7 being severe signs of illness. Changes in appetite and drinking were non-quantified observations of changes in eating or drinking (or a lack of eating or drinking) from the previous day. Body temperature was gauged by feeling the hairless forearm [12]. The global assessment score was then converted to a score ranging from 0-3: 0 and 1 converted to 0; 2 and 3 to 1; 4 and 5 to 2; and 6 and 7 to 3. Unlike the clinical setting, wherein results from the other subscores are “acknowledged” in the final global assessment score, the results from the other subscales were not reflected on final scoring of the global assessment in this study [7].

The Rachmilewitz Endoscopic Index (EI) was used to score observations of the colon, which was scaled differently from the Mayo colonoscopy examination score (Table 2) [13]. Scores from the four subcategories ranged from 0-4, with 4 being severe. The total score was reported, ranging from 0-12, with 12 being the most severe.

2.4. Pharmacological interventions

To test the sensitivity of the outcome measures developed in the current study to changes in colitis due to treatment, two different therapeutic interventions were utilized. Following two full treatment cycles of water with DSS and without DSS and before the start of the third DSS treatment cycle, Mayo scores and endoscopic examinations were conducted (Figure 1).

The first therapeutic intervention utilized prednisolone (Ashi Kasei Corp., Tokyo, Japan). Prednisolone is used to suppress “mild to severe relapse” and “active disease” in IBD patients and is not used as maintenance therapy [14]. As a previous and the current study showed that DSS treatment in nonhuman primates leads to signs of “active disease” as assessed by colonoscopy, prednisolone treatment was initiation at the time of disease induction with DSS (i.e. the third DSS treatment) [8]. Four macaques underwent prednisolone treatment for the two-week duration of disease induction with the third DSS treatment. In parallel, four macaques underwent treatment with placebo. Macaques were not randomized to treatment groups. Prednisolone dosing and the dosing schedule utilized in the current study was similar to that used in clinical studies [14].

Prednisolone was administered twice per day as a 1 mg of tablet embedded in light pink-tinted flavored gelatin (Nanao Confection Co., Kitakyushu, Japan). Light pink-tinted flavored gelatin without prednisolone served as the placebo (control) treatment. Stool consistency and rectal bleeding were evaluated daily. Body weight measurements were performed at the beginning and at the end of DSS treatment.

Vedolizumab was utilized as the second therapeutic intervention. Vedolizumab is used to maintain, in addition to induce, remission in patients with moderate to severe active ulcerative colitis [15]. The serum half-life of vedolizumab is about 25 days, so for the current study, one i.v. dose of vedolizumab (10 mg/kg; Takeda Pharmaceutical Company Limited., Tokyo, Japan). Following treatment with either PBS or vedolizumab, macaques underwent one week of DSS treatment. After the third week of DSS treatment, macaques were treated with water without DSS for two weeks.

In addition to Mayo score and EI measurement in vedolizumab and control-treated macaques, fecal calprotectin, an indicator of mucosal inflammation and a possible biomarker of active ulcerative colitis, was measured with a commercially available ELISA Kit (Calprotectin Mochida, Mochida Pharmaceutical Co., Ltd, Tokyo, Japan) [16]. The lowest level of detection was 10 μg/g. A microplate reader (Bio-Rad Laboratories, Inc., US) was used with optical density set at 450 nm. Fecal samples for calprotectin measurement were collected prior to the start of the fifth DSS treatment, before vedolizumab and control treatment, during the third week of DSS treatment (one week after vedolizumab and control treatment) and during the second week of treatment of water without DSS (three weeks after vedolizumab and control treatment).

2.5. Statistical analysis

A previous study utilized a total of six rhesus macaques to show robust signs of acute DSS-induced colitis and four to six DSS-treated macaques to demonstrate changes in protein expression compared to two untreated macaques and, furthermore, to show protein expression similarity between DSS-treated macaques and patients with ulcerative colitis [8, 17]. The current study is exploratory in that the objective is to adapt the clinically used Mayo score and EI for preclinical use in macaques with experimental colitis and to determine if the outcome measures are sensitive to detect changes in colitis following therapeutic intervention. Thus, the current study used minimal number of animals as in previous studies to achieve the objective of the current study [18, 19, 20, 21]. Because of the development and use of novel outcome measures, an effect size was not determined and, therefore, power analysis was not performed.

