Effect of Dietary Magnesium Manipulation on the Gastrointestinal Microbiome of a Mouse Model of Ulcerative Colitis

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Effect of Dietary Magnesium Manipulation on the Gastrointestinal Microbiome of a Mouse Model of Ulcerative Colitis

A Senior Honors Thesis

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By

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Abstract

Ulcerative colitis (UC) is a disease characterized by inflammation of the GI tract, which disturbs the mucosal lining and hinders magnesium (Mg\(^{2+}\)) absorption. Research has shown that increasing the dietary intake of Mg\(^{2+}\) decreases the severity of the colitis symptoms, but there is no data on the effect this has on the microbiota of the GI tract or the blood. We found that, in DSS-treated mice, the amount of bacteria in the colon increases with a decrease in dietary Mg\(^{2+}\), and that the concentration of bacteria in the spleen does not correlate to symptom severity or to colonic bacterial amounts. Mg\(^{2+}\) could be used as a supplement for UC patients, treating both hypomagnesemia and lowering colonic bacteria closer to healthy levels.
Introduction

Magnesium

Magnesium is a vital ion in regard to physiology. It is the second most abundant intracellular cation, and the fourth in the entire human body (6). It is involved in many pathways of importance, like the degradation of macronutrients, regulation of parathyroid hormone, and excitability of neurons (6). Even further, it has a major role in the process of inflammation. The presence of magnesium limits transport of calcium into cells. Since calcium is known to trigger an inflammatory response, the absence of magnesium would enhance calcium influx and therefore increase the magnitude of inflammation. (3, 5). This interaction is crucial when it comes to inflammatory bowel diseases (IBDs).

IBDs and the mouse model

IBDs are a growing concern in medicine. They are more common than epilepsy or car accidents, and have a comparable prevalence to type 1 diabetes or schizophrenia (8). This paper will focus on ulcerative colitis (UC), which is a bowel disease associated with inflammation of the superficial layer of the colon mucosa (8). It is obviously unethical to induce this condition in humans for research studies, but there are methods to do so in mice. One such procedure uses the chemical agent dextran sodium sulfate (DSS) to bring about comparable UC condition. Such a method is popular for research on therapeutic agents, and is known to be a sensitive screening model. It should be mentioned that this model does not require responses from B or T cells, and that luminal bacteria may have a role in colitis development (4).

Microbiome

Gut microbiome plays a role in the development of IBDs like UC. In humans, the bacteria that are known to have an impact are the presence of the Proteobacteria phylum and the absence of diversity in the Bacteroidetes and Firmicutes phyla. In fact, chronic IBDs are often linked to previous acute inflammation of the gastrointestinal tract incited by bacteria (2). Diet has an effect on the diversity of the gut microbiome. Research has shown that a deficiency in magnesium concentration in the gut decreased the cecal concentration of Bifidobacteria and
Lactobacilli, of which the former is known to enhance mucosal barrier performance (3). The content of the flora of the gut can impact onset and development of IBDs, but at the same time can be impacted by the subject’s diet.

Magnesium and UC

The development of UC is impacted by the intake of dietary magnesium. Subjects with an IBD suffer from a magnesium deficit, which when reverted lessens their symptoms. UC itself creates a cycle of hindering magnesium absorption through intestinal inflammation, which in turn increases the disease activity (7). Research has shown that the presence of magnesium has an impact on the diversity of the gut microbiome. This microbiome also has an impact on the mucosal lining, and therefore UC. Furthermore, the presence of magnesium impacts the virulence of bacteria. Salmonella is the typical model for this research, as it has systems to regulate virulence and allow survival in macrophages. These system also controls magnesium transporters, and it is hypothesized that low magnesium levels signal the virulence pathway (1).

