Symposium

Assessment of *in vivo* Oxidative Stress in Hypertensive Rats and Hypertensive Subjects in Tanzania, Africa

Hiroko NEGISHI, Marina NJELEKELA, Katsumi IKEDA, Miki SAGARA,
Takanori NOGUCHI, Sachiko KUGA, Tomo KANDA, Longjian LIU,
Yasuo NARA*, Motoki TAGAMI**, and Yukio YAMORI

Oxidative stress has been reported to be involved in not only cardiovascular diseases but in hypertension, which is a major risk for cardiovascular diseases. Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) has been recognized as a sensitive biomarker of oxidative DNA damage and also of oxidative stress. In the present study, we assessed the oxidative stress in human subjects with hypertension and in hypertensive rats. In stroke-prone spontaneously hypertensive rats at the age of 14 weeks, the excretion of urinary 8-OHdG was significantly ($p < 0.05$) increased compared with that in age-matched normotensive Wistar-Kyoto rats. Next, we investigated the relationship between oxidative DNA damage and cardiovascular risk factors among Tanzanians aged 46-58 years in a population study carried out in 1998 in at Dar es Salaam, Tanzania, according to the WHO-CARDIAC Study Protocol. Sixty subjects (male/female, 28/32) were selected by SPSS Base 8.0 from those who completed a 24-h urine collection. The 24-h urinary 8-OHdG of the hypertensive subjects (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg) was significantly ($p < 0.05$) higher than that of the normotensive subjects (SBP < 140 mmHg and DBP < 90 mmHg) after adjusting for age and gender (Hypertensives: 17.31 ± 2.0 ng/mg creatinine, $n = 38$; Normotensives: 10.10 ± 2.64 ng/mg creatinine, $n = 22$). Oxidative stress was thought to be involved in hypertensive subjects and in hypertensive rats. (*Hypertens Res* 2000; 23: 285-289)

Key Words: 8-OHdG, hypertension, SHRSP, Tanzania, African, oxidative stress

Introduction

Many findings have indicated that oxidative stress is associated with aging and severe age-related degenerative diseases, including cancer and cardiovascular diseases (1-4). The possible involvement of oxidative stress in hypertension, the most serious risk for cardiovascular diseases, was recently reported both in a hypertensive rat model and in men (5-7). In stroke-prone spontaneously hypertensive rats (SHRSP), which are characterized by severe spontaneous hypertension and the development of cerebrovascular diseases, free radicals are related to the

maintenance of hypertension (7). We demonstrated that free radicals heavily damaged neurons from SHRSP under conditions of hypoxia and oxygen reperfusion (8).

Oxidative stress is a function of imbalance between pro-oxidants and the antioxidants scavenging them. Free radicals, pro-oxidant factors, are atoms or molecules with unpaired electrons, and injury lipids, protein, and DNA. Oxidative damage is therefore the consequence of raised free radical production. 8-OHdG is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base (9, 10), and the monoclonal antibody specific for 8-OHdG has been established (11). Urinary excretion of 8-OHdG can be mea-

From the Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan, *Graduate School of Integrated Science and Art, University of East Asia, Shimonoseki, Japan, and **Department of Internal Medicine, Sanraku Hospital, Tokyo, Japan. Address for Reprints: Hiroko Negishi, Life Science, Environmental Conservation and Development, Graduate School of Human and Environmental Studies, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan.
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sured by an enzyme-linked immunosorbent assay (ELISA) (12). Previous reports have suggested that urinary 8-OHdG can be considered a putative biomarker of the total systemic oxidative stress *in vivo* (13), and oxidative DNA damage has been assessed in non-insulin-dependent diabetes mellitus (14) and other physical conditions (15, 16).

In the present study, the amount of urinary 8-OHdG was assayed in order to investigate the association between oxidative DNA damage and hypertension in SHRSP rats. Further, the 8-OHdGs in 24-h urine samples were assessed in a group of African subjects aged 46–58 years selected from among participants in the World Health Organization coordinated CARDIAC (Cardiovascular Diseases and Alimentary Comparison)/MONALISA (MONEO ALIMENTATIONIS SANAE) Study, which was carried out at Dar es Salaam, Tanzania, in 1998.