For ordinal data, including Mayo and EI scores, a Mann-Whitney test was to compare between control and drug-treated groups (Prizm 4.02, Graphpad Prism, San Diego, CA). A t-test was used for comparisons of calprotectin between control and drug-treated groups. p values less than 0.05 were considered statistically significant. When individual data were not shown, data were expressed as mean ± standard deviation (S.D.).

3. Results

3.1. Effect of DSS treatment on Mayo subscores and endoscopic index subscores

Macaques prior to DSS treatment did not present with signs of colitis as assessed with Mayo score (Figure 2) and colonoscopy (Figure 3). Following one DSS cycle, macaques showed increased Mayo subscores for stool consistency (soft or loose stools), rectal bleeding and Mayo colonoscopy (Figure 3). Only two macaques out of all eight macaques showed increased global assessment scores during the first and then the second DSS treatment, with a score of 1, indicating about 5% weight loss, reduced spontaneous activity, decreased appetite and

| Score | Granulation | Vascular pattern | Vulnerability of mucosa | Mucosal damage |
|-------|-------------|------------------|------------------------|---------------|
|       | No          | Normal           | none                   | None          |
| 0     | –           | Faded/disturbed  | Slightly increased     | –             |
| 1     | Yes         | Completely absent| Generally increased    | Slight        |
| 2     | –           |                   | (contact bleeding)     | Pronounced    |
| 3     | –           |                   | (spontaneous bleeding) |               |
| 4     | –           |                   |                        |               |

A total score was calculated based on the four subscores. Endoscopic Index scores may range from 0-12, with 12 being the most severe. (From Rachmilewicz et al., BMJ, 1989).
drinking or an absence of eating and drinking and either increased or decreased general body temperature. Following the first DW treatment, subscores were generally decreased compared to subscores after the first DSS treatment. Following the second DSS treatment, subscores tended to rise above subscores obtained during the previous DW treatment. In fact, subscores following the second DSS treatment tended to be at the level of that following the first DSS treatment. During the second DW treatment, rectal bleeding and Mayo colonoscopy decreased and tended to return to scores that were obtained before the first DSS treatment. In four macaques, stool consistency was 1 ("soft stools") even two weeks after termination of DSS treatment (Figure 3). Based on Mayo subscores, the findings suggest that repeated treatment cycles of DSS and DW does not lead to worsening of signs of colitis.

Colonoscopic examination prior to the first DSS treatment did not reveal significant signs of colitis (Figure 2, Figure 4). Following the first DSS treatment, EI subscores generally suggests mild to moderately severe ulcerative colitis (Figure 4). Endoscopic index subscores were differentially affected by DSS treatments. Three EI subscores tended to remain elevated following the first and second DSS cycles. First, vascularity

---

**Figure 3.** Effect of prednisolone treatment on Mayo subscores over time in macaques treated with dextran sulfate sodium (DSS) alternated with water without DSS (DW). Mayo subscores, consisting of stool consistency, rectal bleeding, Mayo colonoscopy and global assessment, were taken before the first DSS treatment (Pre) and once every two weeks thereafter. The worst scores obtained within a three-day period of the last day of either DSS or water treatment were used for stool consistency and rectal bleeding. Beginning with the third DSS treatment (grey-shaded area between 8 W to 10 W), four macaques were treated with placebo (Control, yellow bar) and, in parallel, four macaques were treated with 1 mg (p.o., b.i.d.) prednisolone (PSL; green bar) for the duration of the DSS treatment period (between 8 W to 10 W). Data from individual macaques of each treatment group are shown. The horizontal line at each time point represents the group mean.
increased following each DSS treatment and tended to decrease following DW treatment. A total of two macaques out of eight macaques demonstrated elevated vascularity following DW treatment. Friability subscores for five macaques were elevated during the first DW treatment and friability subscores for six macaques were elevated during the second DW treatment. Finally, mucosal lesion subscores were elevated after the first and second DSS treatments. During the first DW treatment, mucosal lesion subscores (score = 2) were elevated in two out of a total of eight macaques. However, following the second DW treatment, mucosal lesion subscores were not elevated in any of the macaques. Colonic granularity did not change at all following either one or two DSS treatments.

