Redox Regulation by Reduced Waters as Active Hydrogen Donors and Intracellular ROS Scavengers for Prevention of type 2 Diabetes

S. Shirahata\textsuperscript{1}, Y. Li\textsuperscript{1}, T. Hamasaki\textsuperscript{1}, Z. Gadek\textsuperscript{2}, K. Teruya\textsuperscript{1}, S. Kabayama\textsuperscript{3}, K. Otsubo\textsuperscript{3}, S. Morisawa\textsuperscript{3}, Y. Ishii\textsuperscript{4}, Y. Katakura\textsuperscript{4}

\textsuperscript{1}Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan; \textsuperscript{2}Centre for Holistic Medicine and Naturopathy, Nordenau, Germany, \textsuperscript{3}Nihon Trim Co.Ltd., Osaka, Japan; \textsuperscript{4}Hita Tenryosui Co. Ltd., Hita, Oita, Japan

Abstract: The analysis using the DBNBS reduction method and the DCFH-DA intracellular reactive oxygen species (ROS) determination method revealed that ERW and diseases-improvable natural waters such as Nordenau water in Germany and Hita water in Japan were all reduced waters (RWs) which could function as active hydrogen donors and intracellular ROS scavengers. RWs suppressed the activity of protein tyrosine phosphatase (PTP), which inactivates insulin receptor, suggesting their anti-type 2 diabetes effects via redox regulation. The clinical test of 356 diabetes patients drinking Nordenau water in Germany resulted in the improvement of the relevant tests parameters after 6 days, suggesting the correlation of these changes with the fluctuation of ROS levels in their blood.

Key words: reduced water, active atomic hydrogen, reactive oxygen species, diabetes

1. INTRODUCTION

Hydrogen-rich electrolyzed reduced water (ERW) scavenged reactive oxygen species (ROS) (Shirahata \textit{et al.}, 1997) and was applied to suppress the oxidative stress of hemodialysis patients (Huang \textit{et al.}, 2003). Some natural mineral waters such as Nordenau water found in Germany in 1992 and Hita water (Hita Tenryousui®) found in Japan in 1997 as well as ERW
scavenged ROS and protected pancreatic β-cells from oxidative stress induced by alloxane (Li et al., 2002). Here, we report redox regulation by reduced waters (RWs) for prevention of type 2 diabetes and clinical trials of type 2 diabetes patients.

2. MATERIALS AND METHODS

2.1 Evaluation of waters as active hydrogen donors and ROS scavengers

A novel colorimetric determination method of active hydrogen in aqueous solution with a spin trap reagent, 3,5-dibromo-4-nitrosobenzene-sulfonic acid sodium salt (DBNBS) was utilized to evaluate the functions of waters as active hydrogen donors. Intracellular ROS levels of rat L6 myotubes were determined using DCFH-DA as described previously (Li et al., 2002).

2.2 Determination of glucose uptake, phosphorylated insulin receptor and activity of PTPase

Glucose uptake into L6 cells was determined using 3H-2-deoxyglucose. Fully differentiated L6 myotubes were incubated with various waters for 72 h. After stimulation with insulin for 20 min, total cell lysates were separated by SDS-PAGE, immunoprecipitated with anti-insulin receptor (IR) β-subunit, and then immunoblotted with anti-phospho-IR (pY1158) antibody. The protein tyrosine phosphatase (PTPase) activity was measured using p-nitrophenyl phosphate as substrate.

2.3 Clinical trials on type 2 diabetes patients drinking Nordenau water

Changes in the relevant tests parameters of 356 type 2 diabetes patients (average age; 71.5 years old) drinking Nordenau water (2 liter per day) were examined after 6 days. The diagnostic parameters such as blood sugar, HbA1c, cholesterol, LDL and serum creatinine concentration were tested twice – at the beginning (MP1) and at the end of the participants stay (MP2) in Nordenau. ROS in randomly sampled bloods of 81 patients were examined by FORT (Free Oxygen Radical) test.