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Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals

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Acute oxidative stress induced by ischemia-reperfusion or inflammation causes serious damage to tissues, and persistent oxidative stress is accepted as one of the causes of many common diseases including cancer. We show here that hydrogen (H_2) has potential as an antioxidant in preventive and therapeutic applications. We induced acute oxidative stress in cultured cells by three independent methods. H_2 selectively reduced the hydroxyl radical, the most cytotoxic of reactive oxygen species (ROS), and effectively protected cells; however, H_2 did not react with other ROS, which possess physiological roles. We used an acute rat model in which oxidative stress damage was induced in the brain by focal ischemia and reperfusion. The inhalation of H_2 gas markedly suppressed brain injury by buffering the effects of oxidative stress. Thus H_2 can be used as an effective antioxidant therapy; owing to its ability to rapidly diffuse across membranes, it can reach and react with cytotoxic ROS and thus protect against oxidative damage.

Oxidative stress arises from the strong cellular oxidizing potential of excess reactive oxygen species (ROS), or free radicals[1–5]. Most of the superoxide anion radical (O_2^{-}•) produced is generated in mitochondria by electron leakage from the electron transport chain and the Krebs cycle[6]. O_2^{-}• is also produced by metabolic oxidases, including NADPH oxidase and xanthine oxidase[7]. Superoxide dismutase converts O_2^{-}• into hydrogen peroxide (H_2O_2)[8], which is detoxified into H_2O by either glutathione peroxidase or catalase. Excess O_2^{-}• reduces transition metal ions such as Fe^{3+} and Cu^{2+} (ref. 2), the reduced forms of which in turn can react with H_2O_2 to produce hydroxyl radicals (•OH) by the Fenton reaction. •OH is the strongest of the oxidant species and reacts indiscriminately with nucleic acids, lipids and proteins. There is no known detoxification system for •OH; therefore, scavenging •OH is a critical antioxidant process[9].

Despite their cytotoxic effects, O_2^{-}• and H_2O_2 play important physiological roles at low concentrations: they function as regulatory signaling molecules that are involved in numerous signal transduction cascades and also regulate biological processes such as apoptosis, cell proliferation and differentiation[7,10]. At higher concentrations, H_2O_2 is converted into hypochlorous acid by myeloperoxidase; hypochlorous acid defends against bacterial invasion[5]. Nitric oxide (NO•), another ROS, functions as a neurotransmitter and is essential for the dilation of blood vessels[11]. Thus, cytotoxic radicals such as •OH must be neutralized without compromising the essential biological activities of other, physiologically beneficial, ROS. Here we demonstrate that molecular hydrogen (dihydrogen, H_2) can alleviate •OH-induced cytotoxicity without affecting the other ROS, and propose that H_2 has potential as an antioxidant for preventive and therapeutic applications.

RESULTS

H_2 selectively reduces •OH in cultured cells

H_2 reduces the •OH that is produced by radiolysis or photolysis of water[12]; however, whether H_2 can effectively neutralize •OH in living cells has not been directly investigated. As the cellular damage produced by spontaneous generation of •OH is not sufficient to be detectable, we induced O_2^{-}• production in PC12 cultured cells. To do this, we treated the cells with a mitochondrial respiratory complex III inhibitor, antimycin A (ref. 13); following such treatment, O_2^{-}• in these cells is rapidly converted into H_2O_2. The addition of antimycin A increased levels of O_2^{-}• and H_2O_2, as judged by the fluorescence signals emitted by the oxidized forms of MitoSOX (**Fig. 1a**) and 2′,7′-dichlorodihydrofluorescein (H_2DCF) (**Supplementary Fig. 1** online), respectively. We dissolved H_2 and O_2 into medium as described in the Methods, and confirmed the prolonged (24 h long) maintenance of H_2 levels (**Supplementary Fig. 2** online). H_2 dissolved in culture medium did not decrease MitoSOX and DCF signals in the cells (**Fig. 1a,b** and **Supplementary Fig. 1**). Additionally, H_2 did not decrease the steady-state level of NO• (**Supplementary Fig. 1**). In contrast, H_2 treatment significantly decreased levels of •OH, as assessed by the fluorescence signal emitted by the oxidized form of 2-[6-(4′-hydroxy)phenoxy-3*H*-xanthen-3-on-9-yl] benzoate (HPF) (refs. 14,15 and **Fig. 1c,d**). When we exposed the cells to antimycin A (30 μg/ml) in the absence of H_2, the HPF signals increased in both the nuclear region and the cytoplasm, probably because H_2O_2 diffused from the mitochondria to produce •OH. Notably, H_2 decreased •OH levels even in the nuclear region (**Fig. 1c**).

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