氢气的选择性抗氧化

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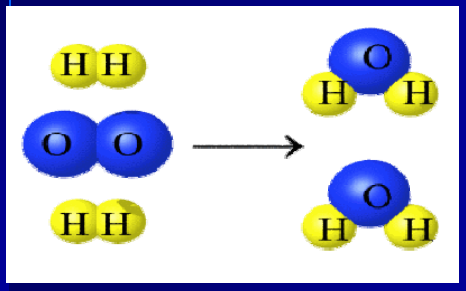
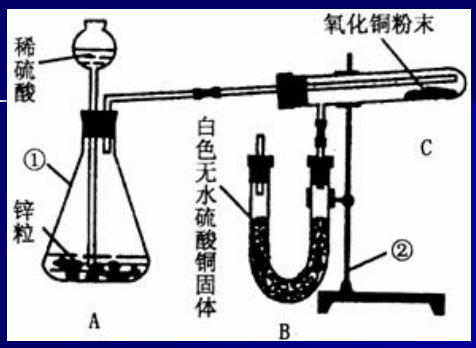
$2H_2 + O_2 = 2H_2O$





氢气还原氧化铜的实验

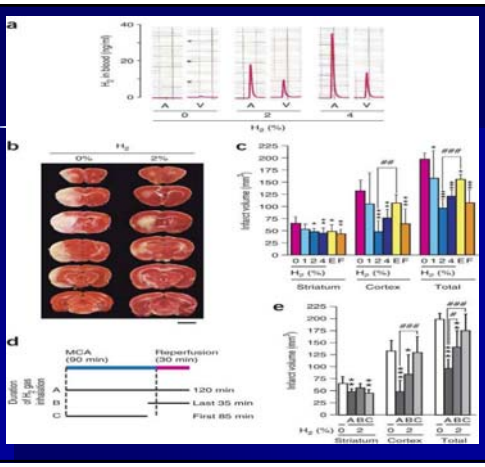
我们所了解的氢气

- 化学上具有还原性
- 生理学上属于惰性气体

一、重新认识氢的作用

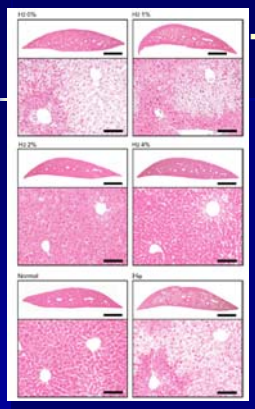
- Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals
- Nature Medicine 2007;13(6):688

nature medicine



氢对肝缺血再灌注损伤的作用

- Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress.Biochemical and Biophysical Research Communications 361 (2007) 670–674



- Fig. 1. Suppression of I/R injury by inhaling hydrogen gas. Under anesthetic gas, mice were subjected to a 90-min ischemic insult, followed by 180-min reperfusion. Ischemic livers were removed for fixing. Paraffin sections were prepared and subjected to HE staining. For mice to inhale hydrogen gas, hydrogen gas was supplied to the anesthetic gas 10 min before reperfusion and continued to be supplied until the end of reperfusion. Instead of hydrogen gas, helium gas (4%) was also inhaled by mice. Representative pictures are shown. Scale bar: 2 mm (upper panels) and 100 lm (lower panels).

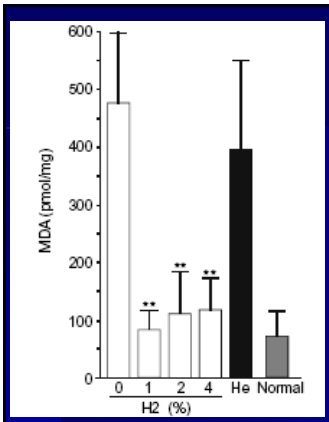
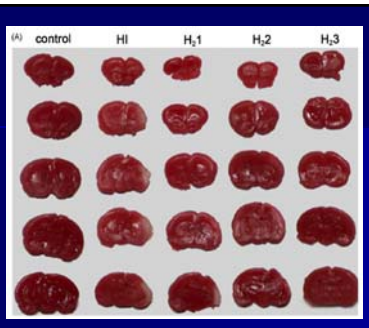


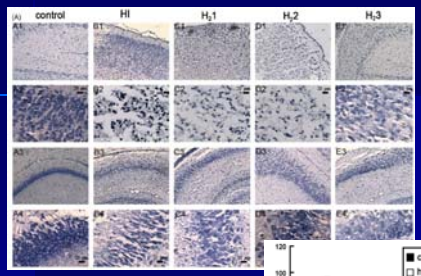
Fig. 4. Hydrogen gas significantly decreased levels of hepatic MDA, a marker of oxidative stress. Mice (n = 6 each group) were subjected to I/R with or without hydrogen gas. Ischemic livers were removed and homogenized in the presence of butylated hydroxytoluene. After centrifugation, free MDA in the supernatant was determined. Mice (n = 6) treated with helium gas (4%) instead of hydrogen gas and normal mice (without any treatment; n = 6) were also analyzed. Free MDA levels were normalized against protein contents in supernatants. The mean is presented with the standard deviation. Statistical analysis was performed using one-way ANOVA. **P < 0.0001, compared with 0% H2 gas.

