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Molecular Hydrogen as a Novel Therapeutic Tool in Situations of Increased Production of Free Radicals

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Abstract

Excessive production of oxygen and nitrogen radicals has been regarded as a causative common denominator of many pathological processes. The increase in the rate of radiation-induced heart disease has emphasized the need to seek for new therapeutic targets to mitigate the negative impact of radiation on the heart.

Scavenging of free radicals can act preventively or therapeutically. A number of substances that preferentially react with free radicals can serve as scavengers, thus increasing internal capacity/activity of endogenous antioxidants.

Our results demonstrate that in the hearts of rats injured by irradiation H2 administration led to a significant decrease in MDA and TNF-α levels as well as to miRNAs return almost to control levels.

Molecular hydrogen (H2) reacts with strong oxidants, such as hydroxyl and nitrosyl radicals in cells, allowing to use its potential for preventive and therapeutic applications in situations with excessive free radicals formation. H2 rapidly diffuses into tissues and cells without affecting signaling reactive species. H2 also reduces oxidative stress by regulating gene expression and functions as an anti-inflammatory and anti-apoptotic agent.

Keywords: Molecular hydrogen; miRNA; ROS; Radiation; Malondialdehyde; Inflammation

Introduction

There are numerous situations with established excessive production of ROS, like radiation, ischemia and reperfusion, inflammation, rheumatoid arthritis, cancer, diabetes, and number of other pathologies including aging [1].

Oxidative stress occurs due to the imbalance between the production of reactive oxygen and nitrogen species and the capability of innate biological systems to eliminate reactive intermediates (Figure. 1).

Unlike superoxide, which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction. Mechanisms of scavenging peroxylradicals to protect cellular structures includes endogenous antioxidants such as melatonin and glutathione, and dietary antioxidants such as mannitol and vitamin E.

Formation of peroxynitrite*in vivo* has been ascribed to the reaction of the free radical superoxide with the free radical - nitric oxide as follows:

$$\cdot O^{-2} + \cdot NO \rightarrow ONO^{-2} \text{ and } H_2O_2 + NO^{-2} \rightarrow ONOO^- + H_2O.$$

In vivo, peroxynitrite generation represents a crucial pathogenic mechanism in conditions such as stroke, myocardial infarction and chronic heart failure.

Oxidative stress and/or nitrosative stress caused by H_2O_2 and •NO (resulting in peroxynitrite formation) induces enzymes involved in anti oxidation and oxidative damage [2,3]. Hydroxyl and nitrosyl radicals represent the major cause of the destruction of biomolecules either by a direct reaction or by triggering a chain reaction of free radicals.

Scavenging of free radicals acts preventively or therapeutically. A number of substances serve as scavengers, protecting cells and tissues against oxidative damage. Early research identified sulfur-containing antioxidants, later on, low molecular weight antioxidants, ascorbic acid, tocopherols, polyphenols, and thiols such as glutathione were used. Recently, gene therapy-mediated over

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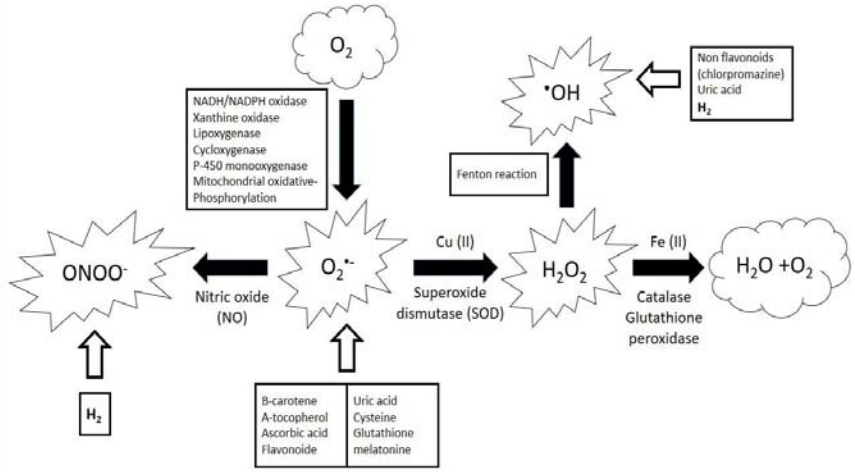

Figure 1: Production of superoxide, peroxynitrite and hydroxyl radicals and some of specific scavangers *in vivo*.

expression of the SOD using recombinant adeno-associated viral vectors and administration of manganese superoxide dismutase-plasmid liposomes (MnSOD-PL) are studied [4].

To attenuate the harmful effects of oxidative stress and ROS, one can eliminate them by medicinal gases (such as carbon monoxide (CO), hydrogen sulphide (H_2S), and molecular hydrogen (H_2), or induce ROS-resistant proteins and antioxidant enzymes to antagonize oxidative stress [5].

