Blood Pressure Effect of Excess Nacl Intake in Young Male Normotensive Africans Living in Tanzania

Negishi H[1], Yasui N[1], Njelekela M[2], Juman S[1], Miki T[1], Mtabaji J[2], Yamori Y[3], Ikeda K[1*]

[1] *School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Japan*

[2] *Department of Physiology, Muhimbilli University College of Health Sciences, Dar es salaam, Tanzania*

[3] *Institute for World Health Development, Mukogawa Women's University, Nishinomiya, Japan*

[*] **Corresponding author:** Katsumi Ikeda, School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya 663-8179, Japan, Tel: +81-798-45-9956; Fax: +81-798-45-9951; E-mail: ikeda@mukogawa-u.ac.jp

Abstract

Objective: Blood pressure effect of excessive salt intake was analyzed in relation to angiotensin I-converting enzyme (ACE) polymorphisms in young male normotensive African Tanzanians.

Design and Methods: We grouped 33 young male normotensive African Tanzanians. Their blood pressure were as follows, systolic blood pressure (SBP) <120mmHg and diastolic blood pressure (DBP) <80 mmHg. Healthy male subjects participated in a 2-week intervention consisting of seven days of salt loading (140 mEq of NaCl) followed by diuretic treatment for a period of one week.

Results: In 33 subjects, ACE genotyped DD, DI and II polymorphisms was 6, 17, 10, respectively. In the base line, there was no significant difference in SBP among the DD, DI and II genotypes. However, SBP among the DD, DI and II genotypes was significantly different after salt loading for 4 and 7days. This tendency was also observed in the diuretic phase. There was no significant difference in DBP and HR among ACE polymorphism genotypes during the experimental period.

Conclusion: We observed an interaction between SBP after salt ingestion and ACE insertion/deletion (I/D) polymorphisms in young male normotensive Tanzanians.

Keywords: Blood pressure, Sodium intake, Polymorphism

Introduction

It has long been recognized that environmental factors such as dietary habits play an important role in the development of hypertension.[1,2] Salt intake is an important environmental risk.[3] A number of epidemiological studies have shown that the prevalence of hypertension increases with habitual salt intake or urinary sodium chloride excretion in various parts of the world whereas some studies have failed to demonstrate such a significant association between salt and hypertension.[4-6] These conflicting observations may be due, in part, to the substantial heterogeneity that is thought to exist in the individual blood pressure response to changes in sodium balance, a phenomenon known as salt sensitivity.[7] Salt sensitivity is presumed to be a result of interactions among a number of modulators, genetic and acquired. Normotensive and hypertensive salt-sensitive subjects tend to exhibit a familial history of hypertension more frequently

than salt-resistant subjects.[8,9] This suggests the existence of genetic determinants that influence BP sensitivity to sodium chloride.

Ethnic differences in the prevalence of hypertension may suggest candidate genes worthy of study. A strong genetic predisposition to hypertension and target organ damage appears to correlate with African ancestry, and is referred to as "the African gene".[10] Sub-Saharan Africans have endured the selective pressure of extreme heat for thousands of generations. Polymorphisms in the renin-angiotensin system, such as the described insertion/deletion polymorphism in the ACE gene, may predispose them to hypertension and related disorders; however, little is known about the relationship between ACE polymorphism and salt sensitivity in normotensive Africans. In the present study, we evaluated the relation between salt sensitive blood pressure changes and ACE insertion/deletion (I/D) in young male normotensive Tanzanians.

Methods

Study population

The study was conducted in the Temeke District, Tanzania, which is a CARDIAC study center.[11] Men aged 25-35 years were invited to participate. They were selected randomly from a list of all men within that age category, who were residents of seven wards in the Temeke district during the study period; 100 subjects were enrolled. Participants underwent a medical examination that included hematological screening tests, urinalysis and a physical examination to exclude serious illness. Subjects were considered eligible for the study if they did not have hypertension, or had not previously been treated with antihypertensive drugs. Patients with hyperglycemia, diabetes mellitus or renal disease were excluded from the study. All subjects gave their written informed consent to participate.

Study design

The study was performed on an outpatient basis. The protocols were approved by the ethics committees of Muhimbili University College of Health Sciences and conformed to the ethics guidelines of these institutions. To evaluate the presence or absence of salt sensitivity among the participants, a dietary method was used as a modification of the original method.[12,13] The procedure involved a two-week intervention consisting of a high salt diet and diuretic treatment for one week each, respectively. Hemodynamic and biochemical measurements and 24 h urine collection were done while subjects maintained their customary diet. Subjects ingested 140mEq of an NaCl supplement as a condensed consommé cube (40 mEq/cube; Ajinomoto Inc., Tokyo, Japan) and 6 tablets of slow sodium (10 mEq/tablet; HK Pharma, Ltd., Hitchin, UK) for a 1-week salt-loading period, and they took diuretics (25 mg/day hydrochlorthiazide) for another week for salt depletion.