Research Aims

This study attempts to find the impact UC and dietary magnesium manipulation has on the gut microbiome of mice. The experimental diets vary in magnesium concentration, from severely depleted to normal feed levels, and UC was induced through the use of the chemical agent DSS. A variety of factors were observed to determine and classify a change in the microbiome of the gut as a result of the UC and dietary magnesium manipulation,

Experimental Procedure

Animal Environment

In house bred black-6 mice [C57BL/6J (Harlan, Madison, WI USA)] were kept in a room of constant temperature and humidity (22-26°C and 56-70% humidity) with a light:dark cycle of 12 hours. Both sexes were used for these experiments, although the number of each in the experimental groups remained constant. Similarly, age and litter were controlled throughout the groups. Males and females were kept in static cages and topped with wire. They were kept with other littermates of the same sex, in order to discourage fights between them. Each cage
contained a small amount of bedding [Sani-chips manufactured by TEklad], shredded paper for nesting, and a section of PVC pipe for enrichment.

**Diet Preparation**

A DSS (colitis grade; MP Biomedicals) solution was made to be as drinking water for experimental mice. This was done with deionized water that was run through a 10-minute liquid cycle in the autoclave. The DSS was then added to be 3% by mass for the bacterial analysis tests, and 2% by mass for the gut permeability tests (to lessen the devastative symptoms of the induced UC). The control diet (0.2% Mg; Teklad TD.130120, Harlan Teklad, Madison, WI, USA) and the depleted diet (0.003% Mg; Teklad TD. 93106, Harlan Teklad, Madison, WI, USA) were given *ad libitum*.

**Anesthesia Preparation**

The anesthesia used within this lab was 4% Avertin. This was prepared by adding 1 g of 2, 2, 2-tribromoethanol to 1 ml 2-methyl 2-butanol. This solution was mixed vigorously with a stir bar in a container covered with aluminum foil to prevent interaction with light. Once the 2, 2, 2-tribromoethanol dissolved fully, 1 ml of the solution was added dropwise to 40 ml of 150 mM NaCl solution. This container was also wrapped in aluminum foil, and the solution was mixed until fully dissolved and presented as clear and precipitate free. The solution was then transferred to an amber glass bottle and stored in the refrigerator.

**Experimental Procedure**

8-10 week old Black-6 mice were weighed and split into 2 groups: magnesium control and magnesium free. The magnesium free received the depleted diet, and the other received the control diet *ad libitum*. Each group was then further divided to determine which received the DSS treated drinking water. Both received purified deionized water (DIW), but the DSS treated mice had the 3% DSS solution. The drinking bottles were autoclaved prior to the start of the experiment, and the DSS solution was changed every two days.

The subjects were given their respective diets and treatments for 6 days, and were weighed and tested for fecal occult blood daily. Feces were collected and smeared on filter
paper. They were then each coated with 120-150 mg of powdered guaiac tablets [Cambridge Diagnostic Products]. Each received two drops of glacial acetic acid followed by two drops of 3% Hydrogen peroxide [CVS]. A positive result presented with blue streaks extending around the sample before two minutes expired, while a negative yielded no color change with the same time limit. On the 6th day, the subjects were transferred to metabolic cages. Each was supplied with a known amount of food and DSS treated or untreated drinking water. After 24 hours, the mice were removed to be weighed, and the remaining amount of food and water was recorded. Urine from each subject was measured with a pipette, and 1 ml was collected for determination of magnesium concentration. Each sample was placed in an accuSpin Micro 17 and centrifuged at 13,000 RPM for 3 minutes. The supernatant from each was transferred to a clean microfuge tube and placed in the freezer for future analysis. After determining the mass of each mouse, they were anesthetized with 4% Avertin by intraperitoneal (IP) injection (300-400 μL). Total anesthesia was evidence by the absence of the pedal reflex. Mice were then dissected via ventral incision, blood samples were collected via intracardiac (IC) puncture, and the spleen and colon were removed. Blood samples were placed in clean microfuge tubes and allowed to clot for 30 minutes. They were then centrifuged for 10 minutes at 6,000 RPM, and the serum samples were transferred to clean microfuge tubes via pipette. These were kept at 4°C in the refrigerator until atomic absorption analysis was performed within one day of collecting the samples.

