SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 16: 2771-2776, 2018

Anti-inflammatory and antitumor action of hydrogen via reactive oxygen species (Review)

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Received November 19, 2017; Accepted June 20, 2018

DOI: 10.3892/ol.2018.9023

Abstract. Hydrogen (H_2) has advantages that lead it to be used as a novel antioxidant in preventive and therapeutic applications. H_2 can permeate into biomembranes, cytosol, mitochondria and nuclei, and can be dissolved in water or saline to produce H_2 water or H_2-rich saline. H_2 selectively reduces oxidants of the detrimental reactive oxygen species (ROS), including hydroxyl radicals (·OH) and peroxynitrite ($ONOO^-$), which serve a causative role in the promotion of tumor cell proliferation, invasion and metastasis, but do not disturb metabolic oxidation-reduction reactions in cell signaling. Compared with traditional antioxidants, H_2 is a small molecule that can easily dissipate throughout the body and cells; thus, it may be a safe and effective antioxidant for inflammatory diseases and cancer, since ROS usually initiates tumor progression. Treatment with H_2 may involve correction of the oxidative/anti-oxidative imbalance and suppression of inflammatory mediators. Therefore the present review will discuss the anti-inflammatory and anti-tumorigenic action of H_2 via ROS.

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Key words: hydrogen, reactive oxygen species, anti-inflammatory, antitumor

1. Introduction

Hydrogen (H_2) occurs safely in the air with a concentration of <4.7%, and can be used as an inert gas at body temperature. H_2 selectively quenches detrimental reactive oxygen species (ROS), and it has become a novel anti-oxidant due to its anti-apoptotic, antioxidant, anti-inflammatory and anti-allergy effects (1,2). ROS increase cell migration and enhance tumor invasion and metastasis (3). Antioxidants have been demonstrated to effectively protect against cell damage, and H_2 effectively decreases radicals (·OH) and peroxynitrite ($ONOO^-$) in living cells without disrupting the ROS that are involved in normal metabolic oxidation reduction reactions in cell signaling. Therefore, H_2 can be used as an anti-inflammatory and anti-tumorigenic agent in clinical practice. The present review focuses on the association between H_2 and ROS in inflammatory disease and cancer.

2. H_2 usage method

Inhalation. H_2 has capability to penetrate biomembranes and diffuse into the cytosol, mitochondria and nuclei due to its distribution characteristics, including being able to rapidly penetrate vessel walls and being able to dissolve in water or saline (Fig. 1) (4,5). By contrast, the majority of hydrophilic antioxidants cannot penetrate biomembranes and remain on the surface. Inhalation of H_2 or the administration of H_2 water can increase the concentration of H_2 in arterial and venous blood (6).

Oral administration. There are several methods to produce H_2 water, including infusing H_2 gas into water up to 0.8 mM (1.6 ppm) under atmospheric pressure or dissolving electrolyzed H_2 into pure water to form H_2 bubbled water. H_2 rapidly penetrates the glass and plastic walls of any vessels, but has a half-time of 0-2 h and almost disappears after 8 h, so aluminum containers with no dead volume are usually used to reserve H_2 gas (7).

Intravenous drip. In contrast to H_2 gas, H_2 saturated in saline (HS) is easy to administer by dissolving H_2 in physiological saline for 6 h under 0.4 MPa pressure to a supersaturated level (8). HS can be stored in an aluminum bag under atmospheric pressure at 4˚C, with a >0.6 mmol/l concentration

of H_2 (8). HS can be infused into the stomachs of rats for experimental and clinical treatments (8,9).

Clinical application of H_2-enriched glucose-electrolyte solution can be used for acute cerebral infarction and in patients treated with t-PA (9). The solution can be produced at 1.6 ppm H_2 concentrations using H_2 adding equipment. Administration of 500 ml intravenous H_2-enriched fluid over 30 min for >3 days could relieve the associated symptoms of fever and pain in patients with acute erythematous skin diseases, but does not change physiological parameters in the blood (10).