Subjects and Methods

Study 1: Model Rat Study

Five male SHRSP and five male Wistar-Kyoto rats aged 14 weeks fed a plain laboratory diet (SP; Funabashi Farm, Chiba, Japan) were used. Twenty-four hour urine samples were collected from rats housed in individual metabolic cages (NALGENE; Nalge, New York, USA). Urine samples were used for the determination of 8-OHdG by ELISA (8-OHdG check, Japan Institute for the Control of Aging, Shizuoka, Japan). The five male SHRSP and five male WKY rats were euthanized under diethylether anesthesia after their blood samples had been taken. The brain, heart, and kidneys were removed and the weight was determined. Blood samples were used for the determination of α - and β -tocopherol in serum. The study protocol was approved by the Animal Ethics Committee of the Graduate School of Environmental Studies, Kyoto University.

Study 2: Human Study

The survey subjects were recruited by the Muhimbili Medical Center, and included a total of 679 subjects (male and female) from three areas (Dar es Salaam, Handeni, Masai) of Tanzania. Sixty subjects were selected randomly from among the subjects living in urban regions of Dar es Salaam who completed 24-h urine collection. Informed consent was obtained from each volunteer after the procedures had been explained.

This study was conducted according to the protocol of the MONALISA study. All participants underwent blood pressure (BP) measurement using an automated BP measurement system, 24-h urine collection using a urine bag (U-container N, Ono Med Co., Osaka, Japan), blood testing after a 10–14 h fast, and a medical interview based

on a standardized questionnaire (17). BP was measured three times, and the lowest systolic BP (SBP) was used for the analysis together with the diastolic BP (DBP) obtained at the same time as the individual's BP. Various biological markers from urine (creatinine and 24-h urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG)) and blood (total cholesterol and high density lipoprotein (HDL) cholesterol in serum) were analyzed centrally in the WHO Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases, at the Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan. Total cholesterol and HDL cholesterol in the serum were determined by enzymic methods (Cholesterol Reagent, Bayer Medical, Kit Determiner L HDL-C, Kyowa Medics, Tokyo, Japan), and urinary creatinine was assessed using Jaffe's method. The body mass index (BMI) was calculated as body weight (kg)/height² (m).

Urine samples were centrifuged at 3,000 rpm for 10 min, and after proper dilution the supernatant was used for the determination of 8-OHdG by ELISA. The urinary 8-OHdG/creatinine ratio was alternatively used in analysis with consistent results.

Statistical Analysis

Values are expressed as mean $\pm$ SEM. Analysis of variance (ANOVA) was used in univariate analyses. In addition, the general linear model (GLM) method was used to estimate 24-h urinary 8-OHdG adjusted for sex and age. Computations were carried out using SPSS, version 8.0, for Windows (USA). A *p*-value of less than 0.05 was considered significant.

Results

Study 1: Oxidative DNA Damage in SHRSP

The organ weight, serum tocopherol, urinary protein, and 24-h urinary 8-OHdG for rats aged 14 weeks are shown in Table 1. The brain and heart weights per 100 g body weight (%BW) in SHRSP were higher than those in WKY rats. The 24-h urinary 8-OHdG in SHRSP was significantly higher than that in WKY rats. No significant differences were observed in the mean serum tocopherol and kidney weight in the %BW between the two groups.

Study 2: Oxidative DNA Damage for Hypertensive Subjects in Tanzania

Table 2 shows the biological, clinical, and demographics of 60 subjects (28 males and 32 females) aged 46–58 years. The mean BMI level was significantly higher in females (28.3 ± 1.0 kg/m²) than in males (24.6 ± 0.9 kg/m²). The rate of obesity (BMI >26.0 kg/m²) was 39% in

Table 1. Organ Weight, Serum Tocopherol, Urinary 8-OHdG, Blood Pressure and Body Weight in WKY Rats and SHRSP