*Disease Activity Index*

A disease activity index (DAI) was created in order to adequately quantify the presence of UC. This was done with known symptoms of UC, such as weight loss, presence of blood in the anus, positive fecal occult blood test, and loose stool. Mice received a score out of five: 1 point for weight loss 3% or greater (in one week), 2 points for weight loss greater than 10%, 1 point for the presence of blood in the anus, 1 point for a positive fecal occult test, and 1 point for loose stool (stuck to sides of the cage).
**Atomic Absorption**

The atomic absorption analysis was performed with a GBC 932 atomic absorption spectrometer. Serum samples were diluted in 1:200 in DI water and compared to a standard curve made with varying concentrations of magnesium chloride solution (0.0025 mmol, 0.005 mmol, 0.0075 mmol, 0.01 mmol and 0.02 mmol). Urine samples were diluted 1:2000 to fit the same standard curve (although the control diet had to be diluted 1:3000 and 1:4000 to fit). Samples were run through the machine until the reading reached a maximum value and stabilized, beginning with the standards and following with the samples.

**Dissections**

The removed spleen was cut into pieces under sterile conditions and homogenized in a solution of .01% Tween and 10% 10X PBS, at a volume nine times that of the spleen mass. Samples were placed in the refrigerator until they could be plated. After the spleen removal, the colon was taken out (cut just above the cecum and at the very end of the rectum) and measured. All were placed in chilled PBS for a photograph.

**Microbiology**

All microbiology procedures were performed by Dr. Pelletier’s lab. After collection, fecal and spleen samples were made into a slurry in 5 ml of 0.010% Peptone and L-cysteine water. These were vortexed, and 100 μl were plated on MRS, Bifidobacteria, and EMB agar. 50 μl of the original slurry was also added to 5 ml of the original mixture (0.010% Peptone and L-cysteine water), of which 100 μl was plated on the BHI agar. The MRS and Bifidobacteria plates were incubated with 5% carbon dioxide at 37°C for 24 hours. The EMB and BHI plates were incubated at 37°C for 24 hours and did not receive the carbon dioxide. The colonies on all plates were then counted and converted to cells per gram. Medium composition are as follows:
A FITC-Dextran (4 kDa, 25 mg/ml; Abbott Laboratories) intestinal permeability assay was used to determine a possible change in the gut permeability due to magnesium and DSS-treatment. The process began with the removal of the mouse food and the transfer of mice to different cages with no bedding, but with the same drinking water.

After 4-6 hours, an oral gavage was performed with 200 µl of a solution of FITC-dextran and sterile 1x PBS (1:2 dilution/0.625 µmol FITC-Dextran for the mice with no DSS treatment, and 1:4/0.3125 µmol FITC-Dextran for the DSS treated group). Mice were put under anesthesia in a container in the fume hood, which had wire above a stir bar and cotton bedding. The
bedding was doused with 300 –400 µl isofluorane upon placement of mouse on the wire.. Anesthesia lasted 2-3 minutes, enough to perform the oral gavage.

After 4 hours, blood was collected through the same process as magnesium determination. 400 µl of the blood sample was placed in a microfuge tube with 6 µl of Na heparin to prevent clotting. This was spun at 6,000 RPM for 10 minutes in a centrifuge, and the plasma was transferred to a clean tube and stored in the refrigerator until testing.

Testing of the fluorescence was done with the use of a Synergy H1Hybrid Multi-Mode Reader (BioTek). A standard curve was created with 110 µl of a solution containing 0.0205 pmol FITC-dextran, 11 µl normal serum, and 98.9 µl PBS placed in the first well. 11 µl was removed from the well and transferred to well 2, which contained 99 µl of PBS and 10% FITC free serum. This continued until 6 wells contained dilutions of the original mixture. One well contained only 99 µl of PBS and 10% FITC free serum to serve as a blank. The plasma of the experimental groups was then placed in following wells in a 1:10 dilution with PBS and kept in the dark until testing could commence.

**Results**

The DAI shows the impact that magnesium has on the progression of UC and its symptoms. Figure 1 shows the variation in the severity of symptoms amongst the groups:
Both the control diet and magnesium depleted subjects without the DSS treatment had an average score of 0.167/5. The DSS treated group shows an actual difference, with the control diet having an average score of 2.50/5 and the depleted 3.50/5. A staggering difference can be seen across treatment groups as well (ANOVA followed by Fisher’s LSD, P<0.05).

