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Hydrogen as a Selective Antioxidant: a Review of Clinical and Experimental Studies

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Oxidative stress is implicated in the pathogenesis of many diseases; however, currently used antioxidants have a high toxicity that constrains administration to a narrow window of therapeutic dosage. There is a clear need for more effective and safer antioxidants. Diatomic hydrogen (H_2) was proposed as a novel antioxidant that selectively reduces levels of toxic reactive-oxygen species. Recently, many studies have reported that H_2 (inhaled or orally ingested, typically as approximately 0.8 mM H_2-saturated water), can exert beneficial effects in diverse animal models of ischaemia–reperfusion injury, and inflammatory and neurological disease. In the clinic, oral administration of H_2-saturated water is reported to improve lipid and glucose metabolism in subjects with diabetes or impaired glucose tolerance; promising results have also been obtained in reducing inflammation in haemodialysis patients and treating metabolic syndrome. These studies suggest H_2 has selective antioxidant properties, and can exert antiapoptotic, anti-inflammatory and antiallergy effects. This review summarizes recent research findings and mechanisms concerning the therapeutic potential of H_2.

KEY WORDS: Oxidative stress; Antioxidant; Hydrogen; Hydrogen-saturated water

Introduction

Oxidative stress is a feature of many conditions including ischaemia–reperfusion (I/R) injury, inflammatory disorders, cancer, cardiovascular and neurological diseases, and ageing.[1] Oxidative stress is generally associated with the production of highly reactive free radicals including reactive oxygen species (ROS). The mitochondrion is the primary source of ROS, and much research has focused on the identification of naturally occurring low-toxicity antioxidants that can target mitochondrial ROS. Although a relatively unexplored field of medicine, increasing attention is now being paid to the potential use of medicinal gases as therapeutic agents.[2,3] This article reviews recent work, in pre-clinical models and clinical trials, on the use of diatomic hydrogen (H_2) – a naturally occurring biomolecule – as a selective antioxidant.

Oxidative stress

Free radicals are atoms, molecules or ions

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with unpaired electrons in an open shell configuration; these unpaired electrons make the radical species highly chemically reactive. ROS include superoxide anion (O_2^-•), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). These different species interconvert via cascade reactions.

Although generally regarded as toxic by-products, physiological roles have been identified for molecules such as H_2O_2 and the nitric oxide radical (NO•),[4] which have been shown to play important roles as effector molecules in immune defence systems against pathogens, and as signalling molecules.[5,6] For this reason, the elimination of all types of ROS through powerful antioxidant therapies is likely to disrupt important cellular functions. Indeed, it has been reported that radical suppression of oxidative stress can promote tumour progression.[7]

Nevertheless, overproduction of several types of ROS has been associated with a range of toxic effects. The majority of these effects have been associated with the hydroxyl radical •OH, one of the most reactive of the ROS species,[8] which can indiscriminately damage cellular components including lipid, protein, carbohydrate and nucleic acid, ultimately leading to cellular necrosis and apoptosis. This raises the prospect of developing selective antioxidants that preferentially remove toxic radicals such as •OH, but not others (such as H_2O_2 or NO•).[4]

The accumulation of ROS is generally counterbalanced by a sophisticated endogenous antioxidant defence system that comprises enzymes – such as superoxide dismutase (SOD), catalase and glutathione peroxidase – and non-enzymes, such as vitamin A, vitamin C, carotene and bilirubin. Although O_2^-• and H_2O_2 can be detoxified by antioxidant defence enzymes, •OH cannot be detoxified by this route. This has emphasized the importance of antioxidants targeting •OH.

Approximately 4000 antioxidants have been described to date, most of which are electron donors that react with ROS to form harmless end-products such as water. Although many have given promising results in animal models of oxidative stress, in most cases the beneficial effects seen in animal studies have not been reiterated in clinical trials.[9] Barriers to the utilization of exogenous antioxidants include low membrane permeability and high toxicity, which constrain administration to a narrow window of therapeutic dosage.[10] The identification of a novel and more effective antioxidant is, therefore, of high priority.

