

MEETING ABSTRACT

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Anti-diabetes effect of water containing hydrogen molecule and Pt nanoparticles

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Background

Electrochemically reduced water (ERW) contains a lot of hydrogen molecule (H₂) and scavenges reactive oxygen species (ROS) to protect DNA from oxidative damage [1]. ERW also contains small amounts of Pt nanoparticles (NPs) and elongates the lifespan of *C. elegans* [2]. Pt NPs are newly recognized multi-functional ROS scavengers [3]. ERW exhibits anti-diabetes effects *in vitro* and *in vivo* [4-6][7]. We proposed mineral nanoparticle active hydrogen reduced water hypothesis to explain the activation mechanism of H₂ to hydrogen atom (H)[4]. Recently, H₂ has been reported to scavenge ROS and suppress a variety of oxidative stress-related diseases [8], however, the action mechanism of H₂ has not been clarified thoroughly. Here, we examined anti-diabetes effects of H₂ and Pt NPs.

Materials and methods

Pt NPs of 2-3 nm sizes were synthesized from H₂PtCl₆ by the citrate reduction method. L6 rat myoblast cells (1.2 x 10⁵ cells) were inoculated into a 35 mm culture dish and a day later, the cells were treated with or without 25mM N-acetylcystein in the presence of BES-H₂O₂, a H₂O₂-specific detection reagent in DMEM for 2 h. After washing the cells, molecular hydrogen treatment was performed in a dark condition by cultivating cells in a fresh DMEM medium in a mixed gas incubator under an atmosphere of 75%N₂/20%O₂/5%CO₂ or 75%(H₂ and N₂ mixed gas)/20%O₂/5%CO₂ for 1.5 h, followed by flowcytometric analysis. In this condition, culture medium contained maximum 0.4-0.5 ppm of dissolved hydrogen. Glucose uptake of differentiated

myotube L6 cells was examined after treating the cells with ³H-2-deoxyglucose for 10 min. Gene expression of catalase (CAT), glutathione peroxidase (GPx) and hemoxygenase (HO-1) was examined using RT-PCR method. Three weeks old type 2 diabetes model mice (KK-A^y) were fed H₂ and/or Pt Nps-containing water *ad lib* for 6 weeks.

Results

H₂ stimulated glucose uptake into L6 cells. Pt NPs catalyzed the activation of H₂ to hydrogen atom (H) to scavenge DPPH radical *in vitro*. The combined use of molecular hydrogen and Pt NPs resulted in extremely stimulated glucose uptake into L6 cells, suggesting that H produced from H₂ by catalyst action of Pt NPs regulated glucose uptake signal transduction. As oppose to the paper by Ohsawa et al.[8], H₂ of 25 to 75% concentration in the mixed gas significantly scavenged intracellular H₂O₂ in rat fibroblast L6 cells (Figure 1) and induced the gene expression of antioxidative enzymes such as CAT, GPx and HO-1 via activation of Nrf2 (Figure 2). H₂, Pt NPs and their combination significantly suppressed the levels of fasting blood glucose and improved the impaired sugar tolerance abilities of obese insulin-resistant type 2 diabetic KK-A^y mice.

Conclusion

H₂, Pt NPs, and their combined use resulted in activation of glucose uptake signal transduction pathways and stimulation of glucose uptake into L6 myotubes. In the groups of H₂, Pt NPs and their combined use groups, blood sugar levels and impaired sugar tolerance of type 2 diabetes model mouse (KK-A^y) were significantly improved, suggesting that H₂, Pt NPs and H are redox regulation factors in animal cells.