Analysis of Mg$^{2+}$ concentration in serum and urine shows how much magnesium is being absorbed and how much is being excreted in different diet and treatment groups. Serum results can be seen in figure 2.
The control diets had the highest average concentration in their treatment groups. Control diet and untreated had an average of 1.395 mmol, and the same diet but with the DSS-treatment had 1.350 mmol. Both are within the normal range of 1.2-1.4 mmol. The depleted magnesium groups, however, did not reach the normal concentration. Magnesium free with no treatment had an average of 0.643 mmol, and the DSS-treated with the same diet had 1.027 mmol. (ANOVA followed by Fisher’s LSD, \( P<0.05 \)). The difference between Mg\(^2+\) control with DSS and Mg\(^2+\) control without DSS is not significant (P-value of 0.114), although the rest of the data is below 0.05.

The Mg output in the urine can be used to determine how much dietary magnesium is excreted by the body. Urine was collected over a period of 24 hours and tested in the same manner as the serum. Figure 3 shows the average concentrations for the different experimental groups.
Magnesium manipulation and UC severely impacts the amount of magnesium excreted in urine. The control diet and without treatment had an average concentration of 41.36 μmol, and the same diet with the DSS treatment contained 18.65 μmol. The depleted diets were very low, at 1.37 and 0.970 μmol, for the untreated and treated respectively (ANOVA followed by Fisher’s LSD, P<0.05). The difference between Mg²⁺ free with DSS and Mg²⁺ free without DSS is statistically insignificant (P-value of 0.928) although the rest of the data is significant.

Figure 3. Mg²⁺ Manipulation and UC Effect on Urine Concentration of Mg²⁺

Urinalysis is vital for observing the path of Mg²⁺. It shows what the body is discarding and how much is being absorbed. The control diets had a much larger concentration than the depleted, and the DSS-treated mice contained much less Mg²⁺ than the untreated.
The mass of the spleen was recorded after its removal, and is shown in figure 4. Spleen mass appears relatively constant between mice that had the same DSS-treatment. The Mg$^{2+}$-control diet with no DSS-treatment reported an average spleen mass of 0.056 g, and the Mg$^{2+}$-depleted diet was 0.054 g. The Mg$^{2+}$-control diet with the DSS-treatment was significantly higher, at 0.105 g than the depleted, at 0.106 g (ANOVA followed by Fisher’s LSD, P<0.05). The difference between DSS untreated groups is not statistically significant (P= 0.818). Differences between Mg-free DSS and Mg-control DSS were equally insignificant (P= 0.380).

Following removal of the spleen, the entire large intestine was dissected from each subject. Their lengths are shown in figure 5.
There appears to be a relationship between induced UC and colon length. Mice that were subjected to depleted dietary magnesium and DSS treatment had a colon shortened to a length of 43 mm. When not subjected to the DSS treatment, the average Mg\(^{2+}\)-depleted diet mouse had a length of 50 mm. The UC seemed to have little effect on the Mg\(^{2+}\)-control diet group, with the DSS-untreated having a length of 52 mm and the DSS-treated 49 mm. There is no significant difference between the Mg\(^{2+}\)-control diet with or without DSS and the Mg\(^{2+}\)-depleted diet without DSS. This difference is most notable from the Mg\(^{2+}\)-depleted DSS-treated group compared to all other groups (ANOVA followed by Fisher’s LSD, P<0.05).

