Preventative oral methylthioadenosine is anti-inflammatory and reduces DSS-induced colitis in mice

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Benight NM, Stoll B, Marini JC, Burrin DG. Preventative oral methylthioadenosine is anti-inflammatory and reduces DSS-induced colitis in mice. Am J Physiol Gastrointest Liver Physiol 303: G71–G82, 2012. First published May 3, 2012; doi:10.1152/ajpgi.00549.2011.— Methylthioadenosine (MTA) is a precursor of the methionine salvage pathway and has been shown to have anti-inflammatory properties in various models of acute and chronic inflammation. However, the anti-inflammatory properties of MTA in models of intestinal inflammation are not defined. We hypothesized that orally administered MTA would be bioavailable and reduce morbidity associated with experimental colitis. We examined clinical, histological, and molecular markers of disease in mice provided oral MTA before (preventative) or after (therapy) the induction of colitis with 3% dextran sulfate sodium (DSS). We found a reduction in disease activity, weight loss, myeloperoxidase activity, and histological damage in mice given preventative MTA compared with DSS alone. We also found that equivalent supplementation with methionine could not reproduce the anti-inflammatory effects of MTA, and that MTA had no detectable adverse effects on control or DSS mice. Expression microarray analysis of colonic tissue showed several dominant pathways related to inflammatory cytokines/chemokines and extracellular matrix remodeling were upregulation by DSS and suppressed in MTA-supplemented mice. MTA is rapidly absorbed in the gastrointestinal tract and disseminated throughout the body, based on a time course analysis of an oral bolus of MTA. This effect is transient, with MTA levels falling to near baseline within 90 min in most organs. Moreover, MTA did not lead to increased blood or tissue methionine levels, suggesting that its effects are specific. However, MTA provided limited therapeutic benefit when administered after the onset of colitis. Our results show that oral MTA supplementation is a safe and effective strategy to prevent inflammation and tissue injury associated with DSS colitis in mice. Additional studies in chronic inflammatory models are necessary to determine if MTA is a safe and beneficial option for the maintenance of remission in human inflammatory bowel disease.

methionine; matrix metalloproteinases; inflammatory bowel disease

METHYLTHIOADENOSINE (MTA), a sulfur containing metabolite, is the precursor of the methionine (Met) salvage pathway (Fig. 1). The majority of MTA is produced via enzymatic reduction of S-adenosylmethionine (SAM) during polyamine synthesis. MTA is the primary precursor of the Met salvage pathway, in which it is converted via a series of enzymatic reactions to form adenine and Met; this process is highly regulated by MTA phosphorylase (MTAP) (26). MTA has been used extensively in experimental systems as a protein methyltransferase inhibitor to study changes in methylation (15, 21, 23, 28, 35). More recently, others have begun to examine the array of beneficial properties this compound exhibits in the treatment of disease. For example, MTA reduces oxidative injury associated with carbon tetrachloride ingestion, a model of liver injury (29). Additional studies indicate that MTA prevents LPS-induced death in a mouse model of septic shock (12). Furthermore, this group has examined the response of MTA in both transformed and nontransformed cells and found MTA has protective effects in nontransformed cells, while it increased apoptosis in transformed cell lines (2). In experimental models of multiple sclerosis, MTA is able to not only prevent the induction of experimental autoimmune encephalomyelitis (EAE), but reverse the EAE phenotype when provided to mice after disease has been established (25).

As MTA has anti-inflammatory properties, we wanted to examine whether it could be used as dietary supplement to impact experimental intestinal inflammation. Our laboratory recently reported a modest increase in plasma MTA in B6-deficient mice that had diminished manifestations of dextran sulfate sodium (DSS)-induced colitis (3). DSS colitis is a model of acute injury-induced colitis that has been used as one model of inflammatory bowel disease (IBD). IBD is a chronic and relapsing condition of intestinal inflammation and injury that affects more than 1 million Americans (18). IBD is more prevalent in Western cultures, and multiple theories have been proposed as to its complex etiology. Current evidence suggests that IBD is due to an exaggerated immune response in individuals with genetic and environmental susceptibilities. The two main disease types of IBD include Crohn’s disease and ulcerative colitis. These life-long illnesses inflict a significant burden on quality of life, and thus there is a need for new therapeutics with lower cost and side-effect risk for these diseases.

To be an effective treatment, MTA needs to be readily absorbed and bioavailable when given orally or ingested, yet little is known about the oral bioavailability of MTA. One recent report in rats showed that the plasma maximal concentration (Cmax; 270 μM) occurred at 20 min after an oral bolus (250 mg/kg) of an MTA salt derivative (24). This study also examined the short-term outcomes of healthy animals supplemented with MTA and found no adverse effects or changes in liver and kidney function. Other studies have examined the safety of MTA administered by intraperitoneal injection and found no changes in liver parameters (1, 12, 25). One additional study examined the effects of MTA on the whole animal (7) through these routes of administration. However, we know of no studies of the native form of MTA that examine the uptake and retention of this bioactive compound.

