It is important that we explore how this new regulatory pathway might be used in a therapeutic setting. One difficulty is that altering retinaldehyde levels might also affect retinoic acid levels. For example, we might consider directly providing retinaldehyde, but in addition to potential difficulties in tissue distribution and pharmacoavailability, long-term treatment might result in higher retinoic acid concentrations. In contrast, inhibiting retinaldehyde catabolism might decrease retinoic acid. In both cases, a careful evaluation of the retinoic acid–mediated consequences on cellular activities will be needed. Given the data presented, it seems likely that retinaldehyde concentrations are tightly controlled. Low retinaldehyde in the fat tissue of obese mice arises from the imbalanced levels of its metabolic enzymes compared with those in lean mice[1]. How these enzyme levels are controlled in the adipose tissue will have to be explored, starting with the question of whether an imbalance in enzyme expression ratio is a cause or a consequence of obesity.

Metabolic syndrome is a highly complex disease that challenges both the fundamentalist, who still hopes for a single causal mechanism, and the clinician, who dreams of a single molecule that would treat all the abnormalities associated with the syndrome. There is no reason to think that retinaldehyde resolves these issues. Rather, the paper by Ziouzenkova *et al.*[1] opens a new avenue of investigation, offering one exciting entry point into the complexity of nuclear receptor networks and their diverse and multifaceted natural ligands.

COMPETING INTERESTS STATEMENT
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The hydrogen highway to reperfusion therapy

Katherine C Wood & Mark T Gladwin

Hydrogen gas debuts as a selective antioxidant with explosive potential as cytoprotective therapy for ischemia-reperfusion injury and stroke.

Just when we thought we had exhausted our tool kit of therapeutic gases, Ohsawa *et al.*[1] provide evidence that inhaled hydrogen gas (H_2) has antioxidant and antiapoptotic activities that protect the brain against ischemia-reperfusion injury and stroke[1].

During the ischemic phase of thromboembolic stroke, a blood clot travels to and lodges in the distal blood vessels in the brain, blocking blood flow to the oxygen-starved tissue for a period of hours. This is followed by the reperfusion phase, when the blood clot is broken down by natural or pharmacological means and blood flow is restored. Although restoration of blood flow is critical, the reintroduction of molecular oxygen triggers a cytotoxic cascade during which reactive oxygen species are generated by the mitochondria. This burst of reactive oxygen species irrevocably drives downstream signaling networks that lead to cellular necrosis and apoptosis. For both stroke and myocardial infarction, there are now highly successful approaches to restore blood flow to the ischemic tissue. So far, however, we have completely failed to relieve this pathological cascade of oxidative damage after reperfusion injury. In this issue, Ohsawa *et al.*[1] report that highly diffusible hydrogen gas can target intracellular sources of reactive oxygen species and dose-dependently inhibit reperfusion-induced oxidative damage.

Numerous studies have consistently demonstrated a burst of reactive oxygen species on restoration of blood flow after a stroke[2,3]. Reactive oxygen species, such as superoxide, have been suggested to be the primary activator of the mitochondrial permeability transition pore, a large multiprotein conductance channel[4]. The opening of this channel causes a loss of membrane potential, mitochondrial swelling with membrane rupture, cytochrome C release and apoptotic cell death.

After ischemic damage to the mitochondrial electron transport chain, there is inefficient transfer of electrons to molecular oxygen, leading to the generation of superoxide. What's more, activation of superoxide-producing enzymes, such as xanthine oxidase and NADPH oxidase, following ischemia-reperfusion injury raises superoxide levels even higher. The central role of reactive oxygen species in reperfusion injury has been further demonstrated in recent studies showing that inhibitors of mitochondrial respiratory complexes I and III prevent reperfusion reactive oxygen species generation and improve cellular viability[5–7].

The lightweight gas diatomic hydrogen (H_2), 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[8].

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[1]. 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.

These studies also indicated that hydrogen gas could reach subcellular compartments