Rationale behind H_2: a selective antioxidant

It has long been known that H_2 – a colourless, odourless and tasteless gas – has antioxidant properties, but the potential exploitation of H_2 as a therapeutic agent has only recently been explored in animal models and in the clinic. The first study, by Dole *et al.*[11] reported that there was significant cancer regression in patients with squamous cell carcinoma exposed to hyperbaric H_2 for 2 weeks. In 2001, hyperbaric H_2 was reported to be beneficial in the treatment of schistosomiasis-associated chronic liver inflammation, and its therapeutic properties were ascribed to scavenging of •OH.[12] These studies were not, however, extended by other researchers, perhaps in view of the explosion hazards associated with hydrogen. Nevertheless, it is important to note that such risks are eliminated when used in H_2/air mixtures of < 4.6% (v/v).[12] Ohsawa *et al.*[13] studied the antioxidant properties of molecular H_2 and reported that it selectively reduces •OH and

$ONOO^-$ but does not affect physiological ROS. Subsequent studies[14–20] confirmed that the beneficial effects of H_2 are principally mediated by •OH scavenging (Fig. 1).

H_2 as a therapeutic agent: review of animal models and clinical trials

The discovery by Ohsawa *et al.*[13] that inhalation of H_2 gas can protect the brain against oxidative stress associated with I/R prompted a series of studies in diverse models, exploring the potential of H_2 inhalation (or oral ingestion of H_2-saturated water) to reduce oxidative damage. These studies, summarized in Table 1,[14–43] have targeted a diverse range of disorders and organ systems including the nervous, digestive, cardiovascular and respiratory systems. In the following sections, reports on H_2 therapy in different disease classes are reviewed.

ISCHAEMIA–REPERFUSION INJURY

Oxidative stress is thought to play a major role in cell damage following I/R injury and several studies have addressed whether H_2 administration can reduce I/R-induced cell

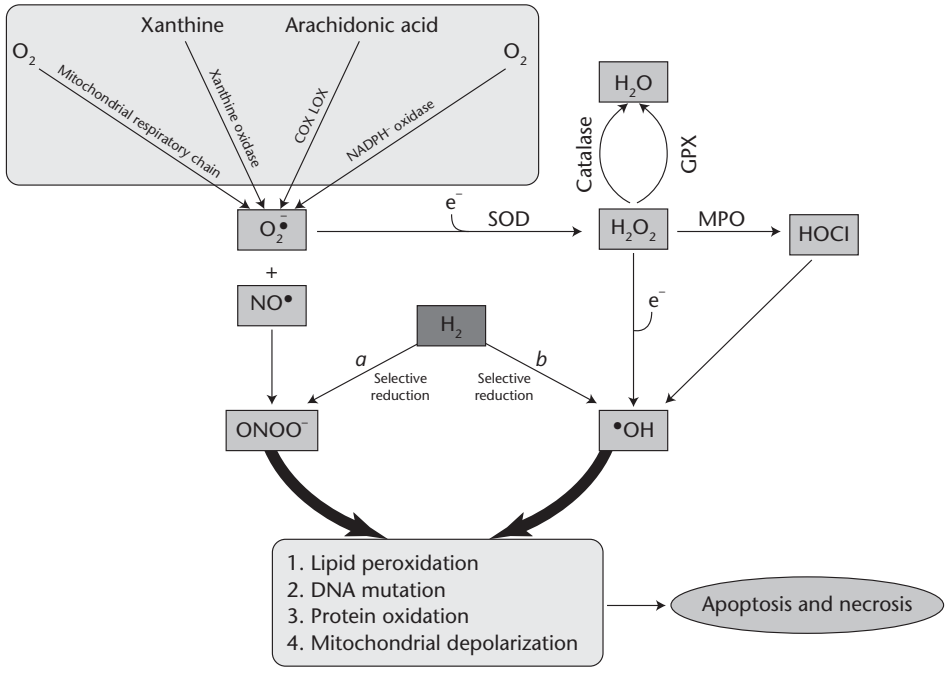

FIGURE 1: The reactive oxygen species (ROS) production pathway and the selective reduction of •OH and $ONOO^-$ by hydrogen, showing that ROS are produced in various ways; one type of ROS can be converted into another type by electron transmission. •OH and $ONOO^-$ are much more toxic than other ROS. Hydrogen selectively reduces •OH (*b*) and $ONOO^-$ (*a*), but does not influence physiological ROS, including O_2^-• and H_2O_2 (COX, cyclo-oxygenase; LOX, 5-lipo-oxygenase; NO•, nitic oxide radical; NAPDH, reduced nicotinamide adenine dinucleotide phosphate; SOD, superoxide dismutase; GPX, glutathione peroxidase 1; MPO, myeloperoxidase; HOCl, hypochlorous acid; O_2^-• superoxide anion; •OH, hydroxyl radical; H_2O_2, hydrogen peroxide; $ONOO^-$, peroxynitrite)