External use. H_2 penetrates the skin easily and is distributed throughout the whole body via the blood in 10 min, as measured by H_2 gas content in expired breath. Submersion in a warm water bath with dissolved H_2 is a method of absorbing H_2 into the body in daily life. Hydrogen-water bathing therapy (hydrogen-water was provided by Shanghai Yiquan Investment Limited Partnership Company, Shanghai, China) has a significant and rapid improvement on disease severity and the quality of life for patients with psoriasis and parapsoriasis en plaques (11). Additionally, H_2-loaded eye drops can be made by dissolving H_2 in saline and can be directly dropped onto the ocular surface (12,13).

3. ROS in inflammatory disease and cancer

Cancer is a multi-stage process defined by initiation, promotion and progression (14-16), and oxidative stress interacts with all three stages of this process. ROS can increase tumor cell proliferation, survival and cellular migration in animal models and humans by inducing cellular signal transduction pathways (17,18).

What are ROS? ROS are formed as a result of an imbalance between free radical and reactive metabolite production, and can potentially exhibit a negative impact on the organism (19). ROS are products of oxygen-derived small molecules involved in normal cellular metabolism, including oxygen radicals such as superoxide anion ($O_2^{\bullet-}$), hydroxyl (·OH), peroxyl ($RO_2^{\bullet}$), and alkoxyl ($RO^{\bullet}$), as well as non-radicals, which can be converted to radicals or function as oxidizing agents, including H_2 peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) and singlet oxygen ($^1O_2$). ROS promote DNA synthesis, cell proliferation, cell survival, cellular migration and invasion, tumor metastasis and angiogenesis (20). Aerobic cells produce ROS, including $O_2^{\bullet-}$, H_2O_2 and ·OH, in endogenous metabolic reactions (21). Mitochondria are constantly exposed to high levels of ROS, which cause mitochondrial DNA damage and increase O and ·OH levels in cellular apoptosis (2).

Reactive nitrogen species (RNS) are formed from nitrogen-containing oxidants such as nitric oxide (NO). The mitochondrial respiratory chain can generate RNS under hypoxic conditions, while RNS can further generate other reactive species (22), and continuous cellular ROS and RNS generation is now known to be a consequence of numerous factors, including carcinogen exposure, inflammation and mitochondrial respiration (23).

ROS initiate tumor progression. Tumor cells generate ROS more abundantly than normal cells and cause elevated oxidative stress (24). Damage to DNA by ROS is involved in chronic inflammatory diseases and in a wide variety of cancer types, including bladder cancer (25), brain tumors (26), breast cancer (27), cervical cancer (28), gastric cancer (29), liver cancer (30), lung cancer (31), melanoma (32), multiple myeloma (33), leukemia (34), lymphoma (35), oral cancer (36), ovarian cancer (37), pancreatic cancer (38), prostate cancer (39) and sarcoma (40).

ROS can initiate tumorigenicity and subsequent tumor progression by inducing DNA damage (41). Oxidative stress interacts with the initiation, promotion and progression of cancer. During the initiation stage, ROS introduce gene mutations and structural alterations into the DNA and produce DNA damage. In the promotion stage, ROS increase cell proliferation or decrease apoptosis of the initiated cell population by causing abnormal gene expression, blocking cell communication and modifying second-messenger systems. Finally, oxidative stress may add DNA alterations to the initiated cell population and promote cancer progression (42).

Impact of ROS on cancer by regulation of gene expression. ROS serve vital roles in stimulating cell signaling pathways in intra- and extracellular environmental conditions (43), regulating gene mutations, and balancing cell proliferation and apoptosis (3,44). Cancer signaling starts from the hypoxic microenvironment of the autocrine and paracrine elements, including vascular endothelial growth factor, hepatocyte growth factor, hypoxia-inducible factor-1α (HIF-1α), NO and H_2O_2, which generate a positive feedback loop to hyper-activate the protein kinase B (Akt) locus. Oxidative stress can activate several transcription factors, including nuclear factor (NF)-κB, activator protein 1, p53, HIF-1α, matrix metalloproteinases, peroxisome proliferator-activated receptor-γ, β-catenin/Wnt and nuclear factor erythroid 2-related factor 2 (Nrf2). These effector molecules are activated under prolonged ROS-related chronic inflammation and alter the malignant transformation and the expression of genes involved in immune, inflammatory responses, carcinogenesis and metastasis.

