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Review

The Clinical Application of Hydrogen as a Medical Treatment

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In recent years, it has become evident that molecular hydrogen is a particularly effective treatment for various disease models such as ischemia-reperfusion injury; as a result, research on hydrogen has progressed rapidly. Hydrogen has been shown to be effective not only through intake as a gas, but also as a liquid medication taken orally, intravenously, or locally. Hydrogen's effectiveness is thus multifaceted. Herein we review the recent research on hydrogen-rich water, and we examine the possibilities for its clinical application. Now that hydrogen is in the limelight as a gaseous signaling molecule due to its potential ability to inhibit oxidative stress signaling, new research developments are highly anticipated.

Key words: hydrogen, antioxidant effect, medical gas, gaseous signaling molecule, clinical tests

In recent years, uses of hydrogen have been greatly anticipated as novel medical treatments [1]. Hydrogen has been applied in different forms to various disease models, and research on its curative effects has progressed at a rapid pace [2,3]. Animal research and clinical trials at research institutions all over the world have shown that hydrogen intake results in a reduction in oxidative stress [4–6]. In particular, Okayama University is one of the most active institutes conducting research on the clinical applications of hydrogen [7–9]. The current evidence leaves little room for doubt as to the benefits of hydrogen.

In this review, we summarize the results of research in the new field of "medical hydrogen," and we make general remarks on medical gases and gas molecules that are being investigated for their effects on living organisms. We hope to contribute to the understanding of hydrogen gas and hydrogen-rich water, as well as to the development of medical hydrogen.

The General Concept of Medical Gas

For many decades, the molecular mechanism of nitroglycerin used for the treatment of angina remained uncertain. In the 1980s, it became clear that the gas molecule nitric oxide (NO) is the true form of the vasodilatation factor in nitroglycerin that originates in the vascular endothelial cells as a result of the activation of guanylic acid cyclase, and it was demonstrated that it is NO that causes vasodilatation [10]. The 1998 Nobel Prize in Physiology and Medicine was awarded for the discovery that even in mammals, gas molecules mediate vasodilatation, neurotransmission, and immune reactions in much the same way as they do

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in plants [11,12]. This discovery greatly contributed to advances in biology and pharmacology.

Since then, research on medical gases progressed rapidly, and it became known that several gaseous molecules are permanently produced within the living body and that important physiological activations due to gaseous molecules thus occur [13]. Broken-down heme-producing carbon monoxide and broken-down methionine-producing hydrogen sulfide, both produced within the living body, have strong anti-oxidative effects and are considered extremely important neurotransmitters. These 3 gas molecules (*i.e.*, NO, carbon monoxide, and hydrogen sulfide) are referred to as gaseous signaling molecules within the body.

NO is already being used clinically to treat heart and lung diseases. Clinical studies have begun on carbon monoxide treatment in kidney transplant and severe respiratory failure patients, as well as hydrogen sulfide treatment for patients with kidney dysfunction and those who have undergone a coronary artery bypass. We are also paying close attention to hydrogen as a fourth molecule with similar features (Table 1).

The Chemistry of Hydrogen

Hydrogen is the lightest of all gas molecules. It has no color and no smell at room temperature. As a chemical element, it exists in abundance throughout space, but for the most part hydrogen gas does not exist on Earth as a simple substance, but rather as a compound substance. Hydrogen is quite flammable and dangerous when it is present with a specific catalyst or in the presence of heat. The tragedy of the Hindenburg disaster in 1937 was a result of hydrogen exploding within the blimp. Taking these factors into account, considering hydrogen as a medical treatment would seem unlikely; however, in the presence of less than 4%, hydrogen will not combust in the air and can be safely used if one simply manages the concentration of hydrogen. In addition, much like oxygen, hydrogen can be dissolved in approx. 0.8 mM of water at one atmospheric pressure.