In contrast to cycling Mayo subscores, some EI subscores were elevated during DW treatment, long after termination of DSS treatment, which suggests signs of low-level, chronic colitis. A general cyclical
pattern of Mayo and EI scores, however, can be observed in Figure 5, from the first DSS treatment to the second DW treatment, up until treatment with either prednisolone or placebo. The cyclical nature of the signs of colitis in the macaques is reminiscent of clinical ulcerative colitis.

3.2. Pharmacological interventions

To determine whether Modified Mayo Score and Endoscopic Index are sensitive to the effects of clinical therapeutics, macaques were treated with prednisolone and then with vedolizumab (see Figure 1 for schedule).

3.2.1. Prednisolone treatment

Macaques were treated with either prednisolone or placebo during the third DSS treatment. By the end of the third DSS treatment, placebo-treated macaques demonstrated increased stool consistency (soft or loose stools), rectal bleeding and Mayo colonoscopy subscores which were similar in magnitude compared to previous DSS treatments (Figure 3). By contrast, prednisolone treatment tended to prevent increased stool consistency and rectal bleeding. While prednisolone treatment prevented increased Mayo colonoscopy subscore compared to placebo treatment, this was not statistically significant ($p = 0.0571$). Changes in global assessment subscores were not observed in either placebo or prednisolone-treated macaques.

At the end of the third DSS treatment, placebo treatment did not appear to prevent DSS-induced increases in vascularity, friability and mucosal lesion EI subscores (Figure 4). These subscores following placebo treatment were comparable to those seen in previous DSS treatments. Prednisolone treatment tended to prevent DSS-induced increases in vascularity, friability and mucosal lesion EI subscores, but these were not statistically significant. Changes in the granularity subscores were not observed in either placebo or prednisolone-treated macaques.

The Mayo and EI scores are typically presented as composites of the subscores (Figure 5). Before either placebo or prednisolone treatment, the mean composite Mayo scores following the first DSS treatment increased to between 3-4, suggesting mild colitis (Mayo scores between 1-5 suggest mild colitis; Mayo scores between 6-12 indicates moderate to severe colitis; Table 1). Subsequent DSS treatments induced mild colitis. Interestingly, despite two weeks of water without DSS treatment after the first DSS treatment, composite Mayo scores were elevated compared to pre-DSS treatment scores, suggesting the presence of a persistent mild colitis (Mayo score = 2).

Following the first DSS treatment, the mean EI composites ranged between 4 and 6, suggesting the presence of mild colitis (EI = 4–6; EI between 7-9 indicates moderate colitis [22]; Figure 5). Following two weeks of treatment with water without DSS, the mean composite EI ranged from 1 to 3, indicating remission or “inactive” colitis. Subsequent treatment with DSS induced mild colitis as indicated by increased EI.
Treatment with prednisolone over the course of the third DSS treatment prevented a DSS-induced increase in both Mayo score and EI ($p < 0.05$, vs. control treatment; Figure 5). While Mayo score was significantly decreased with prednisolone treatment compared to placebo treatment, signs of colitis were not completely eliminated—the mean Mayo score at the end of prednisolone treatment was 2, indicating mild disease. However, the mean EI score at the end of prednisolone treatment was about 2, indicating inactive colitis.

### 3.2.2. Vedolizumab treatment

Mild to moderate colitis was observed in macaques before the fifth DSS treatment period as indicated by the low mean Mayo and EI scores (Pre; Figure 6A, 6B). Prior to either vedolizumab or control treatment, moderate colitis was observed during the fifth DSS treatment, two weeks after the start of DSS treatment. One week after vedolizumab treatment, both Mayo (Figure 6A) and EI (Figure 6B) scores were reduced compared to those of control treatment. While signs of colitis was observed in control-treated macaques during the fifth treatment DW treatment, mild colitis to colitis-in-remission were observed in vedolizumab-treated macaques. As an alternate method of comparing the effect of treatment on Mayo and EI scores, the area under the curve (AUC), between two weeks and five weeks after the start of DSS treatment was calculated. Since treatment began two weeks after the start of DSS treatment and the final data collection time point was five weeks after the start of DSS treatment, these time points were used. The Mayo score AUC of vedolizumab treatment showed a trend of decreased colitis ($p = 0.0571$) compared to control treatment. Similarly, vedolizumab decreased EI score AUC over time, but this was not statistically significant compared to that of vehicle treatment ($p = 0.2286$).