我们的研究》》》》》

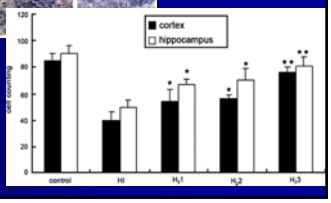
实验3月，3月后文章被接受，3月后被引用3次



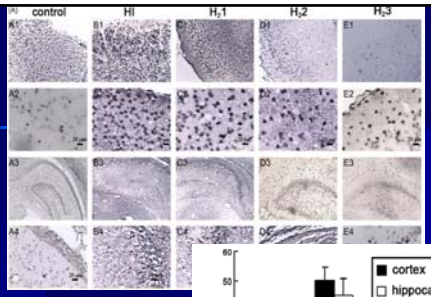
- 呼吸2%氢60 min有显著治疗效果（新生儿缺血模型）



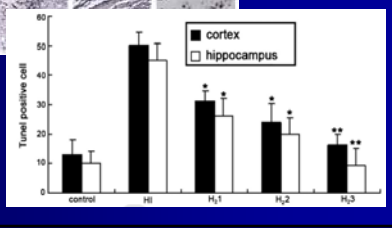
神经染色



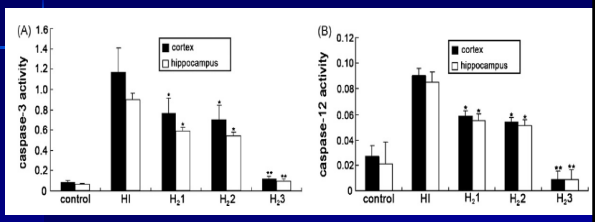
能减少神经细胞损伤



Tunel染色



减少凋亡细胞



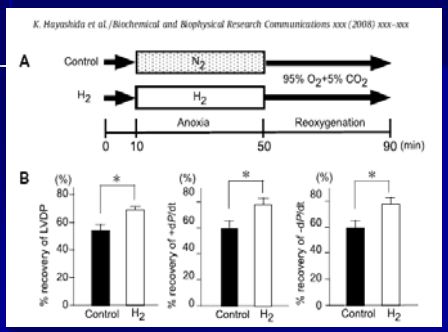
- 控制酶活性

目前该论文被引用8次

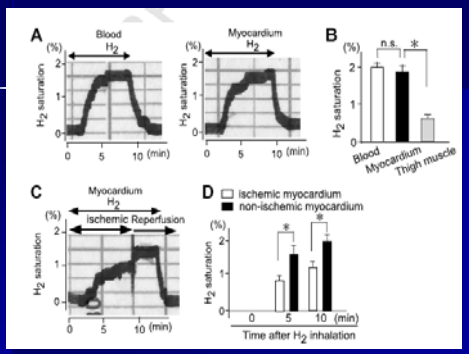
1. Ohsawa I, Nishimaki K, Yamagata K, et al. Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice.Biochem Biophys Res Commun, 2008; 377:1195-8
2. Ohta S. Hydrogen gas and hydrogen water act as a therapeutic and preventive antioxidant with a novel concept Nippon Ronen Igakkai Zasshi. 2008;45:355-62.
3. Cai JM, Kang ZM, Liu K, et al. Neuroprotective effects of hydrogen saline in neonatal hypoxiaischemia rat model. Brain Res. 2009;1256:129-37
4. Taniguchi H, Andreasson K.The hypoxic-ischemic encephalopathy model of perinatal ischemia. J Vis Exp. 2008 in press
5. Nakashima-Kamimura N, Mori T, Ohsawa I, et al. Molecular hydrogen alleviates nephrotoxicity induced by an anti-cancer drug cisplatin without compromising anti-tumor activity in mice.Cancer Chemother Pharmacol. 2009 in press
6. Matchett GA, Fathali N, Hasegawa Y, et al. Hydrogen gas is ineffective in moderate and severe neonatal hypoxia-ischemia rat models, Brain Res. 2009 in press
7. Fu Y, Ito M, Fujita Y, et al. Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease. Neurosci lett. 2009 in press
8. Mao YF, Zheng XF, Cai JM, et al. Hydrogen-rich saline reduces lung injury induced by intestinal ischemia/reperfusion in rats. Biochem Biophys Res Commun.2009 in press

