Black tea reduces uric acid and C-reactive protein levels in humans susceptible to cardiovascular diseases

Theeshan Bahorun a,*, Amitabye Luximon-Ramma a, Teeluck K. Gunness b, Dharmendra Sookar b, Satar Bhoyroob, Rabindranath Jugessurb, Deshmukh Reebeyb, Kreshna Googoolyea, Alan Crozier c, Okezie I. Aruomad

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Abstract

The effect of black tea on the level of uric acid (UA) and C-reactive proteins (CRP) in humans susceptible to ischemic heart diseases was assessed in a prospective randomized controlled study. The study group consumed 9 g of black tea (equivalent to three cups of tea) daily for 12 weeks without additives followed by a 3-week wash-out (with control group consuming equivalent volume of hot water). Black tea consumption induced a highly significant decrease in the high uric acid baseline groups >6 mg/dL by 8.5%; p < 0.05. For men and women in the base line group >7 mg/dL, the decrease was 9.4% and 7.1%, respectively. In the low baseline serum uric acid levels there was a non-significant increase of 3.7% and 15% in men and women, respectively. C-reactive protein in the high risk group >3 mg/L was significantly decreased by 53.4% and 41.1% in men and women, respectively. For the non-supplemented group in this range the changes were 3.7% decrease for men and 2.9% increase for women. Tea supplementation-associated decrease in plasma uric acid and C-reactive protein levels may benefit humans at high risk of cardiovascular events and may augment drug therapy.

1. Introduction

The health benefits of tea and tea extracts have been well documented, especially with respect to chemopreventive effects on cancers, cardiovascular diseases, and inflammation (Widlansky et al., 2005; Stangl et al., 2006; Yang et al., 2006; Yamada and Watanabe, 2007; Tinahones et al., 2008; de Mejia et al., 2009; Sharangi, 2009). The health-promoting effects of regular tea consumption are mainly ascribed to its polyphenol content (35% of their dry weight in the leaves) with the major phenolics being the flavan-3-ols (epicatechins, epigallocatechins and their gallate esters), the flavonols (monogalloylated, digalloylated and trigalloylated conjugates of myricetin, quercetin and kaempferol), the flavones and quinic acid esters of gallic, coumaric and caffeic acids. Black tea has a reduced flavan-3-ol monomer content and higher levels of their polymerized derivatives theaflavins, which account for about 10–30% of the converted catechins, and thearubigins (de Mejia et al., 2009; Rouanet et al., 2010). The pathophysiology of atherosclerosis and other cardiovascular events have associated inflammatory process (Ross, 1999), with the inflammatory components contributing to the instability and rupture of atheromatous plaque leading to atherothrombotic events (Carr et al., 1997; Pasterkamp et al., 1999). The levels of C-reactive protein (an acute phase reactant produced in the liver) are known to rise in inflammation reactions and plasma high-sensitivity (hs)-CRP are widely suggested to be a predictor of coronary events (Koenig et al., 1999; Ridker et al., 2000; Li and Lun, 2007; Mazzone et al., 2008; Brodov et al., 2009; Frazier et al., 2009). Thus inflammatory markers such as hs-CRP may help improve cardiovascular risk prediction and hence complement traditional risk factors including lipid profiles (Kannel et al., 1986; Manson et al., 1992). According to the American Heart Association individuals with hs-CRP level lower than 1.0 mg/L have low risk of developing cardiovascular disease. Those with values between 1.0 and 3.0 mg/L have an average risk whilst hs-CRP values higher than 3.0 mg/L put the individual at higher risk.
Uric acid (UA) may have antioxidant functions in vivo (Waring et al., 2003; Zitnanová et al., 2004; Macchi et al., 2008; Lippi et al., 2008a) but as paradox would have it, epidemiological evidence has linked elevated uric acid with increased cardiovascular risk (Kohn and Prozon, 1959; Bos et al., 2006). For example, UA may promote low-density lipoprotein (LDL) oxidation in vitro (Bagnat et al., 1999), to promote granulocyte adherence to the endothelium (Boogaerts et al., 1983) and liberate peroxide and superoxide free radical (Falasca et al., 1993). Hyperuricemia is considered to be a risk factor in many clinical conditions including hypertension, cardiovascular events, diabetes mellitus, stroke, and metabolic syndrome (Bo et al., 2001; Masuo et al., 2003; Nakashima et al., 2003; Kanellis et al., 2004; Strazzullo and Puig, 2007; Sturm et al., 2008). Further, epidemiological studies indicate that hyperuricemia may be associated with changes in the levels of biomarkers of inflammation, especially CRP and plasma CRP (Ruggiero et al., 2006, 2007; Lippi et al., 2008b). Brodov et al. (2009) investigated prognostic information from a combined assessment of UA and CRP compared with a single marker in patients with coronary artery diseases enrolled in the Bezafibrate Infarction Prevention trial and concluded that the combine markers provide “incremental information for risk of patients with CAD”. The work of Nan et al. (2008) reported that elevated UA could be an independent risk marker for future diabetes in Mauritian men whereas the prediction appears weak for women. It is of interest to record the work of Muntner et al. (2009) which addressed the question of the inclusion of CRP measurement as a useful guide for statin treatment decisions and the observation of Ridker (2003) that “in regard to potential treatment, treatments have been found to reduce hs-CRP levels, and data from statin treatment trials raise the possibility that subjects with elevated hs-CRP levels may derive greater benefit from treatment than do patients without elevated hs-CRP”. The objective of this study was to determine the effects of black tea on fasting uric acid levels and CRP in humans susceptible of cardiovascular diseases.

