Effects of Mineral-rich Solar Sea Salt and Its Heat-treated Salt on Blood Pressure and Endothelial Dysfunction in Dahl Salt-Sensitive Rats

Salt and Health

Keywords: mineral-rich salt, bamboo salt, blood pressure, endothelial dysfunction

Abstract

High salt intake may in some cases cause an increment of blood pressure. Most studies undertaken previously used general salts that contain above 99% of Sodium Chloride (NaCl), with low amount of other minerals. However, some solar sea salts (mineral-rich solar sea salt, MRS) contain various minerals including potassium (K), magnesium (Mg), calcium (Ca), etc. Bamboo salt (BS), which is made by heating MRS in the presence of bamboo at >800°C, is widely used as food material in Korea. In the symposium, we report the effects of various salts [mineral-deficient salt (MDS), MRS, and BS) on blood pressure (BP) in Dahl salt sensitive (DSS) rats. BP of DSS rats increased when various salts which contained the same amount of NaCl were provided. The MRS group showed lower systolic and diastolic BP levels than the MDS group. Surprisingly, the BS group did not appear to increase systolic and diastolic BP levels. Reduced BP by MRS and BS could be due to reduce oxidative stress and angiotensin I-converting enzyme (ACE) activity and increase endothelial cell function of the blood vessel. The MRS group had lower levels of plasma MDA and hepatic protein carbonyls than the MDS group, indicating reduced level of oxidative stress in the MRS group. The reduction of oxidative stress was more significant in BS than MRS. The MRS and BS groups exhibited significantly lower ACE activities and angiotensin II contents than the MDS group. BS inhibited ACE activity through the chelation of zinc ion in the active site of ACE, which was confirmed by *in vitro* assay. In the isometric myograph results, the MRS and BS groups showed significant increase in endothelial cell-derived relaxation to acetylcholine compared to the MDS group. The BS and MRS groups showed lower wall thickness and less expression of endothelial cell dysfunction-related markers compared with the MDS group. These results indicate that MRS and BS reduce BP via amelioration of oxidative stress, angiotensin II production, and endothelial cell dysfunction compared with MDS. Minerals were likely responsible for the reduction of BP. Although the major mineral contents in BS are lower than those in MRS, BS efficiently suppressed the increase of blood pressure induced by a high salt diet compared with MRS. BS seems to contain bioactive compound that inhibits ACE activity.

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1. Introduction

Salt plays a critical role in controlling fluid volume, maintaining acid-base balance, and regulating nervous system (1-2). However, high dietary salt intake is known to increase the risk of hypertension, cardiovascular disease and to impair endothelial function (3-5). There are various types of dietary salts including purified salt, rock salt, solar sea salt, etc. Most dietary salts contain above 99% of NaCl and low mineral contents. However, Mineral-rich solar sea salt (MRS) had been found to contain various minerals such as magnesium (Mg), calcium (Ca), potassium (K), and generate less oxidative stress in rat compared to mineral-deficient salt (MDS) (6). In addition, MRS treated by heat in the presence of bamboo (bamboo salt, BS) has been found to keep lower blood pressure than MDS and also has been reported to exert various health beneficial effects such as anti-inflammation, anti-oxidant, and anti-cancer (7-8).

Endothelial cells, the inner layer of the blood vessel wall, are critical for the regulation of vascular homeostasis. Endothelial dysfunction is considered to represent to reduced bioavailability of nitric oxide (NO), which is a major endothelium-dependent vasodilator. Endothelial dysfunction has been linked to the pathogenesis of atherosclerosis and acute cardiovascular events (9). Moreover, hypertension is associated with endothelial dysfunction and dietary salt may be an important factor which contributes to reduce the endothelial function (10). However, the effects of salt on endothelial function are still unknown. The aim of this study was to investigate effects of MRS and BS on the endothelial dysfunction of a blood vessel in Dahl-salt sensitive rats.

2. Materials and methods

2.1 Materials

The mineral-deficient salt (MDS) and mineral-rich salt (MRS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Taepyung Natural Salt Co, Ltd (Shinan, Korea) respectively. Bamboo salt (BS) was obtained from Choung Soo Food Co. (Muan, Korea).

2.2 Animal studies

Male Dahl-salt sensitive rats were purchased from Harlan (Indianapolis, IN, USA). Rats were housed at the room temperature (25±1°C), humidity (55±5%), and light cycle (12 h: 6.00-18.00) and given ad libitum access to food and water. DSS rats were divided into 4 groups as follows: control group fed the normal diet, MDS group fed a diet containing 3% NaCl of mineral-deficient salt, MRS group fed a diet containing 3% NaCl of mineral-rich solar sea salt, and BS group fed a diet containing 3% NaCl of bamboo salt for 4 weeks.

2.3 Blood pressure measurement

The systolic and diastolic BP were measured by a tail-cuff method using Heater Scanner LE 5650/6 and Storage Pressure Meter LE 5002 (Panlab, sl., BC, Spain).