2％氢对心肌缺血的保护

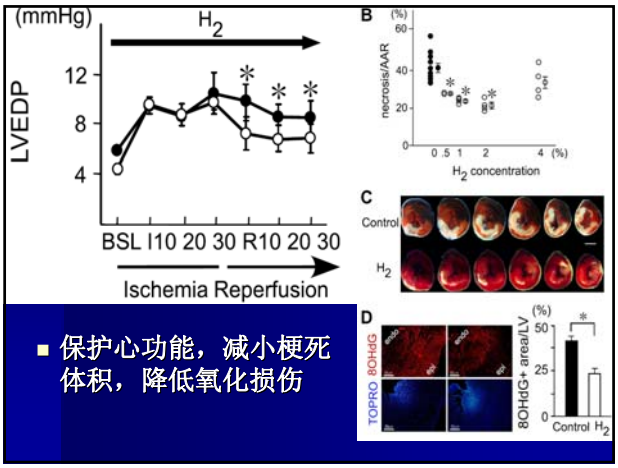
日本随后报道:心肌缺血也有效果



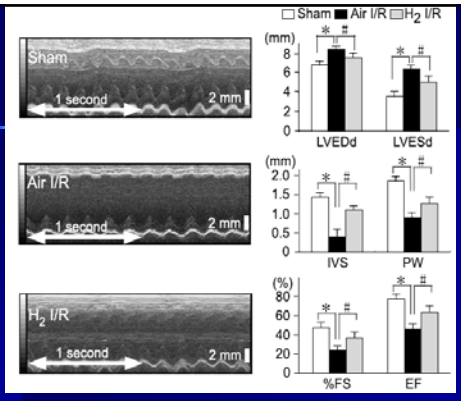
- 体外实验:氢能保护缺氧再复氧心肌损伤



- 可迅速扩散到缺血组织***



- 保护心功能，减小梗死体积，降低氧化损伤

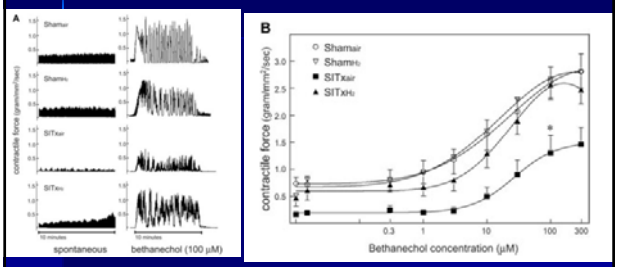


Inhalation of H2 gas reduces adverse LV remodeling. Representative M-mode echocardiographic images of sham-operated (sham), ischemia–reperfusion with air inhalation (Air_I/R), and ischemia–reperfusion with H2 inhalation (H2_I/R).

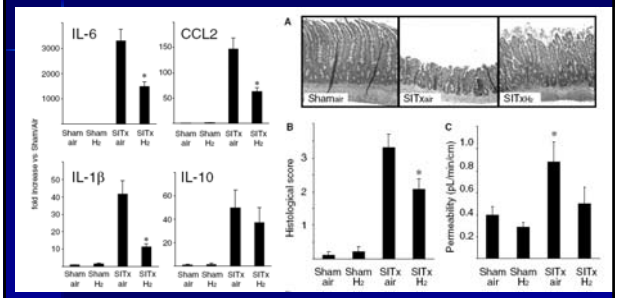
Gas inhalation as disease therapy has received recent interest. There are three endogenous gas signaling molecules, known as gasotransmitters, include nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfate (H_2S). The increased production of these gases under stress conditions may reflect the active involvement of these gases in the protective response. In pre-clinical experimental models of disease, including ischemia–reperfusion injury, the inhalation of exogenous CO or H_2S has produced a favorable outcome for most vital organs [19–22]. However, the inherent toxicity of these gases must be investigated for gas inhalation to be considered an effective therapeutic strategy. It is unknown if the therapeutically effective threshold for CO or H_2S can be attained locally in target organs without delivering a potentially toxic level of the gasses via the lungs.

- 大量研究表明，NO、CO、H_2S都是气体信号分子，具有保护作用，但他们都是毒性很强的分子。氢就不一样了，无毒而且有效！

2%氢对小肠移植后损伤的作用

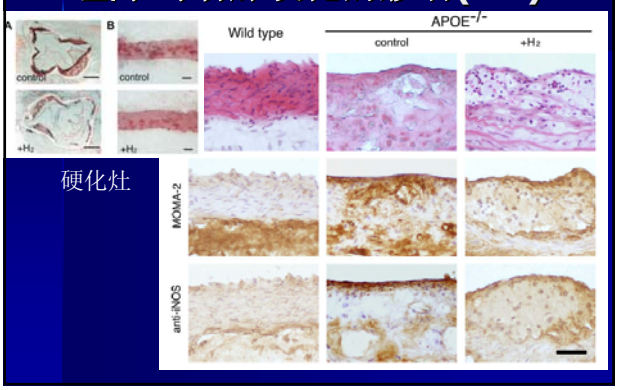


2%氢对小肠移植后损伤的作用



对炎症因子的影响　　形态学改变

氢水对动脉硬化的影响(6M)



硬化灶

饮用氢水8周对患者血糖和胰岛素的影响

Table 3
Changes in glucose and insulin concentrations during a 75-g OGTT before and after consumption of hydrogen-rich pure water for 8 weeks in patients with IGT