	Unit	14 week		<i>p</i>
		WKY rats	SHRSP	
Brain	g	1.988±0.049	1.878±0.042	n.s.
	%BW	0.624±0.009	0.700±0.024	0.0187
Heart	g	0.932±0.018	1.194±0.064	0.0043
	%BW	0.293±0.003	0.445±0.025	0.0003
Kidney	g	2.160±0.075	1.978±0.128	n.s.
	%BW	0.678±0.020	0.736±0.044	n.s.
Serum α -tocopherol	mg/dl	0.396±0.009	0.392±0.041	n.s.
Serum β -tocopherol	mg/dl	0.020±0.000	0.028±0.004	n.s.
24-h urinary 8-OHdG	ng/kg per day	1029.3±61.8	1479.5±150.2	0.0242
Systolic blood pressure	mmHg	138±1.2	226±4.0	0.0001
Body weight	g	315.0±10.86	261.3±4.78	0.0005

Each value is the mean±SE. %BW shows the organ weight per 100 g of body weight. *P* value between WKY rats and SHRSP.

Table 2. Characteristics of the Study Subjects

	Total	Males	Females
<i>n</i>	60	28	32
Age (year)	52.2±0.5	52.3±0.7	52.2±0.7
BMI (kg/m ²)	26.6±0.7	24.6±0.9	28.3±1.0*
Systolic BP (mmHg)	142.7±3.3	143.3±5.0	142.1±4.4
Diastolic BP (mmHg)	81.8±2.1	79.8±2.6	83.6±3.2
Total cholesterol (mg/dl)	191.3±7.3	173.6±10.3	206.7±9.5*
HDL cholesterol (mg/dl)	44.4±2.1	43.8±3.0	45.0±3.0
HR (/min)	87.2±2.1	84.8±2.3	89.6±3.5
8-OHdG (ng/mg)	14.7±1.7	11.6±2.0	17.5±2.5

**p*<0.05.

Table 3. The Urinary Excretion of 8-OHdG/Creatinine

	<i>n</i>	8-OHdG ng/mg, Creatinine	<i>p</i> value
Hypertensives	38	17.4±2.4	0.05
Normotensives	22	10.1±1.1	
Hypercholesterolemic group	16	15.6±3.0	NS
Normal group	42	14.7±2.1	
Obese group	29	11.7±1.4	NS
Normal group	31	17.5±2.9	

males, and 56% in females. The serum total cholesterol was higher in females (206.7±9.5 mg/dl) than in males (173.6±10.3 mg/dl). No significant differences were observed in the means of age, BP, or 24-h urinary 8-OHdG between males and females. The means of SBP in both sexes were at a hypertensive level (≥ 140 mmHg).

The subjects were divided into hypertensives (SBP

≥ 140 mmHg and/or DBP ≥ 90 mmHg) and normotensives (SBP <140 mmHg and DBP <90 mmHg). The 24-h urinary 8-OHdG was significantly higher in the hypertensives than in the normotensives (Table 3). In the same manner, subjects were divided into two groups based on total cholesterol (TC) (hypercholesterolemic group, TC ≥ 220 mg/dl; normal group, TC <220 mg/dl), and on BMI (obese group, BMI >26.0 kg/m²; normal group, BMI ≤ 26.0 kg/m²). However, no significant differences were observed in the 24-h urinary 8-OHdG between the hypercholesterolemic and normal groups, or between the obese and normal groups (Table 3).

Discussion

It is known that reactive oxygen species such as superoxides, hydrogen peroxide, and hydrogen radicals damage cells. It is thought that superoxide is dismutated to hydrogen peroxide and that this hydrogen peroxide can be

metabolized to a hydroxyl radical. A recent study has suggested that an increase in superoxide radical activity may be a causative factor in the maintenance of hypertension because the up-regulation of the NADPH/NADH oxidase system was observed in the aorta of hypertensive rats receiving infusions of angiotensin II (18). McIntyre *et al.* (7) reported that the reduced availability of nitric oxide (NO), a vasodilator, was observed in SHRSP because NO was scavenged by the superoxide radicals and transformed into peroxynitrite, a weak vasodilator with toxic effects. It is suggested that peroxynitrite can be protonated to form peroxynitrous acid, which, in turn, can yield hydroxyl radicals (19).

