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The hydrogen molecule as antioxidant therapy: clinical application in hemodialysis and perspectives

Masaaki Nakayama[1,2*], Shigeru Kabayama[2,3] and Sadayoshi Ito[2,4]

Abstract

Increased oxidative stress and pro-inflammatory conditions, commonly present in chronic dialysis patients, are thought to be enhanced during hemodialysis (HD) and to be associated with the excess morbidity and mortality seen in these patients. The hydrogen molecule (H_2) has a unique biological capacity to act as an antioxidative and anti-inflammatory substance. In light of accumulating evidence from animal studies showing protective effects against organ damage during ischemia and inflammation, development of H_2 treatments for HD patients has become a challenging clinical goal.

An HD system utilizing a water electrolysis technique that renders large amounts of H_2-enriched water has been developed. During HD with an H_2-enriched solution (approximately 50 ppb H_2), markers of increased oxidative stress (such as interleukin-6, myeloperoxidase, methemoglobin, increased lymphocyte apoptosis, and high blood pressure) are suppressed. These findings indicate that the use of an H_2-enriched solution may prove to be a novel approach to ameliorate dialysis-related complications. This manuscript reviews the recent progress in H_2 research and the use of H_2 in HD patients, including a description of a water electrolysis technique that delivers large amounts of H_2-enriched water for use in clinical settings.

Keywords: Molecular hydrogen, Oxidative stress, Hemodialysis, Electrolyzed water

Background

Enhanced oxidative stress and pro-inflammatory conditions are common in chronic dialysis patients and are thought to be associated with the excess morbidity and mortality of these patients [1–3]. Given concurrent underlying clinical conditions, multiple factors play a role in the pathology involved, such as uremic solute accumulation, which enhances the oxidative response [4], including indoxylsulfate [5]; accumulation of advanced glycation end products [6], AOPP [7], methylglyoxal [8, 9], and trans-aconitate [10]; excessive spontaneous respiratory neutrophil apoptosis [11, 12], which causes the release of myeloperoxidase (MPO) into the blood [13]; disturbed antioxidative systems occurring during progressive uremia, including decreased production of hydrogen sulfide [14] and suppressed Nrf2 activation [15]; and activation of monocytes [16] with loss of antioxidative capacity [17] during the hemodialysis (HD) procedure. Therefore, the development of antioxidant therapies has been recognized as a high priority for dialysis patients. Currently available agents are limited to tocopherol [18–20] and *N*-acetylcysteine [7, 21], and evidence of their efficacy has not been established in the clinical setting.

Recently, it has been shown that the hydrogen molecule (H_2) has a unique biological capacity as an antioxidative and anti-inflammatory substance [22]. Evidence that H_2 administration ameliorates organ damage in various models of ischemia and inflammation has been accumulating [23]. For this reason, clinical applications of H_2 for pro-inflammatory disorders are under active investigation, particularly for use during HD therapy [24–29].

This manuscript reviews recent progress in H_2 research and details the applicability of a water electrolysis

* Correspondence: masanaka@fmu.ac.jp
[1]Department of Nephrology and Hypertension, Fukushima Medical University, Hikarigaoka 1, Fukushima City 960-1295, Japan
[2]United Centers for Advanced Research and Translational Medicine, Center for Advanced and Integrated Renal Science, Tohoku University, Sendai, Japan
Full list of author information is available at the end of the article

technique with the capacity for delivering large amounts of H_2-enriched water for the clinical HD setting.

The hydrogen gas molecule as a biological antioxidant

Biological effects of H_2 and primary mechanism of its action

In 2007, Ohsawa et al. [22] first reported that pretreatment with H_2 inhalation ameliorated brain lesions after cerebral infarction in rats. Thereafter, accumulating evidence from animal studies indicated a protective effect of H_2 pretreatment on the progression of organ damage in various types of disease models [30–69], such as ischemia-induced injury and dysfunction of the brain [22, 33, 39, 55, 59, 62–64], heart [34, 45, 59, 65], liver [30], retina [41], and kidney [59, 60]; stress-induced hippocampus dysfunction [38]; cisplatinum nephropathy [37, 44]; transplanted intestinal graft [31, 47]; corneal alkali burn [51]; ouabain-induced auditory neuropathy [66]; lung injury by oxygen toxicity [43, 46]; paraquat [52]; extensive burns [54]; chronic allograft nephropathy [48]; and radiation injuries in various organs [49, 50, 55, 68, 69]. In Parkinson's disease [36, 40] and Alzheimer's disease models [42], H_2 ameliorates neurodegenerative changes in the brain. H_2 suppresses development of hypertension in spontaneously hypertensive rats [67]. Furthermore, H_2 acts on metabolic pathways to suppress the development of atherosclerosis in apolipoprotein E knockout mice [32] and diabetes in db/db mice [53].