	0 min		30 min		60 min		120 min		ΔIRI/ΔBS ratio
	Glucose (mmol/L)	Insulin (pmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	
Case 1									
0 wk	6.00	22.8	9.27	127.0	11.81	433.2	9.33	399.0	0.30
8 wk	6.05	36.6	8.94	182.4	11.77	778.8	6.49	221.4	0.38
Case 2									
0 wk	5.82	10.8	11.32	42.0	15.03	97.2	8.66	175.8	0.06
8 wk	5.66	11.4	11.55	85.8	13.77	337.2	6.72	168.0	0.12
Case 3									
0 wk	5.61	12.6	9.05	29.4	10.29	93.0	8.94	163.2	0.05
8 wk	5.44	12.6	8.05	47.4	9.88	291.6	6.16	267.0	0.12
Case 4									
0 wk	6.05	62.4	9.60	256.4	10.66	600.6	8.33	594.6	0.51
8 wk	5.82	57.0	9.83	307.8	10.32	682.2	7.05	533.4	0.57
Case 5									
0 wk	5.82	23.4	9.49	152.8	12.16	255.6	10.21	195.6	0.33
8 wk	5.55	20.4	10.16	160.8	11.82	288.0	9.05	366.6	0.28
Case 6									
0 wk	5.16	16.2	10.10	178.6	12.82	141.0	10.66	108.0	0.30
8 wk	5.33	27.0	8.94	159.8	10.99	165.0	8.49	108.0	0.37

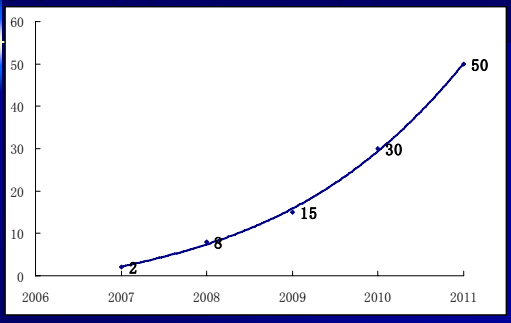
ΔIRI/ΔBS was calculated as follows: (insulin at 30 minutes − insulin at 0 minute)/(glucose at 30 minutes − glucose at 0 minute).

目前进展总体情况

证明氢气可治疗:脑缺血、肝缺血、心肌缺血和小肠移植、应激后神经损伤、动脉硬化和糖尿病氧化损伤。

该领域目前发表的文章共15篇，其中日本发表9篇，美国1篇，我们发表4篇。

将来该领域可能的文章数量



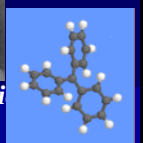
我们要首先占领数量优势

二、氢气选择性抗氧化

与传统抗氧化物质的区别！

1900 - Gomberg:

discovery of triphenylmethyl radi



三苯甲基的制备，确定了自由基的概念

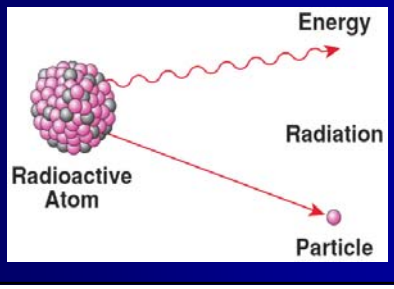
M. Gomberg, *Ber.*, 1900, **33**, 3150; *J. Amer. Chem. Soc.*, 1900, **22**, 7

http://www.chm.bris.ac.uk/motm/triphenylmethyl/main1.html

University of Michigan

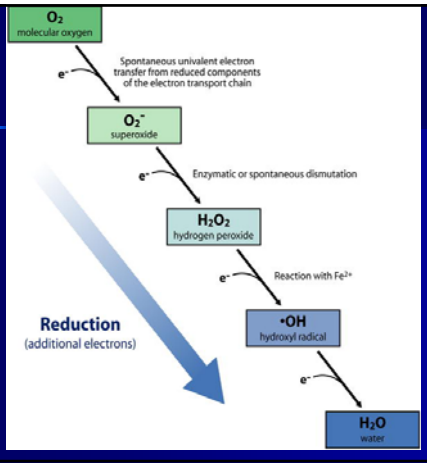
Until 1950/60:

minimal attention was given to biological actions of free radicals and reactive oxygen species



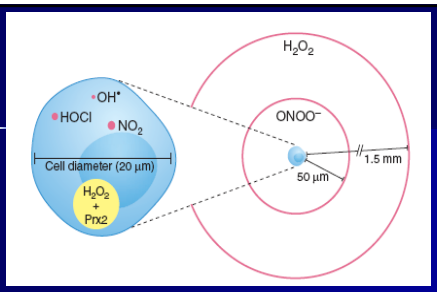
Evidence on the existence of ROS
三个标志性发现

- **1969 - McKord and Fridovich:** ***Discovery of SOD and suggestion of the existence of endogenous superoxide*** *J. Biol. Chem.* 244: 6049-6055 (1969).
- **1973 - Babior et al.:** ***Recognition of the relationship between superoxide production and bactericidal activity of neutrophils***
- **1981 - Granger et al.:** ***recognition of the relationship between local ROS production and ischemia/reperfusion induced gut injury***