TABLE 1:
Evidence for therapeutic use of diatomic hydrogen (H_2) in various diseases and its effects on various markers

Marker		Models of disease	H_2 effect	References
Antioxidase	SOD	Radiation complication, metabolic sepsis, obstructive jaundice	↑	Qian *et al.*,[14] Nakao *et al.*,[15] Xie *et al.*,[16] Liu *et al.*[17]
	CAT	Sepsis, obstructive jaundice	↑	Xie *et al.*,[16] Liu *et al.*[17]
	GSH	Radiation complication	↑	Qian *et al.*[14]
Oxidative stress	MDA	I/R (hepatic, intestinal, neonatal hypoxia, cardiac, renal), Alzheimer's disease, cognitive disorders, pancreatitis, atherosclerosis, obstructive jaundice, radiation and chemotherapy complication, spinal cord injury	↓	Fukuda *et al.*,[18] Zheng *et al.*,[19] Mao *et al.*,[20] Cai *et al.*,[21] Chen *et al.*,[22] Sun *et al.*,[23] Nakao *et al.*,[24] Cardinal *et al.*,[25] Li *et al.*,[26] Nagata *et al.*,[27] Chen *et al.*,[28] Ohsawa *et al.*,[29] Liu *et al.*,[17] Qian *et al.*,[14] Nakashima-Kamimura *et al.*,[30] Chen *et al.*[31]
	4HNE	Atherosclerosis, Parkinson's disease, Alzheimer's disease, cognitive disorders, I/R (renal and retinal), haemorrhagic	↓	Ohsawa *et al.*,[29] Fu *et al.*,[32] Fujita *et al.*,[33] Li *et al.*,[26] Nagata *et al.*,[27] Cardinal *et al.*,[25] Oharazawa *et al.*,[34] Chen *et al.*[35]
	8-OHdG	I/R (cerebral, myocardium, retinal, renal), Parkinson's disease, haemorrhagic, metabolic syndrome, radiation complication	↓	Ohsawa *et al.*,[13] Hayashida *et al.*,[36] Sun *et al.*,[23] Oharazawa *et al.*,[34] Shingu *et al.*,[37] Fu *et al.*,[32] Chen *et al.*,[35] Nakao *et al.*,[15] Qian *et al.*[14]
	MPO	Intestinal I/R injury, sepsis, pancreatitis, haemodialysis, obstructive jaundice, spinal cord injury	↓	Zheng *et al.*,[19] Chen *et al.*,[22] Chen *et al.*,[28] Xie *et al.*,[16] Nakayama *et al.*,[38] Liu *et al.*,[17] Chen *et al.*[31]
	8-oxoG	Parkinson's disease	↓	Fujita *et al.*[33]
	8-isoPGF$_{2\alpha}$	Sepsis, metabolic syndrome	↓	Xie *et al.*,[16] Nakao *et al.*[15]
	DAO	Intestinal I/R injury	↓	Zheng *et al.*[19]
	TBARs	Metabolic syndrome	↓	Nakao *et al.*[15]
	NADPH	Allergic	↓	Itoh *et al.*[39]
Inflammation factor	IL-1β	Intestinal I/R, colon inflammation, obstructive jaundice	↓	Buchholz *et al.*,[40] Zheng *et al.*,[19] Mao *et al.*,[20] Kajiya *et al.*,[41] Liu *et al.*[17]
	IL-6	Intestinal I/R, Alzheimer's disease, renal transplantation, obstructive jaundice	↓	Buchholz *et al.*,[40] Zheng *et al.*,[19] Li *et al.*,[26] Cardinal *et al.*,[25] Liu *et al.*[17]
	TNF-α	Intestinal I/R, Alzheimer's disease, colon inflammation, renal transplantation, obstructive jaundice, hepatitis		Zheng *et al.*,[19] Mao *et al.*,[20] Li *et al.*,[26] Kajiya *et al.*,[41] Cardinal *et al.*,[25] Liu *et al.*,[17] Kajiya *et al.*[42]