Urine and blood parameters

Blood and 24 h urine were sampled was collected before the beginning of the salt-loading phase and on the last day of salt-loading phase. The creatinine coefficient (creatinine [mg/day]/body weight [kg]) of 14.4-33.6 in men was classified as indicating acceptable 24 hour urine collection. Urinary Na and creatinine were measured using our previous methods.[11]

ACE polymorphism determination

Samples for DNA analysis were obtained from frozen peripheral leukocytes, and collected as a buffy coat layer. [14] I/D polymorphism of the ACE gene was assessed by detecting the presence (allele I, insertion) or absence (allele D, deletion) of a 287-base pair sequence in intron 16 of the ACE gene in chromosome 17 by the polymerase chain reaction technique and agar electrophoresis, as described by Rigat et al..[15] In addition, DNA samples typed as DD by the standard amplification procedure were reamplified in the presence of a positive control (ID/II). This allowed the identification of ID genotypes that were mistyped as DDs during the first amplification.[16] Depending on the genotype, the participants were classified as DD, DI or II.

Statistical analysis

The pared t was used to compare the means between baseline data and their after experimental period. One-way ANOVA by SPSS (SPSS Inc., Chicago, IL) for statistical analysis was used to compare the means among different genotype groups (DD, DI, II) at the baseline and in salt-loading and salt-depletion phases. A value of $p<0.05$ was considered significant.

Results

Normotensive africans were defined as follows, SBP<120 mmHg and DBP<80 mmHg. According to these criteria, 33 subjects in 100 subjects aged 25-35 years were selected. All subjects were young, male, non-obese, African who lived in Dar es Salaam, Tanzania. The characteristics of the subjects are shown in Table I.

Their maximal value and minimal value were 119 and 91 mmHg in SBP, 74 and 41 mmHg in DBP. Their SBP, DBP and heart rate (HR) in the experiment periods were presented in Table II. The mean SBP was not changed during experimental period, however the mean DBP and HR were increased in the diuretic treatment, suggesting the effect of the diuretic treatment.

In thirty-three subjects, ACE genotyped DD, DI and II polymorphisms accounted for 18.2(6/33), 51.5(17/33), 30.3% (10/33), respectively. SBP, DBP and HR with high salt intake and diuretic treatment depending on the different ACE polymorphism genotypes (II: n=10, DI: n=17, DD: n=6) shown in Table III. There was no significant difference in SBP among ACE polymorphism genotypes in the basal phase. However, SBP was significantly different after salt ingestion for 4 and 7 days. On the contrary, there was no significant difference in DBP and HR among ACE polymorphism genotypes in the basal phase, salt loading phase, and diuretic treatment. The intake of Na was not different among the DD, DI and II genotype groups in terms of high salt ingestion (DD: 5.67 ± 2.39, DI: 4.93 ± 1.74, II: 4.87 ± 1.13g/day).

Discussion

The relation between ACE gene polymorphism and salt sensitivity has been tested in various studies with controversial results. Kojima et al. showed no relation between ACE gene polymorphism insertion/deletion (I/D) and salt sensitivity in 75 Japanese patients with essential hypertension.[17] Another study by Hiraga et al. found that patients with an insertion allele were more salt-sensitive to the pressure effects of increased salt intake.[18] Giner et al. found a significant association between ID polymorphism of the ACE gene and salt-sensitive hypertension. Patients with the II and DI genotypes were found to have a significantly higher prevalence of salt sensitivity than DD hypertensives in outpatients with essential hypertension in Barcelona, Spain.[19]

In the case of native Africans, there have been few reports on the relationship between salt-sensitive blood pressure changes and ACE polymorphism. Salt-sensitive blood pressure changes have a variety of determinants, such as genetic factors, ethnicity, age, and body mass as well as associated disease states, e.g. hypertension, diabetes and renal dysfunction.[20,21] We thus excluded some determinants of salt sensitivity, and the subjects for the study were young (25-35 years), normotensive Africans. Normotensive subjects were defined as those having a SBP of <120 and a DBP of <80 mmHg, who had not previously been treated with antihypertensive drugs.

In this study, we observed salt-sensitive SBP change, not DBP change in normotensive Africans. Our data suggested that SBP in II and DD genotype show significant increase compared with those in DI genotype.

In hypertensive subjects, the II genotype had the greatest BP sensitivity to sodium, with the DD genotype demonstrating the smallest BP response ant the ID genotype group demonstrating an intermediate BP response.

