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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 ($1.2 \times 10^5$ 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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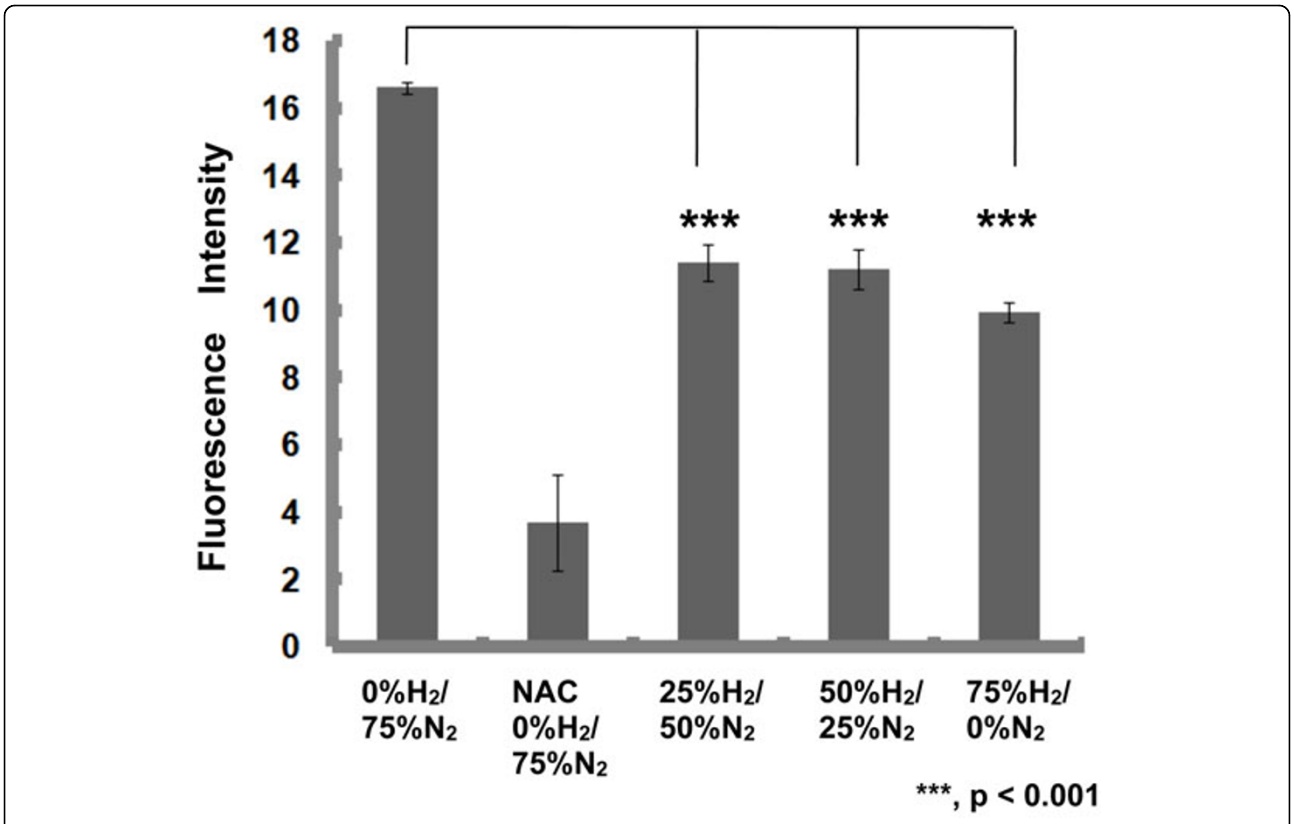


**Figure 1** The scavenging effect of hydrogen molecule on intracellular hydrogen peroxide in rat myotube L6 cells. ***, p<0.001.

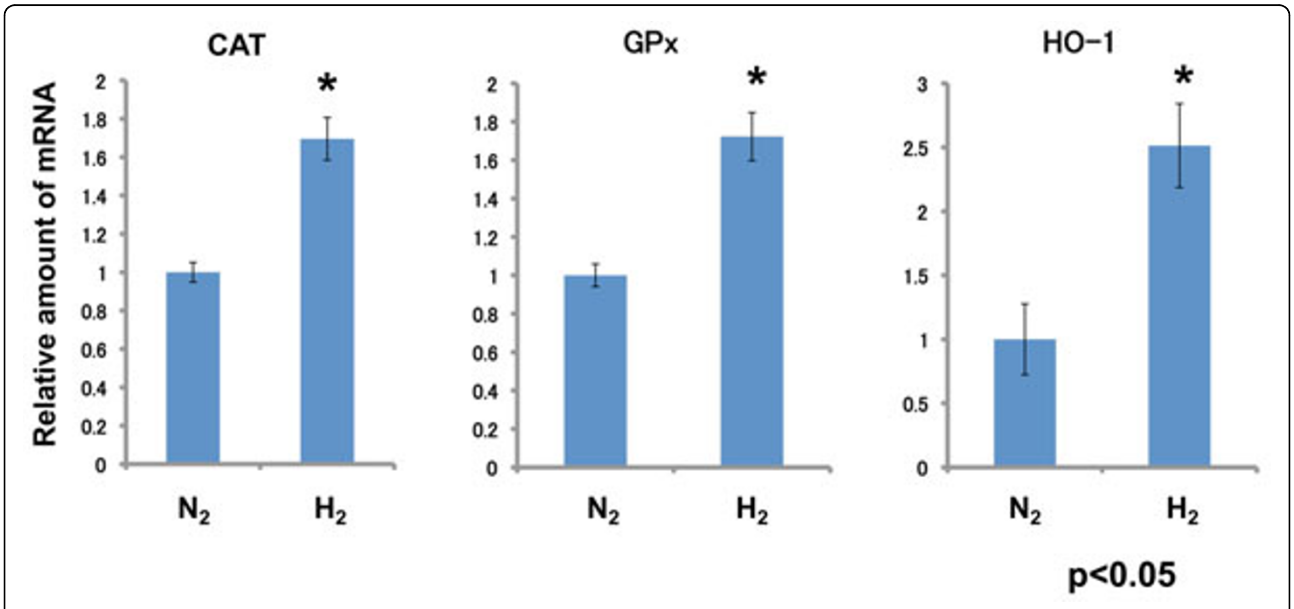


**Figure 2** Induced gene expression of anitoxidative enzymes by hydrogen molecule. L6 myoblast cells were cultivated under an atmosphere of 75%$N_2$ or $H_2$/20%$O_2$/5%$CO_2$ for 2 h and gene expression was analyzed by RT-PCR. *, P<0.05

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