The Role of Physiological Hydrogen

Hydrogen is produced regularly in the process of anaerobic fermentation in order for intestinal bacteria within the body to get energy [14]. The amount of hydrogen produced varies depending on the person and the time of the day, but it is reported to be somewhere between 150 mL and 12 L per day. When making a case for hydrogen, one cannot ignore the role played by intestinal bacteria. Kaijiya *et al.* demonstrated that when hepatitis is induced in mice with the use of drugs and their intestinal bacteria (which produce hydrogen) are eliminated with antibacterial medication, the drug-induced hepatitis is exacerbated [15]. These results show that the hydrogen that is physiologically produced within living organisms plays a very important role in the physiological maintenance of the living body.

Another interesting point is that the gas emitted within the intestines is deeply associated with bowel movements. It has been reported that in the case of

Table 1 Gaseous signaling molecules

	Nitric oxide	Carbon monoxide	Hydrogen sulfide	Hydrogen
Color, Smell	None, None	None, None	None, Irritant odor	None, None
Flammability	−	−	+	++
Toxicity	+	++	++	−
Production in the body	Through NOS, XO	Through HO	Through CBS, CSE	By intestinal bacteria
Vasodilatation	++	+	+	−
Anti-inflammatory effect	+	+	+	+
Anti-apoptotic effect	+	+	+	+

NOS, nitric oxide synthase; XO, xanthine oxidase; HO, heme oxygenase; CBS, cystathionine beta-synthase; CSE, cystathionine gamma-lyase.

irritable bowel syndrome (IBS), patients with intestinal bacteria that emit a high level of methane have remarkably few bowel movements, require longer times for waste to pass through the small intestine, and are more likely to become constipated than patients whose intestinal bacteria emit a very high level of hydrogen [16]. Similarly, it has been reported that when the production of hydrogen was suppressed by antibacterial medication in patients who suffered from diarrhea-type IBS with an overgrowth of hydrogen-producing bacteria, the irregular contractions of the digestive tract were prevented and the diarrhea symptoms became less intense [17]. Thus, the gas produced by the intestinal bacteria greatly contributes to the correct functioning of the intestinal tract, and the importance of intestinal bacterial flora has been reconfirmed.

The Medicinal Treatment Effects of Hydrogen and Its Mechanism

Hydrogen has been in the spotlight as a new antioxidant and has been reported to selectively eliminate the powerfully toxic reactive oxygen species (ROS) hydroxyl radical in culture [2]. While it is true that the stress reaction caused by ROS is suppressed by hydrogen treatment, it was recently confirmed that this phenomenon cannot be explained only by the theory that ROS, which have a strong degree of oxidizing power, directly react with hydrogen molecules, which eliminate ROS. For example, an increase in the level of the anti-oxidative enzyme superoxide dismutase (SOD) was reported in animal experiments with hydrogen intake, as well as in human clinical trials. Our group demonstrated that the strong anti-oxidative enzyme heme oxygenase is induced by hydrogen [18–20]. Moreover, it is now understood that anti-apoptotic molecules including B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL), which suppress cell death by apoptosis, are induced by hydrogen [18]. Hydrogen treatment was also associated with increased ATP levels in the rat heart with ischemia/reperfusion injury and with increased activity of enzymes in the mitochondrial respiratory chain including Complex II/III activity and Complex V activity [21]. Hydrogen has also been reported to restrain a type-I allergy reaction signal brought about by the IgE receptor that exists in fat molecules [16].

Thus, as the molecular system has gradually become clearer, the elucidation of various mechanisms has proceeded to where we are today (Fig. 1).

The Possibility of Hydrogen as a Medical Treatment

Hydrogen can be absorbed as a gas or as an aqueous solution through oral administration, intravenous injection, or as a topical application. Its effectiveness has been observed in various diseases and it is applicable to a number of internal organs.