TABLE 1 (continued):
Evidence for therapeutic use of diatomic hydrogen (H_2) in various diseases and its effects on various markers

Marker		Models of disease	H_2 effect	References
	HMGB1	Sepsis, myocardium I/R, obstructive jaundice	↓	Xie *et al.*,[16] Nakao *et al.*,[24] Liu *et al.*[17]
	PCNA	Pancreatitis, intestinal I/R	↓	Chen *et al.*,[28] Chen *et al.*[22]
	IFN-γ	Hepatitis, renal transplantation	↓	Kajiya *et al.*,[42] Cardinal *et al.*[25]
	CCL2	Intestinal I/R injury	↓	Buchholz *et al.*[40]
	ICAM-1	Renal transplantation	↓	Cardinal *et al.*[25]
	iNOS	Myocardium I/R injury	↓	Nakao *et al.*[24]
	IL-12	Colon inflammation	↓	Kajiya *et al.*[41]
	Iba1	Neonatal hypoxia–ischaemia	↓	Cai *et al.*[21]
Inflammation signals	MAPK	Renal transplantation, obstructive jaundice	↓	Cardinal *et al.*,[25] Liu *et al.*[17]
	MEK-1	Renal transplantation	↓	Cardinal *et al.*[25]
	Lyn-P	Allergic	↓	Itoh *et al.*[39]
	NF-κβ	Pancreatitis	↓	Chen *et al.*[28]
Apoptosis	TUNEL	I/R (myocardium, intestinal, neonatal hypoxia), chemotherapy complication, spinal cord injury, pancreatitis	↓	Hayashida *et al.*,[36] Chen *et al.*,[22] Cai *et al.*,[21] Cai *et al.*,[43] Nakashima-Kamimura *et al.*,[30] Chen *et al.*,[31] Chen *et al.*[28]
	Annexin V	Radiation complication	↓	Qian *et al.*[14]
	Caspase-3	Neonatal hypoxia, myocardium I/R injury, spinal cord injury	↓	Cai *et al.*,[43] Cai *et al.*,[21] Sun *et al.*,[23] Nakao *et al.*,[24] Chen *et al.*[31]
	Caspase-12	Neonatal hypoxia, spinal cord injury	↓	Cai *et al.*,[43] Chen *et al.*[31]

↑, increase of the marker; ↓, decrease of the marker; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; MDA, malondialdehyde; I/R, ischaemia–reperfusion; 4HNE, 4-hydroxynonenal; 8-OHdG, 8-hydroxydeoxyguanosine; MPO, myeloperoxidase; 8-oxoG, 8-oxoguanine; 8-isoPGF$_{2\alpha}$, 8-isoprostane; DAO, diamine oxidase; TBARs, thiobarbituric acid reactive substances; NADPH, reduced nicotinamide adenine dinucleotide phosphate; IL, interleukin; TNF-α, tumour necrosis factor α; HMGB1, high-mobility group box 1; PCNA, proliferating cell nuclear antigen; IFN-γ, interferon-γ; CCL2, chemokine (C–C motif) ligand 2; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible nitric oxide synthase; Iba1, ionized calcium binding adaptor molecule 1; Lyn-P, phosphorylated Lyn tyrosine kinase; MAPK, mitogen-activated protein kinase; MEK-1, MAP-extracellular signal regulated protein kinase-1; NF-κβ, nuclear factor-κβ; TUNEL, terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labelling.

loss in animal models. H_2 was reported to protect against I/R injury in models of cerebral,[13,21,43] myocardial,[24,36] hepatic,[18] intestinal,[22,40] retinal[34] and renal[37] I/R. H_2 was also reported to reduce infarct size in rat models of focal cerebral and myocardial ischaemia,[13,36] maintain the integrity of the blood–brain barrier and reduce haemorrhagic transformation in a rat model of focal ischaemia.[35]

