Original Article

Increased Plasma 8-Isoprostane Levels in Hypertensive Subjects: the Tsurugaya Project

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To examine the relationship between 8-isoprostane and blood pressure, we measured plasma 8-isoprostane concentration and home blood pressure levels in an elderly Japanese population. Our study population comprised 569 subjects aged 70 years and over who were not receiving antihypertensive medication. On the basis of their blood pressure values, the participants were classified into three groups: normotensive (home blood pressure < 135/85 mmHg), hypertensive (home blood pressure 135/85–160/90 mmHg), and severely hypertensive (home blood pressure ≥ 160/90 mmHg). The mean plasma 8-isoprostane level in the severely hypertensive group (21.1 ± 5.2 pg/ml) was significantly higher than that in the normotensive (20.2 ± 4.9 pg/ml) or hypertensive (19.7 ± 5.1 pg/ml) group, and this result was unchanged when we adjusted for possible confounding factors such as age, sex, use of vitamin A, C or E supplements, smoking status, drinking status, body mass index, use of non-steroidal anti-inflammatory drugs, history of diabetes, hypercholesterolemia, home heart rate and serum creatinine level. Thus, the level of plasma 8-isoprostane appears to be elevated in older subjects with severe hypertension. (*Hypertens Res* 2004; 27: 557–561)

Key Words: hypertension, oxidative stress, isoprostanes, home blood pressure measurement, elderly

Introduction

Data from a number of animal experiments and *in vitro* studies in humans support the hypothesis that increased oxidative stress may be related to elevated blood pressure (BP) (1, 2).

However, few studies have investigated the relationship between 8-isoprostane and hypertension in a large sample of human subjects (3).

Isoprostanes are chemically stable lipid peroxidation products of arachidonic acid, and their quantification provides a novel approach to the assessment of oxidative stress *in vivo*

(4). Isoprostanes are detectable in plasma and urine under normal conditions (5), and their levels increase during oxidative stress (6).

Recently, self-measurement of BP at home (home BP measurement) has been reported to have better reproducibility (7, 8) and prognostic value (9) than BP measurement in clinics.

Our objective was to clarify the relationship between plasma 8-isoprostane concentration and home BP measurement in elderly people.

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Methods

Study Participants

Our study population comprised subjects aged 70 years and older who were living in the Tsurugaya area of Sendai, one of the major cities in the Tohoku area of Japan. At the time of the study, there were 2,730 individuals aged 70 years and older living in Tsurugaya. We invited all of these individuals to participate in a comprehensive geriatric assessment, which included medical status, physical function, cognitive function and dental status, and 1,179 of them did so, giving their informed consent for analysis of the data. The protocol of this study was approved by the Institutional Review Board of Tohoku University Graduate School of Medicine. We excluded subjects whose plasma 8-isoprostane levels had not been measured ($n = 29$). Home BP data were obtained from 968 of the remaining subjects, who collected their own data on more than 3 days during the 4-week study period. This criterion was based on our previous observation that average BP values for the first 3 days did not differ significantly from those obtained during the entire study period (7). Furthermore, since antihypertensive medication *per se* would affect the degree of oxidative stress, we excluded subjects who were receiving antihypertensive medication (10). Therefore, the study population comprised 569 subjects (mean age 75.2 ± 4.6 years; men: 45%).

Home BP Measurements

We used the following procedure to ascertain the accuracy of the home BP measurement. First, physicians informed the population about home BP recording and taught them how to measure their own BP. The daily measurement was made within 1 h of awakening and before breakfast, with the subject seated and having rested for at least 2 min. In subjects receiving antihypertensive drugs, home BP was measured before taking the drugs. The home BP of an individual was defined as the mean of all measurements obtained for that person. The mean ($\pm$ SD) number of home BP measurements was 15.3 ± 10.2 (range, 3–48).

BP-Measuring Device

Home BP was measured with an HEM747IC device (Omron Life Science Co. Ltd., Tokyo, Japan), which uses the cuff-oscillometric method to generate a digital display of systolic and diastolic pressures. This device has been validated previously (11), and satisfies the criteria of the Association for the Advancement of Medical Instrumentation (12).

