Hypothesized mechanism of amelioration of colitis by *Clostridium butyricum* as a hydrogen-producing bacterium

Chronic inflammatory disease is an inflammatory bowel disease. Generally, the causes of chronic inflammatory bowel disease, including ulcerative colitis, are unknown.[1] Recently, however, it has become clear that inflammatory colitis is closely related to hydrogen.[2] The human intestine is an anaerobic environment with little or no oxygen from the large intestine to the cecum, although a small amount of oxygen leaks from the capillaries of the intestinal wall.[3-5] There are countless anaerobic bacteria, descendants of primitive life on the ocean floor, that metabolize hydrogen based on the food we ingest.[4] Among these anaerobic bacteria, hydrogen-producing bacteria possessing the enzyme hydrogenase produce more than 10 L of hydrogen gas per day from the human intestine.[6] Hydrogen is an inert substance that does not react with nucleic acids, proteins, or lipids that make up cells, but can only react with hydroxyl radicals to convert them into water molecules.[7] Since hydrogen is an inert substance and the product of the reaction with hydroxyl radicals is a water molecule, it is non-toxic to the cells that make up the organs and has no side effects like drugs.[7] Hydrogen produced by hydrogen-producing bacteria in the intestines is thought to inhibit cell damage caused by hydroxyl radicals by scavenging the hydroxyl radicals produced in the process of aerobic respiration in human cells through this mechanism.[8]

Hypothesis on the association between dietary changes and ulcerative colitis: One of the causes of ulcerative colitis has been attributed to dietary changes.[9] It has been proposed that the reason for the increasing number of patients with ulcerative colitis in Japan is the shift in the Japanese diet from plant-based foods to animal-based foods, mainly meat and dairy products.[10] Retrospective studies in Europe have cited excessive consumption of high-fat diets, including processed meats, fried foods, and snacks, as a risk for the development of inflammatory bowel disease.[11,12] In an epidemiologic analysis in Japan, the incidence of Crohn's disease, which is closely related to ulcerative colitis, was inversely associated with vegetable protein intake ($r = -0.941$, $P > 0.001$), and a multivariate analysis showed that increased animal protein intake was the strongest independent factor in the incidence of Crohn's disease.[13] Multivariate analysis has shown that increased animal protein intake is the strongest independent factor in the development of Crohn's disease.[13] And even after the onset of inflammatory bowel disease, a low-fat diet is important for improving inflammatory bowel disease, as it has been reported that inflammatory biomarkers are significantly improved when a low-fat diet is continuously eaten compared to a high-fat diet intake group.[14] In animal studies, it is known that diet composition modulates the barrier function of the intestinal mucosa in inflammatory bowel disease.[15,16] Thus, although a close relationship between dietary changes and inflammatory bowel disease has been demonstrated, the more detailed mechanisms are not well understood.[17,18] Therefore, we will discuss the mechanism by which the change in diet from phytophthora to animal foods has caused an increase in the number of patients with ulcerative colitis.

Possible improvement of colitis by hydrogen: Fiber intake promotes intestinal fermentation and gas production.[17] In fact, the most important gas among the various gases produced by intestinal fermentation is hydrogen, which has anti-inflammatory properties. Hydrogen-producing bacteria in the gut can generate hydrogen because they possess the enzyme hydrogenase.[8] Since hydrogenase produces molecular hydrogen through the reduction reaction of hydrogen ions, the enzymatic reaction of hydrogenase requires the supply of electrons to hydrogen ions. The electrons required for the hydrogen-producing reaction of hydrogenases are supplied by nicotinamide adenine dinucleotide. In the intestine, nicotinamide adenine dinucleotide is also produced when sugars produced by carbohydrate-digesting enzymes from dietary fiber ingested by humans and animals are converted to pyruvate in a glycolysis system that does not require oxygen, but also through metabolic pathways that utilize substances other than oxygen as electron acceptors.[19] Therefore, the generation of hydrogen by hydrogen-producing bacteria is promoted by the intake of plant foods such as grains and fruits, which contain more polysaccharides and sugars, including dietary fiber, than animal foods.[6]