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http://www.ajpgi.org
Laboratories, Indianapolis, IN). Mice were fed this diet for 2 wk.

Mice were placed on the AIN-93M purified diet (Harlan were purchased from Jackson Laboratories (Bar Harbor, ME) and

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DSS (Met) salvage pathway to form additional Met. SAH, MTA is then metabolized via a series of enzymatic reactions in the methionine

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Spermine from spermidine. Each reaction generates MTA as a by-product. dcSAM is required for the formation of spermidine from putrescine and spermine from spermidine. Each reaction generates MTA as a by-product. MTA is then metabolized via a series of enzymatic reactions in the methionine (Met) salvage pathway to form additional Met. SAH, S-adenosylhomocysteine.

Therefore, we hypothesized that orally administered MTA would be bioavailable and reduce clinical and molecular markers of inflammation in experimental colitis. Using the DSS colitis model, we found that preventive administration of MTA significantly reduced both clinical and molecular indexes of disease. We have further established the availability of MTA in the colon, liver, and plasma and confirm no adverse clinical or molecular responses in the colon of healthy MTA-supplemented animals. However, in studies in which we provided MTA after the onset of disease, there was minimal benefit in reducing colitis.

MATERIALS AND METHODS

Animals and diets. Six-week-old wild-type male C57Bl/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained in standard group housing under a 12:12-h light-dark cycle. Mice were placed on the AIN-93M purified diet (Harlan Laboratories, Indianapolis, IN). Mice were fed this diet for 2 wk before the start of, and continued during, the experiments. The animal protocol was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with National Institutes of Health guidelines.

MTA prevention study. For study 1, we tested the ability of both MTA and Met to impact DSS-induced colitis. Mice were divided into the following treatment groups: control (Con), DSS, DSS + MTA, and DSS + Met. MTA was purchased as 5'-deoxy-5'-(methylthio) adenosine through Sigma Aldrich (St. Louis, MO), and Met through Ajinomoto (Raleigh, NC). The MTA and Met groups were given their metabolites via their drinking water at a dose of 150 mg/kg body wt for 7 days. The amount of Met required for normal growth and development is 5 g/kg diet (32), and the AIN-93M diet we used provides 5.5 g/kg diet, thus meeting the daily requirement. Using our average mouse weight and previous food intake estimates (unpublished), the mice in this study were receiving ~68 mg Met/day from the diet. Using our drinking water estimates, the Met supplementation provided ~8 mg of additional Met, which is ~12% greater than their daily requirement.

Orally administered DSS (molecular mass 36–50 kDa, MP Biomedicals, Solon, OH) induces a reproducible colitis and was administered ad libitum in the drinking water at 3% for 5 days, starting 2 days after the supplements were provided. Mice were weighed every other day before the start of DSS and every day during DSS administration. An established disease activity index (DAI) (36) score was calculated daily during days of DSS administration and used to monitor animal health. This index gives each mouse a score based on daily percent weight loss (0–3), stool consistency (0–2), presence of blood in stool (0–2), and overall appearance (0–3) with a maximum score of 10. Moribund animals or those with >20% weight loss over a single day were euthanized before the end of study.

MTA bioavailability study. In study 2, we determined the uptake of MTA by administering a single oral gavage of 4 mg of MTA. We used the same mice, diet, and housing conditions as described above. Mice were euthanized at 5, 10, 30, and 90 min.

MTA preclinical study. For study 3, 16 mice maintained under the same diet and housing conditions were divided into two groups: Con and Con + MTA. Con + MTA mice were supplemented with 150 mg/kg body wt MTA via the drinking water for 7 days. Con mice were maintained on regular water. We monitored health using the DAI described above, and, on day 7, all animals were euthanized.

MTA treatment study. In study 4, we tested the ability of MTA to treat ongoing DSS-induced colitis. Mice were divided into the following treatment groups: Con, DSS, and DSS + MTA. DSS was administered ad libitum in the drinking water at 3% for 5 days. Mice were returned to regular drinking water for the remainder of the study.

![MTA metabolic pathway](image-url)

**Fig. 1.** Methylthioadenosine (MTA) metabolic pathway. MTA can be created from two pathways, both requiring S-adenosylmethionine (SAM) as a precursor. First, SAM can spontaneously degrade to form MTA. The second pathway is through the use of decarboxylated SAM (dcSAM) in polyamine synthesis. dcSAM is required for the formation of spermidine from putrescine and spermine from spermidine. Each reaction generates MTA as a by-product.

