Modulation of Oxidative Damage by Green and Black Tea: Role of Smoking and Gender in a Randomized Trial

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Abstract

Background: Oxidative stress has been implicated as an important modulator of human health and can play a role in both disease prevention and disease development. Objectives: The overall goal of this study was to determine the effects of high tea consumption on biological markers of oxidative stress that mediate lung cancer risk, including, 8-hydroxydeoxyguanosine (8-OHdG) and F2-isoprostanes (8-iso-PGF2α). Design: We completed a 6-month randomized, controlled, double-blinded trial in a group of former and current smokers who were randomized to receive green or black tea preparations or a matching placebo.

Results: A total of 146 participants (80 females and 66 males) completed the study. At the end of the 6-month intervention, female smokers in the green tea group showed a 35% decrease (p=0.015) in DNA damage while female former smokers in the black tea group showed a 26% decrease (p=0.04) in DNA damage. No significant changes in markers of oxidative stress were observed in men.

Conclusion: This data confirm our previous findings related to the beneficial effect of green tea on oxidative DNA damage among female smokers. The significant beneficial effect of black tea on oxidative lipid damage as well as the gender difference merit further studies.

Keywords: Tea; Gender; Oxidative stress; Clinical trial; Smokers

Abbreviations: 8OHdG: 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2α: 8-isoprostaglandin F2α; GPx: Glutathione Peroxidase; SOD: Superoxide Dismutase; ROS: Reactive Oxygen Species

Introduction

Cigarette smoking is the main risk factor for lung cancers in the United States (USA) despite the recent decline in smoking rates in USA, the age-adjusted mortality of lung cancer has not significantly changed especially among former smokers [1].

Moreover, there are now sufficient data to include female sex as an independent risk factor for lung cancer. In addition to cigarette smoking, epidemiologic evidence suggests increased susceptibility to lung cancer in women [2-5]. Cigarette smoke generates high levels of reactive oxygen species (ROS) [6]. Increased ROS production is involved in a diversity of biological phenomena such as inflammation, carcinogenesis, aging, and atherosclerosis as well as oxidative damage to DNA, lipids and proteins.

The oxidized nucleoside 8-hydroxydeoxyguanosine (8OHdG) is an oxidative adduct form of deoxyguanosine [7]. Urinary 8-OHdG level has been validated as a biomarker of the extent of oxidative DNA modification [8-10] and a large number of studies showed that a high level of urinary 8-OHdG may be a risk factor for chronic diseases such as cancer, cardiovascular diseases and diabetes [7,11,12].

A wide range of biomarkers of lipid peroxidation is available for use in human samples, however, the prostaglandin-like F2-isoprostanes such as 8-isoprostaglandin F2α (8-iso-PGF2α) are formed in vivo through free radical-induced peroxidation of arachidonic acid and are considered by many to be the best indicators of lipid peroxidation as they are not confounded by diet and their levels are increased in many human diseases [13-18].

Tea is one of the most ancient and, next to water, the most widely consumed liquid in the world. In experimental studies, tea preparations exhibited significant inhibitory activity against tumorigenesis [19,20]. Several epidemiological studies suggest that tea drinking may reduce the risk of cancers and cardiovascular diseases [21-23]. However, the differential effects of regular green and black tea intake on urinary markers of oxidative damage by gender and smoking status have not been fully examined in clinical trials. We, therefore, conducted a randomized, controlled, double-blinded trial to test the efficacy of regular green and black tea drinking in reducing oxidative DNA and lipid damage as measured by urinary 8OHdG and 8-iso-PGF2α, respectively, among current and former smokers.

Subjects and Methods

Study population

We recruited current and former smokers between 40 and 80 years of age with 25 or more pack-years of smoking history between September 2003 and December 2007 in Tucson, Arizona, Subjects were screened to exclude pregnant women, regular tea drinkers, persons with a forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) ratio >85%, history of schizophrenia or cancer, current drug or alcohol abusers, individuals with an abnormal liver function blood test or those...
currently being treated with antidepressants. The study was approved by the Institutional Review Board of the University of Arizona, and all of the subjects provided informed consent before enrollment.

**Study Protocol**

The study was a 3-arm double-blinded randomized placebo-controlled tea intervention trial. Each subject was randomly assigned to drink 4 cups (12 oz cup) of green tea, black tea, or placebo tea daily for 6 months.

**Tea products**

All the tea used in the intervention was obtained from the same supplier (Unilever Best foods, NJ, USA). Due to the variability in polyphenol content from lot to lot, a single lot was used to produce the green tea and black tea used in this study. In order to mask the color and taste of the study beverages, a citrus flavor was added to all teas (green, black and control) and all teas were packaged in white tea bags with no labels. Tea analysis was performed in our laboratory and the tea catechin composition of the products used in the study is described in Table 1.