8-Isoprostane Measurement

Total (esterified plus free) 8-iso-prostaglandin (PG) $F_{2\alpha}$ con-

centrations were assayed in plasma by a specific enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, USA) (13, 14).

For total 8-iso-PGF $_{2\alpha}$ measurement, peripheral venous blood was collected in ethylenediaminetetraacetic acid 2Na (EDTA2Na)- and EDTA4Na-coated cold polyethylene tubes containing indomethacin, an inhibitor of cyclooxygenase, and aprotinin, an inhibitor of kallikreins, to prevent any *in vitro* formation of 8-iso-PGF $_{2\alpha}$. After collection, blood samples were immediately cooled at 4°C and transferred to the laboratory within 4 h. In the laboratory, the samples were centrifuged at $3,000 \times g$ at 4°C for 10 min. The plasma fraction was removed and stored at -80°C for later 8-isoprostane assay. The antiserum used in this assay has 100% cross-reactivity with 8-isoprostane, 0.2% with PGF $_{2\alpha}$, PGF $_{3\alpha}$, PGFI, and PGF $_2$ and 0.1% with 6-keto PGF $_{2\alpha}$. Both the intra-assay and interassay variabilities were within 6%. The detection limit of the assay was 4 pg/ml.

Classification of Subjects

On the basis of BP values, participants were classified into three groups: normotensive, home BP <135/85 mmHg; hypertensive, home BP 135/85–160/90 mmHg; and severely hypertensive, home BP 160/90 mmHg.

Data Analysis

Variables were compared by the *t*-test, analysis of variance, the χ^2 test, or analysis of covariance, as appropriate.

We used the following confounders as covariates: age, sex, use of vitamins A, C or E, use of non-steroidal anti-inflammatory drugs (NSAIDs), smoking habit, drinking habit, body mass index (BMI), history of diabetes or hypercholesterolemia, home heart rate (HR) and serum creatinine level. These factors were chosen because it is known that some lifestyle-related factors, such as obesity (3), smoking (15), supplementation with vitamins A, C or E (16), or use of NSAIDs (17), and disease conditions such as diabetes (18) or hypercholesterolemia (19) can affect the plasma 8-isoprostane level. We defined diabetes as a free blood glucose level of 200 mg/dl or over, or current use of antidiabetic medication. Similarly, we defined hypercholesterolemia as a level of total cholesterol of 220 mg/dl or over, or current use of lipid-lowering agents. The drug information was confirmed by a well trained pharmacist. The level of statistical significance was set at $p < 0.05$. Data are given as the mean $\pm$ SD. All statistical analyses were performed with SAS software, version 8.02.

Results

Descriptive Data for Plasma 8-Isoprostane

Table 1 shows the descriptive data for plasma 8-isoprostane.

Table 1. Descriptive Data for Plasma 8-Isoprostane

		<i>N</i>	Plasma 8-isoprostane	<i>p</i> value
Sex	Men	258	20.4 ± 4.9	0.09*
	Women	311	19.7 ± 5.0	
Age	70–79 years	462	20.0 ± 5.0	0.43*
	80 years–	107	20.4 ± 5.1	
Use of vitamins A, C or E supplements	With	74	19.7 ± 5.1	0.47*
	Without	495	20.1 ± 5.1	
Smoking	Current	76	20.7 ± 4.9	0.046**
	Ex	165	20.6 ± 4.8	
	Never	319	19.6 ± 5.2	
Drinking	Current	227	20.0 ± 5.0	0.63*
	Ex or Never	337	20.0 ± 5.1	
Diabetes	With	46	21.0 ± 4.4	0.16*
	Without	523	20.0 ± 5.1	
Hypercholesterolemia	With	243	19.7 ± 5.2	0.11*
	Without	326	20.3 ± 4.9	
Use of NSAIDs	With	92	20.7 ± 4.9	0.18*
	Without	477	19.9 ± 5.1	

NSAIDs, non-steroidal anti-inflammatory drugs. * *t*-test; ** ANOVA.