![Weight change and disease activity index](image-url)

**Fig. 2.** Weight change and disease activity index during MTA supplementation. Mice were provided 3% dextran sulfate sodium (DSS) for 5 days and 150 mg/kg body wt MTA or Met for 7 days via the drinking water [control (Con) n = 16, DSS n = 25, DSS + MTA n = 26, DSS + Met n = 12]. A: MTA supplementation significantly prevented the weight loss associated with DSS colitis compared with both other DSS groups, with DSS + Met having the greatest weight loss [P < 0.05, time, TRT, time * TRT]. B: MTA significantly reduced the disease activity index (DAI) over all days compared with DSS and DSS + Met, which had the highest DAI (P < 0.05, time, TRT, time * TRT). Values are means ± SE.
MTA was added to the drinking water at 150 mg/kg body wt 2 days after the start of DSS administration and was continued until the study ended. This represents the earliest time points at which tissue injury is known to occur in DSS colitis (8) and the earliest time point at which we begin to see indications of disease using our DAI (3). Mice were weighed every other day before the start of DSS and every day during DSS administration. The DAI described in study 1 was scored every day during and after DSS administration and used to monitor animal health. One-third of the study mice were euthanized on days 5, 7, and 9, representing the last day of DSS, day 2, and day 4 of the recovery period, respectively.

**Tissue collection.** Mice were anesthetized with isoflurane, and blood was collected via cardiac puncture; mice were euthanized by isoflurane overdose. Blood was centrifuged to isolate plasma, which was snap frozen in liquid nitrogen for additional analysis described below. Colon was collected and segmented into five equal sections. In studies 1, 3, and 4, sections 2 and 4 were fixed in 10% formalin for histopathology. The remaining colon segments were collected together and snap frozen for further analysis.

**Myeloperoxidase activity assay.** Myeloperoxidase (MPO) activity was measured using the method in Suzuki et al. (33) with modifications. Briefly, whole colon samples were homogenized in PBS and centrifuged at 20,000 g. The pellet fraction was then subject to an additional homogenization and centrifugation at 20,000 g in a buffer containing hexadecyltrimethylammonium bromide (Sigma-Aldrich) to disrupt cell membranes (17). Supernatants were then assayed using a 96-well microplate reader (Molecular Devices, Sunnyvale, CA) for the colorimetric activity of tetramethylbenzidine (Sigma-Aldrich). Activity was calculated based on the standard curve of human macrophage-derived MPO (Sigma-Aldrich) standards at concentrations of 5–100 mU/ml.

**Histology.** Segments 2 and 4 of the colon were fixed in 10% formalin, mounted in paraffin, and 4-μm-thick sections were stained using hematoxylin and eosin. Sections were scored using an established histological DAI (hDAI) (9) by a single individual blinded to the treatment groups. The hDAI gives each section a score based on histological architecture and cytology and are not inflamed. DSS had severe and diffuse destruction of the epithelial layer, neutrophil infiltration in both epithelium and lamina propria, along with edema, affecting most of the tissue. DSS + MTA had focal inflammation in both the epithelium and lamina propria, but the tissue damage was much less severe, with the majority of the epithelial architecture remaining intact. Scale bar = 100 μM.

Fig. 3. MTA supplementation significantly reduces myeloperoxidase (MPO) activity and histological damage. Mice were given 3% DSS for 5 days and 150 mg/kg body wt MTA for 7 days via the drinking water. After euthanasia, colons were collected for analysis of MPO activity (Con n = 15, DSS n = 25, DSS + MTA n = 23) and histological damage (Con n = 10, DSS n = 7, DSS + MTA n = 8). A: MPO was predictably increased in both DSS groups compared with Con, with DSS + MTA significantly reduced compared with the DSS-only group. B: both groups given DSS had the expected increase in histological damage. The MTA-supplemented group was significantly reduced compared with DSS alone. Values are means ± SE. #P < 0.05 vs. Con. *P < 0.05 vs. DSS. hDAI, histological DAI.

![Graph A](http://example.com/graphA.png)

![Graph B](http://example.com/graphB.png)

Fig. 4. Representative histological images. Mice were given 3% DSS for 5 days and 150 mg/kg body wt MTA for 7 days via the drinking water. After euthanasia, colon segments were fixed, stained, and scored for histological analysis (Con n = 10, DSS n = 7, DSS + MTA n = 8). Representative hematoxylin and eosin (H&E) images confirm that Con animals have normal histological architecture and cytology and are not inflamed. DSS had severe and diffuse destruction of the epithelial layer, neutrophil infiltration in both epithelium and lamina propria, along with edema, affecting most of the tissue. DSS + MTA had focal inflammation in both the epithelium and lamina propria, but the tissue damage was much less severe, with the majority of the epithelial architecture remaining intact. Scale bar = 100 μM.
Table 1. Microarray results for study 1 mice comparing fold changes in DSS and DSS + MTA groups relative to the control group