Before the fifth DSS treatment, mean (±S.D.) fecal calprotectin for all seven macaques was $45.4 \pm 30.3 \mu g/g$. As a comparison, human fecal calprotectin level greater than $50 \mu g/g$ has been suggested as an indication of active colonic disease [23]. Fecal calprotectin levels from either healthy macaques or macaques with IBD-like pathology have not been reported elsewhere. The mean value obtained in the macaques prior to the fifth DSS treatment suggests a lack of active disease or remission.

During the third week of DSS treatment (one week after control treatment), mean fecal calprotectin was $114.2 \pm 28.9 \mu g/g$, indicating active colonic disease (Figure 6C). By contrast, during the third week of DSS treatment (one week after vedolizumab treatment), mean fecal calprotectin was $45.4 \pm 30.3 \mu g/g$, indicating inactive colonic disease (Figure 6C).
calprotectin was 61.6 ± 11.8 μg/g (p < 0.05 vs. control). By five weeks after the start of DSS treatment (three weeks after control treatment and during the second week of DW treatment), mean fecal calprotectin was 89.8 ± 9.0 μg/g, decreased from week 3 of DSS treatment but nonetheless indicative of active disease. By five weeks after vedolizumab treatment (three weeks after vedolizumab treatment and during the second week of DW treatment), mean fecal calprotectin was 46.8 ± 27.0 μg/g, below the threshold of active disease (p < 0.05 vs. control).

4. Discussion

The current study adapted methods to observe and quantitate colitis severity in DSS-treated macaques based on clinical assessment methods and tested the sensitivity of these methods to therapeutic intervention. A previous description of the current macaque model of DSS-induced colitis reported signs of inflammation and mild colitis with repeated DSS treatment [8]. With either acute or repeated DSS treatment, histopathological examination demonstrated robust PMN and macrophage infiltration of the colonic mucosa, into the lamina propria and in some cases into the submucosa. Interestingly, the severity of colon mucosal lesioning was such that bacteria normally restricted to the intestinal mucosa was found in distal organs as such as the axillary lymph nodes, suggesting a weakening of the intestinal mucosal barrier, which is thought to be a key mechanism in the pathogenesis of IBD [4]. Hao et al. utilized magnetic resonance imaging (MRI), as well as periodic colonoscopy, to track recto-colonic disease activity over time [8]. Signs of marked inflammation were observed with MRI throughout the length of the colon; signs of inflammation and lesions observed with MRI agreed with histopathological findings [24]. However, whether colonoscopic examination correlated with either MRI or histopathology was not reported.

Understanding of the pathobiology of IBD has greatly expanded with the development and use of chemically-induced and genetically modified rodent models of IBD, but the lack of treatments based on mechanisms elucidated in rodent models is disconcerting [25]. One complication to effective translation from preclinical findings to clinical treatment is that a number of molecular targets identified in rodents could be structurally different compared to other species. For example, vedolizumab, a humanized mouse antibody to the human integrin subunit α4β7, is not cross-reactive to the complementary mouse integrin. Proof of concept of this target's involvement in ulcerative colitis was established in cottontop tamarins with naturally occurring colitis [26]. To close the translatability gap, an important consideration is selection of a preclinical species that approximates the targeted clinical population. As the cynomolgus macaque has the same α4 integrin subunit expression as the human, and reproduces naturally occurring colitis, it could be an important preclinical model.

Cross-reactive to the complementary mouse integrin, α4β7, the macaque model of DSS-induced colitis could be viewed as a more humane alternative. Colonoscopy, along with biopsy, is currently the gold-standard for diagnosing IBD and recently, it appears that colonoscopy has been increasingly used to diagnose IBD and to guide treatment [11]. In the current study, colonoscopy was successfully utilized multiple times in the same animal over time, thereby allowing observation of disease progression and the effects of therapies over time. In terms of animal welfare, no signs of pain or distress were observed in the DSS-treated macaques—global assessment subcores suggested mild symptoms in only some of the macaques during and following repeated DSS treatments. Macaques have been observed with naturally occurring ulcerative colitis, but disease activity is generally severe, resulting in death or necessitating in euthanasia [35, 36]. The lack of severe signs of illness in the DSS-induced macaque model suggests that this model is an improvement, in terms of the 3Rs (“refinement”), compared to the naturally occurring disease and rodent models of DSS-induced colitis [8, 35].