**Study procedures**

Once informed consent has been obtained, participants underwent eligibility evaluation. Each participant completed an Arizona Smoking Assessment Questionnaire, Arizona Food Frequency Questionnaire (AFFQ), Arizona Tea Questionnaire, and Health Assessment Questionnaire and was evaluated for health and respiratory history. A blood sample was collected for CBC with differentials and comprehensive metabolic panel. Spirometry was performed to assess FEV1 and FVC using Nellcor Puritan Bennett Renaissance PB100 spirometer. Those who met all eligibility criteria underwent a 4-week placebo run-in period. All participants received standardized study cups (12 oz cup). Participants were instructed to prepare their tea by brewing 2 tea bags per cup and to drink 4 cups of tea daily (8 placebo tea bags/48 oz). Participants returned unused bags to the study staff during their scheduled monthly visit to the study clinic. Participants who consumed at least 80% of the placebo tea bags underwent the baseline evaluation and were randomized to one of the intervention groups. Randomization occurred using a random permuted block design (block size=6). Randomization lists stratified by gender and smoking status were prepared by the data manager prior to beginning the study and transferred to file-cards in sealed envelopes. The data manager had no contact with study subjects. All study products were assigned a bar-coded specific inventory number that was incorporated into a three-part label. The inventory numbers were linked to specific tea products by a code. None of the staff interacting with subjects knew the link between inventory number and code.

Study participants were asked to maintain the beverage consumption pattern (4 cups/ day) for 6 months, returning to the clinic at monthly intervals. They were telephoned during the week before each follow-up visit to confirm the date and time of the next appointment and to identify any problems or side effects associated with study participation. Blood and urine samples were collected at baseline, mid study (month 3) and end of the study (month 6). Induced sputum was collected at baseline and end of the study. Fasting morning blood samples were collected and serum, plasma, buffy coat, and erythrocyte isolated and stored at -80°C prior to the sample analysis. First morning urine was collected on three consecutive mornings including the morning of the visit. The three urine samples were combined in equal parts and aliquots stored at -80°C prior to the sample analysis.

**Data Collection**

Several techniques were used to maximize participant adherence and retention. Primary adherence to the study intervention was evaluated through self-reporting via monthly intake calendars (tea diaries). For each day, participants recorded the time and number of cups consumed. The monthly tea diaries generated continuous data that allowed identification of problems with the adherence pattern. In addition, participants were instructed to return the unused tea bags to the clinic. A monthly short smoking questionnaire allowed us to identify changes in smoking habits during the period of the intervention.

**Analysis of Urinary 8-Hydroxy-2'-deoxyguanosine (8OHdG)**

Urinary 8OHdG was analyzed using a published HPLC-tandem mass spectrometry method with minor modifications (6). Linear calibration curves have been established from 0.3 to 30 ng/mL. Urinary 8OHdG levels were normalized by urinary creatinine concentrations.
Analysis of Urinary 8-isoprostaglandin F2 (8-iso-PGF2α)

Urinary 8-iso-PGF2α was analyzed using a published HPLC-tandem mass spectrometry method with minor modifications (6). Linear calibration curves have been established from 0.04 to 4 ng/mL. The urinary 8-iso-PGF2α levels were normalized by urinary creatinine concentrations.

Determination of Antioxidant Enzymes in Erythrocytes

Glutathione peroxidase (GPx) activity in diluted erythrocyte lysates was determined using a Cayman Chemical Glutathione Peroxidase Assay kit. Superoxide dismutase (SOD) activity in diluted erythrocyte lysates was determined using a Cayman Chemical Superoxide Dismutase Assay kit. The erythrocyte antioxidant enzyme activity was normalized by hemoglobin concentrations.

Determination of Serum Fat Soluble Antioxidant Vitamins

The serum levels of retinols, carotenoids and tocopherols were determined by HPLC (6). Standard reference material 968c (fat-soluble vitamins in human serum) supplied by the National Institute of Standards and Technology (NIST, Gaithersberg, MD) was used for assigning values to in-house control materials.