This increase in intestinal hydrogen production may be the reason that plant-derived dietary fiber has been shown to suppress inflammation in ulcerative colitis. If the production of hydrogen by intestinal bacteria is promoted through the intake of plant foods, the amount of hydrogen produced in the intestine will also increase, promoting the elimination of hydroxyl radicals, which may prevent chronic inflammation including ulcerative colitis (**Figure 1**). It has been reported that people who eat more fruits and vegetables have a 7–8% lower risk of dying within 20 years than those who do not.[20] This is thought to be caused by the recent dietary shift from plant foods to animal foods, which has resulted in a decrease in the amount of hydrogen produced by hydrogen-producing bacteria in the intestines of modern people. This would also support the hypothesis that the same thing is responsible for the increase in chronic inflammatory diseases such as ulcerative colitis. Thus, in today's society, where diets have shifted from plant-based foods to animal-based foods, an active external intake of hydrogen will be important in preventing the ever-increasing number of chronic inflammatory diseases.

Possible improvement of colitis by *Clostridium butyricum* as a hydrogen-producing bacterium: There have been numerous reports on the improvement of ulcerative colitis by the ingestion of hydrogen in model animal experiments. Mice and rats treated with dextran sulfate sodium (DSS) are known as animal models of ulcerative colitis.[2] When DSS is administered to mice or rats, it binds to medium-chain fatty acids present in the colon, induces inflammation, and causes colitis, thus reproducing a mechanism similar to ulcerative colitis in humans DSS. According to a recent report, administration of hydrogen-rich saline solution to mouse models of ulcerative colitis treated with DSS is reported to improve DSS-induced ulcerative colitis and significantly suppress the anaerobic environment of the colon, strengthening the intestinal barrier by modulating mucosa-associated mucolytic bacteria.[2,21] *Bacteroides* and *Firmicutes* are predominant in a healthy colon, while *Enterobacteriaceae* are the predominant *Enterobacteriaceae* in an unhealthy gut. Hydrogen-producing bacteria metabolize hydrogen and produce energy for vital activities, so administration of hydrogen can increase their populations in the gut.[21-23] *Bacteroides* and *Firmicutes*, which possess hydrogenase genes, increase their populations and dominate by metabolizing the administered hydrogen, thereby inhibiting the

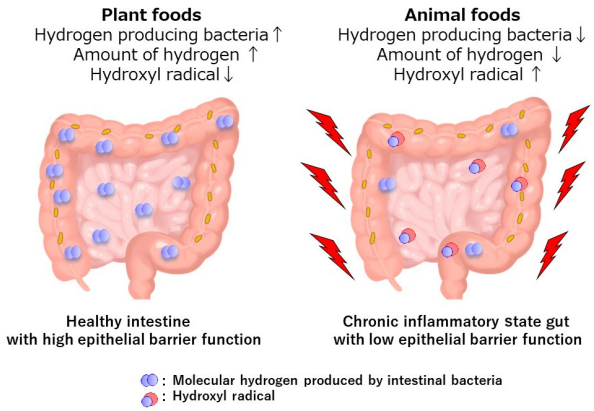

Figure 1: Intestinal environment of humans with plant foods and those with animal foods.