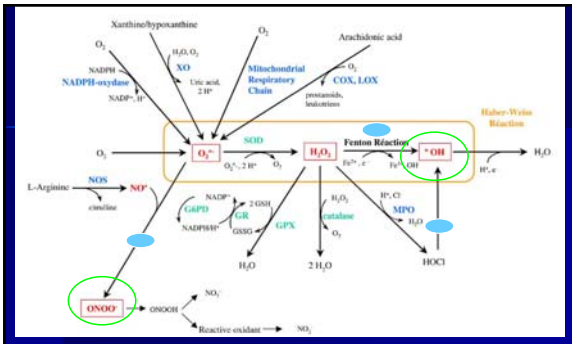


Half-life of some reactive species

Reactive species	Half-life (s)	Physiol conc. (mol/l)
Hydroxyl radical (•OH)	10^{-9}	
Alcoxyl radical (RO•)	10^{-6}	
Singlet oxygen ($^1O_2$)	10^{-5}	
Peroxynitrite anion ($ONOO^-$)	0.05 – 1.0	
Peroxyl radical (ROO•)	7	
Nitric oxide (•NO)	1 - 10	10^{-9}
Semiquinone radical	minutes/hours	
Hydrogen peroxide (H_2O_2)	spontan. hours/days (accelerated by enzymes)	10^{-9} - 10^{-7}
Superoxide anion ($O_2^{\bullet-}$)	spontan. hours/days (by SOD accel. to 10^{-6})	10^{-12} - 10^{-11}
Hypochlorous acid (HOCl)	dep. on substrate	



- 不能简单看种类，应多方面考虑不同类型的特点
- 两类：一是活性分子，如过氧化氢和超氧阴离子，浓度比较高，属于信号分子，扩散距离长；二是毒性分子，如羟自由基和亚硝酸阴离子，属于毒性分子，活性强浓度很低，扩散距离小。



自由基础产生汇总。
一、超氧阴离子是氧气获得一个电子的产物，在细胞内有4个主要途径：(1) 花生四烯酸途径；(2) 线粒体呼吸链；(3)黄嘌呤和次黄嘌呤被黄嘌呤氧化酶氧化；(4)NADPH氧化。
二、超氧阴离子产生后，有两个发展方向，一是可与NO反应产生毒性强的**亚硝酸阴离子**；另一个是在SOD催化下产生过氧化氢。
三、过氧化氢产生后，有三个发展方向，(1)被Gpx或Cat催化，产生水；(2) 在Fe2+存在条件下，产生**羟自由基**；(3)被MPO催化产生次氯酸阴离子后再转化为**羟自由基**。

抗氧化的手段

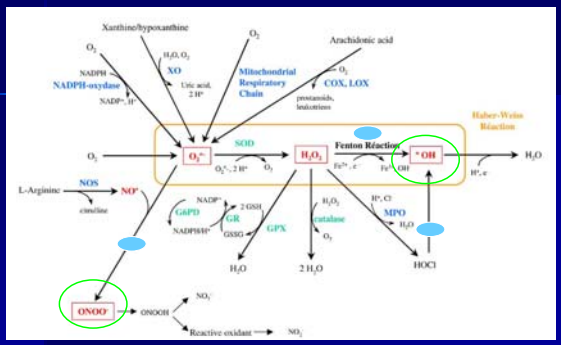
- 提高体内抗氧化能力,NRF2
- 减少体内活性氧的产生，CO等
- 提高清除活性氧的能力，氢气

为什么传统的抗氧化无效?

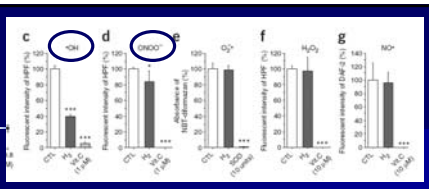
- 还原性太强，敌友不分
- 分子量大，不能自由扩散到任何位置
- 相对浓度低，无法中和毒性强的活性氧
- 有些属于外源性物质，相容性不够
- 不容易降解，影响正常细胞功能

抗氧化的最佳选择：氢气

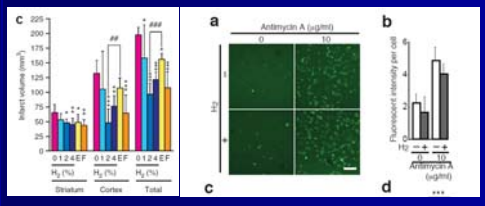
- 还原性相对低，不影响信号类活性氧
- 分子量低，可自由扩散到细胞的任何位置
- 相对浓度高，可中和低浓度毒性活性氧
- 内源性物质，组织相容性强
- 释放快 。



