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MEETING ABSTRACT Open Access

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 (H_2) 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 H_2 to hydrogen atom (H)[4]. Recently, H_2 has been reported to scavenge ROS and suppress a variety of oxidative stress-related diseases [8], however, the action mechanism of H_2 has not been clarified thoroughly. Here, we examined anti-diabetes effects of H_2 and Pt NPs.

Materials and methods

Pt NPs of 2-3 nm sizes were synthesized from H_2PtCl_6 by the citrate reduction method. L6 rat myoblast cells (1.2×10^5 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 H_2O_2-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%N_2/20%O_2/5%CO_2 or 75%(H_2 and N_2 mixed gas)/20%O_2/5%CO_2 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 ^{3}H-2-deoxyglucose for 10 min. Gene expression of catalase (CAT), glutathione peroxidase (GPx) and hemoxoigenase (HO-1) was examined using RT-PCR method. Three weeks old type 2 diabetes model mice (KK-A^y) were fed H_2 and/or Pt Nps-containing water *ad lib* for 6 weeks.

Results

H_2 stimulated glucose uptake into L6 cells. Pt NPs catalyzed the activation of H_2 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 H_2 by catalyst action of Pt NPs regulated glucose uptake signal transduction. As oppose to the paper by Ohsawa et al.[8], H_2 of 25 to 75% concentration in the mixed gas significantly scavenged intracellular H_2O_2 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). H_2, 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

H_2, 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 H_2, 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 H_2, Pt NPs and H are redox regulation factors in animal cells.

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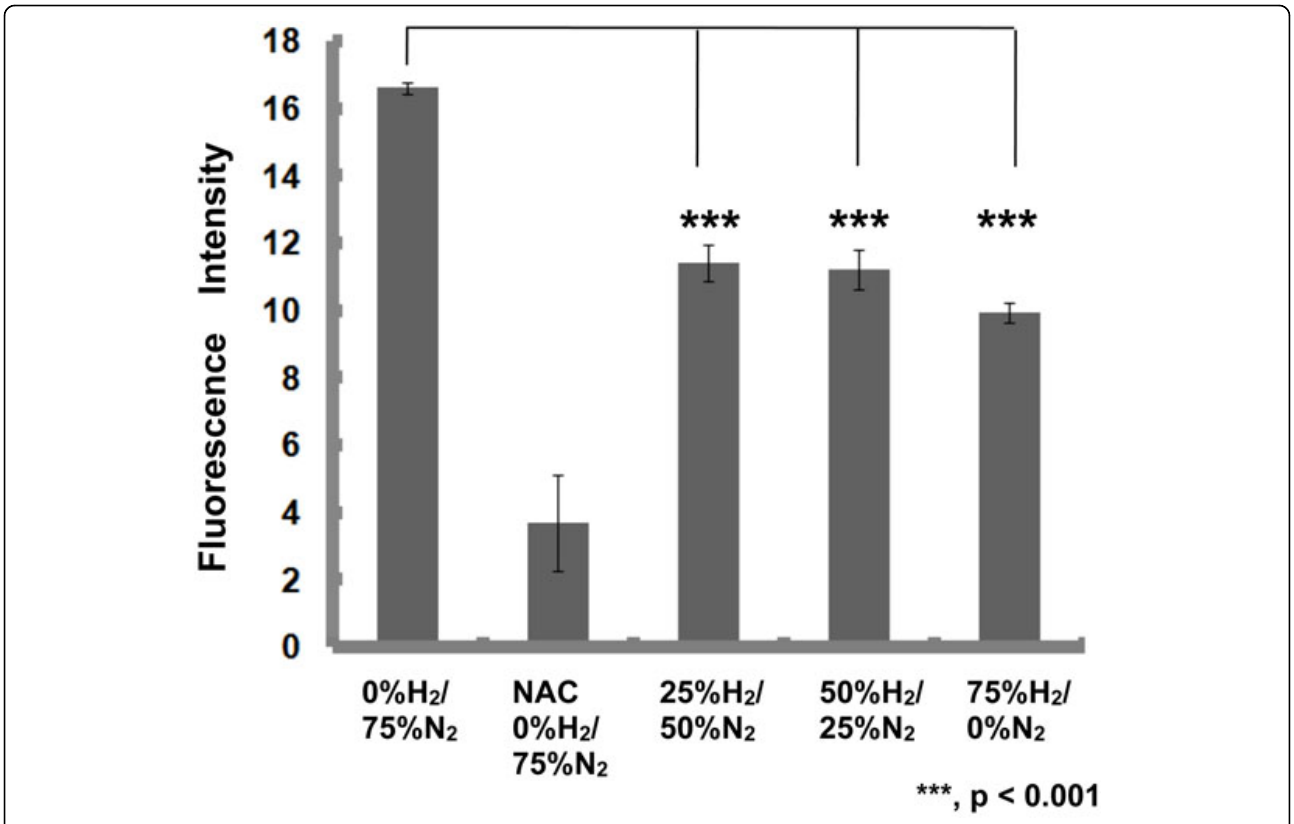

Figure 1 The scavenging effect of hydrogen molecule on intracellular hydrogen peroxide in rat myotube L6 cells. ***, p<0.001.

