# Molecular Hydrogen as Radioprotective Agent

Subjects: Biophysics

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Molecular hydrogen (H2) has the potential to be a radioprotective agent because it can selectively scavenge •OH, a reactive oxygen species with strong oxidizing power. Animal experiments and clinical trials have reported that H2 exhibits a highly safe radioprotective effect.

Keywords: molecular hydrogen ; radiation-induced damage ; medical application ; radioprotective agent ; non-DNA target ; intracellular response ; oxidation ; inflammation ; apoptosis ; gene expression

## 1. Introduction

Ionizing radiation (radiation) is commonly used for medical diagnosis and cancer treatment. Amongst these uses, radiation therapy is known to be one of the most effective treatments for cancer. It is difficult to control radiation-induced damage with conventional radiation therapy; therefore, intensity-modulated radiation therapy (IMRT) has recently been used [1]. However, various radiation damages can also occur with IMRT. The harmful effects of radiation on the living body can be classified into direct and indirect effects. Direct effects are caused by the direct absorption of radiation energy into nucleic acids (DNA), proteins, and lipids [2][3][4][5]. Indirect effects are caused by free radicals, such as hydroxyl radicals (•OH), and molecular products generated in the process of water radiolysis [2][3][4][5]. In addition to the direct damage on DNA, secondary damages to non-DNA targets cannot be ignored because low-dose radiation damage is mainly caused by these indirect effects. Secondary damages include oxidation, inflammation, apoptosis, and effects on gene expression related to intracellular responses.

Medical applications of $H_2$ were first reported by Dole et al. in 1975 [6]. They reported that the inhalation of hyperbaric $H_2$ caused a marked regression in squamous cell carcinoma in mice induced by UV radiation. With the exception of a few studies, however, $H_2$ has not been extensively studied for medical applications. In 2007, Ohsawa et al. reported that the inhalation of $H_2$ gas ameliorated ischemia-reperfusion injury in a rat model with cerebral infarction [7]. In this paper, they showed that $H_2$ is an antioxidant that selectively reduces highly oxidative reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as •OH and peroxynitrite ($ONOO^-$), respectively, but does not react with other ROS such as superoxide anions ($O_2^-$) and hydrogen peroxide ($H_2O_2$). However, we need to reacquaint ourselves with the pioneering paper on the antioxidant effects of $H_2$ by Yanagihara et al. [8], published two years before the study by Ohsawa et al. [7]. They reported that the ingestion of neutral $H_2$-rich water produced by water electrolysis alleviated liver damage in rats induced by chemical oxidants. These papers have led to global research on the medical applications of $H_2$. We recently showed that although $H_2$ is an inactive substance, compared to other antioxidants, it is the only molecule with mitochondrial permeability and an ability to reduce •OH, which is promising for future medical applications [9][10]. Selective •OH scavengers may have potential medical applications as radioprotective agents. The efficacy of $H_2$ against various diseases and disease models have been reported, and there are now more than 1000 papers on the medical applications of $H_2$, including 80 clinical trials.

The use of a safer and more effective radioprotective agent in clinical practice is of great importance. Many drugs have been evaluated in a variety of ways. For instance, the radioprotective effects of many synthetic and natural compounds have been investigated. Cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1, IL-12, and natural compounds such as vitamin C, vitamin D, vitamin E, melatonin, succinate, alpha lipoic acid, and N-acetyl cysteine (NAC) have been reported to exhibit radioprotective effects in animal studies [11][12][13][14][15][16]. Many drugs are in various stages of evaluation, but many are far from being ideal radioprotective agents. However, amifostine (WR2721) has been developed as a radioprotective agent with free radical scavenging properties, such as against •OH, and is the only radioprotective agent approved by the U.S. FDA for clinical use [17][18][19][20][21][22]. However, this drug has not been widely considered as a useful radioprotective agent of choice because of its dose-dependent side effects such as hypotension, nausea, and vomiting [20]. Therefore, it is not an exaggeration to say that there are no clinically usable radioprotective agents with high efficacy and few side effects.