Bacteria was cultured from fecal material to analyze the microbiome of the gut. It was plated on various media to determine amount and composition. This is shown in figure 6.
Fecal samples were collected and grown on different types of media for analysis. BHI (A) is nonselective and nourishing, and allows for all culturable bacteria to grow. Groups that received no DSS treatment had about the same amount growth. The DSS treated control diet group showed a jump in the amount of growth, but the magnesium depleted with DSS had more than 10 times that of any other group. MRS (B) selects for lactobacilli, which seems to be affected more by the lack of magnesium than the BHI. Both control diets were about the same, while there was a slight increase in growth (1.5 times more) in the depleted and untreated group. Depleted diet and DSS treatment showed a huge amount of growth at around $5.18 \times 10^8$ colony forming units (CFUs). EMB (C) selects for gram negative bacteria. This plate follows a similar trend to the BHI plate; the control and depleted diets without treatment have very low CFUs, the control diet with the treatment has 1000 times more, and the depleted and treated has about $4.58 \times 10^8$ CFUs (10 times that of the control diet with treatment). The *Bifidobacterium* (D) plate is self-explanatory. The only group that showed significant growth is the depleted and treated, at $1.99 \times 10^8$. The rest showed no significant growth.
Bacterial analysis of the fecal material sample shows that the group with depleted magnesium and induced with UC contained the greatest amount of varying types of bacteria. The BHI agar is nonselective and permits growth of most bacteria; it is used primarily to compare total amounts. The highest in this media were the ones treated with DSS: the magnesium depleted had a total of 5.18E+08 CFUs, while the Mg^{2+}-control diet had 3.88E+07 CFUs. DSS-untreated groups had about the same values, as the Mg^{2+}-control diet had 1.10E+07 and the Mg^{2+}-depleted had 1.35E+07. The MRS agar selects for lactobacilli, and the highest growth on this plate was from the magnesium depleted with UC treatment with 4.13E+08 CFUs. The next highest was the Mg^{2+}-control diet with treatment at 1.24E+07, followed by Mg^{2+}-control diet DSS-untreated at 8.47E+06, and lastly magnesium free DSS-untreated, at 6.99E+06. The EMB agar is for gram negative bacteria, and does not permit the growth of gram positive. Again, this plate really depended on the UC treatment. Mg^{2+}-depleted without the DSS treatment had 4.58E+08 CFUs, while the control diet with treatment had 1.4E+07 CFUs. The untreated groups are much lower than these values: the Mg^{2+}-depleted diet had 1.3E+04 CFUs, while the control diet had no growth whatsoever. Finally, the *Bifidobacterium* plate selects only *Bifidobacterium*. The magnesium control without DSS treatment had the lowest value, at 6.94E+04. Next was the magnesium depleted without DSS treatment at 3.03E+05 CFUs and the magnesium control with UC treatment at 2.02E+06. The highest was the Mg^{2+}-depleted diet with DSS treatment, which had 1.99E+08 CFUs.

Spleen homogenate was also plated to observe UC disease progression throughout the body. Figure 7 shows the amount of colony forming units from each of the media used.
Figure 7. Effect of UC and Mg on Spleen Bacteria

Bacterial cultures from spleen homogenate were grown to see the effect of UC and Mg on bacteria in the blood. On the BHI agar, Mg-Ctrl with DSS (A) had the highest amount of growth, followed closely by Mg-free no DSS. In the MRS agar (B), Mg-free with DSS had the most growth, with the Mg-ctrl with DSS having about half as much CFUs. The EMB media (C) only displayed growth on the DSS treated plates, with Mg-ctrl having almost 10 times more colonies than Mg-depleted. The *Bifidobacterium* agar (D) had growth in three groups, starting with Mg-ctrl no DSS, followed by Mg-free with DSS at double the first, then Mg-ctrl with DSS doubling that.

Culturing the bacteria from the spleen shows what types and what amount made it into the blood stream. The BHI agar showed that the highest amount of general growth belonged to
the Mg-ctrl with DSS diet, at 7577.27 CFUs. This was followed by Mg-free without DSS (6063.64 CFUs), then Mg-free with DSS (3409.09 CFUs) and lastly Mg-free no DSS (1513.64 CFUs). MRS agar followed a trend similar to that of the fecal MRS plate. The untreated groups had the least amount of growth, with Mg-free displaying no colony forming units, and Mg-ctrl having 759.09 CFUs. Next was the Mg-ctrl with DSS treatment, at 5304.54, which was doubled by the Mg-free with DSS at 11818.18 CFUs. From there, the similarity to the fecal bacteria ends. It is important to note that for these plates the N=3. In the EMB agar, both DSS-untreated groups had no growth. The Mg-free with DSS had 6913.64 CFUs, and the Mg-ctrl had 6135.00 CFUs. The growth of *Bifidobacterium* was stimulated by the presence of magnesium. Mg-depleted without DSS had zero colonies, and the Mg-ctrl without DSS had 15136.36 CFUs. Mg-free with DSS had 30300.00 CFUs, which was nearly doubled by the Mg-ctrl with DSS at 62863.63 CFUs.