The previous studies have suggested high prevalence of D allele among hypertensive patients,[22,23] still there are contradictory reports available.[24] This inconsistency could be due to the genetic and environmental heterogeneity among different ethic groups. Bankir et al. reveals ethnic differences between Black and White individuals in kidney function. [25] Black males have an increased risk of end-stage renal diseases in young adulthood.[26] Renal function and blood pressure are tightly linked. Physiologically, kidneys provide a key mechanism of chronic blood pressure control. Such ethnic difference in renal function may in part due to increased SBP in DD genotype in normotensive Tanzanian Africans. From our finding, we can speculate that the ACE genotypes DD, DI and II show genetic contributions to salt-sensitive SBP change in young male Africans.

In conclusion, the findings of this study suggest an interaction between the ACE I/D polymorphism during salt loading in young Tanzanian men. These findings help to clarify the contribution of ACE gene polymorphism to salt-sensitive hypertension in Africans.

References

1. Miller Iii ER., Erlinger JP., Appel LJ. The Effects of Macronutrients on Blood Pressure and Lipids. An Overview of the DASH and OmniHeart Trials. Curr Atheroscler Rep 2006; 8: 460-465.
2. Njelekela M., Negishi H., Nara Y., Tomohiro M., Kuga S., et al. Cardiovascular risk factors in Tanzania: a revisit. Acta Trop 2001; 79: 231-239.
3. Yamori Y., Nara K., Mizushima S., Sawamura M., Horie R. Nutritional factors for stroke and major cardiovascular diseases: international epidemiological comparison of dietary prevention. Health Rep 1994; 6: 22-27.
4. Elliott P., Stamler J., Nichols R., Dyer AR., Stamler R., et al. Intersalt Revisited: Further analyses of 24 hour sodium excretion and blood pressure within and across populations. BMJ 1996; 312: 1249-1253.
5. Yamori Y., Liu L., Ikeda K., Mizushima S., Nara Y., et al. Different associations of blood pressure with 24-hour urinary sodium excretion among pre-menopausal and post-menopausal women. J Hypertens 1996; 19: 535-538.
6. Smith WC., Crombie IK., Travendale RT., Gulland Sk., Tunstall-pedoe., et al. Urinary electrolyte excretion, alcohol consumption and blood pressure in the Scottish Heart Health Study. BMJ 1988; 297: 329-330.

7. Sullivan MJ. Salt sensitivity: definition, conception, methodology and long term issues. Hypertension 1991; 17: 6-68.

8. Sharma AM., Schorr U., Cetto C., et al. Dietary Versus Intravenous Salt Loading for the Assessment of Salt Sensitivity in Normotensive Men. Hypertension 1994; 7: 1070-1075.

9. Luft FC., Miller JZ., Weinberger MH., Christian JC., Skrabal F. Genetic influences on the response to dietary salt reduction, acute salt loading or salt depletion in humans. J Cardiovasc Pharmacol 1988; 12: S49-S55.

10. Moskowitz DW. Hypertension, thermotolerance, and the "African gene": an hypothesis. Clin Exp Hypertens 1996; 18:1-19.

11. WHO. WHO Collaborating Center on Primary Prevention of Cardiovascular Diseases, and Cardiovascular Unit. CARDIAC study protocol. Izumo/Geneva, 1986.

12. Fujita T., Noda H., Ando K. Sodium susceptibility and potassium effects in young patients with borderline hypertension. Circulation 1984; 69: 468-476.

13. Kato N., Kanda T., Sagara M., Bos A., Moriguchi EH., et al. Proposition of a feasible protocol to evaluate salt sensitivity in a population-based setting. Hypertens Res 2002; 25: 801-809.

14. Leadon SA., Ceruti PA. A rapid and mild procedure for the isolation of DNA from mammalian cells. Anal Biochem 1982; 120: 282-288.

15. Rigat B., Hubert C., Alhenc-Gelas F., Cambien F., Corvol P., et al. An insertion / deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1980; 86: 1343-1346.

16. Shanmugam V., Sell KW., Saha BK. Mistyping ACE heterozygotes. PCR Meth Appl 1993; 3: 120-121.

17. Kojima S., Inenaga T., Matsuoka H., Kuramochi M., Omae T., et al. The association between salt sensitivity and some polymorphic factors. J Hypertens 1994; 12: 797-801.

18. Hiraga H., Oshima T., Watanabe M., et al. Angiotensin I-converting enzyme gene polymorphism and salt sensitivity in essential hypertension. Hypertension 1996; 27: 569-572.

19. Giner V., Poch E., Bragulat E., Oriola J., Gonzalez D., et al. Renin angiotensin system genetic polymorphisms and salt sensitivity in essential hypertension. Hypertension 2000; 35: 512-517.

