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· Expert forum ·

Hydrogen - an endogenous antioxidant in the body

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[**ABSTRACT**] Recently, Ohsawa et al. provide evidence that inhaled hydrogen gas has antioxidant and antiapoptotic activities that protect the brain and liver against ischemia-reperfusion injury. In fact, there is some endogenous hydrogen produced by intestinal bacteria within animal and human. The concentration of hydrogen in some mice tissues reached the antioxidant effect in their paper demonstrates, so we think that hydrogen should be an endogenous antioxidant in the body.

[**KEY WORDS**] hydrogen; antioxidants; reperfusion injury

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The most lightweight gas diatomic hydrogen, a major component of interstellar space and the fuel that sustains the stars, is rare on Earth. Hydrogen gas directly and violently reacts with oxidizing elements such as chlorine and fluorine and is highly flammable, a property evident in the 1937 Hindenburg zeppelin fire and its use as propellant fuel for the space shuttle. Hydrogen gas is highly diffusible and reacts with hydroxyl radical to produce water[1-2].

Ohsawa et al. set out to see if hydrogen gas could be used as a therapeutic mitochondrial antioxidant to neutralize oxidative stress after ischemia-reperfusion injury[2]. To induce the production of reactive oxygen species, the authors treated cultured cells with a mitochondrial respiratory complex I inhibitor or subjected them to oxygen or glucose deprivation. After oxidative damage, cells underwent pathological mitochondrial depolarization, ATP depletion, DNA oxidation, lipid peroxidation, and cellular necrosis and apoptosis. When dissolved in the media, hydrogen gas dose-dependently prevented these events and improved cell viability. In these studies, Ohsawa et al. found the smallest concentration of hydrogen which can increase cell survival significantly is 25 μmol/L(Fig 1).

These studies also indicated that hydrogen gas could reach subcellular compartments such as the nucleus and mitochondria. This is particularly important, as the latter is the primary site of generation of reactive oxygen species after reperfusion and is notoriously difficult to target. Biochemical experiments using fluorescent probes and electron paramagnetic resonance spectroscopy spin traps indicated that hydrogen gas may selectively scavenge the hydroxyl radical. The authors propose this as a unique cytoprotective pathway that specifically quenches the hydroxyl radical while preserving other reactive oxygen and nitrogen species important in signaling.

To test the efficacy of hydrogen gas therapy during oxidative stress, Ohsawa et al. used a rat model of stroke, with middle cerebral artery ligation and reperfusion. Inhalation of 2% hydrogen gas limited the stroke volume if given before the reperfusion phase of injury. Hydrogen gas treatment also reduced brain tissue lipid peroxidation and DNA oxidation, findings that were also noted in cultured cells challenged with reactive oxygen species. The decrease in reperfusion damage improved long term neurological function, such as thermoregulation and weight maintenance, at one week, implying that hydrogen gas can protect cells *in vivo*.

Many antioxidants or enzymes that scavenge reactive oxygen species limit cytotoxicity after ischemia and reperfusion. In the presence of catalytically active metals, however, detoxification of superoxide to hydrogen peroxide by superoxide dismutase generates the more

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potent hydroxyl radical. This radical reacts indiscriminately with and damages molecular targets such as nucleic acids, lipids and proteins. Ohsawa et al. have proposed that selective hydroxyl radical scavenging is how hydrogen gas protects cells from oxidative damage after ischemia-reperfusion[2].

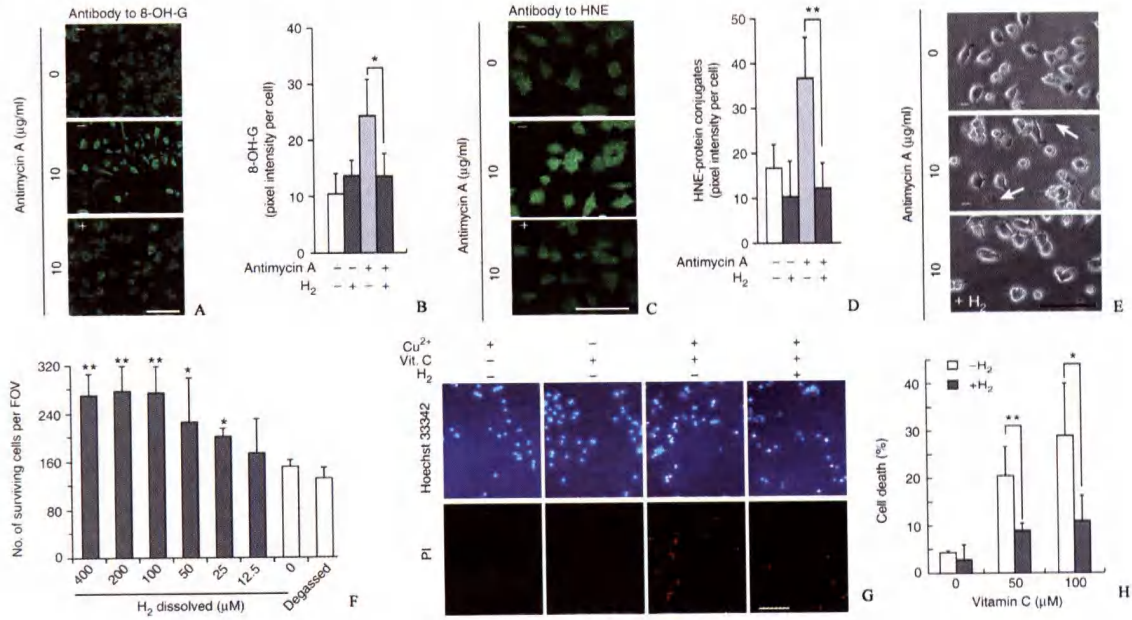

Fig 1　Molecular hydrogen protects cultured PC12 cells by scavenging hydroxyl radicals[2]