Note: A diet centered on plant foods increases the growth of hydrogen-producing bacteria in the intestines, which in turn increases the amount of hydrogen produced in the intestines. The generated hydrogen scavenges hydroxyl radicals in the cells that make up the intestine, inhibiting chronic inflammation of the intestine, which in turn keeps the barrier function of the intestinal epithelial cells high. On the other hand, diets centered on animal foods decrease the amount of hydrogen-producing bacteria in the intestines, and the amount of hydrogen generated in the intestines also decreases. As a result, hydroxyl radicals in the cells that make up the intestine increase and the intestine enters a state of chronic inflammation, resulting in a low barrier function of the intestinal epithelial cells. Created with Microsoft PowerPoint.

growth of *Enterobacteriaceae*.[8,24] Hydrogen-producing bacteria can not only consume hydrogen but also produce hydrogen, as their name implies, so an increase in the number of hydrogen-producing bacteria also results in an increase in the amount of hydrogen in the intestine, thus maintaining a healthy intestinal environment.[25]

Efforts are underway to use fecal transplantation, in which intestinal bacteria collected from the stools of healthy adults are transplanted into patients with ulcerative colitis as a method of treating inflammatory bowel disease such as ulcerative colitis.[26] A good balance of *Bacteroides* and *Firmicutes* composition is an indicator of an excellent microbial environment that healthy adults possess for fecal transplantation.[27] It has been reported that the hydrogen-producing bacteria *Bacteroides* and *Firmicutes* are less abundant in patients with ulcerative colitis, and that the diversity of *Bacteroides* is increased in healthy adults.[28,29] Although these reports do not mention that *Bacteroides* and *Firmicutes* are hydrogen-producing bacteria, they would support the importance of hydrogen-producing bacteria in the control and amelioration of inflammatory bowel disease. Perhaps increasing the number of hydrogen-producing bacteria, *Bacteroides* and *Firmicutes*, is important for the amelioration of ulcerative colitis.

The colon wall is composed of three layers: the mucus layer, the epithelium, and the intrinsic layer.[30,31] The gelatinous mucus layer provides protection against pathogen invasion.[30,31] *Clostridium butyricum*, better known as butyric acid bacteria, is also hydrogen-producing bacteria classified in the phylum *Firmicutes* with hydrogenase.[32] It has been shown that feeding *Clostridium butyricum* to mice increases the antioxidant capacity of their serum, preserves the properties of their mucus layer, and protects them from colitis; and the butyric acid produced by *Clostridium butyricum* is thought to contribute to this mechanism.[33] And it is known that butyrate levels in the stools of patients with ulcerative colitis are lower than in healthy individuals.[27,34-36] However, this is only the result of a reduction in butyric acid bacteria, and, we suspect that enhancement of the antioxidant capacity of the colon by *Clostridium butyricum* may actually be due more to the contribution of hydrogen produced by *Clostridium butyricum* than to butyric acid.

Despite the fact that the mechanism of maintenance of the intestinal environment by hydrogen is known from animal experiments, there are no reports on the improvement of ulcerative colitis through hydrogen inhalation in humans. The improvement of ulcerative colitis by hydrogen can easily be confirmed by observing the abdominal pain and stool condition in patients themselves, without relying on examination by a physician. Therefore, we decided to recruit two patients with ulcerative colitis and confirm whether inhalation of hydrogen gas would show improvement in the patients.

Case reports of improvement of ulcerative colitis by inhalation of hydrogen in humans:

Case 1: The patient was a Japanese male who was 49 years old when he started hydrogen inhalation.

He developed ulcerative colitis in 2004. Symptoms were bloody stools, abdominal pain, and insomnia associated with abdominal pain. He continued going to the hospital to receive medication, but there was no improvement. When abdominal pain prevented him from sleeping, he took a sleep aid.

On October 29, 2019, the patient began inhalation of hydrogen gas for 3 hours per day using a hydrogen gas inhaler (MiZ Company Limited, MHG-2000α, Kamakura, Kanagawa, Japan) with hydrogen concentration 6–7% by volume, converted to 100% hydrogen at 140 mL/min. Inflammation was suppressed and the patient was able to stop taking steroids. Blood stools were eliminated within 1 week of inhalation. Ten days later, the patient was unable to sleep due to abdominal pain, but the abdominal pain subsided and he was able to sleep without taking any sleep aids. Hospital tests showed that there were no problems and the ulcerative colitis had improved on December 4, 2019.