The beneficial effects of H_2 treatment are likely to be due to direct scavenging of •OH, although the kinetic favourability of this direct reaction has not been established. It was observed, however, that in biochemical studies of I/R injury, H_2 treatment was able to reduce the levels of multiple markers of oxidative stress. Notably, H_2 decreased the

levels of: malondialdehyde (MDA), an indicator of ROS-mediated lipid peroxidation;[18 – 20,23] 4-hydroxynonenal, a product of lipid peroxidation;[18,34] myeloperoxidase, a marker of oxidative stress;[20] and 8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA oxidation.[23,34,36] These results argue that H_2 has significant antioxidant effects in I/R injury. Furthermore, I/R injury is commonly associated with cell death in the ischaemic region as a result of apoptosis and/or necrosis. Importantly, it has been reported that H_2 treatment can significantly reduce the numbers of terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labelling (TUNEL)-positive cells in parallel with reductions in the levels of activated caspases 3 and 12,[23,34] arguing that the protective effects of H_2 are mediated in part through inhibition of pathways of programmed cell death.

The above studies, which were all double-blind and placebo-controlled, suggest that the use of H_2 for I/R is effective. Clinical application of H_2 during I/R is slight, so we speculated whether clinical studies would also show encouraging results. In our opinion, the therapeutic window for I/R disease is usually very short. When translated into a clinical application, H_2 must be most frequently applied as early as possible in the treatment of patients with acute infarction. It is particularly important to make sure that H_2 can rapidly reach 'at-risk' ischaemic areas before blood flow in the occluded infarct-related artery is re-established. Consequently, further research is warranted to enhance the application of H_2 for curing I/R injury.

INFLAMMATORY DISEASE

Inflammatory processes are known to be closely linked with oxidative stress, and several studies have addressed the potential of H_2 as an anti-inflammatory therapeutic.

In inflammatory conditions, H_2 treatment was found significantly to reduce levels of interleukin (IL)-6 and tumour necrosis factor-α (TNF-α), as well as levels of other inflammation-associated molecules including intercellular adhesion molecule-1, IL-12, high-mobility group box 1 and interferon-γ[16,23,25,28,40 – 42] in rodent models. Importantly, H_2 partly inhibited mitogen-activated protein kinase signalling pathways, including c-Jun N-terminal kinase (JNK), p38, extracellular signal-regulated protein kinase (ERK1/2), as well as upstream kinase cascades.[25]

In animal models of inflammatory disorders, it has been reported that H_2 can attenuate inflammation in hepatitis,[42] colitis,[41] pancreatitis,[28] obstructive jaundice[17] and sepsis.[16] H_2 treatment was also associated with the downregulation of the expression of proinflammatory cytokines and suppression of inflammatory cell infiltration (summarized in Table 1).

NEUROLOGICAL DISEASE

In a rat model of Parkinson's disease, 6-hydroxydopamine (6-OHDA) induced oxidative stress in dopaminergic neurons, leading to nigrostriatal degeneration.[32] Fu *et al*.[32] first examined the effects of oral administration of 50% H_2-saturated water before intrastriatal injection of 6-OHDA, and found that H_2 significantly reduced the extent of degeneration. More recently, a lower concentration of orally administered H_2 (5% saturated H_2 in drinking water) was found to prevent DNA damage and lipid peroxidation, and to reduce dopaminergic neuron loss in a MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxin model of Parkinson's disease.[33]

In Alzheimer's disease it is thought that amyloid β (Aβ), the major component of

senile plaques, plays a causal role in disease development, and exerts its toxic effects in part via the induction of oxidative stress.[26] Oral administration of H_2-saturated water has been reported to attenuate Aβ-induced deficits, in a rat model.[26] It was also reported that H_2 could improve spatial recognition and memory, and that this was accompanied by reduced levels of proinflammatory cytokines, lipid peroxidation products and glial fibrillary acidic protein immunoreactivity.[26] Furthermore, in a different animal model (restraint-induced stress) H_2 administration was found to alleviate stress-induced deficits in learning and memory.[27]

Oxidative stress, inflammation and apoptosis are also central features of cell loss following acute spinal cord contusion. Using an animal model, H_2 treatment led to improved rates of functional locomotor recovery after spinal cord injury; this recovery was associated with reduced oxidative stress and elevated brain-derived neurotrophic factor levels.[31] Gu *et al.*[44] reported that daily consumption of H_2-saturated water was effective in preventing age-related learning and memory impairments; this effect was attributed to removal of ROS and increased SOD activity.