氢气奇妙的选择性抗氧化作用



溶液水平证据：可选择性中和部分活性氧



细胞水平的证据

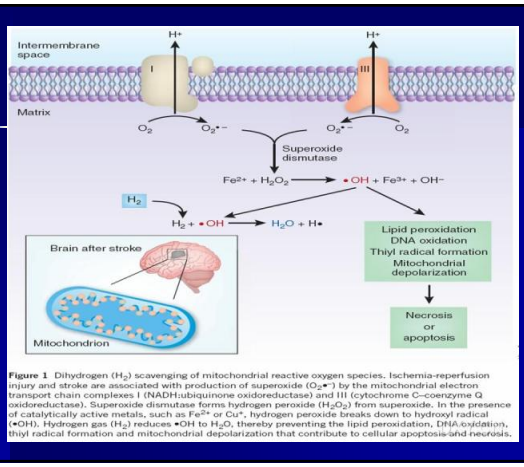


Figure 1 Dihydrogen (H_2) scavenging of mitochondrial reactive oxygen species. Ischemia-reperfusion injury and stroke are associated with production of superoxide ($O_2^{\bullet-}$) by the mitochondrial electron transport chain complexes I (NADH:ubiquinone oxidoreductase) and III (cytochrome C–coenzyme Q oxidoreductase). Superoxide dismutase forms hydrogen peroxide (H_2O_2) from superoxide. In the presence of catalytically active metals, such as Fe^{2+} or Cu^{+}, hydrogen peroxide breaks down to hydroxyl radical ($^{\bullet}OH$). Hydrogen gas (H_2) reduces $^{\bullet}OH$ to H_2O, thereby preventing the lipid peroxidation, DNA oxidation, thiyl radical formation and mitochondrial depolarization that contribute to cellular apoptosis and necrosis.

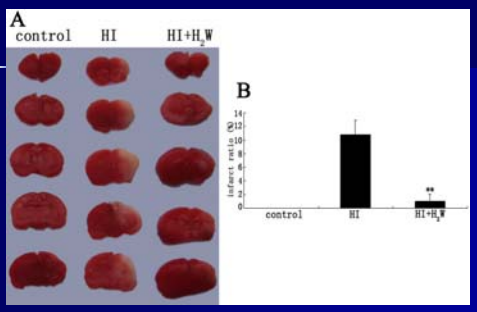
呼吸氢不是一个理想的手段

三、我们的思路：注射氢水

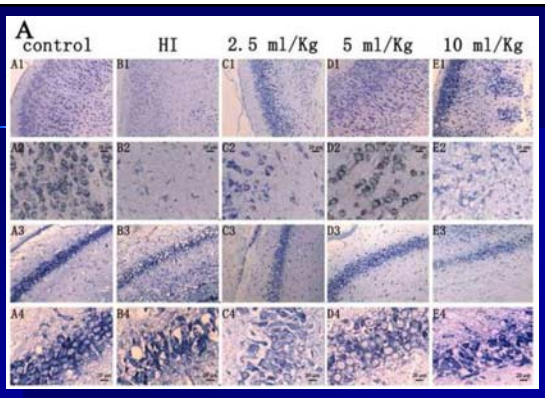
是否可行？

- 呼吸2%，溶解在血液中的水平最高只能溶解2%分压的水平。
- 如果先将100%的氢溶解在液体中（饱和），则只需要注射1/50体液的溶液。
- 饮用饱和氢水也有一定作用。

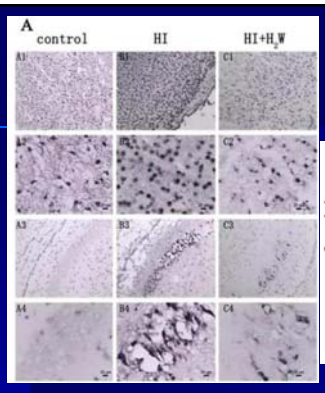
对新生大鼠脑缺血缺氧性脑损伤的治疗作用



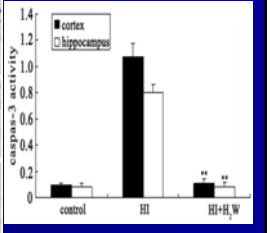
- 注射氢水对脑缺血的治疗作用 (TTC)



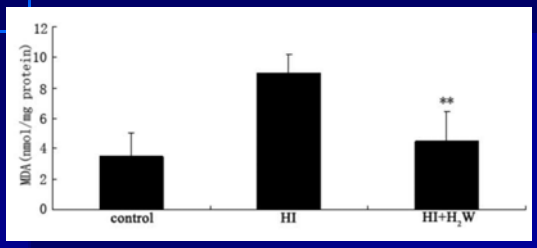
神经元染色结果显示：注射氢水可保护神经损伤



Tunnel染色结果

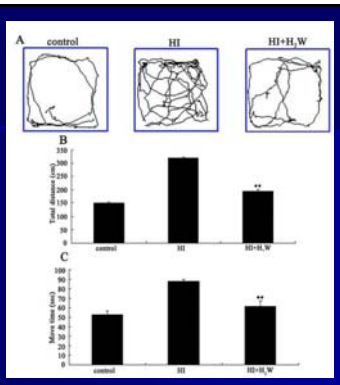


注射氢水可减少细胞凋亡



- 注射氢水可降低氧化损伤（MDA）

水迷宫实验



- 注射氢水可改善学习记忆功能

日本学者来信祝贺，并希望合作申请专利

Re: H2 paper

Dear Xue Jun,

Congratulation!! It is really a great work. Hydrogen saline is really applicable for actual medical uses. I believe that hydrogen saline is more valuable than hydrogen gas for inhalation and hydrogen water for drinking.