Case 2: The patient was a Japanese male, 60 years old, when he started hydrogen inhalation. He developed ulcerative colitis in January 2018. During defecation, there was a 50% chance of hemorrhage. Camera observation showed that the rectal area of the colon was covered with blood. Several polyps were found in the stomach. The patient was anemic and depressed due to bleeding. Body mass loss was observed. He was taking medications (Mesalazine) prescribed by his doctor but did not get any better.

On June 3, 2019, he started to use a hydrogen gas inhaler (MiZ Company Limited, MHG-2000α) with hydrogen concentration 6–7% by volume, converted to 100% hydrogen at 140 mL/min for 3 hours per day. During the 1st week of inhalation, there was no change in his bleeding, but he was able to sleep well. Because of the improved sleep, his energy returned and he felt rejuvenated. The amount of blood loss in his stools decreased and his health improved. The second week, lower bleeding was controlled and there was only a small amount of blood in the stools. The shape of stools also changed from liquid to solid and firm, and diarrhea was cured. The frequency of stools also returned to the state of before the onset of the disease. His mood also returned to the healthy state he was in before the onset of the disease. Two months later, bleeding was almost gone. Three months later, bleeding was gone and weight had increased to his pre-onset healthy state, so hydrogen gas inhalation was discontinued. There was no recurrence of ulcerative colitis after discontinuation of hydrogen gas inhalation. Camera observation also showed that the bloody and rough intestinal wall had healed nicely.

The study has obtained written informed consent from these patients.

Conclusion: If a deficiency of hydrogen produced by intestinal bacteria is the cause of ulcerative colitis, ulcerative colitis can be prevented and improved by actively taking in hydrogen from outside the body to better eliminate the hydroxyl radicals that are generated inside mitochondria in cells throughout the body. Regardless of the disease, the amount of hydrogen ingested by the patient is important in the improvement of diseases by hydrogen. There are rare reports that hydrogen does not improve disease, but this is because the amount of hydrogen ingested is too small.[37] One way to ingest hydrogen is to drink hydrogen water, but there is a limit to the amount that can be consumed. Therefore, prolonged inhalation of hydrogen gas is the best way to take in more hydrogen.[7]

In the two cases we were able to confirm in this study, improvement was observed with inhalation of hydrogen gas for 3 to 4 hours per day. This is the first report of improvement of ulcerative colitis by inhalation of hydrogen gas in humans. The only treatment available for ulcerative colitis in Western medicine is drug therapy. For example, steroids are often used, but steroids cause serious side effects such as adrenocortical insufficiency.[38] Also, transplanting hydrogen-producing bacteria through fecal transplantation may be effective, but some people may resist the idea of having someone else's feces transplanted into their body from a hygienic standpoint. However, since hydrogen is a substance with no side effects, it can be inhaled for a long period of time, and the intestinal microflora can be improved naturally. When clinical trials on hydrogen inhalation for ulcerative colitis are conducted in the future, it is expected that longer inhalation will lead to recovery from ulcerative colitis.

We would like to thank Ms. Yasuko Ginjo (Registered dietitian of Japan), Registered Dietitian, for her useful insights into the history of Japanese food culture. And we would like to thank Ms. Yoko Satoh (MiZ Company Limited) for her excellent advice upon writing this manuscript.

Yusuke Ichiakwa*, Bunpei Sato, Shin-ichi Hirano, Yoshiyasu Takefuji, Fumitake Satoh

Research and Development Department, MiZ Company Limited, Kamakura, Kanagawa, Japan (Ichiakwa Y, Sato B, Hirano S, Satoh F)
MiZ Inc., Balentine Drive, Newark, CA, USA (Ichiakwa Y, Sato B, Satoh F)
Faculty of Data Science, Musashino University, Tokyo, Japan; Keio University, Tokyo, Japan (Takefuji Y)

***Correspondence to:** Yusuke Ichikawa, PhD, y_ichikawa@e-miz.co.jp.
orcid:0000-0002-2526-4681 (Yusuke Ichikawa);
0000-0002-8610-8922 (Shin-ichi Hirano);
0000-0002-1826-742X (Yoshiyasu Takefuji)
doi: 10.4103/2045-9912.390251
How to cite this article: Ichiakwa Y, Sato B, Hirano S, Takefuji Y, Satoh F. Hypothesized mechanism of amelioration of colitis by Clostridium butyricum as a hydrogen-producing bacterium. Med Gas Res 2024;14(3):136-139.