METABOLIC DISORDERS

Metabolic syndrome refers to a common disorder characterized by a combination of obesity, dyslipidaemia, hypertension and insulin resistance. These metabolic disorders are suitable for clinical research with H_2 because of their chronic characteristics. Consequently, clinical studies of H_2 are, at present, mostly limited to metabolic diseases. Nakao *et al.*,[15] in an open-label pilot study involving 20 subjects with potential metabolic syndrome, demonstrated that consumption of 1.5 – 2.0 l/day of H_2-saturated water for 8 weeks reduced the levels of oxidative stress indicators. Moreover, the treated subjects showed significant improvements in liver and kidney function. In addition to direct antioxidant effects, it was reported that H_2 enhanced SOD levels, thereby increasing endogenous antioxidant defence against $O_2^{-}\bullet$. The sample in this study was, however, so small that the finding should be accepted with caution.[15] In a randomized, double-blind, placebo-controlled, crossover study, Kajiyama *et al.*[45] reported that supplementation with 900 ml/day of H_2-saturated water for 8 weeks reduced the levels of several biomarkers of oxidative stress, including plasma oxidized low-density lipoprotein cholesterol and urinary 8-isoprostanes, and also improved glucose metabolism in patients with either type 2 diabetes or impaired glucose tolerance; however, their breath hydrogen levels were not reported in detail after consumption. Supplementation with H_2-saturated water normalized the oral glucose tolerance test in four out of six patients with impaired glucose tolerance.[45] Furthermore, consumption of H_2 *ad libitum* was reported to prevent the development of atherosclerosis in apolipoprotein E knockout mice, partly through its ability to limit oxidative stress in blood vessels.[29] The efficacy of H_2-saturated water in these studies appeared to be significantly greater than other tested antioxidants including folic acid, vitamin E, iron and α-lipoic acid.

CHEMOTHERAPY, HAEMODIALYSIS AND RADIOTHERAPY COMPLICATIONS

Reagents such as cisplatin, which target rapidly proliferating cells, are widely used to treat aggressive neoplastic conditions. Nevertheless, cisplatin use is restricted

because of its major side-effects during therapy, some of which have been ascribed to oxidative stress and/or inflammation. Using an animal model, Nakashima-Kamimura *et al.*[30] reported that both oral consumption of H_2-saturated water, as well as H_2 administration by inhalation, could alleviate cisplatin-induced nephrotoxicity without compromising antitumour activity.

In a non-randomized study with a concurrent control, Nakayama *et al.*[38] found that adding H_2 to haemodialysis solutions after a 1-month run-in period ameliorated inflammatory reactions, decreased plasma oxidative markers and improved blood pressure control during a 6-month trial. The study period was, however, short and the inflammatory conditions of the subjects were not very serious. Thus, long-term studies on patients with more severe inflammation may be optimal for clarifying the impact of H_2.

Excess exposure to ionizing radiation causes a spectrum of tissue damage known as acute radiation syndrome. Importantly, it has been estimated that 60% – 70% of ionizing radiation-induced cellular damage is caused by •OH.[46] However, levels of apoptotic cells, plasma MDA and intestinal 8-OHdG were significantly decreased when cells or mice were treated with H_2 after irradiation.[14] This suggests that H_2 administration could also be of benefit in cancer radiotherapy.

In summary, H_2 can counter side-effects commonly seen during chemotherapy, radiotherapy and haemodialysis, thereby improving the patient's quality of life.

ALLERGIC DISEASES

Immediate-type allergic reactions include pollinosis, bronchial asthma and urticaria, which are conditions that have not been causally associated with oxidative stress. Nevertheless, it is possible that H_2 exerts therapeutic effects that are independent of the removal of •OH. Itoh *et al.*[39] reported that H_2 attenuated degranulation by suppressing FcεRI-mediated signal transduction in a mast-cell culture model. Administration of H_2 down-regulated antigen-induced phosphorylation of FcεRI-associated Lyn – a key regulatory step in the pathway – and suppressed activation of downstream signal targets including Syk, phospholipase C (PLC)-γ1, PLCγ2, Akt, ERK1/2, JNK, p38 and cytosolic phospholipase A_2, without affecting the levels of the immunoglobulin E receptor FcεRIβ. It is, therefore, possible that H_2 can intervene in specific signal transduction pathways.

