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Hydrogen-rich water as a modulator of gut microbiota?

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ABSTRACT

Hydrogen-rich water (HRW) is an innovative functional drink with many professed benefits for human health, including good intestinal viability and gut microbiota upregulation. A source of molecular hydrogen, HRW might be a convenient medium to deliver this bioactive gas to the gastrointestinal tract, and perhaps modulate the activity of both hydrogen-producing and hydrogen-consuming bacteria, abundant members of the intestinal microbiota community. This paper summarizes the findings from previous studies evaluating a response of gut microbiota to HRW intake and discusses possible mechanisms and medical consequences of this interaction. It appears that only a handful of rodent studies and one human randomized-controlled trial investigated how drinking HRW affects gut microbiota, with all studies published from 2018 onwards. HRW-induced protection of the gut barrier integrity and upregulation of butyrate-producing bacteria were seen in most studies, with HRW ameliorated clinical features of gut microbiota disturbances, including diarrhea rate, weight, and fluid loss. However, no well-powered multicentric trial evaluated the effectiveness of HRW consumption so far in common gastrointestinal diseases with gut flora scenario, including inflammatory bowel disease, irritable bowel syndrome, gastroenteritis and colitis of infectious origin. HRW might be an up-and-coming compound that might tune endogenous H_2 homeostasis and modulate gut microbiota but it should still be perceived as an experimental drink and not widely recommended to the general public.

1. Introduction

Hydrogen-rich water (HRW, or hydrogen-infused water) is an emerging functional drink with purported beneficial effects on human health. Over 150 studies with HRW were published in the past decade or so, with human trials reported in 2019–2020 alone have shown advantageous effects of consuming HRW in patients with non-alcoholic fatty liver disease (Korovljev, Stajer, Ostojic, LeBaron, & Ostojic. 2019), metabolic syndrome (LeBaron et al., 2020), in elite athletes to relieve psychometric fatigue (Mikami et al., 2019) and improve performance (Botek, Krejčí, McKune, and Sládečková, 2020), and healthy adults to reduce inflammatory responses and prevent apoptosis (Sim et al., 2020), to quote but a few recent reports. Although many contentious issues surround its medicinal properties (Ostojic, 2019), HRW is an apparent source of molecular hydrogen (H_2), a bioactive gas that is believed to act as a selective antioxidant, anti-inflammatory, antiapoptotic and signaling agent (for more details see Ohta, 2014). A unique molecular target for H_2 remains unknown yet few studies imply its possible role in the fine-tuning of homeostasis (Ishibashi, 2019; LeBaron, Kura, Kalocayova, Tribulova, & Slezak, 2019), perhaps similar to other naturally-occurring gases such as NO, H_2S and CO. Besides other plausible targets, exogenous H_2 delivered by HRW can have an effect on gut microbiota, a complex community of over 100 trillion microbial cells which influence human physiology, metabolism, nutrition and immune function (Guinane & Cotter, 2013). The fact that intestinal microbiota produces and utilizes endogenous hydrogen gas by itself (approximately 12 L of gaseous hydrogen per day) makes HRW performance in the human gut even more convoluted. To address this, I summarized findings from previous studies that evaluated a response of gut flora to HRW intake and discussed possible mechanisms and medical consequences of the interaction.

2. Research studies on HRW and gut microbiota

A handful of rodent studies and one human trial investigated how drinking HRW affects gut microbiota, with all studies appeared from 2018 onwards (Table 1). Arguably the first study, published in January 2018 by a Chinese research group, evaluated whether HRW administration affects radiation-induced small intestine toxicity in an animal model (Xiao et al., 2018). The authors reported that force-fed mice