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>DSS † vs. Con</th>
<th>MTA † vs. Con</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccl1</td>
<td>3.490</td>
<td>1.210*</td>
<td>Chemokine signaling</td>
</tr>
<tr>
<td>Ccl2</td>
<td>4.483</td>
<td>2.470*</td>
<td>Chemokine signaling</td>
</tr>
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<td>Ccl7</td>
<td>8.261</td>
<td>1.169</td>
<td>Chemokine signaling</td>
</tr>
<tr>
<td>Ccl9</td>
<td>2.513</td>
<td>1.777*</td>
<td>Chemokine signaling</td>
</tr>
<tr>
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<td>Chemokine signaling</td>
</tr>
<tr>
<td>Cxcl2</td>
<td>3.451</td>
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</tr>
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</tr>
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<td>Collagen</td>
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<td>Matrix metalloproteinase</td>
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<td>Matrix metalloproteinase</td>
</tr>
<tr>
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<td>1.792*</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
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<td>1.415</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
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</tr>
<tr>
<td>Adatn4a</td>
<td>7.008</td>
<td>1.604</td>
<td>Protease</td>
</tr>
<tr>
<td>Timp1</td>
<td>13.857</td>
<td>4.834</td>
<td>Metalloproteinase inhibitor</td>
</tr>
</tbody>
</table>

Values indicate the fold change upregulation (†) in gene expression compared with control. DSS, dextran sodium sulfate; MTA, methylthiadenosine; Con, control. *For MTA vs. Con, values that are not significantly upregulated are indicated.

RNA Amplification kit (Illumina, San Diego, CA). Quality of cRNA was assessed using an Experion RNA analysis kit (BioRad, Hercules, CA). The cRNA was then hybridized to Illumina MouseRef-8 v2 Beadchips and scanned. Data were preprocessed using Illumina’s BeadChip Studio software and then analyzed using GeneSpring (Agilent Technologies, Santa Clara, CA) with the cutoffs set at P < 0.05 and twofold differences. The resulting data were then placed in the Database for Annotation, Visualization and Integrated Discovery (DAVID) program (13, 14), and relevant pathways were identified.

Several candidate genes were chosen and validated using the protocol described below. Microarray data was deposited in the NCBI GEO repository under GSE34553.

For mRNA expression, RNA was treated with the Ambion DNase protocol (Life Technologies, Carlsbad, CA) to remove any contaminating DNA. Quality and quantity were determined using a micro- sample UV spectrophotometer (Nanodrop, Thermo Fisher Scientific, Waltham, MA). cDNA was synthesized from 2.8 μg of RNA using the ABI High Capacity cDNA Reverse Transcription kit (Life Technologies), according to the manufacturer’s instructions and a PTC-200 thermocycler (Biorad, Richmond, CA). Quantitative PCR (qPCR) was completed using a TaqMan Universal PCR Master Mix kit and TaqMan probes for TNF-α, inducible nitric oxide synthase (iNOS), matrix metalloproteinases (MMP)-3, MMP-13, IL-1β, IL-10, and GAPDH (Life Technologies) on an ABI 7900HT qPCR system (Life Technologies). Data were normalized to GAPDH, and fold change was calculated using the ΔΔCT method.

**Met cycle metabolites.** Plasma and tissue concentrations of Hcys, cysteine, and glutathione were quantified by reverse-phase HPLC with O-phthalaldehyde derivatization using the method described previously (39, 40). Concentrations of adenosine, SAM, S-adenosylhomocysteine (SAH), and MTA were measured by HPLC using the method described in Farrar and Clarke (10) in plasma and tissue. Met concentrations in the plasma and tissue were determined by reverse-phase HPLC of their phenyl isothiocyanate derivatives, as described by Stoll et al. (31).

**Statistical analysis.** All data are expressed as means ± SE. Comparison among all groups for weight change and DAI was performed using two-way ANOVA to compare both time (days of DSS) and treatment effects. All other statistical measures were performed with one-way ANOVA and Tukey posttest. Survival curves were analyzed using Kaplan Meier. All analyses were completed using GraphPad Prism V5 software (La Jolla, CA). Statistical significance was defined as P < 0.05.

**RESULTS**

Preventative MTA reduced disease burden induced by DSS colitis. In study 1, we tested the ability of MTA and Met to prevent colitis induced using 3% DSS for 5 days. Mice supplemented with 150 mg/kg body wt MTA (DSS + MTA) lost significantly less weight over all days compared with both DSS only and DSS + MET (Fig. 2A). Furthermore, DSS + MTA mice had a significantly lower DAI score over all days compared with DSS and DSS + MET (Fig. 2B). DSS + MET had the highest weight loss and highest DAI over all days. As we found no indication that Met supplementation was beneficial in our experimental colitis model, we did not include that group in the remaining analyses.

MTA supplementation reduced inflammatory response to DSS challenge. As an indication of neutrophil infiltration, colonic MPO was measured in study 1. As expected, we found...
that DSS increased MPO in all treated groups. The DSS + MTA group was significantly reduced compared with DSS (Fig. 3A). We also examined histological changes using an established index (Fig. 3B). Histological score was increased significantly in all DSS groups, with DSS + MTA significantly less than the DSS group. Representative images at low and high magnification are presented in Fig. 4.