H_2: advantages over pharmaceutical drugs

Diatomic hydrogen has several potential advantages, compared with pharmaceutical drugs (Table 2). The administration of H_2, either as a low percentage H_2/air mixture or as a solution in water or saline, has several advantages over conventional antioxidants. First, H_2 has not been reported to be toxic at effective dosages, and overdosing is unlikely because excess H_2 is expired via the lungs.[44] This contrasts with antioxidants, such as vitamins C and E, where the effective dosage in humans is higher than the upper limit of tolerated intake. Secondly, H_2 selectively scavenges the most aggressive ROS, •OH, but is far less effective against O_2^{-}• and H_2O_2, which play physiological roles. Moreover, there is no evidence that H_2, a mild antioxidant, is able to disturb metabolic redox reactions or to disrupt cell signalling mediated by less potent ROS. Indeed, H_2 does not influence physiological parameters such as temperature, blood pressure, pH or pO_2. Thirdly, because of its low molecular weight, H_2 can diffuse extremely rapidly into tissue and is likely to reach important target

TABLE 2:
Summary of the therapeutic advantages of the antioxidant, diatomic hydrogen

1	Easily diffuses across the cellular membrane to reach sub-cellular compartments
2	Does not influence physiological parameters in the blood (temperature, blood pressure, pH, pO_2)
3	Does not disturb metabolic oxidation–reduction reactions and cell signalling
4	High concentrations of hydrogen are well tolerated; fewer systemic side-effects
5	Low cost
6	Multiple mechanisms of action:
	(i) antioxidant effect
	(ii) anti-inflammatory effect
	(iii) inhibition of apoptosis
	(iv) antiallergic effect

subcellular compartments, including mitochondria and the nucleus. This is particularly important because mitochondria are the primary sites of the generation of ROS after I/R and are notoriously difficult to target. Nuclear targeting could also be important in protecting DNA from oxidative damage. Finally, it seems likely that H_2 offers significant advantages in terms of cost compared with conventional pharmaceuticals.

Future prospects: many unsolved problems

Although multiple studies have demonstrated that H_2 administration can be effective against a range of disorders, both in animal models and in the clinic, many questions remain. At the molecular level, it is unclear how H_2 achieves chemical selectivity in view of the enormous diversity of potential molecular targets *in vivo*. Moreover, the published rate constant for the reaction of •OH with H_2 to form H_2O and H• is markedly slower than for most radical–radical reactions. Intriguingly, there are some indications that H_2 might not act exclusively as an antioxidant and could also interact directly with specific signalling pathways,[39] as previously discovered for other simple gases including NO, CO and H_2S. Further work will be required to determine the *in vivo* molecular targets for H_2.

The protective effects of H_2 have been established in both animal models and clinical trials; however, none of the published studies has examined the concentration of H_2 in the active site. Further monitoring of H_2 concentrations in injury would be helpful to understand the pharmacokinetics in more detail. In Japan, H_2 therapy has been used clinically in diabetes, the metabolic syndrome and haemodialysis, but further appropriately designed, large-scale, placebo-controlled trials using different dosages and routes of administration will be required to evaluate the therapeutic efficacy of H_2 treatment rigorously in these and other conditions.

Delivery of high concentrations of H_2 gas by inhalation has major drawbacks in view of the risks associated with its flammability and the complex apparatus required for safe delivery. Nagata *et al.*[27] first reported that consumption of H_2-saturated water could afford a safe and effective alternative to inhalation. Nevertheless, the pharmacokinetics of different routes of administration have not been studied in depth, although Fu *et al.*[32] reported that arterial H_2 concentrations following consumption of 0.4 mM hydrogen water were similar to those achieved on inhalation of 2% (v/v) hydrogen gas. It seems

likely that oral administration of H_2 solution will be the route of choice in further studies on the antioxidant properties of H_2.

We have reviewed a large number of studies, principally in animal models, in which H_2 administration reduced tissue injury associated with oxidative stress and inflammation. Despite important differences between animal models and human disease, we argue that the therapeutic potential of H_2 – a non-toxic, convenient, safe and effective antioxidant – warrants further clinical evaluation in oxidative stress-related diseases.

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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