Aldosterone and Telomere Length in White Blood Cells

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Background. Aldosterone accelerates cardiovascular aging by mechanisms that generate reactive oxygen species. Telomere length in white blood cells (WBCs) may be a bioindicator that registers the accruing burden of systemic oxidative stress. The aim of the present study was, therefore, to examine the relationship between plasma aldosterone and telomere length in WBCs.

Methods. We studied 75 normotensive and never-treated mildly hypertensive men whose blood was drawn for the measurements of plasma aldosterone concentration and the terminal restriction fragment (TRF) length in WBCs.

Results. The slope of the TRF–age relationship in the entire cohort showed a decrease in telomere length of 26 ± 5 base pairs per year (r = −0.46, p < .001). Age-adjusted TRF length was the longest in the lowest aldosterone quartile (6.74 ± 0.12 kb) and shortest in the highest aldosterone quartile (6.36 ± 0.11 kb), with intermediate TRF lengths in the second and third aldosterone quartiles (analysis of variance [ANOVA] trend test, p = .025). In telomeric attrition equivalence, participants in the upper aldosterone quartile were 15 years older than their peers in the lowest quartile.

Conclusions. The inverse relationship between aldosterone and WBC telomere length suggests not only that aldosterone is pro-oxidant but that elevated concentrations of this hormone might be linked to a higher rate of telomere attrition and perhaps increased biological aging in humans.

The present consensus is that both oxidative stress (1) and inflammation (2) are key elements in the pathophysiology of age-related cardiovascular disease (CVD) in humans. This tenet is based on studies showing that indices of oxidative stress and inflammation are elevated in individuals prone to and patients with CVD. It is noteworthy, however, that these indices usually express the state of the moment of oxidative stress and/or inflammation. What’s more, it might be difficult to dissociate oxidative stress from inflammation, as oxidative stress evokes inflammation and vice versa. An index that captures the accruing burden rather the acute state of oxidative stress and/or inflammation might be a powerful predictor of cardiovascular risks. For the following reasons, white blood cell (WBC) telomere dynamics (expressed by telomere length and attrition rate) may be such an index. First, telomeres undergo attrition with each cellular replication and their rate of loss is augmented by oxidative stress (3–5). Second, inflammation entails an increase in WBC turnover rate, which would further increase telomere attrition in these cells. Thus, WBC telomere dynamics probably chronicles the burden of oxidative stress and/or inflammation over the individual’s life span. Indeed, several studies (6–10) have demonstrated that WBC telomeres are shorter in persons susceptible to or expressing CVD.

In the present study we focus on the link between circulating aldosterone and WBC telomere length because aldosterone is an established pro-oxidant, generating oxidative stress in the vasculature (11), heart (12), kidney (13), and WBCs (14). Moreover, several clinical results suggest that elevated levels of aldosterone denote a propensity for CVD in humans (15–17).

Materials and Methods

Participants

Seventy-five males undergoing a 5-year periodical health evaluation at the Centre d’Investigations Prévénitives et Cliniques (IPC) were included in this study. The IPC is one of the largest medical facilities in France that provides working and retired persons a free medical examination every 5 years (9). Because antihypertensive treatment can influence aldosterone levels, only participants who had never been treated for hypertension were included in this analysis. Thus, we included 75 consecutive men who were not treated for hypertension and who, in addition to the standard health check-up, had measurements of plasma aldosterone and WBC telomere length. Among these men, 56% were normotensive and 44% had mild to moderate untreated hypertension.

Medical History, Blood Pressure, and Biochemical Measurements

Participants answered a standardized questionnaire, which provided information about demographic background, occupation, medical history, drug use, and personal habits. Thereafter, using a manual sphygmomanometer, the same two physicians measured (under constant temperature of 19°C–21°C) supine blood pressure (BP) in the right arm. Fasting blood samples were then collected after 10–15 minutes of rest in a sitting position. Blood was used for DNA extractions, standard biochemical tests, and plasma aldosterone measurements.