On the other hand, $H_2$ has been reported to show radioprotective effects in many animal studies, and because $H_2$ has also shown to have no side effects in clinical studies, it may be a clinically reliable radioprotective agent. As for its radioprotective effects in clinical trials, Kang et al. reported that $H_2$-rich water improved the quality of life (QOL) of liver cancer patients receiving radiotherapy [23]. We recently reported that the inhalation of $H_2$ gas reduced bone marrow damage in end-stage cancer patients receiving IMRT without compromising the antitumor effects [24][25].

## 2. Biological Effects of Radiation

Exposure to radiation induces many detrimental effects, including genetic mutation, cell death, and carcinogenesis. The most radiation-sensitive organs are in the hematopoietic, digestive, reproductive, and skin systems, consisting of those with high cell proliferation [26][27]. Radiation damage occurs at the cellular level, either directly or indirectly. Thus, harmful effects of radiation on living organisms can be divided into direct and indirect effects [2][3][4][5].

Direct damages occur when radiation energy is directly absorbed by the target molecule, DNA. This direct action excites or ionizes the DNA, making it unstable because of the extra energy that is accumulated. In the process of releasing this extra energy, the ionization of DNA directly breaks chemical bonds in the DNA [2][3][4][5]. On the other hand, there are also indirect effects, which occur when molecules other than the target absorb radiation energy and produce active bodies, such as radicals, which eventually react with the target molecule. In aqueous solutions, radiation is first absorbed by water molecules to produce radicals and molecular products such as •OH, hydrogen radicals (H•), hydration electrons ($e^-_{aq}$), $H_2$, and $H_2O_2$ [4] (Figure 1). These active substances then move through the water and induce chemical reactions with DNA.

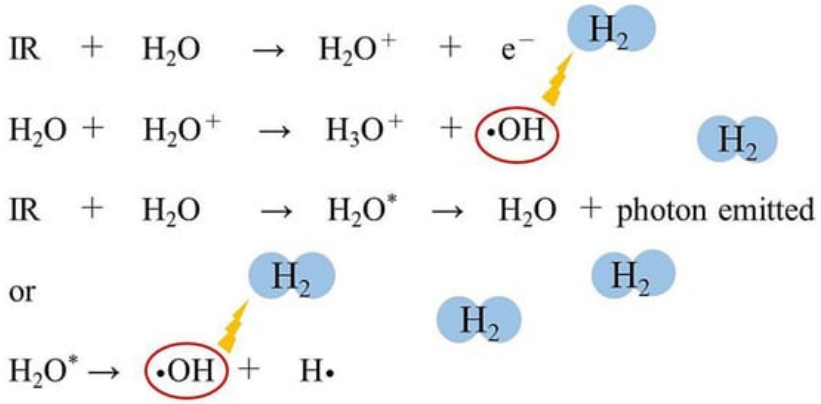


**Figure 1.** Ionizing radiation (IR) acts on water, a component of living organisms, ionizing and exciting the water molecules. Short-lived radical-cations ($H_2O^+$) are very unstable and decompose to produce hydroxyl radicals (•OH) and hydronium ($H_3O^+$). Electronically excited water molecules ($H_2O^*$) cleave to produce •OH and hydrogen radicals (H•). Molecular hydrogen ($H_2$) can selectively eliminate the •OH by the following chemical reaction: $•OH + H_2 \rightarrow H• + H_2O$.

In other words, radiation acts on water, which is a constituent of cells, and causes the ionization and excitation of water molecules. The water molecule ion ($H_2O^+$) is highly unstable and produces •OH and hydronium ($H_3O^+$). Excited water molecules ($H_2O^*$) cleave to produce •OH and H•. The electrons from the water molecules are trapped between other water molecules and produce $e^-_{aq}$ [4] (Figure 1). Approximately 60–70% of DNA damage is induced by the indirect action of free radicals [3].