Fitc-Dextran was used to determine the permeability of the gut and how it is related to UC and magnesium intake. Resulting data is shown in Figure 8.

**Figure 8.** Effect of UC and Mg2+ Manipulation on the Gut Permeability

![An oral gavage of Fitc-Dextran followed by fluorescence of serum shows changing gut permeability. DSS treatment raises the gut permeability, and no effect can be attributed to Mg2+.](image)

When treated with DSS, the gut permeability increases radically. The control diet had an average Fitc concentration of 8.17 pmol/L, while the depleted diet had one of 8.19 pmol/L. No
treatment groups were very low, with the control at 2.14 pmol/L and the depleted at 1.57 pmol/L. None of these groups are statistically significant.

**Discussion**

The present study compares symptoms and features of DSS-induced UC after depleting magnesium intake. It finds that a diet devoid of magnesium causes significant physiological changes and that the content of both the gut and spleen microbiome is altered. After a week of receiving the UC-inducing treatment, mice on a diet of 0.003% magnesium experienced more severe symptoms than those on a 0.2% diet (figure 1). The Mg²⁺-depleted group scored on average 3.5/ 5 DAI, while the Mg²⁺-control diet group scored 2.5/5, showing significantly reduced symptoms. Both Mg²⁺-depleted and control diets experienced the same amount of symptoms without the DSS treatment, allowing the attribution of the symptoms to fall on the amount of magnesium. It has been found in previous studies that magnesium helps to restore mucosal integrity and channel expression, which is what must be occurring here (5). While the increasing intestinal bacterial content mirrors the worsening of symptoms in the DAI, the spleen microbiome points in a different direction.

DSS-treatment altered the amount of magnesium that was absorbed in the body and excreted from it. Mucosal damage from UC occurs in the colon, which is the final location where significant absorption of magnesium and other solutes takes place. Data should show that the serum magnesium is low in the Mg²⁺-depleted DSS-untreated group and even lower in those with the DSS-treatment. Instead, the DSS-treated Mg²⁺-depleted group showed 1.027 mmol/l of serum magnesium, while the DSS-untreated and Mg²⁺-depleted had 0.643 mmol/ of serum Mg²⁺ (Figure 2). Both of these values are below the normal range of 1.1-1.4 mmol. The increase in serum Mg²⁺ observed in the DSS-treated group might be explained by the Mg²⁺-being reabsorbed at the intestine from hemolyzed erythrocytes. Another possibility is that the average 24-hour water consumption in the DSS-treated group was 1.1 ml, which is not even half of the 2.4 ml averaged by the untreated group. Such a small intake would decrease blood volume, driving up the apparent magnesium concentration. This possibility could be tested in
the future by measuring the hematocrit of magnesium deprived mice with or without DSS treatment. The water consumption disparity is seen in the control diet as well, with the untreated drinking 2.8 ml and the DSS-treated drinking 2.0 ml. This could also explain the lack of difference between these two groups in regards to serum magnesium (untreated at 1.395 mmol and DSS-treated at 1.350 mmol).

The amount of magnesium in the urine shows the body’s attempt to harbor the ion, in order to keep levels as close to normal as possible. A more complete picture could be obtained through fecal analysis, but the presence of fecal occult blood would have completely skewed that data. Excretion can only be viewed in this instance by urinalysis. It is easier to see the retaining of magnesium in the control diets, because they absorbed more to begin with. The highest value of μmol excreted in 1 day was 41.36 by the Mg⁡²⁺-control DSS-untreated mice, followed by the control diet with the DSS-treatment at 18.65 μmol/day (figure 3). This shows that the excretion of the ion was limited after DSS-treatment, even in the control diet group. The depleted groups had very low amounts, from 1.37 μmol/day in the untreated and 0.970 μmol/day in the DSS-treated.