4. Anti-oxidative characteristic of H_2

It has been demonstrated that a number of factors, including intense exercise, cardiac infarction (45), cessation of blood flow, organ transplantation and inflammation (46), can cause acute oxidative stress. H_2 is able to reduce the risk of life style-related diseases and cancer (7,47-49), and thus can be used to treat various diseases using its characteristic of protecting nuclear DNA and mitochondria.

H_2 reduces oxidants in ROS. H_2 dissolved in culture medium selectively reduces the strongest oxidants, such as OH and $ONOO^-$, in cell signaling, but does not disturb the cellular levels of $\cdot O_2$, NO· or H_2O_2, as well as ROS involved in metabolic oxidation-reduction reactions in cell-free systems (Fig. 2). As ·OH is strong enough to react with H_2, it can be a marker of the oxidative strength of ROS. It was previously reported that H_2 treatment significantly reduced ·OH produced by radiolysis or photolysis of water and decreased the levels of ·OH in cultured cells, thus protecting the mitochondria from OH (1). Since H_2 penetrates biomembranes

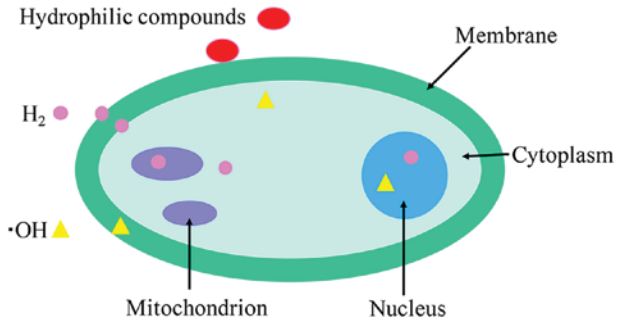

Figure 1. Illustration of H_2 diffusion in a cell. The majority of hydrophilic compounds cannot reach the cytosol and remain at the membranes, but H_2 can rapidly distribute into the cytosol and organelles. H_2, hydrogen.

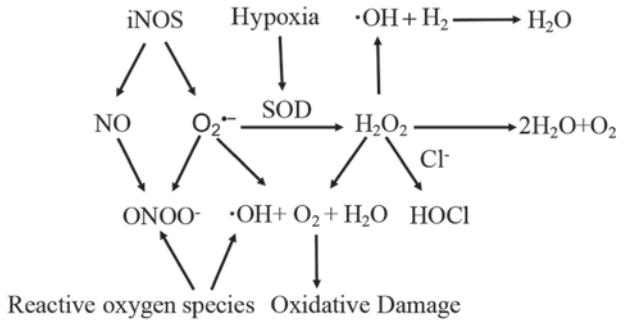

Figure 2. Impact of key oxidants of H_2 in cancer: ·OH and $ONOO^-$ are highly reactive to damaged cells, while $\cdot O_2$, NO· and H_2O_2 have physiological roles as signaling molecules. H_2, hydrogen; OH, hydroxyl radicals; $ONOO^-$, peroxynitrite; $\cdot O_2$, superoxide anion; NO, nitric oxide; H_2O_2, H_2 peroxide; Cl^-, chloride; H_2O, water; iNOS, inducible nitric oxide synthase; SOD, superoxide dismutase.

and diffuses into organelles, it can decrease cellular levels of ATP synthesized in the mitochondria and nucleus (1). Ren *et al* (50) demonstrated that treatment with 5% H_2-rich water led to a significant decrease in the level of ROS, maintained the biomass and polar growth morphology of the mycelium, and decreased the secondary metabolism under acetic acid-induced oxidative stress (50). H_2 also decreased the levels of ROS and promoted the chronic ultraviolet exposure-induced expression of phosphoinositide 3-kinase, Akt and Nrf2 in HaCaT cells (51). Since H_2 treatment exhibited anti-oxidant and anti-inflammatory neuroprotective effects, it essentially decreased cyclooxygenase-2 (oxidative stress markers) in immune-positive neurons (52).