In the present study, SHRSP showed increased 24-h urinary 8-OHdG, suggesting enhanced oxidative DNA damage. Our findings in a previous report suggested that the accumulation of advanced glycation end products (AGEs) in the thoracic aorta from 12-week-old SHRSP was significantly higher than that in age-matched normotensive WKY (20). Several studies have suggested that AGE proteins induce oxidative stress (21, 22), and that hydrogen peroxide may be a candidate for reactive oxygen species that contribute to the oxidative damage of smooth muscle cells (23). In addition, findings in a cell culture study we conducted suggested that AGEs dose-dependently increased the 8-OHdG in a bathing solution of cultured smooth muscle cells from SHRSP (submitted). Therefore, the increased excretion of 8-OHdG in SHRSP may, in part, result from the accumulation of AGEs in the SHRSP arteries.

Further, we conducted a human study according to the protocol of the WHO-CARDIAC/MONALISA study, participated in by middle-aged (46–58 years old) subjects in Tanzania, where people now have more cardiovascular risks, as well as greater risk of infectious diseases, due to urbanization. In Tanzania, hypertension has been noted to be a bigger problem in the capital city (24). It has also been reported that the most common reason for poor medication compliance in Tanzania is drug side effects followed by the high cost of the drugs (for patients who were buying), then by poor doctor-patient communication (25). In this study, the mean of BMI in females was 28.3 ± 1.0 kg/m², within the range of obesity. SBP on average was over 140 mmHg in both sexes. The risks of cardiovascular diseases were considered to be higher in the subjects of this study with respect to the average values for BMI and BP.

A recent report showed that oxidative stress is associated with hypertension (5). It has been reported that subjects with essential hypertension have higher plasma hydrogen peroxide levels as markers of reactive oxygen species than do normotensive subjects. In the present study, we observed that 24-h urinary 8-OHdG excretions were significantly higher in hypertensives than in normotensives. Significant values were still observed after adjust-

ment for age and gender (Hypertensives, 17.31 ± 2.0 ng/mg creatinine, $n = 38$; Normotensives, 10.10 ± 2.64 ng/mg creatinine, $n = 22$; $p = 0.0039$). These results suggest that oxidative DNA damage may be involved in hypertensive subjects. Considering our findings in hypertensive rats, the accumulation of AGEs in vascular systems may contribute to the induction of oxidative DNA damage in hypertensive subjects. Cosentino *et al.* (26), in an investigation using human cultured aortic endothelial cells, reported that elevated glucose levels increased the release of superoxide anions. Moreover, Leinonen *et al.* (14) demonstrated that urinary 8-OHdG increased in patients with non-insulin-dependent diabetes mellitus. However, the mechanism of this increased oxidative DNA damage in hypertension and hypertensive patients is still unknown and requires further clarification.

In conclusion, we demonstrated that oxidative DNA damage increased in hypertensive rats and in hypertensive human subjects in Tanzania. Oxidative stress may be involved in subjects with hypertension in Tanzania, Africa.

References

1. Stephen T, Sinatra MD, Jan Demarco RN: Free radicals, oxidative low density lipoprotein (LDL), and the heart: antioxidants and other strategies to limit cardiovascular damage. *Conn Med* 1995; **59**: 579–588.
2. Flora S, Izzotti A, Walsh D, Degan P, Petrilli G, Lewtas J: Molecular epidemiology of atherosclerosis. *FASEB* 1997; **11**: 1021–1031.
3. Tagesson C, Kallberg M, Klintonberg C, Starkhammar H: Determination of urinary 8-hydroxydeoxyguanosine by automated coupled-column high performance liquid chromatography: a powerful technique for assaying *in vivo* oxidative DNA damage in cancer patients. *Eur J Cancer* 1995; **31A**: 934–940.
4. Erhola M, Toyokuni S, Okada K, *et al*: Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Lett* 1997; **409**: 287–291.
5. Lacy F, O'Connor D, Schmid-Schonbein G: Plasma hydrogen peroxide production in hypertensives and normotensive subjects at genetic risk of hypertension. *J Hypertens* 1998; **16**: 291–303.
6. Russo C, Olivieri O, Girelli D, *et al*: Anti-oxidant status and lipid peroxidation in patients with essential hypertension. *J Hypertens* 1998; **16**: 1267–1271.
7. McIntyre M, Hamilton C, Rees D, Reid J, Dominiczak A: Sex differences in the abundance of endothelial nitric oxide in a model of genetic hypertension. *Hypertension* 1997; **30**: 1517–1524.
8. Tagami M, Yamagata K, Ikeda K, *et al*: Genetic vulnerability of cortical neurons isolated from stroke-prone spontaneously hypertensive rats in hypoxia and oxygen reperfusion. *Hypertens Res* 1999; **22**: 23–29.
9. Loft S, Vistisen K, Ewertz M, Tjønneland A, Overvad