Table 2. Baseline Characteristics

	Normotensive	Hypertensive	Severely hypertensive	<i>p</i> value
<i>N</i>	286	205	78	
Age	74.6 ± 4.0	76.1 ± 5.1	75.1 ± 4.8	0.002*
Sex (% men)	47.6	42.0	46.2	0.46†
Use of vitamins A, C, or E supplements (%)	14.7	11.7	10.3	0.46†
Current smokers (%)	12.2	14.2	15.4	0.82†
Current drinkers (%)	37.4	41.5	44.9	0.18†
Diabetes (%)	8.0	7.8	9.0	0.95†
Hypercholesterolemia (%)	40.9	42.9	48.7	0.037†
BMI	22.9 ± 3.1	24.1 ± 3.5	24.5 ± 3.1	<0.001*
Systolic blood pressure (mmHg)	120.6 ± 9.7	144.1 ± 7.0	167.3 ± 12.4	<0.001*
Diastolic blood pressure (mmHg)	70.5 ± 6.5	77.5 ± 7.7	90.8 ± 8.1	<0.001*
Home heart rate (beat/min)	65.2 ± 7.9	65.2 ± 8.1	67.8 ± 8.9	0.03*
Use of NSAIDs	15.7	17.6	14.1	0.75†
Serum creatinine (mg/dl)	0.76 ± 0.20	0.76 ± 0.44	0.73 ± 0.15	0.71*

Normotensive: home blood pressure <135/85 mmHg; Hypertensive: home blood pressure 135/85–160/90 mmHg; Severely hypertensive: home blood pressure ≥160/90 mmHg. BMI, body mass index; NSAIDs, non-steroid anti-inflammatory drugs. * ANOVA; † χ^2 test.

The plasma 8-isoprostane level tended to be higher in men, elderly subjects and subjects with diabetes. Similarly, subjects who were using vitamin A, C or E supplements showed a lower plasma 8-isoprostane level than those who were not. Current smokers and ex-smokers showed higher levels of plasma 8-isoprostane than subjects who had never smoked.

Baseline Characteristics

Table 2 shows the baseline characteristics of the subjects.

The normotensives were the youngest subjects, and the prevalence of diabetes was highest among the severely hypertensive subjects. The proportions of subjects who were taking antihypertensive medication were higher among subjects with severe hypertension or hypertension than among normotensive subjects. Among the three subject groups, the mean BMI was the highest in severe hypertensives. The plasma 8-isoprostane level in severely hypertensive subjects (21.1 pg/ml) was significantly higher than that in hypertensive (20.2 pg/ml) or normotensive (19.7 pg/ml) subjects.

Table 3. Relationship between Plasma 8-Isoprostane Level and Home BP Levels

	N	Plasma 8-isoprostane (95% C.I.)
All subjects (N = 569)		
Normotensive	286	19.7 (19.1–20.3)*
Hypertensive	205	20.1 (19.4–20.8)
Severely hypertensive	78	21.0 (19.9–22.2)
p for trends		0.041
Men (N = 258)		
Normotensive	136	20.4 (19.5–21.2)
Hypertensive	86	20.0 (18.9–21.0)
Severely hypertensive	36	21.7 (20.0–23.4)
p for trends		0.402
Women (N = 311)		
Normotensive	150	19.1 (18.3–19.9)
Hypertensive	119	20.2 (19.2–21.1)
Severely hypertensive	42	20.6 (19.0–22.2)
p for trends		0.054
Limited population ** (N = 294)		
Normotensive	156	20.3 (19.5–21.0)
Hypertensive	95	20.0 (19.0–21.0)*
Severely hypertensive	43	21.9 (20.4–23.4)
p for trends		0.149

* $p < 0.05$ vs. Severely hypertensive. ** Subjects without HDL < 40 mg/dl, total cholesterol 220 mg/dl, triglyceride 300 mg/dl or free blood glucose 200 mg/dl. Normotensive: home BP $< 135/85$ mmHg; Hypertensive: home BP $135/85$ – $160/90$ mmHg; Severely hypertensive: home BP $160/90$ mmHg. Adjusted for age, sex, use of vitamin A, C or E supplements, smoking habit, drinking habit, body mass index, home heart rate, diabetes, hypercholesterolemia, use of non-steroid anti-inflammatory drugs and serum creatinine level. N, number of subjects; C.I., confidence interval; BP, blood pressure; HDL, high-density lipoprotein.