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gavaged with HRW (H_2 0.80 mM) for 5 days experienced an amelioration of radiation-mediated gastrointestinal toxicity, illustrated by improved tract functions and epithelial integrity, stabilized small intestine MyD88 (myeloid differentiation primary response gene 88, an essential modulator of the innate immune response to gut pathogens), and counterbalanced a radiation-induced lower abundance of *Bacteroidia*, *Betaproteobacteria* and *Coriobacteria,* and a higher relative abundance of *Phycisphaerae*, *Planctomycetia* and *Sphingobacteria* spp. A few months later, Japanese authors have explored the effects of HRW on the intestinal environment, including microbial composition and short-chain fatty acid (SCFA) contents (Higashimura et al., 2018). Six-week-old mice were administered HRW (H_2 0.32 mM) or normal water (H_2 0.00 mM) for 4 weeks in *ad libitum* drinking protocol. At follow-up, HRW-treated animals experienced a significantly increased weight of cecal contents (a marker of intestinal fermentation) as compared to the control group. The animals treated with HRW also produced significantly more certain SCFAs, including propionic acid, isobutyric acid, and isovaleric acid, and exhibited a distinct microbiota composition that clustered separately from that of the control mice. For instance, drinking HRW favored a lower relative abundance of *Bifidobacterium*, *Clostridiaceae*, Coprococcus, Ruminococcus, and *Sutterella*, and a higher abundance of Parabacteroides, *Rikenellaceae*, Butyricimonas, Prevotella, and Candidatus arthromitus, as evaluated by fecal samples 16S rRNA sequencing. Ikeda and co-workers (2018) investigated the impact of HRW therapy as a countermeasure against bacterial translocation in a murine model of sepsis. Either 15 mL/kg of normal saline or super-saturated hydrogen-rich saline (3.5 mM) were gavaged daily for 7 days following cecal ligation and puncture, and hydrogen intervention prevented the expansion of *Enterobacteriaceae* and *Lachnospiraceae,* and ameliorated intestinal hyper-permeability after a ligation. Another trial (Zheng et al., 2018) explored the intestinal microbiota response to 25-day oral administrations of HRW (10 mL/kg body weight; H_2 0.6 mM) and lactulose (a synthetic non-absorbable sugar) in female piglets fed a *Fusarium* mycotoxin-contaminated maize. HRW treatment provoked increased H_2 concentrations in the mucosa of the stomach and duodenum, and decreased the diarrhea rate in *Fusarium* mycotoxin-fed piglets. This was accompanied by higher levels of colon butyrate, and higher levels of acetate, butyrate, and total SCFAs in the caecum of animals treated with HRW. The populations of selected bacteria in different intestinal segments were also affected by HRW treatment, with the abundance of Escherichia coli was lower and Bifidobacterium abundance higher in HRW group in the ileum, as compared to the group that received Fusarium mycotoxin-contaminated diet. In the colon, the abundance of methanogenic Archaea and sulfate-reducing bacteria was higher in HRW versus a contaminated diet. A succeeding study by the same group (Ji, Zhang, Zheng, Yao, 2019) essentially confirmed above findings, with 25-day oral administration of HRW (10 mL/kg body weight; H_2 0.6–0.8 mM) found to remarkably provide beneficial effects against *Fusarium* mycotoxin-induced apoptosis and intestinal leaking of the small intestine in piglets, yet no detailed gut microbiota profiles were outlined. Bordoni and co-workers (2019) have recently explored the effects of HRW on gut permeability and fecal microbiota in a rat model of Parkinson's disease induced by permethrin pesticide. In short, a 15-day treatment with HRW (10 mL/kg body weight; H_2 0.4–0.9 mM) improved intestinal barrier integrity corrupted by permethrin, preserved levels of occludin (a biomarker of tight junction integrity) in the ileum, increased the levels of butyric acid in the feces, and preserved the abundance of Lachnospira and Defluviitaleaceae while inducing a higher abundance of butyrate-producing bacteria (*e.g.*, Blautia, Lachnospiraceae, Ruminococcaceae, Papillibacter). The beneficial effects of HRW consumption on gut microbiota were corroborated in a recent first-in-human trial (Sha et al., 2019). Thirty-eight juvenile female football players were subjected to a 2-month HRW drinking protocol (1.5–2.0 L/day) using a randomized-controlled design. On top of other findings, the

Table 1
The summary of studies evaluating the link between hydrogen-rich water (HRW) and gut microbiota.