2. Methods

2.1. Subjects

Two hundred and sixty three male and female Mauritian volunteers were recruited for the randomized controlled clinical trial. 80 subjects were selected from the waiting list for angiography at the Cardiac Centre, SSRNH Hospital and 90 from the Ministry of Health and Quality of Life National Ethics Committee approved the study protocol and informed written consent was obtained for each subject. The subjects had to be between 25 and 60 years old. The inclusion criteria were: (1) non-smoker or former smokers who had stopped for less than 6 months; (2) alcohol intake less than two standard drinks per day; (3) post-menopausal women not receiving hormone replacement therapy; and (4) cardiac patients with left ventricular ejection fraction < 0.50 (ranged from 28 to 218 arbitrary units at baseline analysis. The mean of 25–60 years old. The compiled diet scores calculated as per the diet during the study. Statistical analysis of these forms consisted of a ranking of the diet during the study. Statistical analysis of these forms consisted of a ranking

2.2. Study protocol

The study was conducted at the Cardiac Centre of the Sir Seewoosagur National Hospital, Pamplemousses, Republic of Mauritius over a 15-week intervention period. The study group (70%) consumed 2.5 mL of black tea infusate per day (three standard cups of 200 mL hot water each containing 3 g of black tea (infused for 5 min) for 12 weeks without additives (milk or sugar) followed by a 3-week wash-out period by consuming same volume of hot water per day), the control group (30%) consumed equivalent volume of hot water for same intervention period. Study and control groups were devised following statistical advice and the subjects therein represented an inclusive population. Subjects were requested to fast for at least 10 h before blood collection. The last tea ingestion before blood collection was 12 h. Blood collection was made at the Cardiac Centre under the guidance of medical doctors.

2.3. Blood collection and preparation for sample analysis

15 mL of fasting blood were collected and dispensed into four different tubes comprising two heparinised tubes (2 × 4 mL), fluoride oxalate tube (2 × 5 mL) and plain tubes. Fresh blood samples were preserved in ice bags for analysis.

2.4. Analysis of biomarkers

Heparinised tubes were centrifuged and plasma removed for determination at 37 °C. Reagent kits for uric acid and CRP analyses were from Human Co. (Wiesbaden, Germany) and Biosystems (Barcelona, Spain).

2.5. Survey of diet forms

Compliance to protocol was assessed by a follow-up of the daily diet of the volunteers. A dietary questionnaire indicating food items consumed daily during the three main meals (breakfast, lunch and dinner) was issued to each subject and was collected daily filled after blood sampling exercise from each volunteer. Information provided by the volunteers enabled the assessment of any possible changes in the diet during the study. Statistical analysis of these forms consisted of a ranking strategy based on the fat/lipid ratio (USDA Food Composition database, 2006) of each food item consumed by both study group and control group (e.g. a food item high in fat content (e.g. red meat) would score 10/10 marks while a vegetable low in fat would be assigned 1/10; the mean value in arbitrary units obtained per day being further averaged on a weekly basis and the trend observed during the 15-week intervention period).