First-line treatments, such as anti-inflammatory corticosteroids, suppress signs and symptoms of colitis as assessed with colonoscopy and semi-quantitative scoring [2, 37]. In the current study, at the end of the third DSS treatment, before the third water without DSS treatment, placebo-treated macaques showed moderate signs of disease activity, suggested by loose stools, occult blood in stool and a moderate endoscopic score. Total EI suggested mild disease activity. By contrast, macaques that were concurrently treated with prednisolone showed no disease activity (suggestive of remission) as indicated by total Mayo score and total EI. The current findings suggest prednisolone treatment prevented or suppressed the onset of DSS-induced colitis. Similar, reductions in Mayo and EI scores following vedolizumab were observed, though the reductions were not statistically significant. The current findings confirm that colonoscopy and semi-quantitative scoring could be used to assess disease severity and treatment outcome in DSS-treated macaques.

Confirmation of colonic disease status could also be obtained via measurement of serum, genetic and fecal markers of inflammation, such as calprotectin [2, 38, 39, 40]. In the current study, during the fifth DSS treatment, fecal calprotectin was markedly reduced one week following vedolizumab treatment compared to that of control treatment. Also, two weeks after termination of DSS treatment, (three weeks after vedolizumab treatment) fecal calprotectin was significantly reduced in vedolizumab-treated macaques compared to that of control-treated macaques—below the 50 μg/g suggested as the threshold for active disease [23]. The current macaque model could be used to determine
whether there is a correlation between other biomarkers of colitis and symptoms of ulcerative colitis and, furthermore, test the effect of treatment on putative biomarkers.

There are non-trivial limitations to the widespread use of macaques for use in biomedical science. Because nonhuman primates are a species phylogenetically and physiologically close to humans, their use in preclinical studies is accompanied by enhanced ethical and welfare scrutiny. In addition, there are the significant costs associated with their care and the need for extensive training of staff in handling macaques for laboratory research. These factors not only limit accessibility but also the number of animals that could be used per study [19, 21]. There are limitations specifically with the current model that should be taken into consideration as well. Chemically-induced ulcerative colitis is believed to be to “self-limiting” and evokes an “acute” inflammatory response, a response that likely differs from the mechanism of chronic colitis [3, 34]. In rodents, DSS treatment is usually of brief duration (e.g. up to a week). The mechanism of action of DSS in rodents is believed to involve disruption of epithelial tight junctions and connection between the epithelia and basal lamina. Epithelial permeability is accompanied by infiltration of PMN, monocytes and macrophages [34]. Interestingly, T cells do not appear to be involved in rodents in the inflammatory response and immune response to DSS [34]. A number of pro-inflammatory and immune activating cytokines released from cells that have infiltrated the colonic mucosa have been identified [34]. Intestinal bleeding aggravated by DSS’s anticoagulant property further evokes inflammatory and immune responses to colonic tissue injury [41]. In some strains of mice, the inflammatory and immune effects of DSS cease following discontinuation of DSS treatment whereas in other strains, robust signs of colitis persist [41]. The basis of the differential duration of responding to DSS between mice strains and its relevance to human colitis is not entirely clear.

The histopathology following acute DSS treatment in nonhuman primate colon has been previously characterized [8]. While colitis in mice generally involves the entire colon, patches of colonic lesions throughout apparently healthy colon were observed in DSS-treated macaques. As observed in rodents, evidence of disruption of intercellular cohesion was observed with the loss of a protein marker for tight junctions and significant cellular infiltration into the colonic lamina propria and submucosa are observed following acute DSS treatment [8]. A number of processes observed in human ulcerative colitis have been observed in DSS-treated macaques, including immune activation, bacterial translocation and changes in short-chain fatty acid metabolism [17]. Given the phylogenetic distance of rodents to primates, there are likely differences in pathophysiology between rodents and macaques following DSS treatment [42, 43]. More detailed comparisons of the disease processes within the current macaque model and human IBD will be needed to further establish the utility of DSS-treated macaques as a preclinical disease model [17]. Perhaps genomic and proteomic comparisons between rodent and macaque DSS models and human colitis could highlight point of similarities and limitations among preclinical models which should be kept in mind when elaborating disease mechanism and developing treatments.

