

CHRONIC EFFECTS OF VARIOUS SALTS ON OXIDATIVE STRESS IN RATS

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

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

Abstract: Heat-treated bamboo salt (HTBS) is a specially processed salt made according to the traditional recipe using solar salt and bamboo. It has been used for the purpose of prevention and treatment of various diseases in Korea. It was reported that HTBS had high radical scavenging power for various radicals such as DPPH, ABTS, and ferric radicals in vitro (Gao T. C. 2008). This study was designed to evaluate chronic effects of various salts on oxidative damage in normal rats. Male Sprague-Dawley rats (610 ± 15 g) were divided into four groups fed diets containing Normal (C), solar salt (SS), purified salt (PS) and HTBS. Seven months-old animals were fed diets containing various salts (1.8 g/kg diet) for seven weeks, then animals were sacrificed and blood samples were collected for assaying plasma biochemical parameters. The livers were excised for evaluating peroxidation products and antioxidant substances, as well as the activities of antioxidant enzymes. The results showed that HTBS diet had significantly ($p < 0.05$) lower plasma levels of the peroxidation product, malondialdehyde.

Key word: solar salt, heat-treated bamboo salts, antioxidant

1. Introduction

It is well-known that high salt diet affects metabolic syndromes such as hypertension and insulin resistance. Also, an excessive salt intake was associated with an increased oxidative stress. Recently, some researchers have studied the relationship among hypertension, insulin resistance, obesity, and oxidative stress in high salt diets. High salt diet in animal models of hypertension induced or aggravated oxidative stress (Tsutsui H et al. 2001; Cheng Z. J., 2001). Banday et al. (2007) reported that high salt diet increased oxidative stress and reduced NO bioavailability in Sprague-Dawley rats. However, the correlations between metabolic syndrome and high salt diet are unclear. Also, this knowledge comes from the researches, which used reagent-grade salt, purified salt or rock salt, all of which are lacking of minerals.

Among various salts, solar salt that is made in tide flat (marsh land) has plenty of minerals such as K, Mg, Ca, etc than other salts (reagent-grade salt, purified salt or rock salt) containing more than 99% NaCl content. The processed salts made from solar salt by a special manufacturing method in some countries have been also used for dietary salt. Some ingredients in these salts are thought to have positive effects hypertension and insulin resistance. We have investigated antioxidative activities of various salts by determining the scavenging ability of various radicals such as DPPH, ABTS and ferric radical. It was found that heat-treated bamboo salt (HTBS) had high antioxidative activity (Gao T. C. 2008). We further evaluated antioxidative effects of various salts on oxidative damage in vivo condition using rats.

2. Materials and methods

2.1 Materials

Natural solar salt was obtained from Tae Pyeong salt Co. (Sinan, Korea) and heat-treated bamboo solar salt was from Choung Soo Food Co. (Muan, Korea). Purified salt, n-butanol, carbon tetrachloride, and thiobarbituric acid were purchased from Sigma-Aldrich Chemical Co. (St, Louis, MO). All other reagents used in this study were reagent grade chemicals.

2.2 Animal treatment

Four weeks old male Sprague-Dawley rat, weighting 200 ± 15 g, were purchased from Damool science Co. (Daejeon, Korea). All rats were raised in plastic cages in a room controlled at 23 ± 1 °C and $55 \pm 5\%$ humidity, respectively under a 12-h dark/12-h light cycles. Rats were fed a standard laboratory diet (Purina chow) and water ad libitum for 6 months after their arrival. Animals (610 ± 15 g) were divided into four groups: normal (C), solar salt (SS), purified salt (PS) and HTBS. Each group has seven to eight rats and they were fed diets containing various salts (1.8 g/kg diet) for seven weeks except C group (0.9% saline intake), then animals were sacrificed and blood samples were collected for assaying plasma biochemical parameters.

2.3 Determination of lipids

Plasma triglyceride (TG), total-cholesterol (T-C) and high-density lipoprotein cholesterol (HDL-C) were determined by using commercial kits (Johnson & Johnson, USA).

2.4 Determination of thiobarbituric acid-reactive substances (TBARS)

TBARS determination was conducted according to the method of Ledwozyw et al. (1986). The calculation was based on standard curve prepared with different dilutions of malondialdehyde (MDA). The result was expressed as nmol of MDA/mg protein. The plasma protein content was determined by Bradford's method (1976).