2.6. Statistical analysis

Simple regression analysis was performed to calculate the dose–response relationship of standard solutions used for calibration as well as test samples. After data cleaning, statistical analyses were carried out using both Microsoft Excel and SPSS 13.0 statistical software. Tests of significance of observed mean differences over the intervention period for two sets of data were performed using Student’s t-test and where data were not normal the non-parametric alternative Mann–Whitney U test was used. Kruskal–Wallis test was used as a non-parametric alternative for one-way ANOVA for comparing more than two sets of data. The critical limits for test of significance were set at 5%, 1% and 0.1% successively. Statistical analyses were also performed to calculate any significant correlations, firstly between individual parameters and secondly, between individual parameters and diet scores. Correlation analyses were made on tea regimens and control data, ignoring gender stratification at first instance and then on gender stratified data. As all the pairs of data for correlation analyses involved at least one non-normally distributed data set, Spearman’s rho correlation coefficient was used. Correlation analyses were undertaken on baseline, week 12 and wash-out data, respectively. Analyses on percentage change data were also performed.

3. Results

The number of subjects at the end of the 15-week intervention period was 232 representing a 12% drop-out from the initial population size. There was no incidence of cardiac events during that period (heart failure, angina pectoris or acute myocardial infarction) and there were no relapses in the patients that have undergone surgery. The final sample population consisted of 137 (59%) male and 95 (41%) female Mauritian citizens in the age group of 25–60 years old. The compiled diet scores calculated as per the lipid/fat contents of the food items consumed during the study varied from 28 to 218 arbitrary units at baseline analysis. The mean data show that the scores remained relatively constant for both the tea and control groups in the combined male and female population from baseline to wash-out period (Fig. 1). Significant positive correlation was obtained between % change in diet scores and % change in C-reactive protein values for female control subjects (r = 0.527, p = 0.036, N = 16). Significant positive correlation was also obtained between % change in diet scores and % change in UA values for female subjects who were on tea regimens (r = 0.302, p = 0.033, N = 50). No other correlation analyses between diet score data and CRP or UA values for any treatment or gender turned out to be significant, nor were there any consistency in the direction of correlations.
<table>
<thead>
<tr>
<th>Gender</th>
<th>Range of values (mg/L)</th>
<th>Tea regimen</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD baseline (mg/L)</td>
<td>Mean ± SD week 12 (mg/L)</td>
</tr>
<tr>
<td>Male</td>
<td>2.5–5</td>
<td>22</td>
<td>4.4 ± 0.6</td>
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<tr>
<td>Female</td>
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<td>3.4 ± 0.4</td>
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<tr>
<td></td>
<td>&gt;7</td>
<td>31</td>
<td>8.4 ± 1.3</td>
</tr>
</tbody>
</table>

Mean values of uric acid levels during the 12-week intervention period and 3-week wash-out period in a male Mauritian population (N= number of subjects) under the tea and control regimens. Percentage increase of values is indicated in brackets. Significance testing between baseline and week 12 values and wash-out period for both study group and control group was performed by Student's t-test comparing mean values of two samples. *p < 0.05 (significance at 5%), **p < 0.01 (significance at 1%), ***p < 0.001 (significance at 0.1%).
Table 3  
Effect of tea on the levels of C-reactive proteins.

<table>
<thead>
<tr>
<th>Gender</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD baseline value (mg/L)</td>
<td>Mean ± SD week 12 (mg/L)</td>
</tr>
<tr>
<td>Male</td>
<td>0–1.5</td>
<td>40</td>
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<td></td>
<td></td>
<td>34</td>
<td>2.1 ± 0.4</td>
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<tr>
<td></td>
<td>&gt;3</td>
<td>30</td>
<td>6.6 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>1.6–3</td>
<td></td>
<td></td>
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<tr>
<td>Female</td>
<td>0–1.5</td>
<td>21</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>&gt;3</td>
<td>15</td>
<td>5.0 ± 1.6</td>
</tr>
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</table>

Mean values of CRP level during the 12-week intervention period and 3-week wash-out period in a male Mauritian population (N = number of subjects) under the tea and control regimens. Percentage change of values is indicated in brackets ( ). Significance testing between baseline and week 12 values and wash-out period for both study group and control group was performed by Student’s t-test comparing mean values of two samples. * p < 0.05 (significance at 5%), ** p < 0.01 (significance at 1%), *** p < 0.001 (significance at 0.1%).

Table 4  
Effect of tea on the levels of C-reactive proteins.