In previous studies, H_2 was administered by inhalation [22, 30, 31, 34, 37, 45, 46, 56, 62–64, 66] or dissolved in water [22, 32–44, 47–55, 57–61, 65, 67–69]. Irrespective of the administration route, H_2 pretreatment could suppress oxidation, inflammation, and apoptosis while enhancing antioxidant reactions in those models.

Thus far, the precise mechanisms of action involved in H_2 protection from organ injury remain unclear, but it is thought that the H_2 molecule, which freely diffuses into cells, can react with toxic radicals, such as excess superoxide anions (O_2^-) generated in mitochondrial respiratory complexes and hydroxyl radical [22, 23, 70], according to the following reactions:

$$O_2^- + H_2 \rightarrow H_2O_2 + e^-$$

$$\cdot OH + H_2 \rightarrow H_2O + \cdot H \circledR H_2O + H^+ + e$$

Suppression of oxidative stress by H_2 could lead to suppression of downstream signaling in pathways such as MAPK [48], MEK-1 [48], NFkB [71], caspase-3 [33, 39], and caspase-12 [33], which would decrease pro-inflammatory molecule production and apoptosis. This unique characteristic of H_2 is thought to protect cells and organs from free radical injury. Figure 1 summarizes the molecular effect of H_2 on oxidative stress, inflammation, and apoptosis.

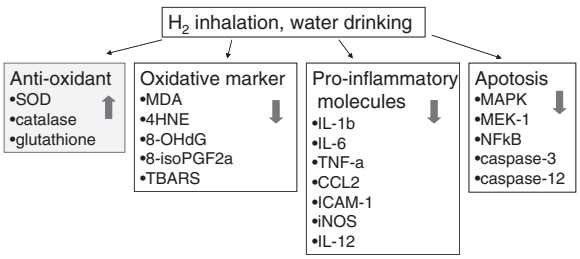

Fig. 1 Summary of the molecular effect of H_2 on oxidative stress, inflammation, and apoptosis. Effects of H_2 as an antioxidant and anti-inflammatory molecule are summarized. Accumulating evidence from animal studies has revealed that H_2 is a biologically active gas, increasing antioxidative molecules, while decreasing oxidative markers, pro-inflammatory molecules, and apoptosis in affected organs and tissues

Quantification of H_2 amount needed to elicit biological effects

By quantifying the amount of H_2 administered in animal experiments, it is possible to speculate on the H_2 dose needed for biological effects in vivo. From studies using H_2-enriched drinking water (0.3 to 0.6 mM H_2), an effective H_2 dose can be calculated roughly as the product of H_2 concentration and amount of daily water intake. Given a model using 200-g animals and 20 ml of enriched water intake daily, the H_2 ingested would be 3–6×10^{-5} mmol/g/day, which would be equivalent to 1.8–3.6 mmol/day for an average-weight (60 kg) human; therefore, this may be the dose needed to achieve biological effects in the clinical setting. Consistent with this speculation, it was reported that drinking 1.5 L of H_2-enriched water (approximately 0.6 mM) daily for 8 weeks (that is, 0.9 mmol of H_2 ingested daily) reduced urinary oxidative product (malondialdehyde) and increased antioxidant (superoxide dismutase) levels in subjects with metabolic syndrome [72]. Accordingly, it is thought that at least this dosage may be required to elicit any clinical effect in humans.

H_2 application for hemodialysis treatment

H_2-enriched water rendered by water electrolysis and biological effects

With the recent progress of H_2 science, therapeutic application of H_2 has become a clinical challenge [73, 74]. However, practical aspects of how to deliver H_2 in a safe and stable manner at the bedside have been a concern. In light of this, we have focused on a water electrolysis technique (Fig. 2).

Water electrolysis gives rise to H_2 enrichment near the electrolysis chamber cathode. The size of an H_2 bubble is thought to be less than 1 μm in diameter [75], and because of this extremely small size, the half-life of stable H_2 in water is approximately 12 h. The H_2 concentration in water depends on the intensity of the electrolysis; it is