Statistical Methods

Primary end points were changes in the level of creatinine-adjusted urinary 8-OHdG and urinary 8-iso-PGF2α from the baseline to 6 months after commencement of intervention. Descriptive analyses were conducted by gender and smoking status of the study population. Associations between baseline characteristics, urinary 8-OHdG, urinary 8-iso-PGF2α, and intervention group were assessed, using a t test or chi square test. Tests for significance of the change (pre-intervention versus post-intervention values) in urinary 8-OHdG and urinary 8-iso-PGF2α were performed by gender and smoking status. Results were expressed as mean ± standard deviation (SD). Multiple linear regression models were used to estimate the main effects of green and black tea intake on creatinine-adjusted urinary 8-OHdG and urinary 8-iso-PGF2α, with or without adjustment for potential confounders. Potential confounders considered were age, BMI, caloric intake, FEV1/FVC (%), pack years, years of smoking, and dietary total carotenoids. Statistical tests were two-sided with a significant level of 0.05. All statistical analyses were conducted using Stata Statistical Software (Stata 12).

Results

The study CONSORT diagram is shown in Figure 1. Of the 319 persons consented, 59 individuals were not eligible, 154 subjects were randomized and 146 current and former smokers completed the 6-month intervention and provided baseline and 6-month samples. The main reason for non-enrollment was loss of interest (n=98), while the reasons for dropout during run in (n=8) and after randomization (n=8) were moving out of Tucson and not having enough time. There were no statistically significant differences by gender, smoking variables, or treatment group between those who completed the study and those who did not. Among those who completed the study, compliance to the tea intervention was >95%.

The mean age of trial participants was 60 years (range, 40–80); 55% were women, and 58% were current smokers. Baseline characteristics of the study population by gender are shown in Table 2. Our data show that although women have relatively lower pack/year of smoking history (p=0.062) compared to men, they have significantly higher levels of urinary 8-OHdG (p=0.035) and urinary 8-iso-PGF2α (p=0.008).
Baseline characteristics were similar across the three study groups (Table 3). There were no significant differences in dietary intake or plasma levels of antioxidants between the three groups (data not shown).

The mean changes in urinary excretion of 8-OHdG by tea group compared to placebo are shown in Table 4. Our data showed a significant decrease in urinary 8-OHdG (-35%) among female smokers after 6 months of drinking green tea (p=0.04). Green tea consumption had no effect on urinary 8-OHdG levels in men or female former smokers. Black tea consumption was associated with a non-significant decrease (p=0.08) in urinary 8-OHdG among male former smokers.

The mean changes in urinary excretion of 8-iso-PGF2α by tea group compared to placebo are shown in Table 5. Our data showed a significant decrease in urinary 8-iso-PGF2α (-26%) among female former smokers after 6 months of drinking black tea (p=0.015). Black tea consumption had no effect on urinary 8-iso-PGF2α levels in men or female smokers. Green tea consumption was not associated with levels of urinary 8-iso-PGF2α.

**Discussion**

Oxidative stress is a key underlying factor in the pathogenesis of many common diseases and is thought to be involved in the initiation, promotion and progression phases of cancer, and its role in each of these phases is complex [24]. It is a major source of chemical-induced cellular injury and is caused by the increased production of ROS and/or decreased efficiency of antioxidant defense mechanisms [25]. Therefore, an oxidant/antioxidant imbalance may play a significant role in lung carcinogenesis among smokers and former smokers.

A number of epidemiologic studies have documented the independent association of gender on lung cancer risk [26-29]. Our data show that although women have relatively lower pack/year of smoking history compared to men, they have significantly higher levels of oxidative damage as measured by urinary 8-OHdG and 8-iso-PGF2α.

Consistent with our findings, several studies [26-28,30,31] reported elevated markers of oxidative DNA damage among female smokers as compared to male smokers, suggesting that women may be more susceptible to oxidative DNA damage induced by cigarette smoking.

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>Green Tea (n=45)</th>
<th>Black Tea (n=47)</th>
<th>Control (n=54)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Former/current smoker</td>
<td>61/85</td>
<td>27/39</td>
<td>34/46</td>
<td>0.846</td>
</tr>
<tr>
<td>Pack-years</td>
<td>42.8 ± 19.4</td>
<td>46.1 ± 21.6</td>
<td>40.1 ± 17.0</td>
<td>0.062</td>
</tr>
<tr>
<td>Years of smoking</td>
<td>33.7 ± 10.2</td>
<td>35.2 ± 9.1</td>
<td>32.6 ± 11.0</td>
<td>0.116</td>
</tr>
</tbody>
</table>