Ref.	Species	*n*	Model	HRW	Control	Study length	Outcomes in HRW
Xiao et al. 2018	Mouse	12	Radiation-induced intestinal toxicity	0.80 mM	Normal water	5 days	↑ epithelial integrity ↓ miR-1968-5p level ∅ abundance of enteric bacteria
Higashimura et al. 2018	Mouse	16	Intestinal environment	0.32 mM	Normal water	4 weeks	↓ serum LDL-C and ALT ↑ propionic, isobutyric, and isovaleric acids ↑ relative abundance of 20 taxa
Ikeda et al. 2018	Mouse	36	Sepsis	3.5 mM	Normal saline	7 days	↑ survival rates ∅ bacterial translocation ∅ intestinal hyperpermeability ↓ intestinal morphologic damage ↓ MDA, TNF-α, IL-1β, IL-6
Zheng et al. 2018	Piglet	24	Mycotoxin-contaminated diet	0.6 mM	Lactulose	25 days	↓ diarrhea rate ↑ acetate, butyrate, total SCFAs ↑ relative abundance of specific taxa
Ji et al. 2019	Piglet	24	Mycotoxin-contaminated diet	0.6–0.8 mM	Hydrogen-free water	25 days	↓ apoptosis and intestinal leaking ∅ abnormal intestinal morphological changes ↑ distribution and expression of CLDN3
Bordoni et al., 2019	Rat	58	Parkinson's disease	0.4–0.9 mM	Permethrin Vehicle	15 days	↑ intestinal barrier integrity ↑ butyric acid ↑ higher abundance of butyrate-producing bacteria ∅ tight junction integrity ∅ abundance of *Lachnospira* and *Defluviitaleaceae*
Sha et al. 2019	Human	38	Exercise training	Unknown	Normal water	2 months	↑ blood hemoglobin, MDA, SOD, TAC ↑ diversity and abundance of specific taxa
Guo et al. 2020	Mouse	30	Intestinal environment	Unknown	Deionized water N2 nanobubble water	5 weeks	↑ species diversity of fecal microbiota ↓ abundance of *Mucispirillum* and *Helicobacter*

Abbreviations: ↑ ↓ ∅ denotes an increase, decrease or no change in a specific variable, respectively. LDL-C – low-density liporotein cholesterol; ALT – alanine tranferase; MDA – malondialdehyde; TNF-α – tumor necrosis factor alpha; IL-1β – interleukin 1 beta; Ili-6 – interleukin 6; SCFA – short-chain fatty acids; CLDN3 – claudin 3; SOD – superoxide dysmutase; TAC- total antioxidant capacity.

authors reported that HRW led to higher abundance and diversity of gut flora, an indicator of favorable microbial balance (Valdes, Walter, Segal, & Spector, 2018).

In summary, it appears that cited research studies typically employed a short- to medium-term duration of the intervention (*e.g.*, five days to eight weeks), and dispensed mostly a moderately saturated HRW ($H_2 < 1.0$ mM) yet in rather heterogenous drinking protocols and across various experimental conditions, which makes the comparison/interpretation of findings complicated. Nevertheless, HRW-driven protection of the gut barrier integrity and an upgrade of butyrate-producing bacteria were seen as pertinent in most studies, with both effects being segment-specific and occurring predominantly in the large intestine. HRW also ameliorated clinical features of gut microbiota disturbances, including diarrhea rate, weight and fluid loss, and kept the intestinal contents close to the normal state-containing stool. Still, no studies evaluated the effectiveness of HRW consumption to modulate intestinal microbiota in common gastrointestinal diseases with a gut flora scenario, including inflammatory bowel disease, irritable bowel syndrome, gastroenteritis and colitis of infectious origin. In addition, no longitudinal large-scale multicentric trials are available thus far, with only one human RCT in healthy girls. Even so, a fact that all studies are published in the past two years indicates that the HRW-gut microbiota case becomes a hot research topic in biomedicine.