20. Franco V., Oparil S. Salt sensitivity, a determinant of blood pressure, cardiovascular disease and survival. J Am Coll Nutr 2006; 25: 247S-55S.

21. Wright JT., Rahman M., Scarpa A., Fatholahi M., Griffin V., et al. Determinants of salt sensitivity in black and white normotensive and hypertensive women. Hypertension 2003; 42: 1087-1092.

22. Fernández-Llama P., Poch E., Oriola J., Botey A., Coll E., et al. Angiotensin converting enzyme gene I/D polymorphism in essential hypertension and nephroangiosclerosis. Kidney Int 1998; 53: 1743-1747.

23. Yudkin JS., Andres C., Mohamed-Ali V., Gould M., Panahloo A., et al. The angiotensin-converting enzyme gene and the angiotensin II type I receptor gene as candidate genes for microalbuminuria. A study in nondiabetic and non-insulin-dependent diabetic subjects. Arterioscler Thromb Vasc Biol 1997; 17: 2188-2191.

24. Beige J., Scherer S., Weber A., Engeli S., Offermann G., et al. Angiotensin-converting enzyme genotype and renal allograft survival. J Am Soc Nephrol 1997; 8:1319-1323.

25. Bankir L., Perucca J., Weinberger MH. Ethnic differences in urine concentration: possible relationship to blood pressure. Clin J Am Soc Nephrol 2007; 2: 304-312.

26. Muntner P., Arshad A., Morse SA., Mandapatra PD., Reisin E., et al. End-stage renal disease in young black males in a black-white population: longitudinal analysis of the Bogalusa Heart Study. BMC Nephrol 2009; 10: 40.

Table I: Baseline characteristics of the subjects (n=33).

Age (years)	28.2 ± 2.8
Height (cm)	167.8 ± 6.5
Bwt (kg)	57.7 ± 8.2
BMI	20.5 ± 3.0
Na(g)/day	2.5 ± 1.3

Note: Bwt: Body weight; BMI: Body Mass Index. Values are the mean ± SD

Table II: Blood pressure and heart rate after salt loading and diuretics treatment.

	Baseline	**Salt4**	**Salt7**	**Diuretics4**	**Diuretics7**
SBP (mmHg)	111 ± 7.8	113 ± 10.3	112 ± 9.1	110 ± 9.6	111 ± 11.4
DBP (mmHg)	61 ± 7.6	62 ± 9.2	63 ± 7.2	67 ± 7.6*	65 ± 9.0*
HR (beats/min)	66 ± 7.4	64 ± 9.4	66 ± 9.6	77 ± 9.2*	71 ± 10.5*

Note: N=33, SBP, DBP and HR response to high salt intake for 4 days (Salt4) and 7 days (Salt7) and diuretic treatment for 4 days (Diuretics4) and 7days (Diuretics7). Pared t was used to compare the means between baseline data and experimental period.*$p<0.05$ from baseline data

Table III: SBP, DBP and HR response to high salt intake for 4 days (Salt4) and 7 days (Salt7) and diuretic treatment for 4 days (Diuretics4) and 7 days (Diuretics7) for the ACEI/D polymorphism.

	Baseline	**Salt4**	**Salt7**	**Diuretics4**	**Diuretics7**
SBP (mmHg)					
DD(6)	114 ± 4.0	121 ± 5.9*	117 ± 8.2*	114 ± 5.6	110 ± 10.3
DI(17)	109 ± 9.1	108 ± 7.4	109 ± 8.2	107 ± 9.0	106 ± 10.0

II (10)	112 ± 6.8	118 ± 11.7*	116 ± 8.8*	113 ± 11.4	118 ± 11.2*
DBP (mmHg)					
DD(6)	60 ± 3.9	61 ± 5.4	60 ± 6.6	64 ± 3.4	60 ± 7.5
DI(17)	60 ± 9.5	59 ± 9.6	62 ± 7.6	66 ± 9.3	64 ± 9.1
II (10)	64 ± 4.6	67 ± 8.1	66 ± 6.2	69 ± 5.3	69 ± 8.8
Heart Rate (beats/min)					
DD(6)	61 ± 4.7	61 ± 6.0	62 ± 5.4	75 ± 4.5	66 ± 10.2
DI(17)	66 ± 5.3	64 ± 8.6	67 ± 10.0	78 ± 10.5	70 ± 8.3
II (10)	69 ± 10.4	64 ± 12.4	69 ± 10.8	78 ± 9.5	75 ± 13.2

Note: II, DI and DD indicates ACE insertion/deletion polymorphism. All values are the mean ± SD. *$p<0.05$ from DI.