Measurements of WBC Telomere Length by Terminal Restriction Fragment Length

DNA samples, extracted from WBCs and DNA samples were digested overnight with restriction enzymes HinfI (10
Table 1. Characteristics of the Population According to the Aldosterone Quartiles (Mean ± SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aldosterone Quartile 1</th>
<th>Aldosterone Quartile 2</th>
<th>Aldosterone Quartile 3</th>
<th>Aldosterone Quartile 4</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>Age (y)</td>
<td>56.9 ± 7.7</td>
<td>55.1 ± 12.8</td>
<td>54.7 ± 10.6</td>
<td>58.0 ± 10.9</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>136 ± 20</td>
<td>147 ± 19</td>
<td>149 ± 19</td>
<td>145 ± 19</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic</td>
<td>85 ± 8</td>
<td>92 ± 10</td>
<td>92 ± 11</td>
<td>90 ± 13</td>
<td>NS</td>
</tr>
<tr>
<td>Mean</td>
<td>102 ± 10</td>
<td>110 ± 12</td>
<td>111 ± 12</td>
<td>108 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>Pulse</td>
<td>51 ± 17</td>
<td>55 ± 14</td>
<td>57 ± 14</td>
<td>55 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>62 ± 7</td>
<td>66 ± 7</td>
<td>73 ± 13</td>
<td>71 ± 11</td>
<td>.003</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 2.7</td>
<td>27.8 ± 4.0</td>
<td>28.4 ± 5.3</td>
<td>25.9 ± 4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium (mmol)</td>
<td>4.32 ± 0.41</td>
<td>4.11 ± 0.31</td>
<td>4.08 ± 0.41</td>
<td>4.26 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/ml)</td>
<td>10.3 ± 0.9</td>
<td>10.6 ± 1.0</td>
<td>11.1 ± 1.0</td>
<td>10.5 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>39.9 ± 9.5</td>
<td>64.0 ± 6.5</td>
<td>91.4 ± 10.0</td>
<td>155.9 ± 47.8</td>
<td>.0001</td>
</tr>
<tr>
<td>Aldosterone range (pg/ml)</td>
<td>17.2–53.3</td>
<td>54.6–75.7</td>
<td>77.5–108.3</td>
<td>110.1–312.7</td>
<td>.0001</td>
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<tr>
<td>TRF length (kb)</td>
<td>6.72 ± 0.47</td>
<td>6.58 ± 0.76</td>
<td>6.49 ± 0.60</td>
<td>6.32 ± 0.35</td>
<td>.03</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; ANOVA = analysis of variance; HR = heart

U) and RsaI (10 U) (Boehringer Mannheim, Mannheim, Germany). Eighteen DNA samples (~5 μg each) and four DNA ladders (1 kb DNA ladder plus λ DNA/HindIII fragments; Gibco, Carlsbad, CA) were resolved on a 0.5% agarose gel (20 cm × 20 cm) at 50 V (GNA-200; Pharmacia Biotech, Piscataway, NJ). After 16 hours, the DNA was depurinated for 30 minutes in 0.25 N HCl, denatured 30 minutes in 0.5 mol/L NaOH/1.5 mol/L NaCl, and neutralized for 30 minutes in 0.5 mol/L Tris, pH 8/1.5 mol/L NaCl. The DNA was transferred for 1 hour to a positively charged nylon membrane (Boehringer Mannheim) using a vacuum blotter (Appligene Oncor, Feasterville, PA). The membranes were hybridized at 65°C with the telomeric probe [digoxigenin 3'-end labeled 5'-CCCTAA]₃ overhang in 5X sodium saline citrate (SSC), 0.1% Sarkosyl, 0.02% sodium dodecyl sulfate, and 1% blocking reagent (Boehringer Mannheim). The membranes were washed 3 times at room temperature in 2X SSC, 0.1% sodium dodecyl sulfate each for 15 minutes, and once in 2X SSC for 15 minutes. The digoxigenin-labeled probe was detected by the digoxigenin luminescent detection procedure (Boehringer Mannheim) and exposed on x-ray film. Each DNA sample was measured in triplicate. The intra-assay coefficient of variation of this measurement is less than 2%.

Measurements were performed blindly; each sample was identified by a number. Results (means of duplicate measurements) were transmitted to CL for statistical analysis.

Plasma Aldosterone Radioimmunoassay

A radioimmunoassay kit (Immunotech SA, Marseille, France) was used to measure plasma aldosterone. Samples (50 μl) and standards (50 μl) were incubated with ¹²⁵I-labeled aldosterone, as tracer, in antibody-coated tubes. After 3 hours of incubation at room temperature with shaking, the liquid content of each tube was aspirated and bound radioactivity was measured. Sample values were determined against a standard curve. The values were expressed in pg/ml. The intra-assay coefficient of variation evaluated for this assay is less than 9.5%.

Statistical Analysis

The association between plasma aldosterone concentration and terminal restriction fragment (TRF) length (age-adjusted) was tested using univariate and multivariate analyses. Aldosterone concentration was log-transformed (because of a skewed distribution) and treated as a categorical entity, expressed in quartiles, or as a continuous entity. Multivariate models were adjusted for age and other covariates. The association between aldosterone quartiles and TRF length was tested using a trend test analysis of variance. A two-sided p value of less than .05 was considered to be statistically significant. All statistics were performed using SAS statistical software (SAS Institute, Cary, NC). Data in Table 1 are presented as mean ± standard deviation. Adjusted mean values are presented as mean ± standard error of the mean.