The •OH produced during water radiolysis causes the oxidation of DNA, lipids, amino acids, and saccharides, and the oxidation of these biological materials leads to the formation of various secondary free radicals [26][27]. DNA is one of the major targets of free radicals. The compound 8-hydroxydeoxyguanosine (8-OHdG) is produced by •OH from deoxyguanosine in DNA and is considered to be one of the biomarkers of DNA damage and carcinogenesis [28][29]. Structural changes in proteins are induced by •OH and other free radicals, leading to functional changes in proteins [30]. Lipids in cell membranes are one of the major targets of •OH and other free radicals. Lipid peroxides such as malondialdehyde (MDA) and 2-thiobarbituric acid reactive substances (TBARS) are indicators of lipid damage [31]. These lipid peroxides induce changes in cell membrane permeability [32].

On the other hand, as an indirect effect of radiation, the molecular products generated by water radiolysis, such as $e^-_{aq}$, $H_2$ and $H_2O_2$, also cause chemical reactions in biomolecules [4]. In particular, low doses of radiation induce modifications of intracellular molecules, leading to effects on oxidation, inflammation, apoptosis, and gene expression. It has been reported that there is also a bystander effect, in which information can be transmitted from exposed cells to unexposed cells, transferring radiation damage to these unexposed cells [33], as well as an abscopal effect, in which the local radiation therapy of a tumor can also shrink distant untreated tumors [34]. The involvement of radiation in cellular

responses and the immune system has also been considered. Furthermore, the effects of radiation on epigenetic effects, i.e., changes in gene expression or cellular phenotypes that are inherited after cell division without changes in DNA sequence, have also been pointed out [35].

## 3. Radioprotective Effects of $H_2$ in Animal Models

As for the radioprotective effects of $H_2$ in animal models, protective effects on cognitive function, the immune system, lungs, heart, digestive organs, hematopoietic organs, testis, skin, and cartilage disorders have been reported [36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53]. An inhibitory effect on thymic lymphoma caused by radiation has also been reported [54]. The following is a summary of the literature that reports specific examples of the protective effects of $H_2$ against different radiation disorders (Table 1).

**Table 1.** Radioprotective effects of $H_2$ in cell free system, cells, animal models and clinical trials.

| Damages/Damage Models | Species/Cells | Effects of $H_2$ | Ref. No. |
|---|---|---|---|
| Cell-free system | | •OH is produced by the Fenton reaction and water radiolysis, and it was reduced by $H_2$. | [48] |
| Cognitive impairment | Rats | Radiation-induced cognitive dysfunction was protected by $H_2$-rich water. | [36] |
| Immune dysfunction | AHH-1 cells | Pretreatment with $H_2$-rich PBS prior to radiation reduced the levels of MDA and 8-OHdG. | [37] |
| | AHH-1 cells | Pretreatment with $H_2$-rich saline increased the viability of AHH-1 cells and inhibited apoptosis. | [38] |
| | AHH-1 cells | Pretreatment with $H_2$-rich medium reduced •OH induced by radiation. | [39] |
| | Mice | $H_2$-rich saline protected immunocytes from radiation-induced apoptosis. | [39] |
| | Mice | $H_2$-rich saline protected against radiation-induced immune dysfunction. | [40] |
| Lung damage | A549 cells | $H_2$-rich PBS suppressed ROS production, and improved oxidative stress and apoptosis markers. | [41] |
| | Mice | $H_2$ gas inhibited not only acute lung damage, but also chronic lung damage. | [41] |
| Myocardial damage | Mice | $H_2$-rich water protected against radiation-induced myocardium damage. | [42] |
| | Rats | $H_2$-rich water protected against radiation-induced myocardium damage. | [43] |