By the way, I wish to discuss with you regarding a patent. A partner of mine, Mr. Murota CEO of a venture company, applied a world wide patent, including China, on the hydrogen saline two years ago. I think Mr Murota's patent is basic and your work supports and strengthens his patent. If we collaborate with you regarding the patent, all of you, Mr Murota and I will be very happy. Any world wide expenditure will be paid by Mr. Murota. I propose that you keep all rights as an inventor and you transfer a right of application to Mr Murota. I will assist the patent application. If we collaborate, the patents, both of the basic patent of Mr Murota and your work, will be more valuable. Certainly you will be able to receive royalty.

Could you consider my proposal? As your paper has already accepted, if we would apply a new patent, we have to apply the patent before the publication. Cold you give me a rely as soon as possible? If you have apply a patent, there must be no problem. If you have questions, please feel free to contact me.

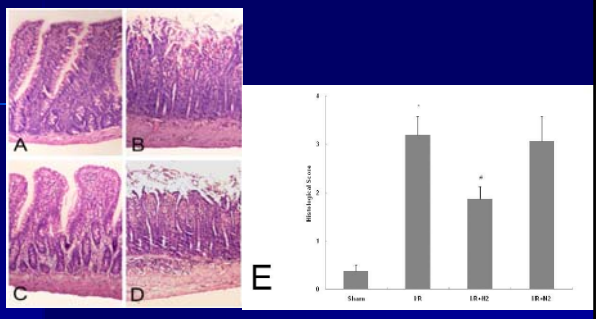
I hope your success.

Best regards

Shigeo Ohta

Congratulation!! It is really a great work. Hydrogen saline is really applicable for actual medical uses. I believe that hydrogen saline is more valuable than hydrogen gas for inhalation and hydrogen water for drinking.

对小肠缺血再灌注损伤的作用



- **Figure 1.**Morphologic observation of the intestinal tissue in sham-operated animals, I/R animals, and I/R animals treated with H2 water.

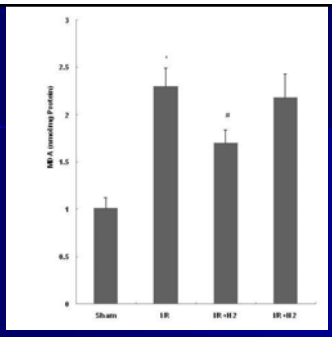


Figure 3 Intestinal mucosa malondialdehyde (MDA) levels in sham-operated animals, I/R animals,and I/R animals treated with hydrogen-rich saline or nitrogen-rich saline. Data are expressed as means Â± SEM for at least triplicate independent experiments (n = 8 per group). *P < 0.01 vs. Sham; #P < 0.05 vs. I/R group

Table 1. Serum TNF-α, IL-1β and IL-6 concentrations in sham-operated animals, I/R animals, and I/R animals treated with hydrogen-rich saline or nitrogen-rich saline.

	TNF-α	IL-1β	IL-6
Sham	21.6 ± 5.0	9.1 ± 2.4	30.7 ± 4.5
I/R	137.2 ± 26.0 *	37.6 ± 5.8 *	134.3 ± 18.0 *
I/R+H_2	75.7 ± 10.8 #	22.8 ± 2.9 #	82.5 ± 13.3 #
I/R+N_2	127.9 ± 16.3	41.3 ± 5.5	141.2 ± 22.1

Data are expressed as means ± SEM for at least triplicate independent experiments (n = 8 per group). *P < 0.01 vs. Sham; #P < 0.05 vs. I/R group

Results of arterial blood gas analysis.

Parameters	Sham		Saline-II/R		Hydrogen-rich saline-II/R	
	Baseline	I/R	Baseline	I/R	Baseline	I/R
PaO_2 (mm Hg)	96.7 ± 4.3	96.2 ± 5.9	97.3 ± 6.4	83.5 ± 11.9**	97.2 ± 10.1	89.8 ± 13.1**†
O_2Sat (%)	95.4 ± 2.7	94.9 ± 2.5	97.0 ± 2.0	95.7 ± 3.4	94.5 ± 2.9	96.2 ± 2.5
$PaCO_2$ (mm Hg)	41.3 ± 5.2	43.2 ± 5.7	41.2 ± 3.5	37.6 ± 4.3	39.3 ± 5.0	38.3 ± 7.1
HCO_3^- (mmol/L)	24.5 ± 4.7	21.4 ± 5.3	24.1 ± 4.9	14.3 ± 4.2**	24.7 ± 4.4	17.8 ± 3.6**†

Data are expressed as mean ± SD. n = 10. *P < 0.05, **P < 0.01 compared with the corresponding baseline; †P < 0.05 compared with saline-II/R group.