2.4 Oxidative stress

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The lipid peroxidation content in plasma was determined by TBARS method. The malondialdehyde content in the butanol layer were measured by a spectrophotometer. Protein carbonyl contents were measured using the modified method of Reznick and Packer (11).

2.5 Determination of plasma nitric oxide (NO) content by Griess reaction

Nitric oxide end products (nitrite) in plasma were determined by Griess assay following Han Moshage (12).

2.6 Determination of angiotensin I converting enzyme (ACE) activity and Ang II

ACE activity in serum was assayed by spectrophotometric determination using hyppuryl-histidyl- leucine (HHL, Sigma-Aldrich Co, MO, USA). Ang II levels in the aorta were analyzed by the immunohistochemical method.

2.7. Vascular reactive test and wall thickness

The arteries were constricted followed by re-equilibration two times using a high-potassium Krebs solution. The arteries were then constricted to phenylephrine and arteries were exposed to acetylcholine and sodium nitroprusside for determination of endothelium-dependent relaxation and endothelium-independent relaxation respectively. The sections of aorta were stained by hematoxylin and eosin staining to determine the thickness of vessel wall.

2.8 Western blotting

The protein solutions were analyzed by SDS-PAGE gels and incubated with primary antibodies overnight. Visualized protein bands and their intensities were photographed and analyzed.

2.9Statistic analysis

Data are expressed as a means ± SEM using One-way ANOVA followed by Tukey's test. $P < 0.05$ was considered as significant.

Results and discussion

We investigated the effects of MDS, MRS, and BS in the DSS rats fed the same amount of NaCl for 4 weeks. The DSS rats showed the increase of systolic and diastolic BP. The MRS and BS groups showed lower systolic and diastolic BP than the MDS group, suggesting that the lower BP of the MRS and BS groups could be due to the mineral contents in those salts. In addition, the further reduction of BP by BS could be due to the ability of BS to inhibit the angiotensin I-converting enzyme (ACE) activity, which constricts the vessels and leads to increase of blood pressure. Moreover, MRS and BS also reduced oxidative stress and ameliorated the endothelial cell function.

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Oxidative stress is associated with high blood pressure, in response to vessel stimulation. Malondialdehyde (MDA) and protein carbonyls (PCO) are important markers of lipid peroxidation and protein oxidation respectively. The DSS rats fed with MRS and BS had lower levels of MDA in the plasma and protein carbonyl in the liver than DSS rats fed MDS. Moreover, we have found that the reduction of oxidative stress was more effective in BS than MRS.

The renin-angiotensin-aldosterone system (RAAS) plays the important role in controlling blood pressure in the body. The major cause of high blood pressure is the conversion by angiotensin I converting enzyme (ACE) of angiotensin I to angiotensin II, which is known as the most potent stimulator of blood pressure by constriction of blood vessel. The MRS and BS groups exhibited significantly lower ACE activity than the MDS group. Interestingly, BS inhibited ACE activity through the chelation of zinc ion (Zn^{+}) in active site of ACE. In addition, the angiotensin II expressions in the blood vessel was significantly increased in MDS group compared to control group. Moreover, the MRS and BS showed lower angiotensin II expressions when compared to MDS.

The endogenous nitric oxide (NO), which is a vascular smooth muscle relaxant, is released by endothelial cells of blood vessel through NO synthesis, possibly resulting in lowering blood pressure. We found that the DSS rats fed MDS diet showed a tendency to have lower plasma nitric oxide contents than those fed MRS and BS diets. However, angiotensin II increases blood pressure through stimulation of arginase-I and inhibition of eNOS and other relaxation-related factors. In the DSS rats fed diets for 4 weeks, which contained 3% of NaCl of different types of salts (MDS, MRS, and BS), the isometric myograph results revealed that the artery segments of MDS group showed significantly decreased endothelial cell-derived relaxation to acetylcholine (Ach). The MRS and BS groups showed the improvement of endothelial cell-derived relaxation to Ach compared to MDS group. In addition, the BS and MRS groups showed thinner wall thickness of blood vessel than the MDS group. Moreover, we have found that the MRS and BS groups showed less protein expressions of endothelial cell dysfunction-related markers. The MRS and BS groups showed higher protein expressions of eNOS and p-eNOS ser^{1177} than MDS group, and decreased the protein expression of arginase-I compared to MDS group, which correlated with increasing plasma nitric oxide levels.

Conclusion

In this study, we demonstrated that MRS and BS ameliorated endothelial dysfunction induced by dietary salt in DSS rats by reducing oxidative stress, ACE activity, and angiotensin II production compared to MDS. The minerals, which were contained in MRS and BS, were likely to be responsible for the reduction of blood pressure. Although, the major mineral contents (Mg, K, Ca) in BS were lower than those in MRS, BS was found to efficiently suppress the increasing of blood pressure in DSS rats compared with MRS. BS seems to contain a bioactive compound, which was effective in inhibition of ACE activity and reduction of blood pressure. The amelioration of endothelial dysfunction was observed in MRS and BS-treated DSS rats. Therefore, the MRS and BS are expected to be excellent dietary salts that can reduce BP in the body induced by excessive sodium intake.

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