**Systemic oxidative stress**

<table>
<thead>
<tr>
<th>Systemic oxidative stress</th>
<th>Green Tea (n=45)</th>
<th>Black Tea (n=47)</th>
<th>Control (n=54)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary 8OHdG (ng/mg creatinine)</td>
<td>4.2 ± 7.1</td>
<td>2.8 ± 2.4</td>
<td>5.4 ± 9.6</td>
<td>0.035</td>
</tr>
<tr>
<td>Urinary 8-iso-PGF2α (ng/mg creatinine)</td>
<td>423.3 ± 297.3</td>
<td>344 ± 250</td>
<td>477± 304</td>
<td>0.008</td>
</tr>
<tr>
<td>Erythrocyte GPx (nmol/min/g Hb)</td>
<td>24097.1± 8371</td>
<td>24,558 ± 9,488</td>
<td>23,591 ± 7,350</td>
<td>0.5</td>
</tr>
<tr>
<td>Erythrocyte SOD (U/g Hb)</td>
<td>5332.5 ± 1894</td>
<td>5,464 ± 1,985</td>
<td>5,182 ± 1,821</td>
<td>0.348</td>
</tr>
</tbody>
</table>

Table 3: Overall characteristics of the study population by gender (means ± SD).
Our study used urinary 8-iso-PGF2α as a biomarker for oxidative lipid damage. 8-iso-PGF2α is biologically active and may play a role in pulmonary pathophysiology [41] and increased lung cancer risk [24,42]. Consistent with our data, previous studies have also shown that female smokers have higher urinary 8-iso-PGF2α [43,44].

<table>
<thead>
<tr>
<th>Urinary 8 OHdG</th>
<th>Green Tea Mean change (95% CI)</th>
<th>p</th>
<th>Black Tea Mean change (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.50 (-0.32; 1.42)</td>
<td>0.21</td>
<td>-0.33 (-1.10; 0.60)</td>
<td>0.54</td>
</tr>
<tr>
<td>Smokers</td>
<td>0.75 (-0.41; 1.98)</td>
<td>0.19</td>
<td>0.05 (-1.06; 1.23)</td>
<td>0.88</td>
</tr>
<tr>
<td>X-smokers</td>
<td>0.18 (-1.10; 1.40)</td>
<td>0.79</td>
<td>-1.32 (-2.60; 0.17)</td>
<td>0.08</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>-0.83 (-2.70; 1.40)</td>
<td>0.53</td>
<td>1.6 (-0.09; 3.90)</td>
<td>0.06</td>
</tr>
<tr>
<td>Smokers</td>
<td>-1.82 (-4.1; -0.06)</td>
<td>0.04</td>
<td>1.46 (-0.76; 3.22)</td>
<td>0.22</td>
</tr>
<tr>
<td>X-smokers</td>
<td>0.72 (-2.7; 5.8)</td>
<td>0.46</td>
<td>1.79 (-1.30; 6.70)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 4: Mean change (95% CI) in urinary excretion of 8-OHdG (ng/mg creatinine) by tea group compared with placebo.

<table>
<thead>
<tr>
<th>Urinary 8 8-iso-PGF2α</th>
<th>Green Tea Mean change (95% CI)</th>
<th>p</th>
<th>Black Tea Mean change (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>113.2 (-13.8; 257.1)</td>
<td>0.08</td>
<td>90.5 (-39.5; 237.4)</td>
<td>0.16</td>
</tr>
<tr>
<td>Smokers</td>
<td>132.3 (-90.7; 256.2)</td>
<td>0.34</td>
<td>102.3 (-126.6; 232.0)</td>
<td>0.55</td>
</tr>
<tr>
<td>X-smokers</td>
<td>78.7 (-73.3; 407.1)</td>
<td>0.16</td>
<td>71.6 (-80.4; 400.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>-90.7 (-285.3; 140.5)</td>
<td>0.50</td>
<td>31.9 (-154.9; 255.4)</td>
<td>0.63</td>
</tr>
<tr>
<td>Smokers</td>
<td>-137.3 (-448.6; 265.9)</td>
<td>0.61</td>
<td>143.2 (-149.8; 528.0)</td>
<td>0.27</td>
</tr>
<tr>
<td>X-smokers</td>
<td>-20.9 (-176.9; 116.5)</td>
<td>0.68</td>
<td>-176.6 (-332.6; -39.2)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 5: Mean change (95% CI) in urinary excretion of 8-iso-PGF2α (ng/mg creatinine) by tea group compared with placebo.

Conclusion

Our study as well other published data showed that the cellular effects of tobacco smoke may be more pronounced in women than in men. Women may be more susceptible to tobacco smoke and potentially more vulnerable to lung cancer development [48] and therefore are more likely to benefit from a tea intervention. The beneficial effects of regular green tea consumption seem to be more likely in participants exposed to oxidative challenge while the beneficial effects of regular black tea consumption seem to be more likely in former smokers and/or non-smokers.

This data confirm our previous findings related to the beneficial effect of green tea on oxidative DNA damage among female smokers. The significant beneficial effect of black tea on oxidative lipid damage as well as the gender difference merit further studies.

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