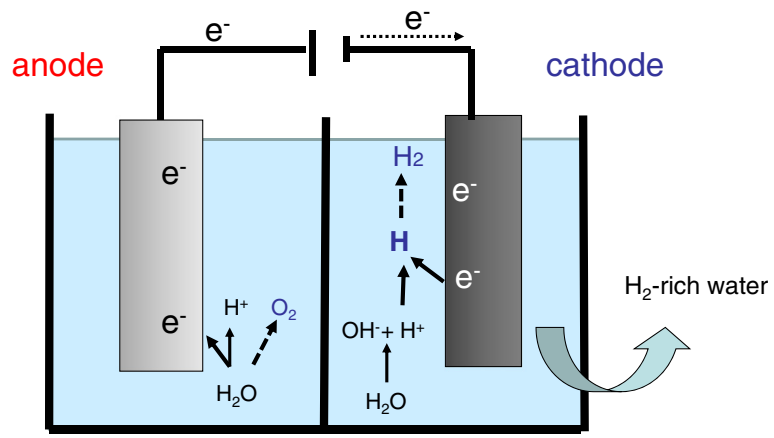

Fig. 2 Principle of water electrolysis and chemical properties of electrolyzed water at the cathode side. Water electrolysis supplies electrons to the cathode side. Electrons at the cathode surface react with hydrogen ions (H+) in water to produce theoretical intermediate hydrogen atoms and the final product, i.e., hydrogen molecules (H_2), by a chemical process. Chemical properties of electrolyzed water are characterized by alkalinity and the presence of dissolved hydrogen molecules (H_2)

possible to deliver water having 0.3–0.5 mg/L using presently available commercial electrolysis equipment [58, 59]. Shirahata et al. [76] demonstrated the unique chemical characteristics of electrolyzed water near the cathode, such as its antioxidant capacity. The hypoxanthine-xanthine oxidase system generates superoxide anions (O_2^-). In H_2-enriched electrolyzed water, concentrations of O_2^- and, furthermore, of hydrogen peroxide (H_2O_2) are lower when compared with control water. DNA breakage in a mixture of Cu(II) and ascorbic acid was suppressed by this water. Considered together, these results indicate that H_2-enriched water rendered by an electrolysis system could elicit chemical reactions in a similar way to the H_2 molecule, as described above.

Studies designed to test the biological activity of electrolyzed water have demonstrated tumor antiproliferative effects [77–79] and antidiabetic actions in a diabetic model by amelioration of beta cell oxidative injury [80–82]. Drinking enriched water ad libitum ameliorated disuse muscular atrophy after paralysis in rats [58] and protected against cardiac and kidney fibrosis by ischemic/reperfusion of kidney [59] and aging [83] in Dahl SS rats. Furthermore, chronic ad libitum drinking could reduce lipopolysaccharide-induced neuroinflammation by down-regulation of TNF-α and upregulation of IL-10 in the brain, to promote recovery from sickness behavior in mice [84].

Manufacture and delivery of H_2 dialysate using water electrolysis technique

The clinical application of electrolyzed water rendered at the cathode as HD therapy was reported in 2003 by Hung et al. in Taiwan [24]. The primary system employed in that report and our present study is shown in Fig. 3. In studies from the Taiwanese group, after 6 months of regular treatments with the HD solution manufactured in this manner, reductions in serum IL-6 and CRP levels were seen in 26 patients enrolled in the study [24]. Furthermore, reductions in levels of methemoglobin [25] and apoptotic lymphocytes [26] were reported in subsequent studies. These results indicated that clinical application of electrolyzed water could have some clinical impact. However, the precise nature of the involvement of H_2 in these studies has remained unclear, since the concept of H_2 therapy was not considered at that time.

This issue was clarified by our recent study [29]. The amount of H_2 obtained using this technique depends primarily on the intensity of water electrolysis; however, the maximum levels of H_2 are limited to approximately 200 ppb in the original system because of the increase in alkalinity with intensification of electrolysis. As shown in Fig. 3, H_2 levels after electrolysis exceeded 200 ppb, followed by a decline during reverse osmosis to 50 ppb in the final HD solution. Since the H_2 in the HD solution moves completely into blood through the dialyzer membrane, the delivered dose depends on the flow rate of the solution. Given that the blood and dialysis solution flow remain constant, e.g., 200 ml/min for the blood flow, and 500 ml/min for the solution flow, it would be possible to achieve a 1–3 mmol H_2 load in a single HD session.