Global gene expression indicates changes in immune signaling and tissue remodeling. Using colonic mRNA, we examined global changes in gene expression using Illumina BeadChip platform. After initial normalization, data were transferred to GeneSpring GX v10. With cut-off values of $P > 0.05$ and changes twofold or greater, we found 1,611 genes that were differentially expressed with DSS or DSS + MTA. This data were analyzed using DAVID software to identify pathways. We found enriched pathways for cytokine/chemokine signaling, collagen, and MMPs (Table 1).

We selected three representative genes to validate our microarray using qPCR (Fig. 5). As expected, DSS significantly increased expression compared with Con. For IL-1β, DSS increased 48-fold compared with Con, and DSS + MTA was 75% less. The MMPs, MMP-3 and MMP-13, were upregulated 75% less. The MMPs, MMP-3 and MMP-13, were upregulated compared with Con (103-fold and 330-fold, respectively). The DSS + MTA group was 80–85% lower compared with DSS. We also found increased inflammatory gene expression with DSS treatment. TNF-α was increased 10-fold with DSS, but...
this was significantly reduced by 29% in DSS + MTA. iNOS was increased 66-fold in DSS, but significantly reduced by 53% in the DSS + MTA. For IL-10, DSS and DSS + MTA were significantly upregulated compared with Con, and DSS + MTA was 50% lower than DSS only.

Met metabolites showed only minor changes during MTA supplementation. Using HPLC, we measured metabolite-associated Met cycle (Table 2). In plasma, Met was significantly decreased with DSS. However, it was restored to Con levels in the DSS + MTA mice. Other Met metabolites measured in plasma were not different among the groups.

In the colon, Met was significantly increased in DSS and DSS + MTA compared with Con. Homocysteine was significantly reduced in DSS and restored to Con levels in DSS + MTA. SAM was significantly reduced in DSS and DSS + MTA. All other measured metabolites were unchanged in the colon. In the liver, we found significantly higher homocysteine in DSS mice. Homocysteine was reduced to near Con levels in DSS + MTA mice. SAM was significantly different among all groups, with SAM concentrations increasing in DSS compared with Con and a further increase in DSS + MTA. SAH was decreased in DSS and DSS + MTA compared with Con. These changes in SAM and SAH lead to a change in the SAM-to-SAH ratio, which is steadily and significantly increased from Con to DSS to DSS + MTA. Interestingly, we were unable to measure a change in MTA concentration in plasma, colon, and liver, even after 7 days of MTA supplementation in DSS + MTA group.

Oral MTA was rapidly absorbed into circulation. As we were unable to measure an increase in MTA in mice given a supplement for 5 days, we designed a study to determine the kinetics of MTA absorption when given as an oral bolus. Mice were given a single gavage of 4 mg of MTA and euthanized over a 90-min period. We found the bolus MTA rapidly appeared in plasma, colon, and liver within 5 min of gavage (Fig. 6). Both plasma and liver peaked at 5 min, with plasma $C_{\text{max}}$ of 125.8 $\mu$M and while liver $C_{\text{max}}$ was 268.7 $\mu$mol/mg. The large increase in both the plasma and liver was rapidly cleared, and MTA was restored to baseline levels within 90 min. In the colon, $C_{\text{max}}$ was achieved at 30 min (22.5 pmol/mg), but remained above baseline at 90 min. As the conversion of MTA to Met is thought to be 1:1, we expected to see a large increase in plasma Met following the spike in MTA. However, Met concentration fluctuated near baseline values for all time points measured (baseline 106 $\mu$M and post-gavage 86–120 $\mu$M).

Acute MTA supplementation had no adverse affects. We examined the same parameters measured in our DSS colitis mice in a group of healthy Con mice supplemented with MTA for 7 days in the drinking water. No mortality occurred in either group of mice (data not shown). Weight gain was not different between the Con and supplemented groups (Fig. 7A).

![Fig. 7](http://ajpgi.physiology.org/)

Fig. 7. Short-term MTA supplementation did not adversely affect healthy Con mice. Mice were given 150 mg/kg body wt MTA in the drinking water for 7 days (Con $n = 9$, Con + MTA $n = 8$). There was no difference in weight gain (A), disease activity index (B), or MPO activity (C). Values are means ± SE. D: representative H&E images show that both groups had no histological score, normal architecture, and no inflammation or crypt hyperplasia. Scale bar = 100 $\mu$M.
Furthermore, the DAI was less than one for all time points for both groups (Fig. 7B). In examining inflammatory cell infiltrate, we found no differences in MPO activity in the supplemented group (Fig. 7C). Finally, normal histological architecture with no evidence of crypt hyperplasia was seen (Fig. 7D).

MTA treatment delays, but does not reduce, DSS-induced mortality. As study 1 indicated that MTA given before the onset of DSS colitis is protective, we tested whether MTA remained effective when provided as a treatment after the onset of colitis. As shown in Fig. 8B, treating mice with MTA after the onset of colitis delayed DSS-induced mortality, but, at the conclusion of the study, there was no difference between DSS and DSS + MTA, with 40% mortality in both groups.