3. Possible mechanisms of HRW action

HRW could modulate gut flora by assorted means (Fig. 1). The primary effect of drinking HRW is likely driven by delivering extra H_2 to the metabolite milieu of the gut, a dynamic environment already rich in this simple gas. Normally, intestinal H_2 is produced continuously by several classes of hydrogen-releasing bacteria (*e.g.*, *Firmicutes* and *Bacteroidetes* phyla), with a daily yield of approximately 13 L of hydrogen gas (Hylemon, Harris, & Ridlon, 2018). On the other side, cross-feeding microbes or hydrogenotrophs (*e.g.*, methanogens, acetogens, sulfate-reducing bacteria) sustain to utilize intestinal H_2 for its growth and metabolism (Smith, Shorten, Altermann, Roy, & McNabb, 2019), with H_2-consuming microbes usually located in the distal intestine (Rey et al., 2013). HRW may thereby provide a supplemental substrate for hydrogenotrophs, leading to a higher abundance of these members of gut flora, and possible increase of their metabolites in the gut (including CH_4, acetate, and H_2S). Zheng and co-workers (2018) confirm this speculation by finding an abundance of colonic methanogens and sulfate-reducing bacteria (*e.g.*, *Methanobrevibacter smithii,* Desulfovibrio spp.) after 25-day HRW intervention, accompanied by higher levels of acetate in the caecum of animals treated with HRW. This effect might be a duration-dependent since short-term HRW gavage (*e.g.*, 5 days) had no significant effect on enteric microbiota abundance, at least in total abdominal irradiation model (Xiao et al., 2018). Besides reinforcing hydrogenotrophs, a rise in hydrogen partial pressure after HRW consumption could dampen the redox potential in the intestinal lumen (Million & Raoult, 2018), favoring the growth of anaerobes and butyrate-producing bacteria. This has been proven in previous studies where HRW facilitated intestinal fermentation by increasing the relative abundance of various anaerobic phyla (e.g., Deferribacteres, *Bacteroidetes, Firmicutes*) (Higashimura et al., 2018; Bordoni et al., 2019). The above effects likely happen at once but could push cascade reactions of HRW-triggered microbiota to mass-produce various biologically active compounds (*e.g.*, propionic acid, butyric acid, acetate, H_2S) that can further modulate gut microbiota metabolism *per se*, perhaps as a secondary upshot of HRW. For instance, propionic acid and H_2S are known to have many physiological functions that might be relevant for both intestinal and systemic immunomodulation, gene expression and cell signaling (Al-Lahham, Peppelenbosch, Roelofsen, Vonk, & Venema 2010; Blachier et al., 2010). This indirect effect might be accompanied by another possible impact of HRW that occurs after a proportion of exogenous H_2 passes through the gut mucosa wall into the circulation and being transported to various organs; this by itself may produce effects relevant to the gut microbiota that are mediated by gastrin modulation (McCarty, 2015). Besides, hydrogen from HRW may also act as a signaling agent and alter gene expression of several gut-specific metabolic genes including proliferator-activated receptor-gamma coactivator-1alpha and fibroblast growth factor 21 (Kamimura, Ichimiya, Iuchi, & Ohta, 2016), and reactome pathways related to collagen biosynthesis and heat shock response (Nishiwaki et al., 2018). However,

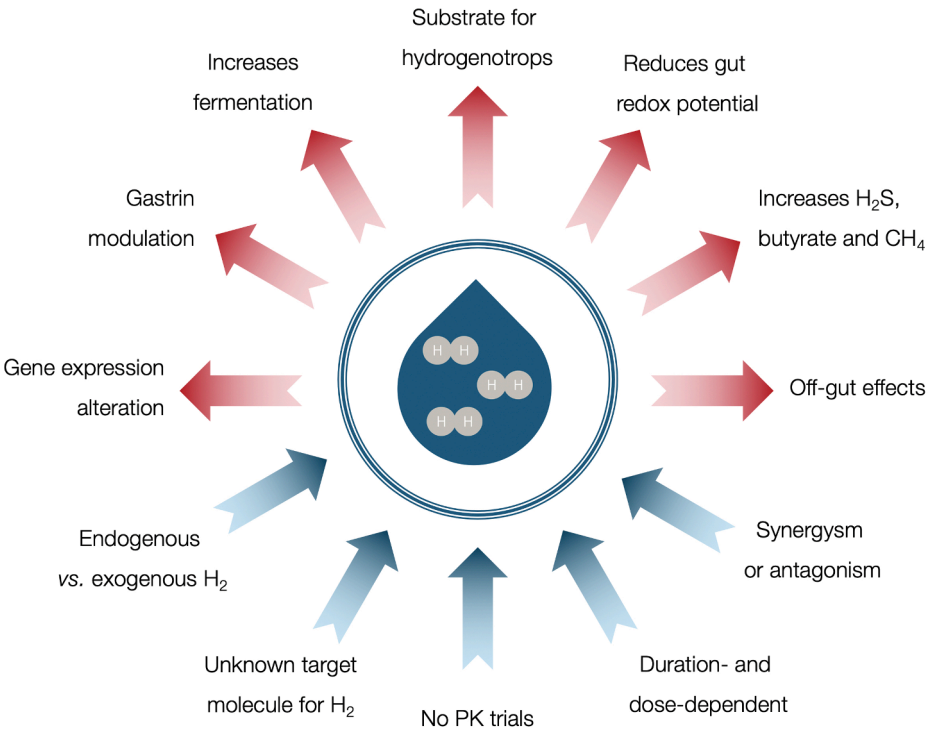

Fig. 1. Possible mechanisms (red arrows) and open questions (blue arrows) of hydrogen-rich water action on gut microbiota. PK – pharmacokinetics.