Clinical experiences of electrolyzed HD and mechanistic hypothesis of clinical effects by H_2 delivery

Reported clinical signs and symptoms delivered by H_2-enriched HD solution and possible mechanistic role of H_2 delivery

Since the start of collaborative project over development of novel hemodialysis system between Nihon Trim Co., Ltd. and Tohoku University Graduate School of Medicine at 2007, case series to suggest clinical significance

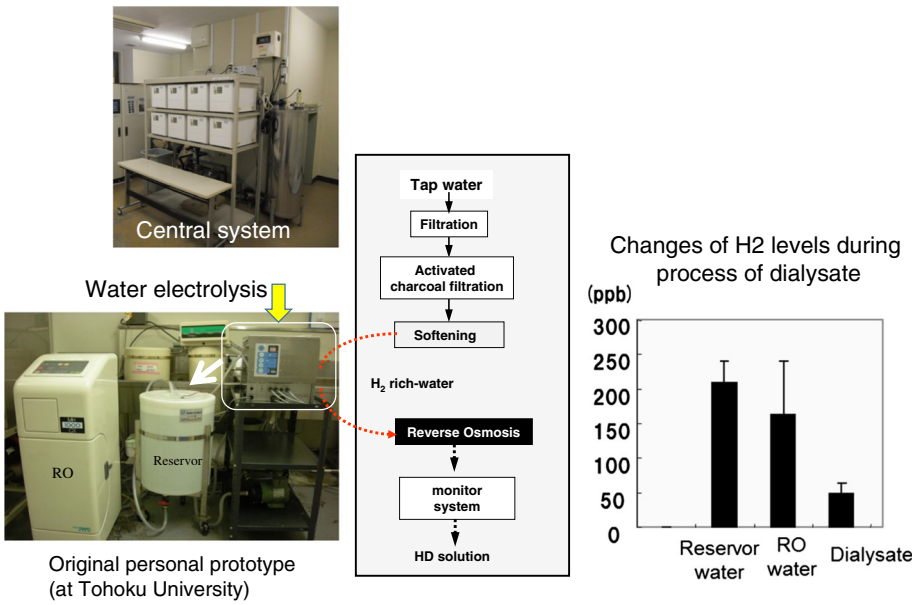

Fig. 3 Original manufacturing process of hemodialysis solution. Tap water is processed by filtration, activated charcoal filtration, and softening, followed by water electrolysis to deliver H_2-rich water for reverse osmosis. There is a progressive decline in H_2 levels by this process, and the final H_2 content in dialysate solution is approximately 50 ppb (*right figure*). Images show the original personal prototype of the electrolyzed HD system with a single water electrolyzer (*left below*) and the recent central system for multiple patients that incorporates multiple electrolyzers (*left upper*)

of this system have been accumulating. We and our collaborators have so far observed various clinical benefits in patients receiving this therapy [29, 85]. H_2-enriched HD solution corrected intra- and inter-dialytic high blood pressure (Fig. 4a–c), prevented blood flow reduction by function of dialysis, and increased skin temperature in cases with peripheral arteriosclerosis (Fig. 5a, b), enhanced wound healing in a case with ischemic lower limb lesion (Fig. 5c).

The role of H_2-enriched HD solution in these effects has remained speculative; however, it seems clear that the delivery of H_2 during the HD session is involved with the mechanism. There is a close relationship between endothelial dysfunction and oxidative stress. Generation of oxygen radicals in dialysis patients, e.g., iron infusion and uremic oxidants, such as indolyl sulfate, could disturb endothelium-dependent vascular relaxation [86, 87] and accelerate atherosclerosis by enhancing expression of cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) of endothelium [88]. During the process of NOS uncoupling, a characteristic feature of patients with chronic kidney disease, the superoxide anion reacts with nitric oxide to inactivate the bioactivity of NO and to generate peroxynitrite, a potent vasoconstrictive substance [89]. These processes could be involved in the pathological mechanism of increased blood pressure and decreased lower limb peripheral blood flow, which result in exaggeration of arteriosclerosis obliterans (ASO) and uncontrolled hypertension. Thus, we speculate that H_2 as antioxidant may have suppressed these pathological processes.

There is also a close relationship between enhanced oxidative stress/inflammation and clinical symptoms which associate with dialysis treatment. Those include dialysis hypotension, fatigue, and pruritus. Interestingly, we have observed substantial effects of H_2-enriched HD solution on ameliorating dialysis-related hypotension and subjective symptoms of dialysis-related fatigue and uremic pruritus.

Dialysis hypotension, which is defined as intra-dialytic hypotension, is a critical indicator of poor outcome. Frequent episodes of hypotension may induce a noxious inflammatory response mediated by the oxidative stress [90, 91]. It is supposed that abrupt fall in blood pressure accompanies systemic pathological condition which mimic acute ischemia reperfusion, inducing inflammatory type M1 macrophage activation [92]. Furthermore, elevated levels of serum IL-6 in patients with fatigue [93], and skin micro-inflammation in patients with uremic pruritus [94, 95], have been reported. Therefore, it is possible to speculate that H_2 may interact with the underlying pathology of enhanced oxidative stress or inflammation by inactivating oxygen radicals, leading to amelioration of clinical conditions.