MTA treatment does not reduce clinical indexes of experimental colitis. In the mice who survived to the end of the study, there was no difference in weight loss between DSS and DSS + MTA (Fig. 8C), with both groups losing ~20% of their baseline body weight at the end of the study. With regard to DAI (Fig. 8D), DSS + MTA mice tended to have a slightly lower DAI during the first 6 days of the study, and this difference was significant on day 7 compared with DSS. During the remaining days of the recovery phase (days 8 and 9), DSS indicated a faster recovery than DSS + MTA, with a 1.4 unit drop to 3.4 in DAI from its maximal of 4.8 on day 7 compared with DSS + MTA, which continued to score around 3.2.

MTA treatment does not reduce the inflammatory response. We tested MPO activity of colonic tissue extracted from mice during the study. Figure 9 shows that MPO was increased in DSS and DSS + MTA compared with Con, with the DSS + MTA group tending to be higher on days 7 and 9. To further examine inflammation, we scored histological sections using an established index. We found a significant increase in DSS and DSS + MTA compared with Con on days 7 and 9, with no difference between DSS and DSS + MTA (Fig. 10). For DSS, mild inflammation is evident on day 5. By day 7, there is complete destruction of the epithelial layer, and the lumen is filled with cellular debris and inflammatory cells. On day 9, we still see large regions of focal inflammation in both the epithelium and lamina propria, but the tissue damage was much less severe. For the MTA-supplemented mice, there is also evi-
MTA in Experimental Colitis

Fig. 9. MTA supplementation tends to increase MPO activity. Mice were provided 3% DSS for 5 days and 150 mg/kg body wt MTA, starting on day 2 of DSS via the drinking water. After euthanasia (days 5, 7, 9), colons were collected for analysis of MPO activity (Con n = 8, DSS n = 18, DSS + MTA n = 18). MPO was predictably increased in both DSS groups compared with Con; however, DSS + MTA tended to be higher than the DSS-only group on days 7 and 9 (P < 0.05, time, TRT, time × TRT). Values are means ± SE. *P < 0.05 vs. Con.

dence of inflammation on day 5, with slightly larger patches of inflammatory infiltrate and tissue damage in the epithelium and lamina propria. Similar to DSS, day 7 shows major tissue destruction and inflammatory infiltrate. By day 9, we still see regions of focal inflammation; however, there is evidence of tissue repair, as indicated by the darkly staining epithelial layers that are properly organized to restore a barrier to luminal contents. We went on to quantify changes in infiltrating F4/80 positive monocytes. Although we found a large increase in positively staining cells, there was no difference between DSS and DSS + MTA (data not shown).

In our MTA prevention study (study 1), we found changes in inflammatory and extracellular matrix remodeling gene expression with MTA supplement. However, the present study indicates little change between DSS and DSS + MTA. As expected, induction of colitis upregulated all measured genes. For IL-1β, there was no difference between DSS or DSS + MTA on days 5, 7, or 9 (Fig. 11). We found the same results for iNOS and MMP-13. For TNF-α measured on day 5, we measured a significant increase in message expression in DSS + MTA compared with DSS. Although day 9 had the same trend, the difference was not significant. For MMP-3, we measured a significant increase in message in DSS compared with DSS + MTA on day 5, with no differences on days 7 and 9.

DISCUSSION

MTA is a metabolite produced from SAM primarily during polyamine synthesis. MTA has been used experimentally as a methyltransferase inhibitor in cell culture systems for many years (15, 21, 23, 28, 35), with limited investigation as to its effects in vivo in animal models. Recently, other groups have begun to explore the potential of MTA as a therapeutic treatment for diseases (1, 7, 12, 24, 25, 29). However, little is known about the bioavailability of orally administered MTA, including MPO activity, inflammatory gene expression, and histological damage, indicated that MTA supplementation was protective against DSS colitis. These results are similar to other studies of MTA in animal models of inflammation, including experimental autoimmune EAE (24, 25), LPS-induced septic shock (12), and various liver injury models, including carbon tetrachloride (29) and hepatobiliary fibrosis (19). Taken together, these results provide strong support for MTA as a therapeutic candidate for the treatment of acute and chronic inflammatory conditions.

We conducted a microarray study to identify the cellular mechanisms affected by preventative MTA administration. We found two dominant pathways that were upregulated by DSS and then downregulated by MTA supplementation. As expected, one of those pathways was chemokines and cytokines, both integral elements of tissue inflammation. Interestingly, the other major pathway affected was tissue remodeling genes, including proteases, collagen family members, and MMPs, with MMPs most frequently represented in the microarray. MMPs are a large class of genes responsible for extracellular matrix remodeling and have been classified based on substrate specificity (22). Our data set contains members from most groups of MMPs: collagenases (MMP-13), gelatinases (MMP-9), stromelysins (MMP-3, MMP-10), as well as membrane-bound MMPs (MMP-14). Other studies have previously shown that MMPs are upregulated with DSS colitis (34). The extensive downregulation of these genes provides a strong indication that MTA is affecting extracellular matrix remodeling during DSS colitis.