Anti-inflammatory and antitumor activity of H_2. H_2 anti-inflammatory and anti-allergic features that function via the induction of inflammatory cytokines and the inhibition of cell signal factors. H_2 has been shown to decrease the expression of a number of pro-inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, IL-10, IL-12, chemokine ligand 2 (CCL2), intercellular adhesion molecule 1, NF-κB, high mobility group box 1 protein and prostaglandin E2. Furthermore, H_2-rich saline reduced serum diamine oxidase, TNF-α, IL-1β, IL-6, tissue malondialdehyde, protein carbonyl and myeloperoxidase activity, and also inhibited pro-apoptotic players, including JNK and caspase-3 (53,54).

Table I. Summary of various preventive and therapeutic effects of hydrogen by clinical examinations or by animal experiments.

Category	Disease/condition	Preventive treatment	(Refs.)
Metabolic syndrome	Diabetes	Improve the impaired sugar tolerance abilities of obese insulin-resistant type 2 diabetic mice.	(47)
	Hypertension and dyslipidemia	Increase the level of antioxidant enzyme SOD and decrease total cholesterol/HDL-cholesterol level.	(7)
Ischemia-reperfusion injuries	Cerebral infarction	Protect brain ischemia and reperfusion injuries against inflammation and oxidative stress.	(1)
	Liver cirrhosis	Prevent ROS-induced cell death and inflammation in the liver.	(53)
	Myocardial infarction	Reduce infarct size in the rat model of myocardial ischemia-reperfusion injury.	(45)
	Organ transplantation	Reduce ischemia-reperfusion injury in the intestinal graft injury.	(46)
Neuroprotection	Parkinson's disease	Reduce dopaminergic neuronal loss in 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine in Parkinson's disease.	(60)
	Cognitive impairment	Ameliorate cognitive impairment in senescence-accelerated mice.	(59)
Inflammation	Septic appendicitis	Reduce early and late pro-inflammatory cytokine levels in the serum and tissues of appendicitis rats.	(57)
	Intestine disease	Reduce ischemia-reperfusion injury in the intestinal graft injury.	(61)
Cancer therapy	Fibrosarcoma	Inhibit tumor invasion of human fibrosarcoma cells.	(48)
	Tongue carcinoma	Inhibit clonal growth of human tongue carcinoma cells.	(48)
	Ehrlich ascites tumor	Erase the ROS in Ehrlich ascites tumor types.	(49)

SOD, superoxide dismutase; HDL, high-density lipoprotein; ROS, reactive oxygen species.

In a previous study, H_2 gas inhalation significantly reduced the number of total cells, eosinophils and lymphocytes in the bronchial alveolar lavage fluid, and increased the level of IL-4, IL-13, TNF-α and chemokine (C-X-C motif) ligand 15. The IL-4 serum level was significantly decreased following inhalation. H_2 gas inhalation markedly upregulated the activity of superoxide dismutase and significantly attenuated the increased level of malondialdehyde and myeloperoxidase in allergic asthmatic mice (55).

H_2 can function as an anti-tumorigenic agent due to its preventive effect against tumor progression and invasion. Accordingly, neutral pH H_2-enriched electrolyzed (NHE) water as an anti-oxidant was previously shown to counteract ROS, inhibiting tumor cell proliferation and invasion together with scavenging of intracellular oxidants. NHE water preferentially inhibited clonal growth of human tongue carcinoma cells, inhibited tumor invasion of human fibrosarcoma cells concurrently with intracellular oxidant repression, and scavenged intracellular oxidant H_2 peroxides (48). Additionally, nano-bubble H_2 water with platinum colloid is more attractive as a novel antitumor regiment, as it reduces the side effects in normal tissues; it was reported that decreased cell numbers, cell shrinkage, cell apoptosis, cell deformation and microvilli on the membrane surface were observed in Ehrlich ascites tumors, as H_2 water erased the ROS that were indispensable for cell growth. These antitumor effects were promoted by combination with hyperthermia at 42˚C (49) (Table I).