Hydrogen inhalation. Hydrogen can be used at a safe density through a ventilation circuit. Since hydrogen concentration monitors can be purchased at a low price, administering and managing hydrogen is relatively easy. Ohsawa *et al.* reported a reduction of cerebral ischemia lesions due to vascular occlusion after administering a safe 2% concentration of hydrogen to rats [2]. They also reported a reduction of ischemic heart disease in rats that were administered 2%-density hydrogen for heart ischemia [22]. The efficacies of inhaled hydrogen were seen in the rats' ischemia/reperfusion (I/R) injury to the small intestine [19] and lung [18], as well as hemorrhagic shock [23], ventilator-induced lung injury [24], and hyperoxic lung injury [20].

The intravenous injection & local administration of hydrogen-rich water. It is possible to use isotonic solutions dissolved with hydrogen for an intravenous injection and/or local injection. Cai *et al.*

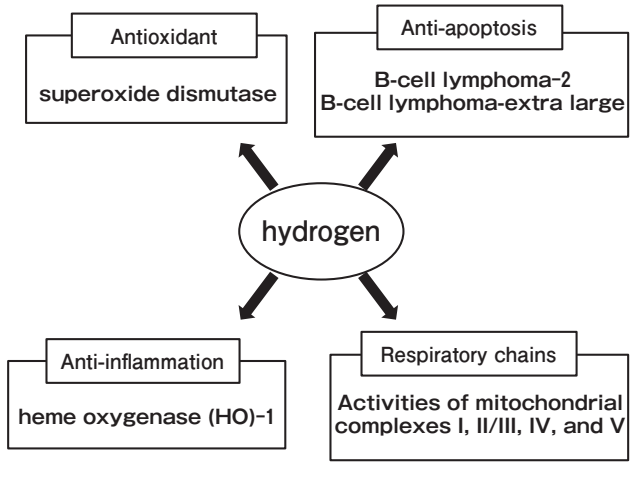

Fig. 1 Molecules that are upregulated by hydrogen treatment.

succeeded in curing cerebral ischemia in rats by administering a normal saline solution containing hydrogen into their abdominal cavity [25]. Oharazawa *et al.* induced ischemia in the retinas of rats by increasing the intraocular pressure and administering hydrogen eye drops, which were shown to protect the retinas [26,27]. The results of these experiments suggest the possibility of the future clinical application of hydrogen in forms such as eye drops and intravenous drips.

Hydrogen-rich drinking water. Hydrogen is also commonly taken orally as an aqueous solution. Different methods of making hydrogen-rich water include bubbling hydrogen gas and melting hydrogen gas, breaking it down electrically, and using magnesium to release hydrogen. Kajiya *et al.* [15] reported that symptoms of intestinal inflammation prominently improved in mice with inflammatory bowel disease given hydrogen-rich drinking water. Fujita *et al.* succeeded in reducing the symptoms of drug-induced Parkinson's disease by having mice drink hydrogen-rich water [28]. Using a kidney transplant mouse model, our group also reported that kidney graft functions were maintained and imperfect grafts caused by chronic rejection were prevented in mice that drank hydrogen-rich water every day [29]. Kawai *et al.* found that hydrogen-rich drinking water reduced hepatocarcinogenesis in a nonalcoholic steatohepatitis-hepatocellular carcinoma mouse model [7]. Hydrogen water is also effective in dentistry as shown by its effects on periodontitis, which is marked by gingival bleeding, the development of periodontal pockets, the destruction of connective tissue, and the loss of alveolar bone [8,9].

Hydrogen-rich organ perfusate or organ preservation solution. Improving organ preservation techniques to minimize I/R injury can potentially improve transplant outcomes by better preserving donor-organ quality and increasing the number of organs available for transplantation. Graft preloading with hydrogen showed remarkable morphologic and functional graft protection in rodent intestinal transplantation, ultimately promoting recipient survival. Antioxidant property and an upregulation of muscularis heme oxygenase-1 are possible protective mechanisms [6]. Noda *et al.* demonstrated that the incubation of a donor organ in a hydrogen-rich bath significantly prevented I/R injury in a rat heart transplant model [30].