The current study adapted clinical measures to assess disease activity in a nonhuman primate preclinical model of ulcerative colitis. Mild disease activity was observed, based on Mayo scores and EI, following each DSS treatment cycle. In between DSS treatments, no or minimal disease activity was observed, based on Mayo scores and EI, following in a nonhuman primate preclinical model of ulcerative colitis. Mild disease remission as observed in IBD patients. Signs of DSS-induced ulcerative colitis are amenable to treatment, as concurrent treatment with anti-inflammatory steroid prednisolone suppressed the emergence of colitis and administration with vedolizumab during active colitis suppressed symptoms of colitis. The current findings indicate that the macaque DSS-induced colitis model, in combination with clinical outcome measures, could be a useful preclinical translational bridge to clinical studies.

Declarations

Author contribution statement

N. Takahashi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

C. Kitazawa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

I. Hayashi and H. Takamatsu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Y. Itani and Y. Awaga: Performed the experiments.

A. Hama: Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported Hamamatsu Pharma Research, Inc.

Competing interest statement

All authors are employees of Hamamatsu Pharma Research, Inc.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors thank the HPR Animal Care Group for expert animal care and assistance. Also, the authors thank Dr. Naoko Matsuura and Mr. Takashi Ochi for technical assistance.

References

[1] G.R. Lichtenstein, S.B. Hanauer, W.J. Sandborn, G. Practice, Parameters committee of American college of, management of crohn’s disease in adults, Am. J. Gastroenterol. 104 (2) (2009) 465–483, quiz 464, 484.

[2] W.J. Sandborn, S. Hanauer, G. Van Assche, J. Panes, S. Wilson, J. Peterson, et al., Treating beyond symptoms with a view to improving patient outcomes in inflammatory bowel disease, J Crohns Colitis 8 (9) (2014) 927–935.

[3] V. Valatas, M. Vokas, G. Kolios, The value of experimental models of colitis in predicting efficacy of biological therapies for inflammatory bowel disease, Am. J. Physiol. Gastrointest. Liver Physiol. 305 (11) (2013) G763–785.

[4] V. Valatas, G. Bamas, G. Kolios, Experimental colitis models: insights into the pathogenesis of inflammatory bowel disease and translational issues, Eur. J. Pharmacol. 759 (2015) 253–264.

[5] T.R. Schoeb, D.C. Bullard, Microbial and histopathologic considerations in the use of mouse models of inflammatory bowel disease, Inflamm. Bowel Dis. 18 (8) (2012) 1558–1565.

[6] Food and Drug Administration, Ulcerative Colitis: Clinical Trial Endpoints Guidance for Industry (Silver Spring, Center for Drug Evaluation and Research (CDER), MD), 2016, p. 19.

[7] K.W. Schroeder, W.J. Tremaine, D.M. Istrup, Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study, N. Engl. J. Med. 317 (26) (1987) 1625–1629.

[8] X.P. Hao, C.M. Lucero, B. Turkbey, M.L. Bernardo, D.R. Morcock, C. Deleage, et al., Experimental colitis in SIV-uninfected rhesus macaques recapitulates important features of pathogenic SIV infection, Nat. Commun. 6 (2015) 8020.

[9] M. Perse, A. Gerar, Dextran sodium sulphate colitis mouse model: traps and tricks, J. Biomed. Biotechnol. 2012 (2012) 718617.

[10] S.J. Lewis, K.W. Heaton, Stool form scale as a useful guide to intestinal transit time, Scand. J. Gastroenterol. 32 (9) (1997) 920–924.

[11] G. Pineton de Chambrun, L. Peyrin-Biroulet, M. Lemann, J.F. Colombel, Clinical implications of mucosal healing for the management of IBD, Nat. Rev. Gastroenterol. Hepatol. 7 (1) (2010) 15–29.

[12] M.M. Laffins, N. Mellal, C.L. Almlie, D.E. Regalia, Evaluation of infrared thermometry in cynomolgus macaques (Macaca fascicularis), J. Am. Assoc. Lab. Anim. Sci. 56 (1) (2017) 84–89.

[13] D. Rachmilwitz, Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial, BMJ 298 (6666). (1989) 82–86.

[14] M.L. Carter, A.L. Lobo, S.P. Travis, B.S.o.g. Ibd Section, Guidelines for the management of inflammatory bowel disease in adults, Gut 53 (Suppl 5) (2004) V1–16.

[15] U.S.A. Takeeda Pharmaceuticals, Highlights for Prescribing Information, 2019. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/125476s024lbl.pdf.