Many attempts have been made to inhibit ROS production and to limit the extent of reperfusion injury. However, the administration of ROS scavengers at the time of reperfusion has brought conflicting results that can be partially explained by the dual role of ROS in I/R [6], since ROS play an important role as signaling molecules as well.

Molecular Hydrogen (H_2)

Therapeutic effects of molecular hydrogen for a wide range of disease models and human diseases have been investigated thoroughly since 2007 and the positive effects were reported in essentially all organs covering many disease categories and disease models [7,8].

One of the main effects of H_2 is hydroxyl radicals scavenging by reaction: $H_2 + \cdot OH = H_2O + H\cdot$ and followed by $H\cdot + O_2^- = HO_2^-$ reaction [9,10]. This way H_2 is able to provide cytoprotection by increasing other antioxidant enzymes such as superoxide dismutase and catalase [11].

H_2 is superior to some antioxidants since it can selectively scavenge the deleterious hydroxyl radical while preserving other important reactive oxygen and nitrogen species for signaling. Emerging evidence has demonstrated the pleiotropic therapeutic effects of molecular hydrogen in a variety of animal disease models as well as in many human diseases [12,13,14,15].

Ways of H_2 applications

Hydrogen can be applied in form of hydrogen-rich water, hydrogen gas, by administration of hydrogen-rich saline and/or by dropping H_2-saline into the eyes. Hydrogen water is mostly given *ad libitum*. Hydrogen gas of less than 4 % is given by inhalation.

Hydrogen-rich water (prepared by different procedures like dissolving molecular hydrogen into water under high pressure or dissolving hydrogen generating tablets in water) generally shows a more prominent effect than hydrogen gas, although the amount of hydrogen taken up by hydrogen water is ~100 times less than that given by hydrogen gas [16].

H_2 reduces oxidative stress not only by direct reactions, with strong oxidants, but also indirectly by regulating various gene expressions and modulating cell signaling [7,8,17].

Effects of hydrogen on various diseases can be summarized and have been attributed to four major molecular mechanisms:

1 - specific scavenging activity of hydroxyl radicals

2 - scavenging activity of peroxynitrite

3 - alterations of gene expressions

4 - signal modulating activities.

Methods

As a source of free radicals, we used radiation that interacts with the water present in the cells, resulting in its hydrolysis and formation of free radicals (especially hydroxyl OH).

Wistar rats at the age of 12 weeks, weighing approximately 250 g, were irradiated with a single dose of 25 Gy (6-7 Gy / min) and/or 10 Gy (4-5 Gy / min.) focused on the mediastinal area. Therapeutic application of molecular hydrogen has been performed by different delivery methods including inhalation, drinking hydrogen-rich water prepared by dissolving molecular hydrogen into water under high pressure and/or infusion of hydrogen-saturated solutions. Concentrations of malondialdehyde (MDA) in rat serum were determined by spectro photometric method using a commercially available TBARS assay kit (Cayman Chemical, Michigan, USA). TNF-α expression was analyzed from the left ventricle tissue using the Western blot method. The effect of molecular hydrogen in irradiated rat myocardium based on the changes of expression of miRNA 1, 15b and 21 levels were measured by RT-qPCR.

Results

The results of our experiments in irradiated rats demonstrated that $H_2$was followed by a decrease in MDA and TNF-α levels as well as miRNAs return almost to control levels.

Discussion

Possible molecular mechanisms of H_2 action

Although the underlying mechanisms were initially proposed as selective extinctions of hydroxyl radical and peroxynitrite, the signaling pathway regulation effect of molecular hydrogen by modulating a various molecules expressions/activities, gene expression and microRNA may also account for the ultimate effects on ischemia-reperfusion injury, infammation, apoptosis, metabolic disorders, allergy, radiation injury, dementia as well as aging [8,17-22].

H_2 was shown to exhibit multiple functions including anti-inflammatory, anti-apoptotic, anti-allergic, and antioxidant properties, as well as regulation of cell differentiation and involvement in energy metabolism. Molecular hydrogen modulates various signal transduction pathways and the expression of many genes [14,23] and regulates more components of MAPK signal transduction pathways [24]. It was shown that H_2 modulates Ca^{2+} signal transduction and regulates gene expression [15].

As demonstrated in our experiments, molecular hydrogen modulates in irradiated rats miRNA expression of miR-1, miR-15b and miR-21 [17] and returns the values of these miRNAS to control levels.