| Damages/Damage Models | Species/Cells | Effects of $H_2$ | Ref. No. |
|---|---|---|---|
| Gastrointestinal damage | HIEC | $H_2$-rich PBS inhibited apoptosis and increased the cell viability of HIEC. | [37] |
| | Mice | $H_2$-rich saline protected against radiation-induced gastrointestinal disorders. | [38] |
| | Mice | $H_2$ water ameliorated radiation-induced gastrointestinal toxicity. | [44] |
| | IEC-6 cells | $H_2$-rich medium improved survival and inhibited ROS production. | [45] |
| | Mice | $H_2$-rich saline improved mouse survival and intestinal mucosal damage and function. | [45] |
| Hematopoietic cell injury | Mice | $H_2$-rich water ameliorated radiation-induced hematopoietic stem cell injury. | [46] |
| Spermatogenesis and hematopoiesis disorders | Mice | $H_2$-rich saline protected spermatogenesis and hematopoietic functions of irradiated mice. | [47] |
| Testicular damage | Rats | $H_2$-rich saline protected against radiation-induced testicular damage. | [49] |
| Skin damage | HaCaT cells | $H_2$-rich medium protected HaCaT cells from radiation injury by improving the survival rate. | [50] |
| | Rats | $H_2$-rich saline reduced the severity of dermatitis, accelerated tissue recovery, and inhibited weight loss. | [50] |
| | Rats | Prior inhalation of $H_2$ gas mitigated radiation-induced skin damage. | [51] |
| | Rats | $H_2$-rich water promoted wound healing in radiation-induced skin lesions. | [52] |
| Cartilage damage | BMSC | $H_2$-rich medium increased cell viability and differentiation potential. | [53] |
| | Rats | $H_2$-rich saline protected against the osteonecrosis of jaw cartilage induced by radiation. | [53] |
| Thymic lymphoma | Mice | $H_2$-rich saline protected against radiation-induced thymic lymphoma. | [54] |
| Impaired QOL | Humans | $H_2$-rich water improved side effects of poor QOL by radiation therapy. | [23] |

| Damages/Damage Models | Species/Cells | Effects of $H_2$ | Ref. No. |
|---|---|---|---|
| Bone marrow damage | Humans | $H_2$ gas inhalation protected bone marrow damage in cancer patients receiving IMRT. | [24][25] |

$H_2$: molecular hydrogen; •OH: hydroxy radical; AHH-1: human lymphocyte cell; MDA: malondialdehyde; 8-OHdG: 8-hydroxydeoxyguanosine; ROS: reactive oxygen species; HIEC: human intestinal crypt cell; IEC-6: intestinal crypt epithelial cell; HaCaT: human keratinocyte cell; BMSC: marrow-derived mesenchymal stem cell; QOL: quality of life; IMRT: intensity-modulated radiation therapy; Ref.: references.

## 4. Radioprotective Effects of $H_2$ in Humans

### 4.1. Improvement of Decreased QOL in Cancer Treatment

Cancer patients who have been irradiated often experience fatigue and decreased QOL. Radiation damage is attributed to radiation-induced oxidative stress and inflammation. Therefore, Kang et al. investigated the effects of $H_2$-rich water on the improvement of QOL in patients with liver cancer who received radiation therapy [23]. The study was a randomized controlled trial with 49 patients. The placebo group ($n$ = 24) ingested placebo water, and the $H_2$ group ($n$ = 25) ingested $H_2$-rich water (1.2 ppm) for six weeks each.

The results revealed that the $H_2$ group showed an improvement in the index related to oxidative stress compared to the placebo group. In addition, compared to the placebo group, the $H_2$ group showed a significant improvement in QOL scores such as anorexia and taste disorder. Assuming that •OH is produced during and after irradiation and that $H_2$ scavenges it, the antitumor effects of radiation may be impaired by $H_2$. Therefore, Kang et al. investigated the effects of a placebo and $H_2$ on tumor response. The results showed that the tumor responses of the placebo and $H_2$ groups were similar, suggesting that the intake of $H_2$-rich water did not impair the antitumor effects of radiation. They reported that $H_2$-rich water improves the side effects of poor QOL without compromising the antitumor effects [23] (Table 1).

