EFFECTS OF MINERAL-RICH SOLAR SALT ON INSULIN RESISTANCE AND INSULIN SIGNALING

Ekkapon Lertthanakornkul, Tian-Cheng Gao, Mi-Ae Bang, Jeong-Yong Cho and Kyung-Sik Ham

Department of Food Engineering and Solar Salt Biotechnology Research Center, Mokpo National University, Jeonnam 534-729, Republic of Korea

Abstract: Insulin resistance is known to be induced by high-salt consumption. This knowledge comes from researches which used mineral-deficient salts (MDS) such as reagent-grade salt, purified salt and rock salt. Insulin resistance contributes significantly to the pathophysiology of type 2 diabetes mellitus. One mechanism that mediates insulin resistance involves phosphorylation of serine residue in insulin receptor substrate-1 (IRS-1), leading to impaired ability of IRS-1 to degrade insulin signaling pathways. We have investigated whether the effects of mineral-rich solar salt (MRS) on insulin resistance and insulin signaling are different from those of MDS. Male Sprague-Dawley rats were fed diets containing 8% NaCl of MRS (94% NaCl) and MDS (more than 99% NaCl) for four weeks. Our research suggests that MRS intake is preferable to MDS intake in reducing the risk of insulin resistance by improving glucose tolerance and insulin-stimulated glucose uptake. Moreover, MRS group enhanced insulin signaling by diminishing Ser^{307} phosphorylation of IRS-1 and the expression of NF-κB and IκB that are involved in inflammation compared to MDS group.

Key words: mineral-rich salt, insulin resistance, serine phosphorylation, glucose uptake

1. Introduction

It is known that excessive sodium intake can cause the increment of blood pressure and also induce insulin resistance. However, this knowledge comes from the researches which used mineral-deficient salts (MDS) such as reagent-grade salt, purified salt and rock salt, all of which are lacking of minerals. There are various types of salts for food consumption. Mineral-rich solar salt (MRS), which is made in tide flat (marsh land), has a plenty of minerals such as potassium, calcium, magnesium, etc than other salts (purified salt and rock salt). Type 2 diabetes mellitus is affecting more than 150 million people worldwide. It is characterized by insulin resistance, impaired glucose tolerance (IGT) and reduced glucose uptake from blood into adipose tissue and skeletal muscle. One mechanism that mediates insulin resistance involves phosphorylation of serine residue in insulin receptor substrate-1 (IRS-1) as known to be promoted by elevated circulating levels of several metabolites, including free fatty acid and glucose. Moreover, adipose-derived cytokines like TNF-α also activate Ser^{307} phosphorylation of IRS-1 by inhibiting PTB domain, which uncouples IRS-1 from insulin receptor, leading to diminish the recruitment of PI 3-kinase and to stimulate the IRS-1 degradation pathway. Insulin-resistant states and Ser^{307} phosphorylation of IRS-1 are associated with the activation of NF-κB. However, the precise molecular mechanisms by which NF-κB contributes to the development of insulin resistance remain unclear. In this study, we have investigated whether effects of MRS on insulin resistance and signaling are different from those of MDS.

2. Materials and methods

2.1 Materials

Anti-phospho-IRS-1 (Ser^{307}) and antibody against NF-κB (p65) were purchased from Upstate Biotech, Inc., USA. Monoclonal antibody anti-IκBα and rabbit anti-GAPDH polyclonal antibody were purchased from AB frontier, Korea. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobin G was purchased from Millipore, USA.

2.2 Animals

Four-week-old male Sprague-Dawley rats (Damool Science Company, Daejeon, Korea) were fed standard rodent diet (2018S, Halan Teklad) or high-salt diets containing 8% NaCl of mineral-rich salt (MRS group) or mineral-deficient salt (MDS group) from Sigma-Aldrich (St. Louis, Mo., USA) during 4-week experiment period. The rats were housed in a room at constant humidity (55±5%), temperature (25±1°C), and light cycle (12 hours: 0600 to 1800). Food and water were available ad libitum throughout the study.

2.3 Analytical methods

The systolic and diastolic blood pressures were measured in conscious rats by tail-cuff method using Heater Scanner LE 5650/6 and Storage Pressure Meter LE 5002 (Panlab sl., Barcelona). Plasma glucose concentration was determined by using glucometer ACCU-Chek Active (Roche Diagnostics, Basel, Switzerland). Plasma insulin concentration was determined by sandwich technique of enzyme immunoassay using rat insulin ELISA kit (Shibayagi Co., Gunma, Japan).

