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Title: Hydrogen gas alters the production of reactive oxygen species in alveolar epithelial cells in vitro

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Body: [Aim] The pulmonary toxicity of high concentration of oxygen during mechanical ventilation relates to reactive oxygen species (ROS). Hydrogen gas (H_2) has potential as eliminating highly reactive ROS. We therefore expected that H_2 could reduce the adverse effects of the oxygen exposure. The aim of the present study was to determine the protective effects of H_2 against various oxidative stresses on epithelial cells in vitro. [Methods] Human alveolar epithelial cells (A549) were incubated with antimycin A which enhances the generation of superoxide anions (O_2^-) in mitochondria, with menadione which exogenously generates O_2^- and H_2O_2 in the cells, or with Cu^+ (converted from Cu^{2+} by ascorbic acid) which exogenously generates hydroxyl radical by the Fenton reaction with added Cu^+ and endogenous H_2O_2. The viability of the cells as well as the levels of O_2^- and highly reactive ROS in the cells was evaluated with or without 2% H_2. [Results] The viability of the cells incubated with menadione or Cu^+ decreased or did not change in the presence of H_2, respectively, while that with antimycin A significantly increased in the presence of H_2 ($n=12$, $P<0.01$). The production of O_2^- induced by antimycin A significantly decreased with the addition of H_2 ($n=11$, $P<0.01$), while highly reactive ROS induced by menadione significantly increased in the cells incubated with H_2 ($n=5$, $P<0.01$). [Conclusions] H_2 protects alveolar epithelial cells against injury induced by antimycin A probably due to the decrease in the production of O_2^- in mitochondria, while H_2 cannot act protective against ROS induced by menadione or the Fenton reaction, meaning that H_2 cannot overcome the effects of exogenously provided ROS.