Mechanistic hypothesis of clinical effects delivered by H_2-enriched HD solution

As already mentioned, the primary action involved in H_2 protection from organ injury is thought to quench toxic

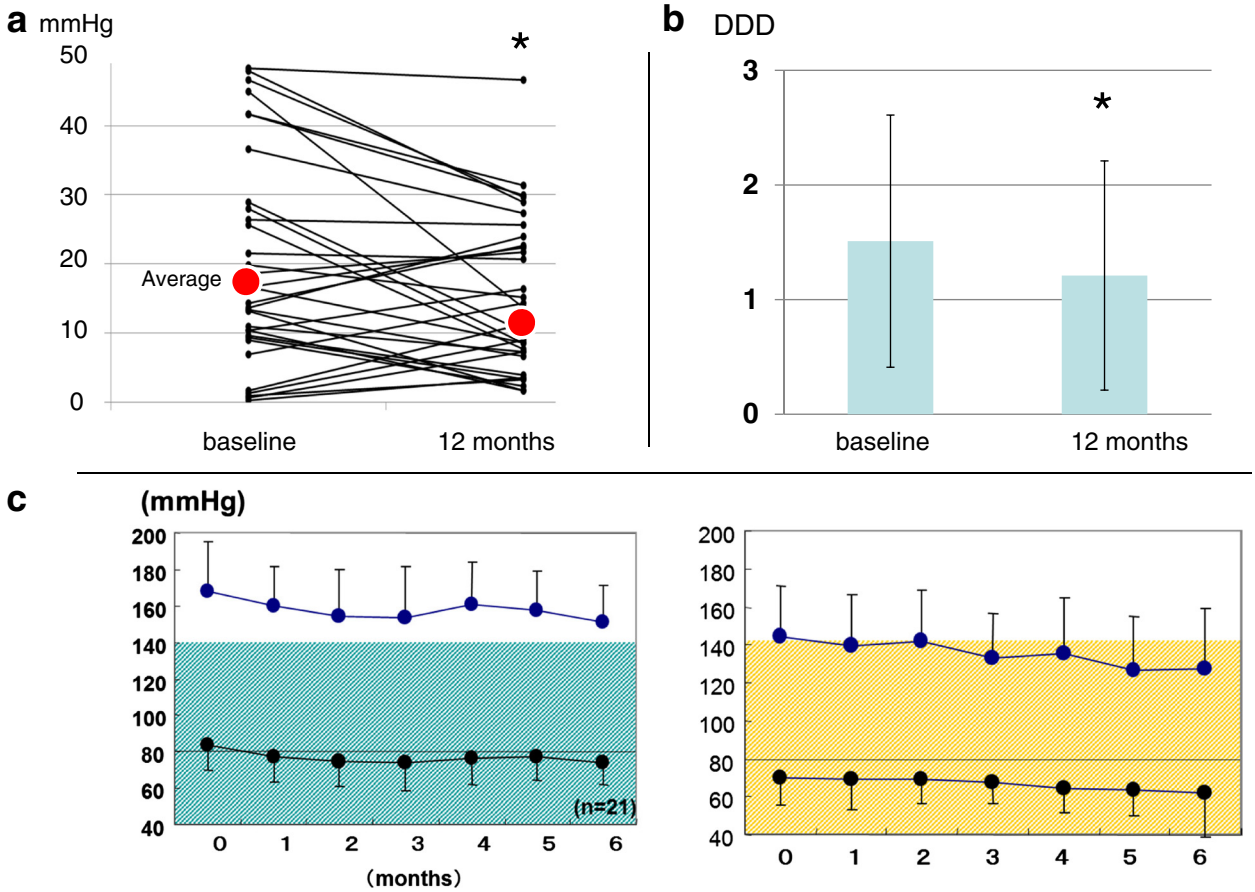

Fig. 4 Effect of H_2-enriched HD solution on blood pressure (BP) profiles. Changes in diurnal blood pressure differences (*ΔBP* BP at the start of dialysis BP in the evening) on hemodialysis day (**a**) and defined daily dose (*DDD*) of antihypertensive agents (**b**). There were significant reductions in the two parameters at 12 months, compared to baseline, following treatment with H_2-enriched HD solution (*$p < 0.05$) (courtesy of Dr. Hodaka Suzuki, Higashi-Horai Clinic, Fukushima). Changes in BP before (*left*) and after (*right*) regular HD sessions during the 6-month trial of H_2-enriched HD solution (**c**). There were significant reductions of BP, ($p < 0.05$, each) (modified from ref. [29])

radicals, such as excess superoxide anion and hydroxyl radical (Fig. 6). Nevertheless, the observed clinical effects were not confined to the time period of HD sessions of three times a week. Upon the fact that elevated H_2 level during HD returns to baseline as soon as the HD stops, it is therefore difficult to expect prolonged clinical effects from the chemical point of view of the H_2 molecule. Thus, we think additional mechanism(s) should be indicated in mediating the clinical effect of H_2-enriched solution.