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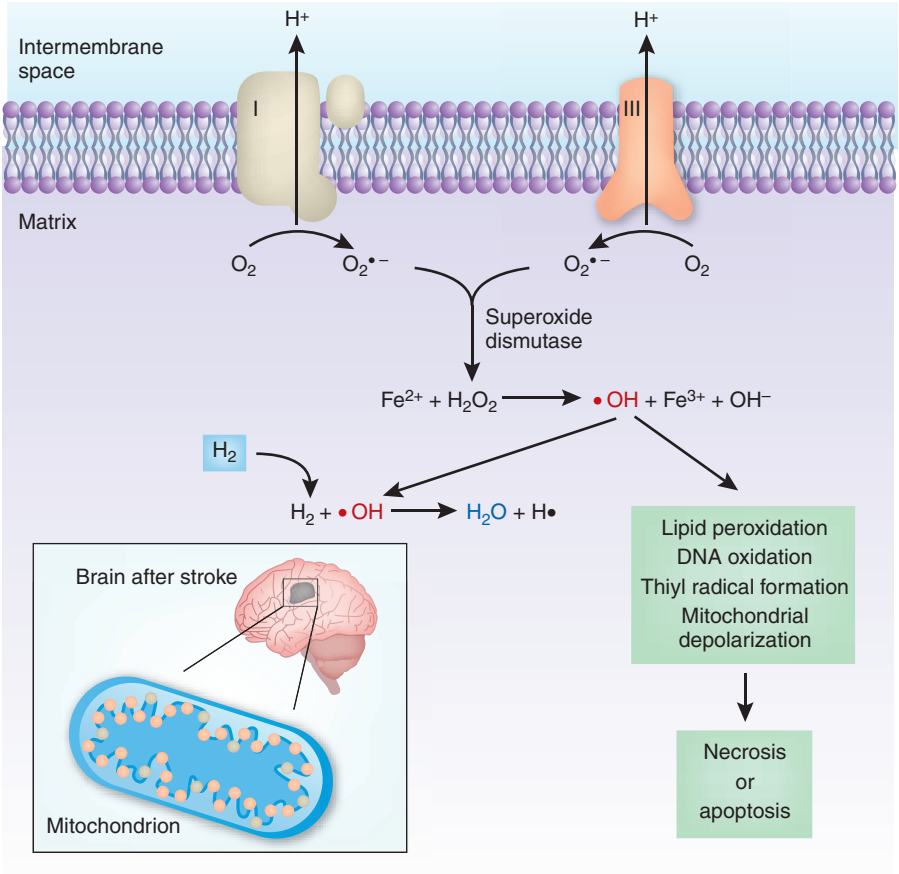

Figure 1 Dihydrogen (H_2) scavenging of mitochondrial reactive oxygen species. Ischemia-reperfusion injury and stroke are associated with production of superoxide ($O_2^{\bullet-}$) by the mitochondrial electron transport chain complexes I (NADH:ubiquinone oxidoreductase) and III (cytochrome C–coenzyme 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 (•OH). Hydrogen gas (H_2) reduces •OH to H_2O, thereby preventing the lipid peroxidation, DNA oxidation, thiyl radical formation and mitochondrial depolarization that contribute to cellular apoptosis and necrosis.

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[1]. Inhalation of 2% hydrogen gas limited the stroke volume if given before the reperfusion phase of injury. This effect was comparable to that of FK506, an immunosuppressive and neuroprotective drug used in preclinical trials for the treatment of cerebral infarct[8]. 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[9]. In the presence of catalytically active metals, however, detoxification of superoxide to hydrogen peroxide by superoxide dismutase generates the more potent hydroxyl radical (•OH). This radical reacts indiscriminately with and damages molecular targets such as nucleic acids, lipids and proteins (**Fig. 1**). Ohsawa *et al.* have proposed that selective hydroxyl radical scavenging is how hydrogen gas protects cells from oxidative damage after ischemia-reperfusion[1]. There is some question, however, whether these concentrations of hydrogen gas could compete effectively with the numerous cellular targets of the hydroxyl radical; membrane lipids and thiols are in far greater abundance than the hydrogen gas molecules successfully used in these experiments. Furthermore, the published rate constant for the reaction of •OH with H_2 to form H_2O and H• is drastically slower than most radical-radical reactions (4.2×10^7 $M^{-1}sec^{-1}$ versus 10^9 $M^{-1}sec^{-1}$)[10]. More study will be necessary to identify the precise chemical mechanism by which hydrogen gas quenches oxidative stress.

Though it is a particularly novel approach with evident potency in cell culture and in the rat preclinical model, hydrogen gas faces a difficult path toward therapeutic use, a track littered with the virtual corpses of other therapies to neutralize reactive oxygen species. Previously, other therapeutic strategies for scavenging reactive oxygen species seemed promising in animal models but then failed in human clinical trials[11]. Challenges facing any successful reperfusion therapy include identifying the dose, duration and site at which an agent will be optimally effective. Of particular importance is targeting the site of reactive oxygen species generation, such as the mitochondria[11]. Ohsawa *et al.* addressed several of these issues in their study with hydrogen gas, defining the therapeutic window (before reperfusion), the dose (similar in cell culture and with inhalation) and the ability of the gas to target the mitochondria[1], a critical feature of successful reperfusion therapies[12].

COMPETING INTERESTS STATEMENT
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