RESULTS

Table 1 displays relevant clinical and laboratory data (without adjustment for age), stratified according to the quartiles of plasma aldosterone concentrations. Age, body mass index, BP, plasma creatinine, and serum potassium were similar in the aldosterone quartiles, whereas the heart rate (HR) was higher and the TRF was lower in participants belonging to the higher aldosterone quartiles. Age was negatively correlated with telomere length; the slope of TRF–age relationship showed a mean decrease in telomere length of 26 ± 5 base pairs per year (r = −0.46, p < .001; Figure 1). After adjustment for age, TRF length (Figure 2) was the longest in the lowest aldosterone quartile (6.74 ± 0.12 kb) and the shortest in the highest aldosterone quartile (6.36 ± 0.11), with intermediate TRF lengths in the second and third aldosterone quartiles (6.55 ± 0.12 and 6.45 ± 0.12, respectively) (analysis of variance trend test, p = .025).

The association between age-adjusted TRF length and aldosterone concentration was also observed when aldosterone was treated as a continuous variable (log aldosterone vs age-adjusted TRF length r = −0.28, p = .02; Figure 3). TRF length was not associated with either HR or BP levels in either univariate or multivariate analyses.

DISCUSSION

Oxidative stress and inflammation are central to theories of aging (18,19). WBC telomere length may be a bioindicator of aging if it records the cumulative burden of oxidative stress and inflammation (20). Our study shows that WBC telomere length is shorter in men with high plasma aldosterone levels. From the biological standpoint, individuals with shorter age-adjusted telomere length might be regarded as older than their chronological age would indicate. The difference in TRF length between the lowest and the highest aldosterone quartiles was 0.38 kb (380 base pairs). As the telomere attrition for the entire cohort was 26 base pairs per year, this difference in telomere length between the highest
and lowest aldosterone quartiles corresponds to approximately 15 years in telomere attrition equivalence, meaning that from the standpoint of telomere dynamics, participants in the highest aldosterone quartile are considerably older than their peers in the lowest quartile.

Recently, Vasan and coworkers (15) reported that higher aldosterone levels are associated with predilection to hypertension. In the present study we did not find a significant association between plasma aldosterone and BP levels, though as shown in Table 1, BP was lower in participants belonging to the lowest aldosterone quartile. In addition, participants with higher aldosterone had higher HR. However, the association between aldosterone and telomere length cannot be explained by these hemodynamic differences in the aldosterone quartiles, because neither BP nor HR were associated with TRF in univariate analyses. Adjustment for BP or HR in multivariate analyses did not modify the association between aldosterone and telomere length.

Aldosterone is a determinant in the pathogenesis of CVD and in accelerating cardiovascular aging in that it is a major stimulator of cardiac and vascular collagen synthesis (21). The presence of aldosterone receptors in large arteries, particularly the aorta (22), and the endogenous vascular synthesis of aldosterone (23) are in line with these findings. Pharmacological blockade of the aldosterone receptors reduces arterial fibrosis associated with aging in normotensive (24) and genetically hypertensive (25) rats, providing further support for the role of aldosterone in vascular aging.

Figure 1. Relation between age and terminal restriction fragment (TRF) length. TRF length in kilobase pairs (kb) = −0.026 * Age (y) + 7.97; $R^2 = 0.211$, $p < .001$.

Figure 2. Age-adjusted terminal restriction fragment (TRF) length stratified according to aldosterone quartiles. Quartile 1 represents the lowest plasma aldosterone levels and Quartile 4 corresponds to the highest aldosterone levels. The trend analysis of variance test showed an inverse relationship between the aldosterone quartiles and TRF length; $p = .025$. MAP = mean arterial pressure.

Figure 3. Relationship between plasma aldosterone and age-adjusted terminal restriction fragment (TRF) length. TRF length in kilobase pairs (kb) = −0.609 * LogALDO (aldosterone) (pg/mmol) + 7.60; $R^2 = 0.072$, $p = .02$. 

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ALDOSTERONE AND TELOMERES

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in laboratory animals. In addition, aldosterone blockade significantly decreases cardiovascular morbidity and mortality in heart failure and in postinfarction patients (16,17). Collectively, these studies suggest that increased aldosterone levels may be linked to an accelerated biological aging of the cardiovascular system. As aldosterone is a powerful pro-oxidant in a variety of tissues and cells (11–14), it is reasonable to propose that an increase in oxidative stress and perhaps inflammation are the likely causes for the shortened WBC TRF length in participants with increased aldosterone levels.

Conclusion
This study suggests that persons with higher plasma aldosterone levels have a more advanced biological age than do their peers of the same age with low aldosterone levels—a phenomenon that may be the outcome of a greater increase in the cumulative burden of oxidative stress. Longitudinal studies that include measurements of oxidative stress in conjunction with aldosterone and WBC telomere length will be essential to gaining a better understanding of the links between aldosterone and telomere dynamics.

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