Increase in hydrogen produced by intestinal bacteria. By obstructing the alpha-glucosidase of the small intestinal mucous membranes, the oral hypoglycemic agent acarbose (Glucobay®) delays the digestion and absorption of disaccharides and improves hyperglycemia after eating. However, because a great degree of hydrogen is emitted as a result of non-broken-down saccharides in the small intestine being carried to the large intestine and as a result of being broken down by the intestinal bacteria at the same time, side effects such as abdominal distension occur. Actually, the amount of hydrogen gas in the breath of patients using acarbose is significant. Acarbose was previously known to suppress cardiovascular events, but a hypothesis has been proposed: with the consumption of acarbose, hydrogen gas emitted within the intestines shows anti-oxidative properties and suppresses cardiovascular events. These results are all of great interest [31].

Orally administered lactulose can be bacterially fermented in the colon and induce dramatic amounts of endogenous hydrogen. Intragastrically administered lactulose can prevent the development of colitis and alleviate oxidative stress in the colon [32] and cerebral I/R injury, probably by increasing the endogenous hydrogen production that is attributed to hydrogen produced by the fermentation of lactulose through intestinal bacteria [33]. Interestingly, the oral intake of lactulose accelerates liver regeneration in rats and is associated with increases of regeneration markers such as proliferating cell nuclear antigen and cyclin D1 [34].

Results of Recent Clinical Research

In order to use hydrogen in clinical situations, it is essential to implement systematic clinical testing. These systems are either already in place or are under consideration at the leading Japanese universities and medical institutions. The results of several clinical studies of hydrogen-rich drinking water were recently released. Hydrogen-rich water is expected to be easily used in place of regular everyday drinking water and will effectively treat chronic maladies such as lifestyle-related diseases.

To the authors' knowledge, hydrogen inhalation has not yet been tested in humans; however, to prevent

decompression and nitrogen sickness, hydrogen is mixed into gas cylinders for deep-sea divers. The safety of the intake of hydrogen into the human body has already been confirmed.

Kajiyama *et al.* [35] reported the results of a randomized double-blind study of type 2 diabetes patients who drank 900 mL of hydrogen-rich water every day for 2 months. The study found that their abnormal glucose tolerance improved. In our study of a group of 20 men and women with preliminary metabolic syndrome symptoms (such as light obesity, dyslipidemia, abnormal glucose tolerance, and high blood pressure) who continuously drank 1,500–2,000 mL of hydrogen-rich water every day for 8 weeks, we observed an increase in the antioxidant enzymes SOD and HDL cholesterol [36]. Additionally, through our joint research with a South Korean group, we found that hydrogen-rich water significantly alleviated the side effects of radiation, such as fatigue and digestive symptoms, in 25 patients undergoing radiation therapy for malignant tumors of the liver who were given hydrogen-rich water or a placebo every day [37].

Although the mechanism by which hydrogen is effective against radiation is still unclear, the fact that the oxidative stress marker within the blood was significantly reduced at the very least leaves no doubt that one type of oxidative stress that is induced by radiation is reduced by drinking hydrogen-rich water. NASA (the U.S. National Aeronautics and Space Administration) recognized the results of this study and began researching the possible application of hydrogen to protect astronauts from space radiation [38]. The above findings show that the status of hydrogen in various fields is gradually increasing, and one can very much feel the anticipation of the benefits of hydrogen in the future.

Aoki *et al.* determined that hydrogen-rich water is suitable hydration for athletes. Adequate pre-exercise hydration with hydrogen-rich water lessened blood lactate levels and ameliorated an exercise-induced decline of muscle function [39]. Yoritaka *et al.* performed a placebo-controlled, randomized, double-blind, parallel-group clinical pilot study to assess the efficacy of hydrogen water in Japanese Parkinson disease patients medicated with levodopa. The participants drank 1,000 mL of hydrogen-rich water or pseudo water every day for 48 weeks. The total Unified Parkinson's Disease Rating Scale (UPDRS) scores in the hydrogen-rich water group improved, whereas the UPDRS scores in the placebo group worsened [40]. In China, the oral intake of hydrogen-rich water by hepatitis B virus (HBV) patients was tested. After hydrogen water treatment (1,200–1,800 mL/day, twice daily, for 6 consecutive weeks), oxidative stress did not change in the routine-treatment group but markedly improved in the hydrogen-treatment group, and it was associated with reduced HBV DNA levels [41]. Thus, the results from a number of human trials indicate that drinking hydrogen-rich water is safe and well-tolerated and significantly improves various diseases.

