ELECTROLYZED AND NATURAL REDUCED WATER EXHIBIT INSULIN-LIKE ACTIVITY ON GLUCOSE UPTAKE INTO MUSCLE CELLS AND ADIPOCYTES

M. Oda, K. Kusumoto, K. Teruya, T. Hara, T. Maki, S. Kabayama, Y. Katakura, K. Otsubo[1], S. Morisawa[1], H. Hayashi[2], Y. Ishii[3] and S. Shirahata
Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka, Japan; [1]Nihon Trim Co. Ltd., Osaka, Japan; [2]Water Institute, Tokyo, Japan; [3]Hita Aqua Green Co. Ltd., Hita, Japan)

Abstract

In the type 2 diabetes, it has become clear that reactive oxygen species (ROS) cause reduction of glucose uptake by inhibiting the insulin-signaling pathway in muscle cells and adipocytes. We demonstrated that electrolyzed-reduced water (ERW) scavenges ROS and protects DNA from oxidative damage[1)]. Here we found that ERW scavenges ROS in insulin-responsive L6 myotubes and mouse 3T3/L1 adipocytes. Uptake of 1-deoxy-D-glucose (2-DOG) into both L6 cells and 3T3/L1 cells was stimulated by ERW in the presence or absence of insulin. This insulin-like activity of ERW was mediated by the activation of PI-3 kinase, resulting in stimulation of translocation of glucose transporter GLUT4 from microsome to plasma membrane. These results suggest that ERW may be useful to improve insulin-independent type 2 diabetes.

1. Introduction

Reactive oxygen species (ROS: $^1O_2$, $O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$ *etc.*) are known to cause irreversible damage to macromolecules such as nucleic acids, proteins and lipids. Since ROS are produced in many processes in many tissues, there are many diseases caused by ROS. Cancer, diabetes and arteriosclerosis are representative those.

Diabetes is mainly grouped into two types; IDDM (insulin-dependent diabetes mellitus) and NIDDM (insulin-independent diabetes mellitus). Insulin stimulates blood glucose uptake into muscle and adipocytes which are main tissues in the body. IDDM is caused by deficiency of insulin secretion from pancreas. NIDDM is caused by lowered responses of cells against insulin (insulin-resistance). Recently participation of ROS has been noted in both IDDM and NIDDM. Since oxidative damage in insulin signaling pathway (hypeoxia[2)], high glucose[3,4)]) has been reported, we tried to improve glucose uptake by shifting redox state of muscle cells and adipocytes to more reduced one by reduced water.

A. Bernard et al. (eds.), Animal Cell Technology: Products from Cells, Cells as Products, 425–427.

Devices to reform tap water by way of electrolysis were produced in Japan before half a century ago and now very popular in Japan. Daily intake of electrolyzed alkaline reduced water produced near cathode by electrolysis is believed to be beneficial for health. The ministry of Health and Welfare In Japan authorized in 1965 that the intake of electrolyzed reduced water is effective for restoration of unusual fermentation of intestinal flora. However, the action mechanism of electrolyzed reduced water was unknown for a long time. Recently we found electrolyzed reduced water (ERW) contains a lot of hydrogen, scavenges ROS and protects DNA from oxidative damage[1]. We proposed active hydrogen water hypothesis that active hydrogen in reduced water may be ideal radical scavenger to scavenge ROS[1]. Cancer cells cultured in electrolyzed reduced water exhibited suppressed growth, drastic morphological changes, telomere-shortening, lowered activities of telomere binding proteins, suggesting that cancer cells lost tumor phenotypes in reduced water[5]. Since sooner decline in blood sugar level in diabetic patients by daily intake of electrolyzed reduced water or of natural reduced water drawn from deep underground in some districts has been reported, we examined effects of those reduced water on glucose uptake into muscle and adipocytes.

2. Materials and Methods

Preparation of reduced water Electrolyzed reduced water (ERW) was obtained from ultrapure water containing 0.01% NaCl by using an electrolyzing device (type TI-7000S, Nihon Trim Co., Osaka). Natural reduced water drawn from 1000 m underground was provided from Hita Aqua Green Co. Ltd.

Cell Culture Rat skeletal muscle L6 cells were differentiated in DMEM containing 2% FBS and experiments on myotubes were usually performed between days 9 and 11 after the initiation of differentiation[6]. Differentiation from 3T3/L1 fibroblasts into adipocytes were accomplished as previously described[7,8]. Mature 3T3/L1 adipocytes were used between days 10 and 12 after the initiation of differentiation.

Assay of intracellular redox state Intracellular redox state levels were measured using a fluorescent dye, 2',7'-dichlorofluorescein-diacetate(DCFH-DA).

2-Deoxyglucose uptake After incubation in serum-deprived DMEM for 5 h at 37 °C prior to incubation with or without insulin, cells were rinsed twice with HEPES-buffered saline, followed by determinations of transport of 2-deoxy-D-[^{3}H]glucose(1μCi/ml) for 10 min in the same solution, and the associated radioactivity was determined by a liquid scintillation counter.

3. Results and Discussion

First we observed intracellular redox state of L6 myotubes treated with ERW. After incorporation into cells, DCFH-DA changes to fluorescent substance by oxidation with H_2O_2 accumulated in cells. We found ERW-treated cells shifted to more reduced redox state compared to control cells. Even when the cells were more oxidized in the presence of