this is a fairly simplified overview of how HRW may alter intestinal flora while many questions remained unanswered, including a relative contribution of each possible mechanism to the net-effect of HRW. It remains particularly puzzling exactly how a relatively small quantity of exogenous H_2 from HRW drives notable changes in gut microbiota since only up to 0.04 L of hydrogen gas is supplied daily by drinking 2 L of HRW while at least 300 times more H_2 is produced endogenously. This could be due to a rather steep rise in gut H_2 levels that happens almost immediately after HRW consumption (Zheng et al., 2018), a pattern that might trigger acute mechanism(s) and/or cascade reactions described above (or another unknown pathway), while endogenous H_2 is released more gradually and perhaps being unable to elicit this response. A clinical biotransformation study to describe the disposition of H_2 after drinking isotopically labeled HRW is highly warranted to better understand the possible mechanism(s) and behavior of exogenous hydrogen within the human body.

4. Can HRW be used as a prebiotic?

Prebiotics are food substances that could instigate the growth and activity of healthy gut bacteria. A typical prebiotic is a non-digestible specialized plant fiber that purportedly enhances the fermentation in the colon by being a substrate for *Bifidobacteria* and *Lactobacillus,* protective endogenous enteric bacteria that may have favorable effects on the host digestion and immunity (for a review see Holscher, 2017). Although HRW has been found to modulate intestinal microbiota in the above pilot studies, it should not be categorized either as a prebiotic or probiotic (*e.g.*, live microorganisms claimed to improve or restore the gut flora) owing to the more complex and different behavior of H_2 in the gut. HRW could be rather named as '*hydrobiotic*', a unique compound that aims to compensate and stabilize H_2 levels in the gut. A disbalance in the intestinal cycling of hydrogen gas is recognized as a risk factor for several diseases, including irritable bowel syndrome, inflammatory bowel disease (IBD), obesity, and Parkinson's disease (Ostojic, 2018; Smith et al., 2019). For instance, a low abundance of hydrogen-producing bacteria has been demonstrated in patients with irritable bowel syndrome (Pozuelo et al., 2015), with an abundance of several bacterial taxa (*e.g.*, Bacteroides, Ruminoccocus, Prevotella) correlates negatively with the sensations of flatulence and abdominal pain. An apparent H_2 deficiency may thus advance HRW as an experimental therapeutics in those disorders. As a matter of fact, HRW was found to be protective against IBD in an animal model (Shen et al., 2017). Although gut microbiota profiles were not evaluated to confirm flora-specific modulation, 7-day HRW effectively alleviated the symptoms of food toxin-induced IBD (*e.g.*, change in weight, blood in the stool, and stool consistency), ameliorated diarrhea, macroscopic and microscopic damage of the colon, and protected colonic cells from oxidative stress and inflammation. Along with this, drinking ionized water (presumably rich in hydrogen) for eight weeks improved quality of life in patients with irritable bowel syndrome (Shin et al., 2018); no information has been provided does ionized water modulates gut microbiota although the authors suggested acceleration the growth of anaerobic bacteria (Lactobacilli and Bifidobacteria) after the intervention. Besides assumed beneficial effects on gut microbiota, drinking HRW could induce less favorable consequences. For instance, HRW encourages sulfate-reducing bacteria to produce hydrogen sulfide. Hydrogen sulfide (H_2S) is a biologically active gas that is normally produced in small amounts and has a number of signaling functions; if present in large amounts it may show pro-inflammatory properties on the colonic mucosa and negatively affect epithelial barrier in the colon (Blachier et al., 2010). Does HRW induce over-production of H_2S in the gut and how HRW-driven H_2S output alter intestinal ambiance remain currently unknown.

5. Conclusion

Preliminary findings suggest that HRW may positively affect the gut microbiota. However, this claim is still of very limited scope due to a small body of work done in this area, and many unresolved biomedical attributes of HRW consumption. The promising results from the pilot studies, however, justify further scientific endeavors in this direction. Further trials are highly warranted to detail mechanism(s) of HRW action, its pharmacokinetics and pharmacodynamics, and HRW medium- and long-term safety, while accounting for diverse microbial profiles among different individuals (both healthy populations and clinical patients), and HRW treatment dosages/protocols. HRW might be an up-and-coming functional drink that could finely tune endogenous H_2 homeostasis and adjust gut microbiota but it should still be perceived as an experimental drink and not widely recommended to the general public.

Ethical statement

This is a review paper, which doesn't include animal or human experiments.

Author contributions

SMO solely contributed to all aspects of this paper. The corresponding author had final responsibility for the decision to submit for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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