Hydrogen is licensed as a food additive in Japan, and hydrogen-rich water is already being sold as a safe drinking product in Japan. Magnesium sticks and hydrogen-rich water made by electrolysis are also being tested for acute/sub-acute toxicity, mutagenicity, etc. with the goal of using them for medical treatments. The safety of these methods has been confirmed and reported [36,37].

Problems with Hydrogen Research

As the effects of hydrogen have become more and more evident from a scientific standpoint, hydrogen-rich water is readily available for purchase by consumers. Although sold as a health supplement, the scientific evidence of the effects of these hydrogen-rich water products is lacking, and products that may or may not have any beneficial properties are being sold. Because hydrogen-rich water became commercially available before it was scientifically explained and systematically analyzed, claims of its beneficial effects have become the subject of prejudiced scrutiny, as no evidence of its effects has been made available. Although the field of research on hydrogen has dramatically increased in recent years, expectations are high regarding research on the physiology of hydrogen and the biology of hydrogen gas molecules. Many questions remain, such as the effects of hydrogen-rich water on living organisms and the mechanism by which the hydrogen molecule works, in addition to the suppression of oxidative stress.

Future Developments

As noted in this review, the effects of hydrogen

have been examined in studies of not only animals but also humans, and this research has opened a new chapter for hydrogen grounded in science. To pave the way for future developments in the proper clinical applications of hydrogen, it is thus vital to obtain a thorough and clear understanding of the mechanisms of hydrogen, and to conduct more systematic clinical trials.

To date, hydrogen has been recognized for its effectiveness as an ROS-eliminating antioxidant; however, a great deal of observed phenomena cannot be explained (only) with eliminating ROS. In fact, a large amount of hydrogen is produced in the human digestive tract, an amount so large that it is not comparable to the amount obtained through hydrogen-rich water. Therefore, explaining the benefits of hydrogen in terms of density is extremely difficult.

In addition, the antioxidant effect of hydrogen is qualitatively less than 1/100th of that of vitamins C and E; thus, it is no longer reasonable to narrowly ascribe the benefits of hydrogen to only its antioxidant functions. On the other hand, we have no doubt about the results of research showing the effectiveness of hydrogen as a gas or an aqueous solution used to treat or prevent various diseases. Within living organisms, super-low-molecular-weight chemical compounds such as hydrogen easily diffuse within cells and may physically work more efficiently than other antioxidants already in existence. Further research is necessary regarding the movement and distribution of hydrogen within the body. As mentioned earlier, the history of medical hydrogen research is still young. Even from the perspective of "medical gase", in which gas molecules are known to have some sort of effects on living organisms, continued research on the molecular makeup of hydrogen as a signaling gas is considered vital.

Conclusion

We have made general remarks about the results and findings of the latest fundamental hydrogen-related basic science and clinical research. Though the possibilities of the clinical application of hydrogen gas are many, hydrogen is not yet approved as a medicine, and at present cannot be described as "effective" or "beneficial" under the Pharmaceutical Affairs Law in Japan.

However, today's scientific developments in hydrogen research are outstanding. It is absolutely necessary for both clinicians and researchers to deepen their understanding of this enticing gas molecule, to logically examine it from both clinical and fundamental perspectives so that it will be used properly, and to conduct systematic clinical experiments from the standpoint of preventive medicine. We have an earnest desire to see those involved in medicine take more of an interest in hydrogen and promote a correct understanding of this molecule and its future applications.

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