Physiological changes that are associated with this model of UC include colon shortening and the increased size of the spleen. Colon shortening occurs only in UC mice on a depleted diet. Spleen mass is different only across treatment groups; the mice receiving the DSS-treatment had a similar sized spleen to the others on the DSS-treatment, regardless of their diet. The same can be said for those not on the treatment (Figure 4 and 5). This data confirms results seen in previous research (5).

Induced UC on its own, but especially when paired with depleted magnesium conditions, increases the amount and the type of bacteria within the microbiome of the colon. The removal of magnesium, without UC, does not show a marked increase in the general bacterial amount. Introducing UC in the control diet, however, more than doubles growth. Removing magnesium in a DSS treatment group raises the number of colony forming units more than 10 times that of the control diet with treatment (Figure 6). This data correlates to
the DAI and the severity of UC symptoms: the higher the DAI score, the higher the amount of bacteria present in the intestines.

The combination of a lack in dietary magnesium with DSS-induced UC increases not only the amount of colony forming units in the colon, but also every type of bacteria tested for in this study: lactobacilli, *Bifidobacterium*, and gram-negative bacteria. This is something that has not been seen before in scientific journals. Previous research has shown that a decrease in magnesium decreases the presence of *Bifidobacterium* and lactobacilli, although there is no data on what happens in the presence of DSS-induced UC. It is known that the aforementioned lactobacilli enhances mucosal barrier performance, which would decrease the movement of bacteria into the bloodstream (2). It should be viable to observe such a movement through the microbiome of the spleen and the permeability of the gut.

UC inflames the colon, which decreases magnesium absorption and even further compromises the mucosal lining. This compromise would theoretically increase the gut permeability, allowing for the movement of intestinal bacteria into the bloodstream. Gut permeability analysis in this study found that there is no significant increase in permeability due to magnesium manipulation.

The presence of bacteria in the spleen would show the amount and type that survived transport there by macrophages. An increase in splenic bacteria in the Mg-free DSS-treated group would prove that bacteria has a large role in the infection of UC and IBDs. What is seen instead is data that is contradictory and paradoxical, and does not match that of the intestinal bacterial analysis. The BHI plate is the easiest explanation of such a paradox. The lowest amount of growth is seen in the Mg-ctrl DSS-untreated group, which is expected. Next highest is Mg-free DSS-treated, followed by Mg-free DSS-untreated. The greatest amount of bacterial colonies in this media is the Mg-ctrl with DSS. This data does not allow for any conclusions regarding magnesium or UC to be drawn from it. The control diet without UC had the lowest value, but the control diet with UC had the highest. If this were the only data, it would be possible to assume that bacterial growth depends more on UC. The depleted magnesium groups refute this possibility, since the untreated was higher than the treated. Except for the
MRS agar, the data from the spleen analysis does not match that of the intestine. The MRS shows that the presence of lactobacilli is encouraged mostly by the lack of magnesium and the presence of UC. Without the UC, however, the bacteria require magnesium to grow. One of the only viable extrapolations from the other plates is that the gram-negative and *Bifidobacterium* require magnesium or UC to grow, and the combination of both increases growth. The data gained from the spleen refutes the idea that bacteria has an impact on the symptoms of UC, as the groups that had the highest amount of bacteria did not have the worse symptoms.

This study attempted to find a link between the alteration of magnesium intake and presence of UC with changes in gut microbiota. There was a change observed in the intestinal flora as a direct result of this manipulation, as the increase in bacterial types (gram-negative, lactobacillus, and *Bifidobacterium*) mirrored that in groups of worsened UC symptoms. The presence of magnesium in subjects that were induced with UC expressed less intense symptoms related to the disease and had a decreased amount of bacteria in the colon. This means that bacteria interact with magnesium and that directly impacts the progression of IBDs.

Splenic bacterial analysis did not show any relation between magnesium and UC, nor did the gut permeability. This is due to the very few trials actually performed as the experimental process is very lengthy. Future research will try to expand on spleen analysis, to see the impact magnesium has on virulence and the ability to survive in macrophages. In patients with UC or other IBDs, magnesium supplements would help to return hypomagnesemia to normal levels, decrease severity of symptoms, and also lower colonic bacterial amounts closer to normal levels. Further research should also look at developing a specific magnesium supplement for patients with IBDs.
References