The redox state is determined by the balance between the extent of oxidative stress and the activity of antioxidative mechanisms. This is crucially influenced during the course of an HD session, since there is an enhancement of free radical generation from polymorphonuclear cells during HD [16], which indicates excess apoptosis and disturbance of the physiological function of these cell populations. High plasma MPO, released from injured neutrophils, is an independent risk factor for patient survival [96]. In chronic kidney disease, monocyte heterogeneity is widely acknowledged, and a growing body of circumstantial evidence suggests that intermediate monocytes (CD14(++)CD16(+)) is predisposed to secrete pro-inflammatory cytokines [97] and that polarization of monocyte and macrophage is disturbed, e.g., polarization of becoming dominant macrophages is impaired; enhanced pro-inflammatory (M1); and impaired anti-inflammatory (M2) phenotypes, which corresponds to the progression of inflammation [98, 99].

Considering these facts, it is important to suppress excess immune cell injury within the dialyzer, while preserving normal cellular functions of these circulating cells, which exaggerate the inflammation-prone pathological process of disorders such as atherosclerotic and ischemic lesions of the vasculature.

H_2-enriched solution could benefit patients on HD in this regard. There is an increase of reduced/oxidized albumin ratio by single HD session using H_2-enriched

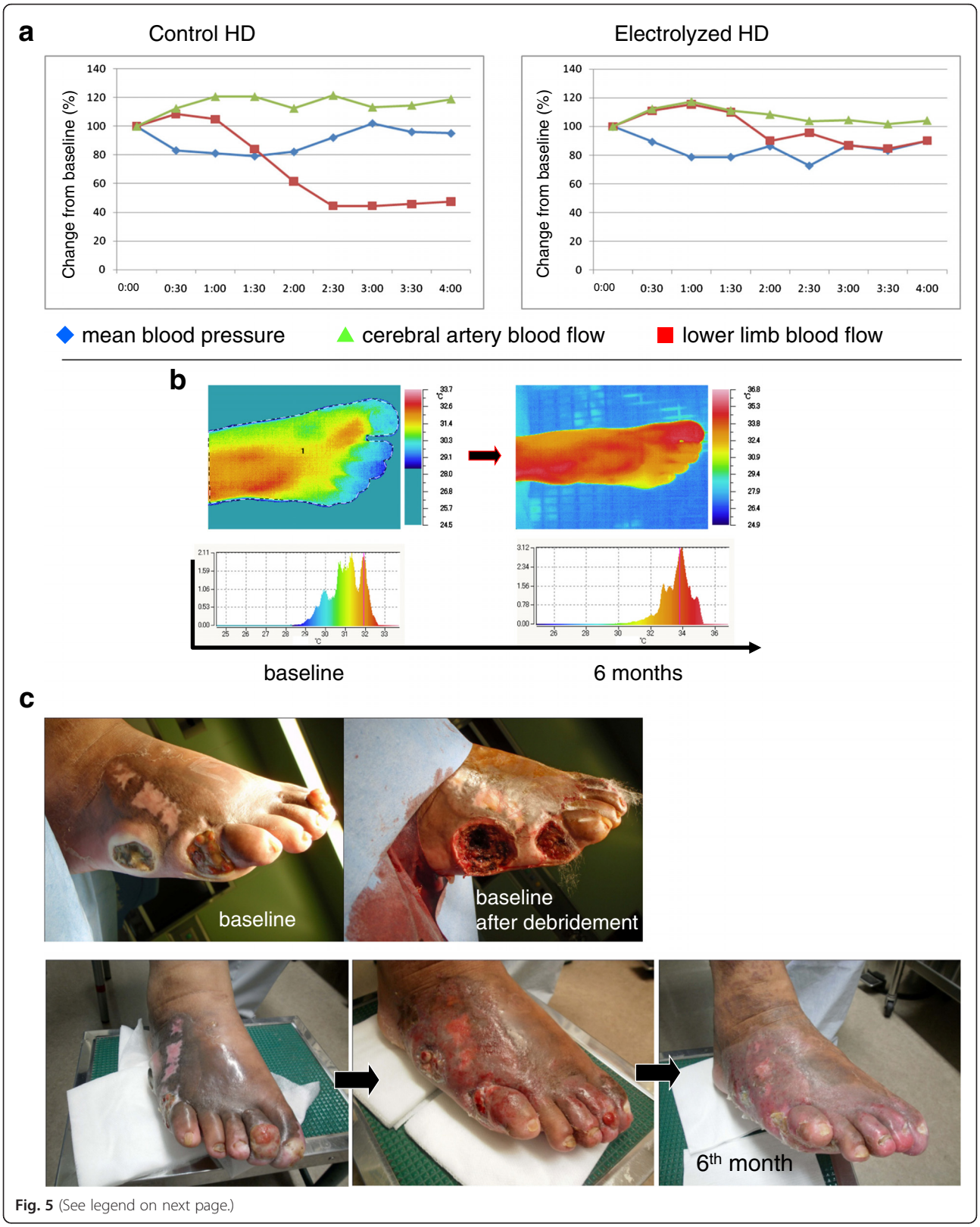

Fig. 5 (See legend on next page.)

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Fig. 5 Effect of H_2-enriched HD solution on changes in blood flow and skin temperature of the lower limb: a clinical case. A case with progressive decline in lower limb blood flow during regular HD session (*left*); the decline was suppressed by introduction of H_2-enriched HD solution (*right*) (courtesy of