H_2 treats disease via an antioxidant effect. In previous studies, the beneficial effects of treatment with H_2 on organ damage were associated with decreased oxidative product levels, increased antioxidant enzyme activities, and reduced early and late pro-inflammatory cytokine levels in the serum and tissue. Brain damage followed by cerebral ischemia/reperfusion (I/R) injuries generated ROS, while the antioxidant effect of H_2 gas inhalation was able to reduce brain, liver and heart ischemia-reperfusion injury, and intestinal graft injury (1,45,46,56). H_2 protected neurons from ischemia and reperfusion, and was efficacious for cerebral infarction. Furthermore, H_2 gas suppressed the progression of hepatic ischemia and reperfusion injury (1). Inhalation of H_2 gas significantly lessened the damage to the organs of septic mice with moderate or severe appendicitis by reducing early and late pro-inflammatory cytokine levels in the serum and tissues, thus increasing the survival rate (57).

Ingestion of H_2 water can eliminate ROS and confer antitumor activity (48); it represents a novel method of H_2 administration and has greater advantages over other forms of antioxidant therapy. Consumption of H_2-enriched water has beneficial effects in clinical practice, including the treatment of atherosclerosis, metabolic syndrome, type 2 diabetes, and cognitive impairment during aging and Parkinson's disease (7,47,58-60).

It was previously reported that HS protected brain ischemia and reperfusion injuries against inflammation and oxidative stress, as well as improving function in a neonatal hypoxia-ischemia rat model (59). HS prevented early pathological changes in acute hepatic injury and was able to prevent ROS-induced cell death and inflammation in the liver by inhibiting the processes of liver cirrhosis and hepatocyte compensatory proliferation (53). Additionally, HS has protective effects on small intestine ischemia/reperfusion injuries (8). These advantages of HS elucidate the clinical potential for preventive and therapeutic anti-oxidative applications (Table I).

Therapeutic and protective function of H_2 in chemotherapy and radiotherapy. Radiotherapy and chemotherapy are major treatment types for cancer. H_2 diffuses rapidly to reduce cytotoxic radicals and inflammation in tissues. H_2 gas or H_2 water has been shown to improve the quality of life (QOL) of patients during chemotherapy via its antioxidant properties. Inhalation of 1% H_2 gas or drinking H_2 water alleviated the nephrotoxicity, mortality and body-weight loss caused by cisplatin. Drinking H_2 water also decreased the level of apoptosis in the kidney. Despite possessing protective effects against cisplatin-induced toxicity, H_2 did not compromise the antitumor effects of cisplatin against cancer cell lines *in vitro* and in tumor-bearing mice *in vivo* (4,61).

It was hypothesized that the majority of radiation-induced symptoms associated with increased ROS and inflammation during radiotherapy would significantly affect the patient's QOL (62). The biological reaction to radiation-induced oxidative stress is reduced by the consumption of H_2-rich water, without antitumor activities being impaired. In one study, consumption of H_2-rich water for 6 weeks during radiotherapy significantly improved the QOL scores of patients with malignant liver tumors, and the levels of reactive oxygen metabolites in the blood were reduced (63).

Overall, H_2 reduces the risk of life style-related oxidative stress by reacting with strong reactive oxygen/nitrogen species in cell-free reactions. It is easily to apply H_2 in cases of oxidative stress, inflammation and tumors. Due to the lack of adverse effects and the high efficacy for the majority of pathogenic statuses involved, H_2 gas, H_2 water and HS are increasingly being accepted as promising candidates for therapeutic approaches. We hypothesize that H_2 gas inhalation and oral administration of H_2 water could protect against inflammation in oxidative stress-related cancer, and thus improve the antitumor effect in the clinical management of cancer.

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