References

1. Campieri M, Gionchetti P. Bacteria as the cause of ulcerative colitis. *Gut*. 2001;48:132-135.
2. Ge L, Qi J, Shao B, et al. Microbial hydrogen economy alleviates colitis by reprogramming colonocyte metabolism and reinforcing intestinal barrier. *Gut Microbes*. 2022;14:2013764.
3. Tashiro T, Ishida A, Hori M, et al. Early trace of life from 3.95 Ga sedimentary rocks in Labrador, Canada. *Nature.* 2017;549:516-518.
4. Fukai Y. *Molecular hydrogen for medicine: the art of ancient life revived*. Springer. 2020.
5. Konjar Š, Pavšič M, Veldhoen M. Regulation of oxygen homeostasis at the intestinal epithelial barrier site. *Int J Mol Sci*. 2021;22:9170.
6. Levitt MD. Production and excretion of hydrogen gas in man. *N Engl J Med.* 1969;281:122-127.
7. Hirano SI, Ichikawa Y, Sato B, Satoh F, Takefuji Y. Hydrogen is promising for medical applications. *Clean Technol.* 2020;2:529-541.
8. Ichikawa Y, Yamamoto H, Hirano SI, Sato B, Takefuji Y, Satoh F. The overlooked benefits of hydrogen-producing bacteria. *Med Gas Res.* 2023;13:108-111.
9. Timmer A. Environmental influences on inflammatory bowel disease manifestations. Lessons from epidemiology. *Dig Dis.* 2003;21:91-104.
10. Watanabe T, Nagawa H. Epidemiology of ulcerative colitis--comparison between Japan and western countries. *Nihon Rinsho.* 2005;63:750-756.
11. Preda CM, Manuc T, Chifulescu A, et al. Diet as an environmental trigger in inflammatory bowel disease: a retrospective comparative study in two European cohorts. *Rev Esp Enferm Dig.* 2020;112:440-447.
12. Poullis A, Foster R, Shetty A, Fagerhol MK, Mendall MA. Bowel inflammation as measured by fecal calprotectin: a link between lifestyle factors and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2004;13:279-284.
13. Shoda R, Matsueda K, Yamato S, Umeda N. Epidemiologic analysis of Crohn disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn disease in Japan. *Am J Clin Nutr.* 1996;63:741-745.
14. Fritsch J, Garces L, Quintero MA, et al. Low-fat, high-fiber diet reduces markers of inflammation and dysbiosis and improves quality of life in patients with ulcerative colitis. *Clin Gastroenterol Hepatol*. 2021;19:1189-1199.e30.
15. Martinez-Medina M, Denizot J, Dreux N, et al. Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. *Gut.* 2014;63:116-124.
16. Stenman LK, Holma R, Eggert A, Korpela R. A novel mechanism for gut barrier dysfunction by dietary fat: epithelial disruption by hydrophobic bile acids. *Am J Physiol Gastrointest Liver Physiol.* 2013;304:G227-234.
17. Khalili H, Chan SSM, Lochhead P, Ananthakrishnan AN, Hart AR, Chan AT. The role of diet in the aetiopathogenesis of inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol*. 2018;15:525-535.
18. Davis SC, Yadav JS, Barrow SD, Robertson BK. Gut microbiome diversity influenced more by the Westernized dietary regime than the body mass index as assessed using effect size statistic. *Microbiologyopen.* 2017;6:e00476.
19. Kucera J, Lochman J, Bouchal P, et al. A model of aerobic and anaerobic metabolism of hydrogen in the extremophile acidithiobacillus ferrooxidans. *Front Microbiol.* 2020;11:610836.
20. Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D. Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the United States. *JAMA*. 2017;317:912-924.
21. He J, Xiong S, Zhang J, et al. Protective effects of hydrogen-rich saline on ulcerative colitis rat model. *J Surg Res*. 2013;185:174-181.
22. Hopkins MJ, Macfarlane GT. Changes in predominant bacterial populations in human faeces with age and with Clostridium difficile infection. *J Med Microbiol.* 2002;51:448-454.
23. Wurm P, Spindelboeck W, Krause R, et al. Antibiotic-associated apoptotic enterocolitis in the absence of a defined pathogen: the role of intestinal microbiota depletion. *Crit Care Med.* 2017;45:e600-e606.
24. Ikeda M, Shimizu K, Ogura H, et al. Hydrogen-rich saline regulates intestinal barrier dysfunction, dysbiosis, and bacterial translocation in a murine model of sepsis. *Shock.* 2018;50:640-647.