Dr. Kazumasa Usami, Taigenkai Hospital, Ichinomiya). **a** A case showing improved aggregated lower limb coldness with uncontrollable ulceration on stubbed toe after the introduction of H_2-enriched HD solution (6 months). **b** A case showing improved intractable lower limb ulceration due to obstructive arteriosclerosis after the introduction of H_2-enriched HD solution (6 months) (**b**, **c** courtesy of Dr. Hirofumi Nakano, Kashima Hospital, Iwaki)

solution [100]. In an *ex vivo* study, there was an increased oxidative injury of polymorphonuclear leukocytes during HD, but the injury was reduced with the use of H_2-enriched solution [27]. Furthermore, available data indicated that induction of inflammatory M1 macrophages is suppressed by H_2 [101]. Taken together, we speculate that induction of pro-inflammatory conditioning of immune cells which enhance oxidative stress during the HD session plays a crucial role for the dialysis-related adverse effects and that amelioration of injured circulating immune cells by H_2-enriched solution could contribute to the appearance of clinical effects. This point needs to be further elucidated in future studies.

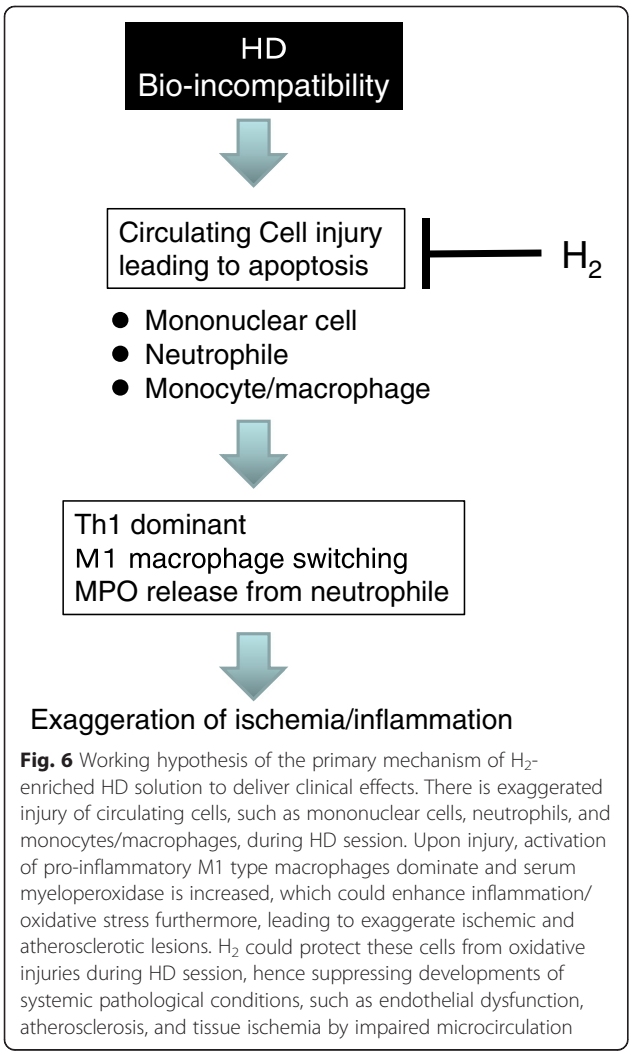

Fig. 6 Working hypothesis of the primary mechanism of H_2-enriched HD solution to deliver clinical effects. There is exaggerated injury of circulating cells, such as mononuclear cells, neutrophils, and monocytes/macrophages, during HD session. Upon injury, activation of pro-inflammatory M1 type macrophages dominate and serum myeloperoxidase is increased, which could enhance inflammation/oxidative stress furthermore, leading to exaggerate ischemic and atherosclerotic lesions. H_2 could protect these cells from oxidative injuries during HD session, hence suppressing developments of systemic pathological conditions, such as endothelial dysfunction, atherosclerosis, and tissue ischemia by impaired microcirculation