2.4 Oral glucose tolerance test

The rats were fasted overnight and then were given oral glucose solution. Whole blood was obtained from tail vein. Plasma glucose and insulin concentration were immediately determined at 0, 30, 60 and 120 min, respectively, after glucose intake. The total area under the curve (AUC) was calculated from 0 to 120 min.

2.5 Glucose uptake into isolated tissues

Based on nonradioisotope assay, rats were anesthetized by diethyl ether. The adipose tissue and skeletal muscle were isolated after exsanguinations. Fresh tissues were individually incubated for 15 min in KH-buffer, then incubated with insulin and 2-deoxyglucose. One hundred milligram of each frozen sample was homogenized and used for the glucose uptake assay. The fluorescence with ex: 530 nm and em: 590 nm was measured by FL-4500 fluorescence spectrometer (Hitachi, Japan).

2.6 Immunoblotting

The rats were anesthetized by injection of ketamine (45 mg/kg body wt. i.p.), after 15 min the abdominal cavity was opened, and 4 ml of normal saline with or without 10^{-5} M insulin was injected into portal vein. Adipose tissues were separated and homogenized. The extracts were centrifuged to remove insoluble material. Supernatants containing equal amounts of protein were used for SDS-PAGE and transferred to nitrocellulose membranes. Membranes were incubated with the primary antibodies (as described in Material) and then incubated with HRP secondary antibody. Proteins were visualized with enhanced chemiluminescence (ECL) and autoradiogram method.

2.7 Statistical analysis

Data are expressed as mean ± SE. Comparisons were made using the one-way analysis of variance (ANOVA). $P<0.05$ was considered a statistically significant difference.

3. Results and discussion

3.1 Characterization of rats studied

Male Sprague-Dawley rats, at 4 weeks of age, were fed normal diet (Con group), high mineral-rich solar salt (MRS group) or high mineral-deficient salt diet (MDS group) for 4 weeks (n=8 for each group). The body weight, weight gain, food intake, water intake, food efficiency ratio (FER) and plasma parameters of the rats were determined. The body weight and weight gain were significantly lower in high-salt groups than in the Con group. Food intake per rat and FER did not differ between three groups (Con, MRS or MDS group) but both of high-salt groups had significantly higher water intake than Con group. Fasting plasma glucose and insulin concentration were similar among

those groups. Systolic and diastolic blood pressures were significantly higher in high-salt group than in Con group. Moreover MRS group had significantly lower systolic and diastolic blood pressures than those of MDS group.

3.2 Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed with eight SD rats in each group. Whole blood was obtained from tail vein. The plasma glucose concentrations were not significantly different among groups in the period between 0 min to 60 min. At 120 min, the plasma glucose concentration of MDS group was significantly higher than MRS group. The total area under the curve (AUC) was calculated from 0 to 120 min. The AUC of MRS group was significantly lower than MDS group. However, the AUCs of plasma insulin were not significantly different between those three groups.

3.3 Insulin-induced 2-deoxy glucose (2DG) uptake in adipose tissue and skeletal muscle

For comparing the glucose uptake of each tissue, adipose tissue and skeletal muscle that were separated from anesthetized rats were incubated with insulin and 2DG, and then 2DG uptakes into the adipose tissue and skeletal muscle were assayed. All of the adipose tissues treated with insulin showed increased 2DG uptake. However, insulin-induced 2DG uptake was reduced more significantly in MDS group than MRS group. In skeletal muscle, insulin-stimulated 2DG uptakes were also reduced in both of MRS group and MDS group compared with Con group.

3.4 Serine phosphorylation of IRS-1 and the inhibition of NF-κB and IκBα

Ser^{307} phosphorylation of IRS-1 and the activation of NF-κB and IκBα expression levels in adipose tissue of MDS group were elevated more than those in MRS and Con group. Interestingly, insulin-stimulated Ser^{307} phosphorylation was reduced in MRS group compared with that of MDS group.

4. Conclusion

The data in this study indicate that mineral-rich solar salt plays an important role to enhance insulin signaling compared with mineral-deficient salt. Dietary supplement of mineral-rich solar salt may be preferred to mineral-deficient salts for the reduction of insulin resistance.

5. Reference

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