Levels of TNF-α, IL-1β and NF-κB 65 in rat lung tissues.

Parameters	Sham	Saline-II/R	Hydrogen-rich saline-II/R
TNF-α (pg/mg protein)	6.01 ± 2.57	21.9 ± 5.74**	15.34 ± 4.81**†
IL-1β (pg/mg protein)	3.55 ± 1.10	11.82 ± 3.75**	8.08 ± 3.34**†
NF-κB 65 (pg/mg protein)	41.22 ± 10.31	94.65 ± 21.35**	69.12 ± 15.49**†

Data are expressed as mean ± SD, $n = 10$. *$P < 0.05$, **$P < 0.01$ compared with sham group; †$P < 0.05$, ††$P < 0.01$ compared with saline-treated II/R group.

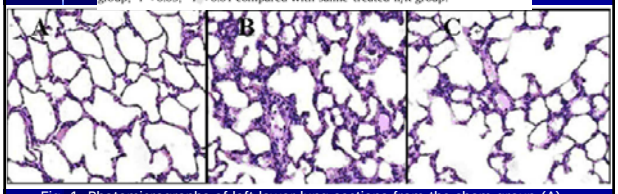
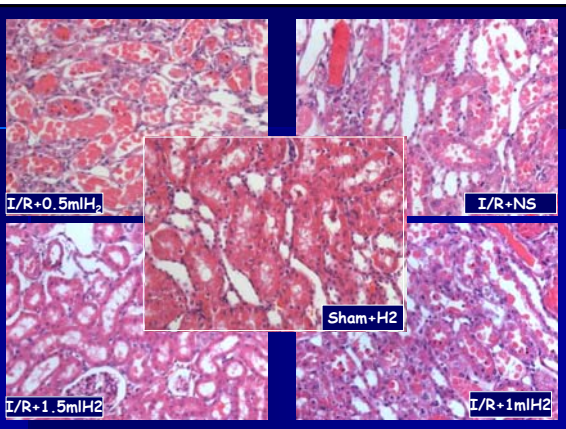


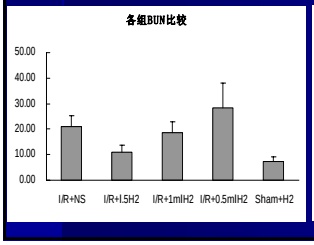
Fig. 1. Photomicrographs of left lower lung sections from the sham group (A), saline-treated II/R group (B) and hydrogen-rich saline-treated II/R group (C) after 90 min of intestinal ischemia followed by 4 h of reperfusion. (A) Normal histopathology, (B) the lung tissues revealed edema, alveolar hemorrhage and inflammatory cell infiltration, (C) moderate edema, hemorrhage and inflammatory cell infiltration. Routine hematoxylin and eosin stained (200).

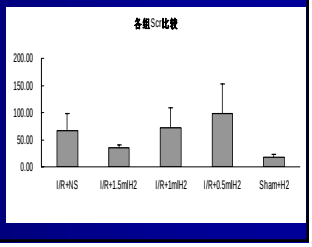


HE染色×400

血生化结果显示氢生理盐水组可显著改善肾功能

	I/R+ 1.5mlNS	I/R+1.5mlH_2	I/R+1mlH_2	I/R+0.5mlH_2	Sham+1.5mlH_2
BUN (mmol/l)	21.09 ± 4.13	11.12 ±2.47	18.63 ± 4.30	28.34 ± 9.80	7.46 ± 1.70
Scr (umol/l)	66.76 ± 30.88	34.98 ±5.97	71.12 ± 37.05	98.82 ±54.06	18.08 ± 4.43





四、有关思考

1、内源性氢的作用

- 体内含有一定水平的氢
- 体内氢水平可达到抗氧化水平
- 需要直接证据
- 文章发表在校学报2008.3

2、电针和直流电

- 电流可使组织电解，产生氢气，其作用机制是否与氢有关？值得关注。

3、具体机制

- 选择性抗氧化证据不足，特别是在体证据不足，是目前的主要问题。
- 可能的情况是：相对选择性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小结

- 奇：能清除自由基！
- 妙：治疗疾病很有效！
- 绝：潜在应用范围广泛！

意义重大，不可忽视

我们发表的有关氢的论文

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5. Matchett GA, Fathali N, Hasegawa Y, et al. Hydrogen gas is ineffective in moderate and severe neonatal hypoxia-ischemia rat models. Brain Res. 2009 in press
6. Mao YF, Zheng XF, Cai JM, et al. Hydrogen-rich saline reduces lung injury induced by intestinal ischemia/reperfusion in rats. Biochem Biophys Res Commun.2009 in press

致谢

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