Future directions for H_2 therapy in chronic dialysis patients

Thus far, clinical studies of H_2 therapy have been limited; however, therapeutic intervention with H_2 has great potential to benefit patients on chronic dialysis treatment. Comorbidities like renal anemia, malnutrition, vascular calcification, and dialysis hypotension are potential targets for H_2 therapy, since all are associated with enhanced oxidative stress. Currently, whether uremic micro-inflammation is a cause of erythropoietin-resistant renal anemia is a matter of debate [102, 103]. Inflammation stimulates hepcidine production, which suppresses iron utilization and worsens renal anemia. Malnutrition observed during long-term dialysis treatment often accompanies inflammation, as the so-called malnutrition-inflammation atherosclerosis (MIA) syndrome [104]. Development of vascular calcification is connected with the transformation of vascular smooth muscle cells, in which oxidative stress plays a crucial role [105, 106].

Further studies designed to ascertain the clinical effect of H_2 on these disorders are warranted. Currently, the

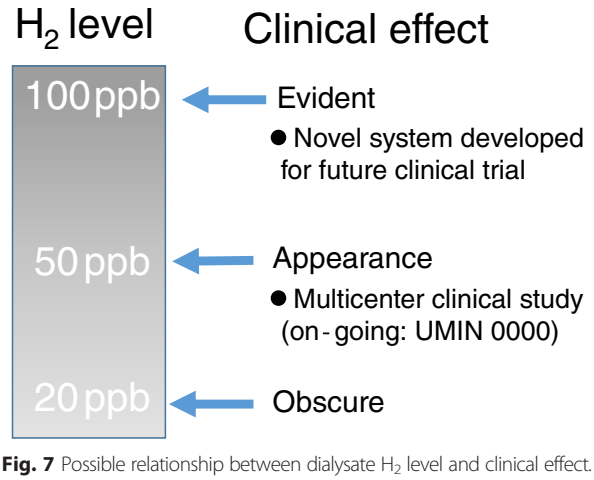

Fig. 7 Possible relationship between dialysate H_2 level and clinical effect. Clinical studies conducted using dialysate solution of approximately 50 ppb H_2 showed some clinical effects (UMIN000004857). These effects become more evident at >100 ppb H_2, were diminished at lower levels, and become obscure at <20 ppb. A novel water electrolyzer capable of supplying HD solutions with H_2 exceeding 200 ppb has been developed for assessment in a future clinical trial

optimal effective clinical H_2 dose is not known. Moreover, the existence of a dose-response relationship between the amount of H_2 delivered in each HD session and clinical benefit remains unclear (Fig. 7). This issue needs to be addressed in order to fully explore the clinical applications of H_2 in future trials.

Conclusions

Recent studies have revealed that H_2 has a unique biological capacity to act as an antioxidative and anti-inflammatory substance. In light of accumulating evidence from animal studies showing protective effects against organ damage during ischemia and inflammation, development of H_2 treatments for HD patients has become a challenging clinical goal. An HD system utilizing a water electrolysis technique that renders large amounts of H_2-enriched water has been developed. Accumulating findings indicate that the use of an H_2-enriched solution may prove to be a novel approach to ameliorate dialysis-related complications.

Competing interests

MN and SI have no competing interests. SK is an employee of Trim Med Institute.

Authors' contributions

MN surveyed the published manuscript and finalized this review. SK helped to draft the manuscript and to collect the clinical data of the hemodialysis applying electrolyzed water for this review. SI organized the comprehensive study project of the clinical use of electrolyzed water and gave comments to MN and SI. All authors read and approved the final manuscript.

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Author details

[1]Department of Nephrology and Hypertension, Fukushima Medical University, Hikarigaoka 1, Fukushima City 960-1295, Japan. [2]United Centers for Advanced Research and Translational Medicine, Center for Advanced and Integrated Renal Science, Tohoku University, Sendai, Japan. [3]Trim Medical Institute Co., Ltd., Osaka, Japan. [4]Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan.

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