Three genes from the represented pathways in the microarray were validated using real-time qPCR. The genes representing tissue remodeling were MMP-3 and MMP-13, both of which were highly upregulated in the microarray. The qPCR analysis confirmed that MMP-3 and MMP-13 were highly upregulated in the DSS mice compared with Con (103-fold and 330-fold, respectively). Both genes were reduced >80% in the DSS + MTA group. This reduction made the mRNA expression of MMP-3 and MMP-13 in the DSS + MTA group similar to what was measured in the untreated Con. These data are similar to that reported in a model of hepatobiliary fibrosis, in which MTA administration significantly reduced fibrosis. This study also found a significant decrease in MMP-13 mRNA expression (19). While all MMPs have been implicated in human IBD (reviewed in Refs. 22, 27), both MMP-3 (38) and 13 (37) are specifically upregulated, indicating that MTA is targeting a process that occurs in both human and animal models of IBD.

Our results from the oral MTA bolus experiment show that it is rapidly absorbed from the gut. We found that MTA concentration was increased in the plasma, liver, and colon immediately, with Cmax at 5 min in the liver and plasma pools, which is earlier than that reported in rats, where Cmax occurs at 20 min (24). This suggests that absorption of MTA occurs in the stomach and small intestine, and MTA is rapidly disseminated throughout the body. The mechanism of MTA transport...
across the epithelium is not clear. Studies in human erythrocytes (5) and isolated rat liver (41) indicate that MTA is absorbed via facilitated diffusion. However, studies in a leukemic cell line (HL-60) suggest MTA is transported by a nonspecific nucleoside transporter that is shared with adenosine (30). Given the potent anti-inflammatory properties of MTA, studies examining its transport and metabolism in intestinal epithelial cells would provide valuable insight into the bioavailability. Our results suggest that the strong anti-inflammatory effect of MTA occurs during the relatively brief time when MTA is elevated in the body. The declining concentrations in the plasma and liver pool indicate that MTA is rapidly cleared, presumably by MTAP, the only known enzyme for MTA disposal. The rapid clearance of MTA is of interest because loss of MTAP has been associated with cell transformation via accumulation of MTA (16). However, our data suggest that even large quantities of MTA could be handled by the body and disposed of efficiently with no apparent adverse side effect. These results are similar to those reported by other groups, where they examined MTA given by intraperitoneal injection or orally to mice and saw no changes in weight, behavior, or kidney and hepatic function (1, 7, 12, 24). Taken together, these studies suggest that short-term oral MTA supplementation has a favorable pharmacokinetic and safety profile, and thus longer term studies are warranted.

In our preventative colitis study, we also included a group of Met-supplemented mice to determine whether the effects of MTA were working through the Met salvage pathway. We choose to test Met supplementation because, in our metabolite data, we found a significant increase in plasma and colonic Met in the DSS + MTA mice compared with DSS only. Clinical indexes suggest that the effect of MTA is not mediated by Met, as the Met-supplemented group had the greatest weight loss and highest DAI. Furthermore, metabolite analysis in our MTA availability study did not show an increase in plasma Met concentration at any time point measured. These data suggest that the effect of MTA is direct and not mediated by its conversion to Met via the Met salvage pathway.

We also tested the ability of MTA to treat established DSS-induced colitis by starting MTA treatment 2 days after onset of DSS and continued MTA during recovery. The clinical and molecular markers indicated that MTA treatment was ineffective at reducing the inflammatory response associated with DSS colitis, and that MTA seemed to delay the recovery
phase. Studies of additional time points past day 9 would be necessary to confirm this; however, these studies would be challenging, given the high mortality rate in both groups at the later time point.

In considering the different responses during the preventative and treatment study, it is possible that the severity of the colitis, which was induced by 3% DSS in our system, obscured any positive benefits of MTA when given after colitis induction. While a dose of 3% DSS is not excessive, it is likely that, in our study, colitis is aggravated by the administration of the purified diet to the animals before and during DSS. A recent study of DSS-induced colitis in mice showed that the degree of weight loss and disease activity was much higher in mice fed a purified diet compared with those fed standard chow, and the authors postulated that this differential response was due to the lower fiber content of the purified diet (11). Indeed, our purified diet (AIN-93M) contains only cellulose as a fiber source, whereas nonpurified, rodent chow diets contain multiple fiber sources and are typically higher in fiber content. It is possible that the overriding damage to the colonocytes, as indicated by histology, was insurmountable by any treatment and that with a less severe colitis, either from a reduction in DSS or feeding a nonpurified diet, we would see the protective effect of MTA after the induction of disease. We also suggest that the efficacy of MTA in treatment of established colitis in higher dose-seeking studies is feasible, given that our pharmacokinetic results showed that MTA is rapidly absorbed by the intestine and has a limited half-life in circulation.