As shown in our experiments, H_2 can protect myocardium from radiation-induced injury and decrease lipid peroxidation of myocardium demonstrated by decrease of malondialdehyde (MDA) levels, and increase myocardial endogenous antioxidants *in vivo*. Results are in consent with work of [25] who have investigated the cardio protective properties of molecular hydrogen by pre-treating mice with hydrogen-rich water prior to irradiation where 90% of the mice without hydrogen-rich water pretreatment died, while 80% of the mice with hydrogen survived. Hydrogen pre-treatment proved to exert cardio protective properties by decreasing malondialdehyde (MDA) and eight-hydroxydeoxyguanosine (8-OHdG) levels as opposed to the non-treatment counterparts, which showed increased levels of those oxidative stress markers.

It was shown that $ONOO^-$ has the potential to regulate gene expressions through the nitration of factors involved in transcriptional regulation [26]. Drinking hydrogen water suppresses the nitration of proteins; thus, it is possible that small amount of H_2 consumed by drinking hydrogen-rich water influences nitration in *in vivo* experiments and results in regulatory as well as anti-oxidative effects. These results also agree with the finding of Cardinal et al. that H_2 suppressed the nitration of proteins [27].

Hydrogen was shown to act also by inhibiting LPS/IFN γ-induced nitric oxide production in macrophages, resulting in decreased inflammation. Hydrogen was able to modulate signal transduction, which suggests that hydrogen is a signal modulator [28]. Shi has proposed that molecular hydrogen may be able to affect signal transduction by interacting with metallo proteins, since metal ions can be a possible binding site for hydrogen [29]. H_2 can reveal cytoprotection by preventing the activation of caspase-3 and reduce apoptosis [30].

As shown in our experiments, molecular hydrogen is able to inhibit inflammation caused by irradiation and the TNF-α/NF-κB pathway [31] as well as the Ras-ERK1/2-MEK1/2 and PI3K/Akt pathways [32, 33]. Huang demonstrated that NF-κB is activated by hydrogen administration and this can be correlated with the elevated levels of the anti apoptotic protein Bcl-2. The protective effects of hydrogen was reversed by a chemical inhibitor of NF-κ B[34].In consent with this study is a work of showing that treatment with hydrogen rich saline restored expression of the anti-apoptotic factor, Bcl-2, and decreased protein expression of Bax, a pro-apoptotic factor [35].

A study performed by Sun, demonstrated that hydrogen-rich saline was able to be effective against myocardial ischemia and reperfusion injury in rats. The findings of the study were that there were significant decreases in infarct sizes, MDA concentrations and 8-OHdG levels in at risk areas, as well as evidence that hydrogen was able to inhibit the caspase-3, and attenuate apoptosis [30].

Zalesak published results of myocardial ischemia/reperfusion experiment where H_2 saturated Krebs–Henseleit solution was administered in a setting of myocardial hypoxic post conditioning and significantly decreased infarct size. Application of hydrogen appears to be beneficial in a setting of hypoxic post conditioning and to facilitate its cardio protective efficiency [22].

As demonstrated in our experiments, in irradiated rats, molecular hydrogen modulates miRNA expression of miR-1, miR-15b and miR-21 [36] and miR-9, miR-21, and miR-199 [37,38], and possibly many others. Analysis of miRNA profiles of hippocampal neurons during I/R injury revealed that hydrogen inhibits I/R-induced expression of the miR-200 family by reducing ROS production, which has led to suppression of cell death [38]. However, modulation of miRNA expression cannot solely explain all the biological effects mediated by hydrogen. Mechanisms underlying modulated miRNA expression remain to be elucidated.

miRNAs show different expression patterns in the normal and diseased heart and some data indicate that miRNA expression may represent an efficient diagnostic marker of heart disease [39]. Ikeda showed that the specific class of heart disease can be predicted using miRNAs expression with the probability of 69% [40]. As a great advantage for the use of miRNAs as biomarkers or potential therapeutic targets is its stability under many conditions [41].

In this regard microRNAs (miRNAs) have received considerable interest. MiRNAs regulate post-transcriptional gene expression by their ability to target various mRNA sequences because of their imperfect pairing with mRNAs. It has been recognized that miRNAs modulate a diverse spectrum of cardiac function with developmental, patho physiological, and clinical implications. This makes them promising potential targets for diagnosis and treatment [17].

Conclusion

Targeted application of molecular hydrogen (H_2) as one of the potent scavengers may be used in the prevention and treatment of ROS-related diseases.H_2 reacts with strong oxidants, such as hydroxyl and/or nitrosyl radicals that makes it possible to utilize its potential for preventive and therapeutic applications. H_2 is able to reduce oxidative stress also by regulating gene expression, and acts as an anti-inflammatory and anti-apoptotic agent.

miRNAs represent potential targets for modulation and manipulation to be used as biomarkers and therapeutic instruments.

Application of molecular hydrogen in situations with excessive production of free radicals and, in particular, hydroxyl and

nitrosyl radicals increases internal capacity/activity of endogenous antioxidants. It is simple and efficient method that deserves special attention.

H_2 may be novel effective clinical tool for treating oxidative stress-related diseases.

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