2.5 Statistical analysis

Statistical analyses were performed using the SPSS statistics package (v.12.01). All data were expressed as mean \pm standard deviation (mean \pm SD) for each group. The probabilities of 5% or less ($p < 0.05$) were considered significant.

3. Results and discussion

3.1 Plasma lipids

After six months rats were fed diets containing various salts (1.8 g/kg diet) for seven weeks, triglyceride and cholesterol contents of their blood samples were determined. HTBS group showed a significantly decreased plasma TG levels compared to other groups (Table 1). The total-C and HDL-C contents were not significantly different between salt diet groups. This result was in agreement with the previous report that plasma TG and Total-C were decreased by sodium chloride intake (Catanozi S., 2001; Reaven G.M., 1991). Therefore, this result indicated that the different salt diet could lipid metabolism.

Table 1: Triglyceride, total-C and HDL-C of plasma in rats fed with various salts for 7week.
(mg/dl)

Group	TG	Total-C	HDL-C
Control	$116.38 \pm 6.23^{a1)}$	85.28 ± 4.48^{ns}	$37.95 \pm 3.49^{ns2)}$
SS	102.71 ± 7.96^a	71.71 ± 5.44	42.37 ± 3.26
PS	101.57 ± 9.20^a	72.50 ± 3.36	44.83 ± 4.37
HTBS	77.34 ± 5.31^b	80.88 ± 4.03	38.00 ± 5.87

¹⁾ Values with the different letters in the same column are significantly different ($p < 0.05$).

²⁾ No significant difference.

3.2 Effects on lipid peroxidation

Six-month rats were fed diets containing various salts (1.8 g/kg diet) for seven weeks

and TBARS contents of their blood samples were determined. HTBS group showed the significantly lower TBARS content than other salt groups ($p < 0.05$). In contrast, TBARS content was significantly higher in purified salt and solar salt groups compared to normal group ($p < 0.05$). The high antioxidative

activity of HTBS by the in vitro assay using radical scavenging has been previously reported (Gao T. C. 2008). Ha et al. (1999) reported that bamboo salt had the lower autooxidation than SS, PS and other salts, especially PS caused the highest autooxidation rate.

- Goto-Kakizaki rats. Hypertension, 37, 433-439.
- Dean, R. T., Fu, S., Stocker, R. & Davies, M. J.. (1997). Biochemistry and pathology of radical-mediated protein oxidation. Biochemical J, 324, 1-18.
- Chapter 1 Gao T. C., Yang X., Bang Mi. A. & Ham K. S.. (2008). Evaluation of antioxidative activities of Korean solar salt and its processed salts using various assay method. The Korean society of food science and nutrition J., P8-165.
- Ha J. O. & Park K. Y.. (1999). Comparison of autooxidation rate and comutagenic effect of different kinds of salt. J of Korean association of cancer prevention, 4, 44-51.
- Chapter 2 Halliwell B. & Gutteridge J. M. C.. (1999). Free radicals in biology and medicine (3rd ed). Oxford University Press Oxford.
- Chapter 3 Ledwożyw, A., J. Michalak, A. stepień A. & Kadzioka A.. (1986). The relationship between plasma triglycerides, cholesterol total lipids and lipid peroxidation products during human arteriosclerosis. Clinica Chimica Acta, 155, 275-283.
- Reaven G. M., Twersky, J. & Chang, H.. (1991). Abnormalities of carbohydrate and lipid metabolism in Dahl rats. Hypertension, 18, 630-635.
- Traverso, N., Menini S., Maineri E. P., Patriarca, S., Odetti, P., Cottalasso, D., Marinari, U. M. & Pronzato, M. A.. (2004). Malondialdehyde, a lipoperoxidation-derived aldehyde, can bring about secondary oxidative damage to protein
- s. The J of Gerontology Series A: Biological sciences and medical sciences, 59, 890-895.
- Tsutsui H, Ide T., Hayashidani S., Kinugawa S., Suematsu N., Utsumi H. & Takeshita A.. (2001). Effects of ACE inhibition on left ventricular failure and oxidative stress in Dahl salt-sensitive rats. J. Cardiovasc Pharmacol, 37, 725-733.

**SALT PROCESSING AND QUALITY
ASSURANCE**