- K, Poulsen E: Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* 1992; **13**: 2241-2247.
10. Zhang D, Okada S, Yu Y, Zheng P, Yamaguchi R, Kasai H: Vitamin E inhibits apoptosis, DNA modification, and cancer incidence induced by iron-mediated peroxidation in Wistar rat kidney. *Cancer Res* 1997; **57**: 2410-2414.
11. Toyokuni S, Tanaka T, Hattori Y, et al: Quantitative immunohistochemical determination of 8-hydroxy-2'-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. *Lab Inv* 1997; **76**: 365-374.
12. Witherell HL, Hiatt RA, Replogle M, Parsonnet J: *Helicobacter pylori* infection and urinary excretion of 8-hydroxy-2'-deoxyguanosine, an oxidative DNA adduct. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 91-96.
13. Shigenaga M, Gimeno C, Ames B: Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proc Natl Acad Sci USA* 1989; **86**: 9697-9701.
14. Leinonen J, Lehtimäki T, Toyokuni S, et al: New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Lett* 1997; **417**: 150-152.
15. Fraga C, Shigenaga M, Park J, Degan P, Ames B: Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci USA* 1990; **87**: 4533-4537.
16. Pilger A, Germadnik D, Formanek D, Zwick H, Winkler N, Rudiger HW: Habitual long-distance running does not enhance urinary excretion of 8-hydroxydeoxyguanosine. *Eur J Appl Physiol* 1997; **75**: 467-469.
17. Gao M, Ikeda K, Hattori H, Miura A, Nara Y, Yamori Y: Cardiovascular risk factors emerging in Chinese populations undergoing urbanization. *Hypertens Res* 1999; **22**: 209-215.
18. Fukui T, Ishizaka N, Rajagopalan S, et al: p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. *Circ Res* 1997; **80**: 45-51.
19. Munzel T, Heitzer T, Harrison D: The physiology and pathophysiology of the nitric oxide/superoxide system. *Herz* 1997; **22**: 158-172.
20. Mizutani K, Ikeda K, Kawai Y, Yamori Y: Biochemical properties and chemical composition of the aorta in genetic hypertensive rats. *J Hypertens* 1999; **17**: 481-487.
21. Nagai R, Ikeda K, Higashi T, et al: Hydroxyl radical mediates N epsilon-(carboxymethyl)lysine formation from Amadori product. *Biochem Biophys Res Commun* 1997; **234**: 167-172.
22. Fu S, Fu MX, Baynes JW, Thorpe SR, Dean RT: Presence of dopa and amino acid hydroperoxides in proteins modified with advanced glycation end products (AGEs): amino acid oxidation products as a possible source of oxidative stress induced by AGE proteins. *Biochem J* 1998; **330**: 233-239.
23. Sakata N, Miyamoto K, Meng J, et al: Oxidative damage of vascular smooth muscle cells by the glycated protein-cupric ion system. *Atherosclerosis* 1998; **136**: 263-274.
24. Mtabaji JP, Nara Y, Moriguchi Y, Yamori Y: Diet and hypertension in Tanzania. *Cardiovas Pharmacol* 1990; **16** (Suppl 8): S3-S5.
25. Maro EE, Lwakatare J: Medication compliance among Tanzanian hypertensives. *East Afr Med J* 1997; **74**: 539-542.
26. Cosentino F, Hishikawa K, Katusic ZS, Luscher TF: High glucose increased nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 1997; **96**: 25-28.