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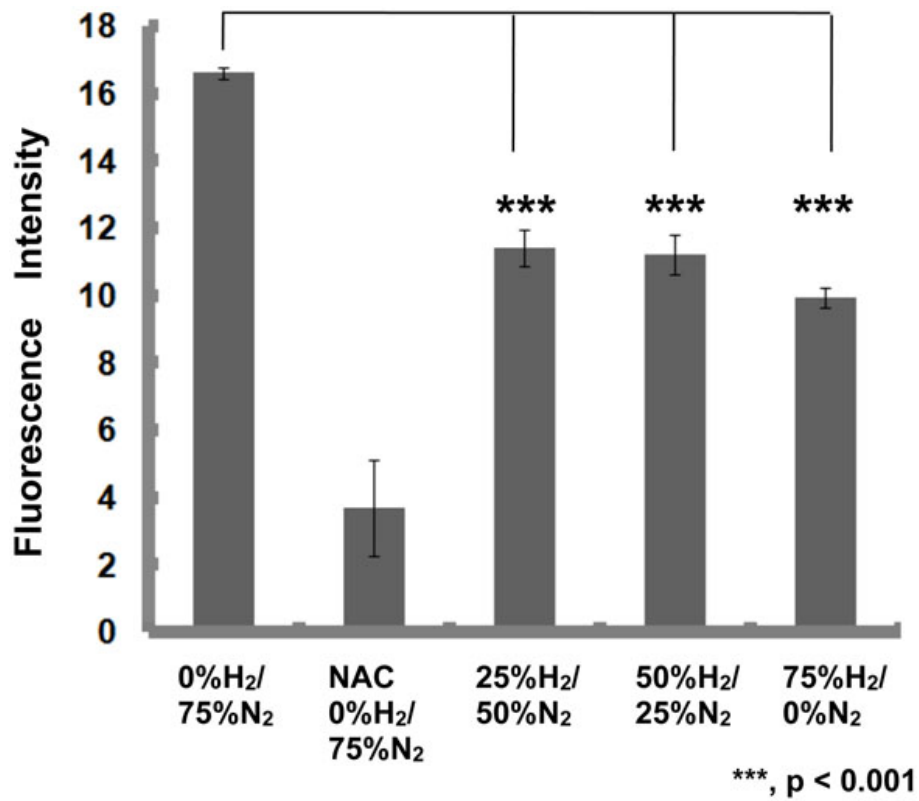


Figure 1 The scavenging effect of hydrogen molecule on intracellular hydrogen peroxide in rat myotube L6 cells. ***, p<0.001.

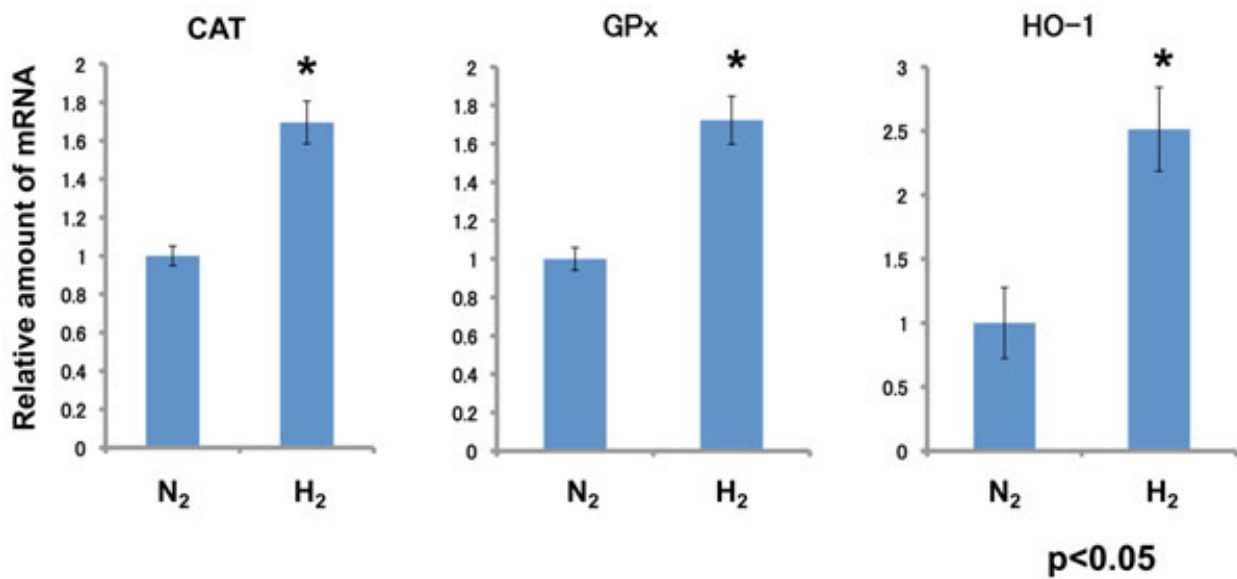


Figure 2 Induced gene expression of antioxidant enzymes by hydrogen molecule. L6 myoblast cells were cultivated under an atmosphere of 75%N₂ or H₂/20%O₂/5%CO₂ for 2 h and gene expression was analyzed by RT-PCR. *, P<0.05

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