Fig. 11. MTA reduces upregulation of inflammatory genes. Mice were provided 3% DSS for 5 days and 150 mg/kg body wt MTA, starting on day 2 of DSS via the drinking water. After euthanasia, colonic RNA was extracted for gene expression changes using TaqMan probes for real-time PCR (Con n = 8, DSS n = 12, DSS + MTA n = 10). All genes increased with DSS and DSS + MTA compared with Con. For IL-1β, iNOS, and MMP-13, there was no difference between DSS and DSS + MTA on any days. DSS + MTA was significantly upregulated compared with DSS for TNF-α on day 5, while MMP-13 was significantly reduced in DSS + MTA compared with DSS on day 5. Values are means ± SE. #P < 0.05 vs. Con. *P < 0.05 vs. DSS.
The dominant presence of remodeling genes in the prevention microarray indicates that MTA may be exerting its beneficial effect by reducing the number of inflammatory cells that extravasate into the site of tissue injury. These results are corroborated by our findings of reduced MPO activity in MTA-supplemented mice, as well as reduced histological damage and neutrophil infiltration by histological scoring. Furthermore, the large increase in F4/80-positive staining monocytes in DSS-treated groups in the treatment study indicate that an influx of inflammatory cells occurs in the colon in response to DSS colitis. The microarray also indicated other genes associated with neutrophils were downregulated by MTA. These include FLOT1, a gene important for neutrophil migration (20), and LEN2, a molecular marker of neutrophil infiltration (4). These results are similar to those reported by Cerri et al. (6), where they examined MTA-induced changes in adhesion molecules in isolated endothelial cells. This work indicated a reduction in TNF-α production from endothelial cells, which led to a reduction in intercellular adhesion molecule 1 and a concomitant reduction in polymorphonuclear cell adhesion. Likewise, our preliminary unpublished results show that MTA treatment suppresses flagellin-induced IL-8 secretion in cultured HT-29 cells, suggesting that MTA directly affects the colonic epithelial inflammatory response (data not shown). However, in our study, the possibility exists that the effects of MTA on adhesion molecules is secondary to the reduction in inflammation. Therefore, future studies are warranted using in vivo intravital microscopy to examine the temporal changes in inflammatory cell adhesion during MTA treatment, as well as additional in vitro studies to determine the contribution of epithelial cells to the phenotype seen in vivo.

A limitation of these studies is the use of the DSS model. DSS induces a chemical injury in the colon in a reproducible and predictable manner, but this inflammatory response only involves the innate immune system. Human IBD is a chronic, relapsing condition that involves both B- and T-cell responses, as well as the innate immune system. Therefore, the DSS model does not completely mimic the physiological responses seen in human IBD. Therefore, it is unclear whether similar benefit would be seen in human IBD. However, as reports indicate that similar changes in extracellular matrix remodeling occur in human IBD (37, 38), additional studies examining MTA supplementation in other animal models of chronic inflammation and fibrosis are warranted to confirm the positive findings reported here.

In conclusion, we demonstrated that oral supplementation of MTA for 1 wk before and during DSS colitis is protective, as indicated by improved weight maintenance and a reduced DAI in mice supplemented with MTA (DSS + MTA) compared with DSS alone. The protective effect was mediated by a reduction of the inflammatory response, as indicated by reduced MPO activity and histological damage, including reduced neutrophil infiltration. The anti-inflammatory action of MTA was also confirmed by evidence of reduced expression of genes involved in inflammation and tissue remodeling. MTA is rapidly absorbed into the circulation after oral administration, but cleared promptly, suggesting that the anti-inflammatory effects of MTA occur during the transient rise in MTA. However, when MTA is administered after the onset of colitis, it is ineffective at reducing mortality rate, the amount of weight loss, and DAI. Furthermore, markers of inflammation indicate that, after the onset of colitis, MTA is unable to control the inflammatory response. Timing of MTA administration may not be the direct cause of this loss of response, as other studies have seen the anti-inflammatory benefits of MTA administered after the onset of disease. Our results extend the list of reports showing the protective, anti-inflammatory of MTA and provide compelling evidence for further studies aimed at maintaining a quiescent state in existing IBD. Our evidence of rapid bioavailability and clearance with no adverse effects makes MTA a potentially attractive therapy for management of remission of IBD patients. This issue is relevant since some immunosuppressive drugs, such as methotrexate and 6-mercaptopurine, used to treat IBD are ineffective at inducing remission, but are efficacious at maintaining remission (16).

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DISCLAIMER

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

N.M.B. and D.G.B. conception and design of research; N.M.B., B.S., J.C.M., and D.G.B. interpreted results of experiments; N.M.B. analyzed data; N.M.B. prepared figures; N.M.B. drafted manuscript; N.M.B., B.S., J.C.M., and D.G.B. edited and revised manuscript; N.M.B., B.S., J.C.M., and D.G.B. approved final version of manuscript.

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