25. Suzuki A, Ito M, Hamaguchi T, et al. Quantification of hydrogen production by intestinal bacteria that are specifically dysregulated in Parkinson's disease. *PLoS One.* 2018;13:e0208313.
26. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology.* 2015;149:102-109.e106.
27. Barnes D, Ng K, Smits S, Sonnenburg J, Kassam Z, Park KT. Competitively selected donor fecal microbiota transplantation: butyrate concentration and diversity as measures of donor quality. *J Pediatr Gastroenterol Nutr.* 2018;67:185-187.
28. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients.* 2012;4:1095-1119.
29. Antharam VC, Li EC, Ishmael A, et al. Intestinal dysbiosis and depletion of butyrogenic bacteria in Clostridium difficile infection and nosocomial diarrhea. *J Clin Microbiol.* 2013;51:2884-2892.
30. Ahmad R, Sorrell MF, Batra SK, Dhawan P, Singh AB. Gut permeability and mucosal inflammation: bad, good or context dependent. *Mucosal Immunol.* 2017;10:307-317.
31. Hunyady B, Mezey E, Palkovits M. Gastrointestinal immunology: cell types in the lamina propria--a morphological review. *Acta Physiol Hung*. 2000;87:305-328.
32. Long M, Yang S, Li P, et al. Combined use of C. butyricum Sx-01 and L. salivarius C-1-3 improves intestinal health and reduces the amount of lipids in serum via modulation of gut microbiota in mice. *Nutrients.* 2018;10:810.
33. Stoeva MK, Garcia-So J, Justice N, et al. Butyrate-producing human gut symbiont, Clostridium butyricum, and its role in health and disease. *Gut Microbes.* 2021;13:1-28.
34. Manichanh C, Rigottier-Gois L, Bonnaud E, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut*. 2006;55:205-211.
35. Fujimoto T, Imaeda H, Takahashi K, et al. Decreased abundance of Faecalibacterium prausnitzii in the gut microbiota of Crohn's disease. *J Gastroenterol Hepatol.* 2013;28:613-619.
36. Machiels K, Joossens M, Sabino J, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut.* 2014;63:1275-1283.
37. Ichikawa Y, Satoh B, Hirano SI, Kurokawa R, Takefuji Y, Satoh F. Proposal of next-generation medical care "Mega-hydrogen Therapy". *Med Gas Res*. 2020;10:140-141.
38. Hanai H, Iida T, Takeuchi K, et al. Intensive granulocyte and monocyte adsorption versus intravenous prednisolone in patients with severe ulcerative colitis: an unblinded randomised multi-centre controlled study. *Dig Liver Dis.* 2008;40:433-440.

Date of submission: January 12, 2023
Date of decision: February 21, 2023
Date of acceptance: October 12, 2023
Date of web publication: March 20, 2024