### 4.2. Improvement of Bone Marrow Damage in Cancer Treatment

Compared to conventional radiotherapy, IMRT has been developed to reduce side effects and is used clinically, but the reductions in side effects are insufficient. Therefore, we investigated the efficacy of $H_2$ gas inhalation on bone marrow damage in end-stage cancer patients receiving IMRT [24][25]. The study was conducted as a retrospective observational study of 23 patients. Patients received IMRT for 1–4 weeks according to the irradiation protocol. Patients in the control group ($n$ = 7) received 30 min of mild-pressure (1.35 atm) air inhalation in a chamber after each IMRT. On the other hand, patients in the $H_2$ group ($n$ = 16) also inhaled mild-pressure (1.35 atm) air and 5% $H_2$ gas for 30 min in the chamber. The number of irradiations and total exposure doses of radiation in the control and $H_2$ groups were almost the same. When bone marrow damage was compared before and after IMRT, the control group showed a significant decrease in WBC ratio and PLT ratio, while the $H_2$ group significantly improved these decreases seen in the control group. Tumor response to IMRT in the control and $H_2$ groups was similar, and the inhalation of $H_2$ gas improved bone marrow damage without compromising the antitumor effects in cancer patients. Although this study examined the effects of mild-pressure $H_2$ gas inhalation on radiation damage in cancer patients, we confirmed that the inhalation of $H_2$ gas equivalent to mild-pressure $H_2$ gas (1.35 times) in a normal pressure environment had the same radioprotective effects. Inhalation of $H_2$ gas may be a new therapeutic strategy for bone marrow damage induced by IMRT [24][25] (Table 1).

## 5. Mechanism of the Radioprotective Effects of $H_2$

As described in the previous section, there are both direct and indirect effects of radiation. Direct effects are damages to biomolecules such as DNA [2][3][4][5]. Indirect effects include oxidative damages caused by •OH, which is produced during water radiolysis, where •OH causes oxidation of various biological substances, and the oxidation of these biological substances leads to the generation of further secondary free radicals [2][3][4][5]. $H_2$, on the other hand, is an inert substance, but it can protect living organisms from radiation-induced oxidative damage by selectively scavenging the large amounts of •OH generated in the living body. Although the radioprotective effects of $H_2$ have been confirmed in the past literature, there are few that report the detailed mechanisms of $H_2$ [36][37][38][39][40][41][42][43][44][45][46][47][48][49][50][51][52][53][54]. In this section, we will discuss the possible mechanisms of the radioprotective effects of $H_2$ from these reports.

### 5.1. Antioxidant Effects

$H_2$ selectively scavenges •OH, which is produced in large quantities during irradiation, and the scavenging of •OH can be considered as a direct effect of the radioprotective effects of $H_2$. Chuai et al. showed that •OH is produced by the Fenton reaction and water radiolysis in cell-free systems, and it can be reduced by $H_2$ [48]. Yang et al. [39], Zhang et al. [46] and Chuai et al. [48]. showed that $H_2$ significantly reduces the •OH produced by radiation in in vitro and in vivo experiments. On the other hand, at the level of total ROS, Zhao et al. [40][54], Terasaki et al. [41], Qiu et al. [45] and Chen et al. [53] showed that $H_2$ significantly reduces radiation-induced ROS production in in vitro and in vivo experiments, suggesting that the radioprotective effects of $H_2$ involve the selective elimination of •OH by $H_2$.

On the other hand, some studies have evaluated 8-OHdG as an indicator of DNA oxidation, MDA as an indicator of lipid oxidation, and both SOD and GSH activities as indicators of free radical scavenging systems to maintain the redox balance. Namely, the reduction in 8-OHdG and MDA levels by $H_2$ has been reported by many authors [36][37][38][42][49][50][51][52][54]. In addition, the increase in SOD and GSH levels by $H_2$ has been reported by many authors [37][40][42][49][50][52]. From these reports, we can assume that the radioprotective effect of $H_2$ is largely due to the inhibition of oxidative stress.

