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Hydrogen, the next neuroprotective agent?

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Annually, in the United States, approximately 795,000 people suffer a stroke, of which 610,000 are first attacks [1]. Treatment of acute ischemic stroke has made significant progress that involves medical or endovascular recanalization of the blocked artery [2]. In spite of this, stroke continues to be a major cause of disability and mortality. An estimated 7.2 million Americans age 20 or more report a history of stroke [1].

It has been observed that cellular damage does not end with restoration of blood flow. Although several stroke animal models have shown promising results in neuroprotection studies, all interventions in humans have failed to identify a viable molecule. Due to the complexity of the pathological processes, single molecules may not be able to stop the apoptotic and cytotoxic cascades. Additionally, the effect may be hampered by limited diffusion of the neuroprotective agent within the penumbra. Therefore, a successful drug has to be easily available, diffuse rapidly, act on multiple pathological intertwined mechanisms and have few side effects. In recent years, there has been an increasing interest in studying the neuroprotective effects of hydrogen.

The 'oxygen paradox', or reperfusion injury as it is known today, is the accelerated cell death following reperfusion of an ischemic tissue with oxygenated blood [3]. Within seconds to minutes of reperfusion, dysfunctional mitochondria produce excessive amounts of radical oxygen species (ROS), six-times higher than in normal tissue and two-times higher than during the ischemic phase [4,5]. Of these, hydroxyl is the most reactive, being several hundred-times stronger than hydrogen peroxide [6].

The most important ROS are singlet oxygen, hydroxyl, superoxide and peroxynitrite. They are involved in the cascade pathways of cell death and apoptosis [7]. Peroxynitrite, the result of nitric oxide reaction with superoxide anion, triggers necrosis and apoptosis. It is therefore involved in inflammation, autoimmunity, atherosclerosis and neurodegenerative conditions [8].

The ROS have a high propensity for chain reaction, creating new reactive molecules. There are multiple layers of defense against ROS consisting of antioxidant molecules. Alpha-tocopherol protects the lipid membrane from peroxidation. This is further reduced by ascorbate and thiols present in cytosol. Cholesterol, plasmalogens and carotenoids interact with the more aggressive singlet oxygen and hydroxyl radical. As the highly reactive radicals have a short half-life, the location of the antioxidant molecules is important for their interception and neutralization. Singlet oxygen is transformed into superoxide by superoxide dismutase, then into hydrogen peroxide, which is further converted into water by glutathione peroxidase and catalase. Hydroxyl anion is produced from hydrogen peroxide, under the catalytic influence of iron and copper [9].

In Earth's atmosphere, hydrogen is present as an inert, colorless and odorless gas, at about 0.5 ppm. It can be produced by electrolysis of water during production of oxygen, by bacterial digestion of sugars in the gut or as a by-product of batteries. There is no evidence of toxicity due to sudden or continuous exposure other than asphyxia, if present in a concentration high enough to displace oxygen. This would occur at a concentration significantly higher than the threshold for explosion, which is 4.1% [10]. The mixture of 49% hydrogen, 50% helium and 1% oxygen (Hydreliox), is used by deep-sea divers without notable toxicity [11]. Hydreliox has been used safely to diminish the neurological symptoms of high-pressure nervous syndrome in divers working at depths up to 500 m for 27 h [12].

Hydrogen has many of the required characteristics for a successful neuroprotectant: is widely available, is easy to produce, diffuses rapidly through the lipid membranes, is inert and safe to administer and reacts only with the most aggressive ROS. At the same time, it mediates multiple pathophysiologic pathways leading to apoptosis and cell death.

In cultured cells, hydrogen protects the PC12 cell line derived from the neural crests by reducing the hydroxyl radical. At the same time, it spares other ROS which may have important physiologic roles [13]. Due to its quenching capability, hydrogen has been shown to decrease lipid peroxidation [14], prevent DNA oxidation [15] and reduce the glutamate toxicity-induced death of neurons [16].

In addition to the scavenger properties, hydrogen modulates the cellular response to oxidative stress, inflammation and apoptosis pathways. Mitophagy, the removal of dysfunctional mitochondria that produce excessive amounts of ROS is enhanced by hydrogen through the PINK1/Parkin signaling pathway [17]. Hydrogen also increases expression of the Nrf2, which mediates the cellular response to oxidative stress via antioxidant enzymes [18]. Moreover, hydrogen attenuates the reduction of activity of the antioxidant enzymes superoxide dismutase and catalase seen in ischemic rats [19].

The anti-inflammatory effect of hydrogen is highlighted by the decrease of the number of microglia and astrocytes found in injured brain tissue [15,20]. Hydrogen increases the number of the regulatory T cells. The level of proinflammatory cytokines such as TNF- α , IL-6, IL-1 β concentrations are decreased while the anti-inflammatory cytokines such as TGF- β are increased. Finally, hydrogen activates the NF- κ B that regulates many antiapoptotic factors and cellular inhibitors of apoptosis proteins [21].

Human exposure to hydrogen has been shown to be remarkably innocuous. One limiting factor of using hydrogen gas is the risk of explosion at concentrations above 4%. Safer storage technologies are being developed, in particular hydride [22]. The risk of explosion can also be overcome by dissolving hydrogen in water or normal saline which can be administered orally or intravenously, respectively. Administration of lactulose which by fermentation in colon produces hydrogen is an alternative in situations that do not require a rapid administration [23].

The present challenge is translating the cellular protective mechanisms of hydrogen into clinical applications. The animal models studied include occlusion of the middle cerebral artery, common carotid arteries or four vessels occlusion and induced cardiac arrest followed by resuscitation.

In the transient focal ischemia model, administration of 2% hydrogen gas during ischemia-reperfusion or only during reperfusion reduced infarction 1 day later and behavioral deficits 7 days later [15]. Moreover, the spontaneously hypertensive stroke-prone rats who received hydrogen-rich water had fewer hemorrhagic and ischemic infarcts in cortex and hippocampus. The mechanism appears to be protection of the blood–brain barrier and suppression of matrix metalloproteinase activity in the hippocampus [24]. Following global cerebral ischemia, hydrogen increased the 7-day survival rate of mice from 8.3 to 50% [25]. Another study showed that the improvement of functional outcome after cardiac arrest is comparable to therapeutic hypothermia [26].

Human studies have also been conducted. An open-label, prospective, nonrandomized study in which 38 patients received hydrogen and edaravone showed that laboratory, ECG and radiographs of patients did not deteriorate [27]. Neutral-pH hydrogen-enriched water did not produce genetic mutations, clinical symptoms or laboratory changes. The no-observable-adverse-effect level was greater than 20 ml/kg per day. For a 60-kg person, the equivalent amount of neutral-pH hydrogen-enriched water is at least 1.2 l/day [28].

In a clinical trial of 25 patients with cerebral ischemia, 3% hydrogen was administered by inhalation for 1 h of twice daily for 7 days. Hydrogen concentration reached a plateau at 20 min, then it decreased to 10% of the plateau after 6–18 min of cessation of administration, in arterial and venous blood, respectively. The vital signs and usual blood tests were unchanged, except for an improvement of O₂ saturation in the hydrogen group, which improved clinically more rapidly than the control group [29,30].

There is increasing evidence from cellular, animal and human studies suggesting that hydrogen can be administered safely as a neuroprotector during revascularization. The encouraging preliminary data call for larger randomized double-blind studies to further evaluate the role of hydrogen in the treatment of acute stroke.

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