A-D: PC12 cells were maintained with 10 μg/ml antimycin A, with (+) or without (−) 0.6 mmol/L hydrogen, for 24 h in a closed flask, and immunostained with antibodies to 8-OH-G or HNE. Fluorescence signals in response to 8-OH-G and HNE were quantified using 100 cells from each independent experiment ($n=4$). * $P<0.05$, ** $P<0.01$; E: Phase-contrast pictures of PC12 cells 24 h after the exposure to antimycin A, with (+) or without (−) 0.6 mmol/L hydrogen. Arrows indicate dead cells; F: Cell survival was assessed by manually counting the cells ($n=4$). * $P<0.05$, ** $P<0.01$ (compared with 0 mmol/L hydrogen); G: PC12 cells were exposed to intracellular hydroxyl radical produced by the Fenton reaction, with or without 0.6 mmol/L hydrogen. Cells were preincubated with 1 mmol/L $CuSO_4$, washed, and exposed for 1 h to 0.1 mmol/L ascorbate in order to reduce intracellular Cu^{2+} to Cu^{+}. The cells were costained with propidium iodide (for dead cells) and Hoechst 33342 to visualize the nuclei; H: Cell survival was assessed by manually counting the cells as in F ($n=5$). * $P<0.05$, ** $P<0.01$. Scale bars: 50 μmol/L in A, C, E; 100 μmol/L in G. Histograms represent $\bar{x}\pm s$. Fig 1 was cited from the papers by Ohsawa et al [Nat Med, 2007, 13, 688-694]

It is important to pay attention to the smallest effect concentration of Ohsawa et al's paper. *In vitro* experiment shown in Figure 1F in their paper demonstrates that 25 μmol/L hydrogen is still effective in cultured cells. *In vivo* experiment, 2% gas gives 32 μmol/L hydrogen in water. Hydrogen is dissolved in lipids more than water, thus may give 60 μmol/L in the brain.

In fact, there is production of hydrogen within animals and human. Carbohydrates that are incompletely absorbed by the small intestine within animals reach the colon where they are fermented by intestinal bacteria [3-4]. These floras are primarily anaerobes present in animal faeces or in the colon and perform hydrogen producing reactions associated with short chain volatile fatty acid production. Along with these fatty acids the gases hydrogen and CO_2 are produced. These gases associated with fermentations are not utilized by the host, but are primarily either lost in faeces or flatus, or assimilated by methaneproducing bacteria[4-5]. Some studies indicate that a significant proportion of the hydrogen produced by the colonic flora is absorbed into the bloodstream and can even be detected on the breath[6-7]. For example, an estimated 14%[6] or 20%[4] of the total colonic hydrogen production was reported to be carried through the human bloodstream and then released into the lungs.

Some researchers reported that there is plenty of hydrogen in stomach and liver tissues. Olson and Maier determined the average hydrogen content of the mucus layer of the mouse stomach to be 43 μmol/L[8] (Tab 1). These measurements were taken on different days and at different times during the day and ranged in concentrations from 17 to 93 μmol/L[8]. It may be ex-

pected that the type of diet of the animal would affect the colonic flora fermentation responses; diet would then affect the hydrogen concentrations in tissues, but was not studied in this paper. Maier et al also found the average hydrogen concentration is over 53 μmol/L (Tab 2). Molecular hydrogen levels ranged from 118 to 239 μmol/L in the small intestine of live mice (the mean value for 12 determinations was 168 μmol/L), and spleen and liver tissue hydrogen levels were approximately 43 μmol/L[9-10].

Tab 1 Hydrogen concentrations in mouse stomach[8]

Mouse No.	Hydrogen range c_B/μmol · L^{-1}	Site measured
1	25-93	8
2	35-88	8
3	17-29	7
4	19-77	8

A 50 mm size microelectrode probe was used to measure hydrogen in the mucus lining area of the stomach in mice

Tab 2 Microelectrode-determined hydrogen concentrations in mouse liver[9]

Mouse No.	Hydrogen range c_B/μmol · L^{-1}	$\bar{x}\pm s$	Sites measured*
1	43-63	54±9	10
2	29-89	53±18	12
3	43-68	57±11	12

* The sites measured included all lobes of the liver in mice

These studies show that the concentration of hydrogen, at least in the liver, stomach, small intestine and spleen, reached the concentration which is needed to neutralize oxidative stress after ischemia-reperfusion injury. So we think the hydrogen should be an endogenesis antioxidant in the body.

Although exogenous hydrogen can act as a therapeutic antioxidant as described by Ohsawa et al, there is also a gap between the endogenous and exogenous hydrogen. Further studies are required to elucidate whether endogenous hydrogen is an antioxidant. In order to verify our proposal, we can induce the intestinal bacteria to produce hydrogen by some special drugs, and examine the oxidative stress index in some tissues.

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氢——一种内源性抗氧化剂

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[摘要] 最近 Ohsawa 等报道,呼吸氢气能通过抗氧化作用,减少动物脑和肝缺血再灌注引起的细胞凋亡。实际上,人和动物消化道内许多正常细菌能产生氢气,这些氢气可被消化道吸收进入血液循环。考虑到小鼠部分器官内氢气水平已达到产生抗氧化作用的浓度,我们认为,氢是一种内源性抗氧化物质。

[关键词] 氢;抗氧化剂;再灌注损伤

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