We need to consider the mechanism of radioprotection by $H_2$. •OH reacts non-specifically with many substances. The reaction rate of •OH with $H_2$ in aqueous solution is much slower than with DNA, amino acids, sugars, and GSH [55]. However, Ohsawa et al. [7], Terasaki et al. [41] and Chuai et al. [48] reported that the amount of •OH in the medium produced by the Fenton reaction was reduced by $H_2$, using electron spin resonance (ESR) methods. They also reported that the fluorescence of •OH was attenuated by $H_2$ in an experiment using hydroxyphenyl fluorescein (HPF), a specific fluorescent dye for •OH [7][41][48]. Theoretically, for $H_2$ to react with •OH, a higher concentration of $H_2$ is required in the nucleus than for other solutes. Although future detailed studies are needed to resolve these contradictions, in aqueous solutions containing a large amount of solute, such as culture medium and buffer solutions, it may be necessary to consider factors, such as high intracellular diffusion rates of $H_2$. It is also possible that the reaction rate of •OH and $H_2$ is different in the nucleus.

If we assume that the only mechanism of the radioprotective effects of $H_2$ is the selective elimination of •OH, the antitumor effects of radiation may be attenuated. However, in both Kang et al. and our reports of clinical trials examining radioprotective effects in cancer patients, $H_2$ did not attenuate the antitumor effects by radiation [23]. Kang et al. showed that $H_2$ improved the oxidative stress-related index, suggesting that the radioprotective effects of $H_2$ may be due to its antioxidant effect, but that other biological defense systems, including hormones and enzymes involved in radiation protection, may also be at work [23]. We also reported that the radioprotective effects of $H_2$ may involve not only the direct scavenging of •OH, but also indirect effects through the activation of host-mediated antioxidant and anti-inflammatory systems [24][25]. The possibility that the radioprotective effects by $H_2$ involves an indirect effect, rather than a direct effect, on •OH is supported by the study schedule in which patients inhaled $H_2$ gas after IMRT, but not before.

### 5.2. Anti-Inflammatory Effects

Chronic inflammation caused by radiation exposure is closely related to oxidative damage. Yahyapour et al. reported in their review that the long-term effects of radiation exposure accidents include increased risk of cancer, but also many inflammation-related diseases and autoimmune diseases [56]. They reported that cytokines including IL-1, TNF-α and interferon-γ (IFN-γ) play an important role as indicators of chronic inflammatory damage and oxidative damage after radiation exposure [56]. Indeed, in a report by Kura et al. that examined the protective effect of $H_2$ on a rat model of myocardial injury induced by irradiation, $H_2$ significantly reduced MDA and TNF-α levels in the myocardium [43]. Zhou et al., who examined the radioprotective effects of $H_2$ on a rat skin damage model, showed that $H_2$ significantly reduced MDA and IL-6 levels in the damaged skin [52]. In a recent review, we reported that •OH generated in mitochondria induces oxidative stress in mitochondrial DNA (mtDNA), and that oxidized mtDNA triggers a cascade of inflammatory cytokine release from nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) to IL-1 and IL-18 [57]. The mechanism of $H_2$-induced amelioration of chronic inflammatory diseases may involve the scavenging of •OH generated in mitochondria [57].