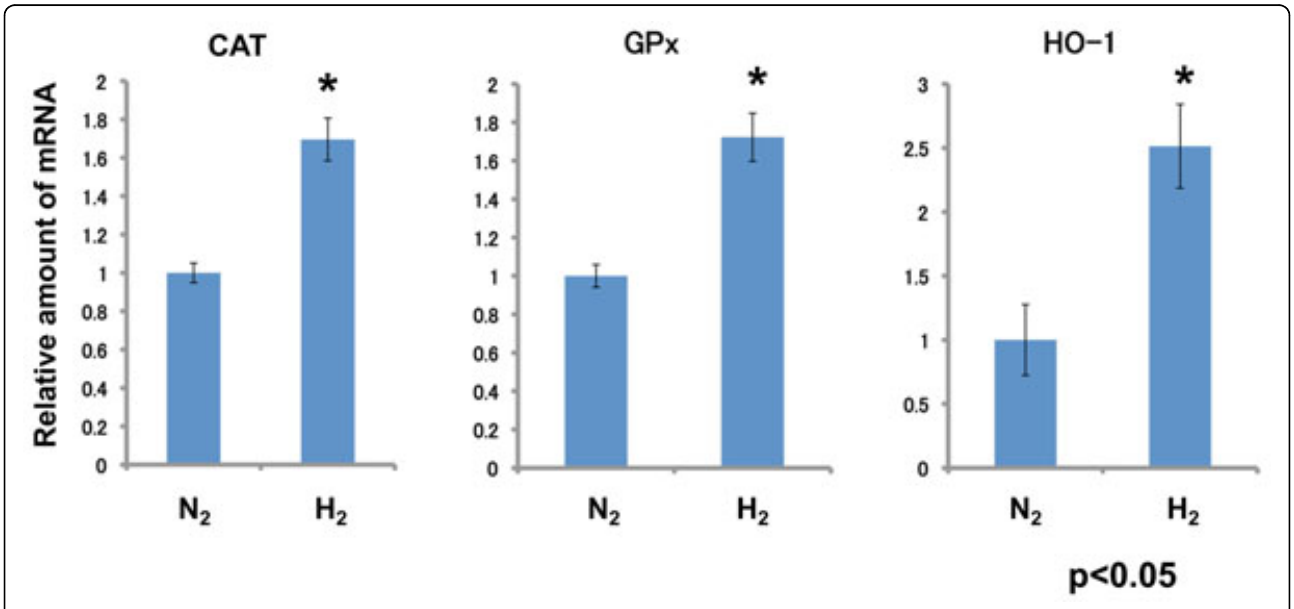

Figure 2 Induced gene expression of anitoxidative enzymes by hydrogen molecule. L6 myoblast cells were cultivated under an atmosphere of 75%N_2 or H_2/20%O_2/5%CO_2 for 2 h and gene expression was analyzed by RT-PCR. *, P<0.05

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References

1. Shirahata S, Kabayama S, Nakano M, Miura T, Kusumoto K, Gotoh M, Hayashi H, Otsubo K, Morisawa S, Katakura Y: **Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage.** *Biophys Biochem Res Commun* 1997, **234**:269-274.
2. Yan H, Tian H, Kinjo T, Hamasaki T, Tomimatsu K, Nakamichi N, Teruya K, Kabayama S, Shirahata S: **Extension of the lifespan of *Caenorhabditis elegans* by the use of electrolyzed reduced water.** *Biosci Biotech Biochem* 2010, **74**:2011-2015.
3. Hamasaki T, Kashiwagi T, Imada T, Nakamichi N, Aramaki S, Toh K, Morisawa S, Shimakoshi H, Hisaeda Y, Shirahata S: **Kinetic analysis of superoxide anion radical-scavenging and hydroxyl radical-scavenging activities of platinum nanoparticles.** *Langmuir* 2008, **24**:7354-7364.
4. Shirahata S, Hamasaki H, Teruya K: **Advanced research on the health benefit of reduced water.** *Trends Food Sci Tech* 2011, DOI 10.1016/j.tifs.2011.10.009.
5. Li Y–P, Nishimura T, Teruya K, Maki T, Komatsu T, Hamasaki T, Kashiwagi T, Kabayama S, Shim S–Y, Katakura Y, Osada K, Kawahara T, Otsubo K, Morisawa S, Ishii Y, Gadek Z, Shirahata S: **Protective mechanism of reduced water against alloxan-induced pancreatic β-cell damage: Scavenging effect against reactive oxygen species.** *Cytotechnology* 2002, **40**:139-149.
6. Li Y–P, Hamasaki T, Nakamichi N, Kashiwagi T, Komatsu T, Ye J, Teruya K, Abe M, Yan H, Kinjo T, Kabayama S, Kawamura M, Shirahata S: **Suppressive effects of electrolyzed reduced water on alloxan-induced apoptosis and type 1 diabetes mellitus.** *Cytotechnology* 2010, DOI 10.1007/s10616-010-9317-6.
7. Kim M–J, Kim H–K: **Anti-diabetic effects of electrolyzed reduced water in streptozotocin-induced and genetic diabetic mice.** *Life Sciences* 2006, **79**:2288-2292.
8. Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura K, Katayama Y, Asoh S, Ohta S: **Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals.** *Nature Med* 2007, **13**:688-694.

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