Adjustment for Possible Confounders

Even after adjustment for confounding factors, there was no change in the finding that the plasma 8-isoprostane level in severely hypertensive subjects was higher than that in hypertensive or normotensive subjects (p for trend = 0.041) (Table 3).

When we performed separate analyses for men and women, the finding that the plasma isoprostane level among severely hypertensive subjects was higher than that in normotensives or hypertensives was unchanged. Furthermore, even when we excluded the subjects with a high-density lipoprotein (HDL) cholesterol level < 40 mg/dl, or with a level of total cholesterol 220 mg/dl, or with a high triglyceride level 300 mg/dl or a free blood glucose level 200 mg/dl, the tendency for the plasma isoprostane level in severely hypertensive subjects to be higher than that in normotensive or hypertensive was also unchanged.

Discussion

The plasma 8-isoprostane level in elderly subjects with severe hypertension was modestly but significantly higher than that in normotensive or hypertensive subjects, even when we adjusted for possible confounders.

Our study had several methodological advantages. First, the use of home BP measurement made it possible to obtain multiple measurements over a long observation period under well-controlled conditions. This approach has been reported to have better reproducibility (7, 8) and prognostic value (9) than casual BP measurement, because it avoids observer bias, regression dilution bias (8) and the white-coat effect. Second, we adjusted for possible confounders such as age, sex, use of vitamin A, C or E supplements, smoking habit, drinking habit, BMI, use of NSAIDs, history of diabetes, history of hypercholesterolemia, home HR, and serum creatinine level, since these factors could affect the level of 8-isoprostane or BP.

Although many animal experiments have indicated a positive relationship between high BP levels and 8-isoprostane, few studies have supported such a relation in humans (3). Keaney *et al.* examined 2,828 subjects in the Framingham Heart Study and measured urinary creatinine-indexed 8-isoprostane as a marker of systemic oxidative stress (3). However, they did not find any meaningful positive association between oxidative stress and hypertension.

The difference between their findings and ours may be explained as follows. First, their diagnosis was based on clinic BP measurements, whereas we used home BP measurements. Therefore, our approach may have reduced the number of misclassifications (8).

Second, Keaney *et al.* considered that the proportion of individuals with oxidative-mediated hypertension, such as salt sensitivity, may have been too small to drive an association between hypertension and oxidative stress in their sample (3). On the other hand, our population comprised elderly Japanese individuals. The proportion of individuals with sodium sensitivity is known to be higher in Japanese than in Caucasian populations (20). Similarly, BP becomes salt-sensitive with age (20). Therefore, the proportion of subjects with salt sensitivity might have been higher in our subjects than in theirs, and this might have at least partly accounted for the difference between our results and those of Keaney *et al.* (3).

Our study also had some limitations. First, most of the participants were sufficiently active and healthy to participate in the survey, and this might have led to small inter-individual differences in the study effects. Second, since this study was a cross-sectional study, we cannot conclude that oxidative stress causes hypertension or that higher BP leads to increased oxidative stress. Third, we used EIA rather than gas chromatography/mass spectrometry, the gold standard for isoprostane analysis, and plasma obtained by centrifuga-

tion was stored at -80°C within 1 to 4 h—rather than immediately—after collection, because large numbers of samples had to be processed in a timely manner. Finally, we used plasma samples rather than urine samples. Although the plasma samples were prepared carefully (peripheral venous blood was collected in polyethylene tubes containing 1 mmol/ml indomethacin, cooled immediately at 4°C and transferred to the laboratory within 4 h; plasma obtained by centrifugation was aliquoted and stored at -80°C for later 8-isoprostane assay in the laboratory), some autoxidation might have occurred.

In conclusion, we have demonstrated that plasma 8-isoprostane levels are elevated in elderly subjects with severe hypertension. This is the first study to clarify the relationship between isoprostanes and hypertension in elderly individuals. However, as the difference in plasma 8-isoprostane levels among the three groups was modest, further study will be needed to clarify the clinical significance of this difference.

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