### 5.3. Anti-Apoptotic Effects

Apoptosis, or cell death caused by radiation, is also closely related to oxidative damage and inflammation. It has been reported in the literature that $H_2$ has a radioprotective effect on radiation-induced cell or animal models through its anti-apoptotic effects [37][39][40][41][45][48][49][50][51]. The TUNEL assay and the quantification of caspases (caspase-3, caspase-8, and caspase-9), which are essential proteases for apoptosis, have been used to evaluate the anti-apoptotic effects of $H_2$ on radiation injury models. It can also be assessed by examining the expression of Bcl-xL and Bcl-2, proteins that inhibit apoptosis, and Bax, a protein that induces apoptosis. For example, Watanabe et al. measured the percentage and staining level of apoptotic keratinocytes in irradiated skin by TUNEL and 8-OHdG staining in an experiment to evaluate the

efficacy of $H_2$ on a radiation-induced skin damage model and showed that these were reduced by $H_2$ [51]. In addition, in cell experiments using IEC-6, an intestinal crypt epithelial cell line, Qiu et al. showed that $H_2$ inhibits mitochondrial depolarization, cytochrome c release, and the activities of caspase-3, caspase-9, and PARP [45]. They further reported that $H_2$ exerts an anti-apoptotic effect by recovering from the decreased expression of Bcl-xl and Bcl-2 and inhibiting the increased expression of Bax [45].

### 5.4. Regulation of Gene Expression

Nuclear factor erythroid 2-related factor (Nrf2), an endogenous antioxidant regulator, is closely correlated with the enhancement of SOD and catalase (CAT). In addition, Nrf2 has biological protective effects such as enhancing heme oxygenase-1 (HO-1) activity, which exhibits cytoprotective effects such as anti-inflammation and antioxidation. Many studies have reported that $H_2$ promotes the expression of Nrf2 and bioprotective responses through HO-1 and other bioprotective proteins [58][59][60]. Xiao et al. examined the mitigating effects of $H_2$ on gastrointestinal disorders in a model created by irradiating mice [44]. They reported that $H_2$ down-regulated MyD88 expression in a microarray analysis of the small intestine [44]. Furthermore, Kura et al. reported experimental results showing that $H_2$ regulates the expression of miRNAs involved in myocardial oxidation, hypertrophy, or fibrosis in a rat model with myocardial injury induced by radiation [43]. These results suggest that $H_2$ not only has a direct radioprotective effect by scavenging •OH, but also indirect effects by regulating gene expression and exhibiting antioxidant, anti-inflammatory, and anti-apoptotic effects (Figure 2).

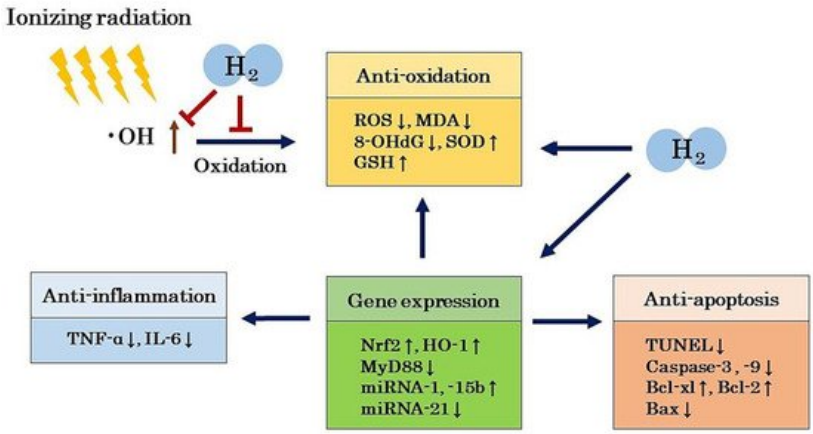


**Figure 2.** $H_2$ not only has a direct radioprotective effect by scavenging •OH, but also indirectly by regulating gene expression, exhibiting antioxidant, anti-inflammatory, and anti-apoptotic effects, which may lead to radioprotective effects. $H_2$: molecular hydrogen; •OH: hydroxy radical; ROS: reactive oxygen species; GSH: glutathione; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; Nrf2: nuclear factor erythroid 2-related factor; HO-1: heme oxygenase-1; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; Bcl-xl: B-cell lymphoma-extra-large; Bcl-2: B-cell lymphoma-2; Bax: BCL2-associated X protein; MyD88: myeloid differentiation factor 88; miRNA: microRNA.

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