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<td></td>
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<td>15</td>
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Mean values of CRP level during the 12-week intervention period and 3-week wash-out period in a female Mauritian population (N = number of subjects) under the tea and control regimens. Percentage change of values is indicated in brackets ( ). Significance testing between baseline and week 12 values and wash-out period for both study group and control group was performed by Student’s t-test comparing mean values of two samples. * p < 0.05 (significance at 5%), ** p < 0.01 (significance at 1%), *** p < 0.001 (significance at 0.1%).
The levels of biomarkers measured under both tea and control regimens at the beginning of the study, its percentage change at the end of the intervention period and after a three week wash-out period were recorded. The data were stratified in three ranges of values indicating below normal, normal and above normal categories. Plasma UA is an endogenous low-molecular weight antioxidant. However values >7 mg/L is considered pathological and is considered as a risk factor in cardiovascular diseases. Subjects were categorized in groups that varied from 2.5 to 5 mg/L (group 1), 5.1–7 mg/L (group 2) and >7 mg/L (group 3). Baseline uric acid levels varied from 2.9 to 12.3 mg/L in males (Table 1) and from 2.5 to 7.8 mg/dl in females (Table 2). Male subjects were categorized in group 1: 2.5–5 mg/L, group 2: 5.1–7 mg/L and group 3: >7 mg/L while female volunteers were categorized as group 1: 2.5–4 mg/L, group 2: 4.1–6 mg/L and group 3: >6 mg/L. After 12 weeks, black tea intake decreased uric acid levels by 9.4% (p < 0.01) and 7.1% (p < 0.001) in both male and female groups with highest baseline values (group 3). The wash-out treatment produced non-significant decreases in all the groups. The levels generally non-significantly decreased in the controls (12-week period and wash-out).

Baseline C-reactive protein data varied between 0.9 and 6.6 mg/L in men (Table 3) and from 1 to 5 mg/L in women (Table 4). The subjects were categorized into three groups corresponding to (1) low risk 0–1.5 mg/L, (2) average risk 1.6–3 mg/L and (3) high risk >3 mg/L. The levels in the high risk group (>3 mg/L) were significantly decreased by 53% (p < 0.01) and 41.1% (p < 0.01) in men and women, respectively. For the non-supplemented group in this range the changes (non-significant) were 3.7% decrease for men and 2.9% increase for women. Significant decreases were also observed in average risk group: −43.4% (p < 0.001) for males and −21% (p < 0.05) for females. Non-significant decreases of 23.4% and 1.7% were observed in corresponding controls for both genders. Wash-out treatments produced non-significant increases and decreases in both study and control groups.

There were no significant correlations between UA and CRP levels for baseline, week 12 and wash-out data for subjects on either tea regimen or control. Further ventilation of data by gender did not reveal any correlations between UA and CRP values. No significant correlations were found for calculated percentage changes.

4. Discussion

Daily intake of 9 g of black tea (corresponding to 1.5%; w/v) represented a supplementation of 738 mg of polyphenols per day. This tea dose was within the range (0.7–1.6%; w/v) used in studies that assessed the effects of Oolong, black and green teas on plasma antioxidant capacity and a number of risk factors in patients with coronary artery disease (Langley-Evans, 2000; Shimada et al., 2004). The effect of smoking and alcohol on levels of the measured biomarkers was minimized by the exclusion criteria applied to the patient population. Diet questionnaire applied during the study period indicated that the fat/lipid content of the population diet did not change significantly during the intervention trial. Furthermore, statistical analysis did not show any significant correlation between diet scores and UA/or CRP levels.

Uric acid has been reported to traverse dysfunctional endothelial cells and accumulate as crystal within atherosclerotic plaques (Patetsios et al., 1996), so the protective antioxidant effects of uric acid may be obscured by detrimental effects associated with cardiovascular disorders. Interestingly Strazzullo and Puig in 2007 have suggested that elevation of UA often observed in patients with uncomplicated obesity, insulin resistance and/or hypertension, deriving from an altered renal handling of sodium and uric acid, might be of no major concern. As such, there is no solid evidence to recommend pharmaceutical treatment of this asymptomatic mild form of hyperuricemia (up to a UA level <10 mg/dl). There appears to be a consensus of opinion that UA elevation in hypertensive and/or diabetic patients at high cardiovascular risk (particularly in those with overt ischemic conditions) may represent a marker of oxidative stress as well as underscoring inflammatory and degenerative alterations in the cardiovascular system (Ferroni et al., 2006). In this study, low baseline of plasma UA levels showed a non-significant increase of 3.7% and 15% in men and women, respectively, while significant decreases by 9.4% and 7.1% were noted in both genders with high baseline uric acid levels (>7 mg/dl). Statistical data showed significant correlation between CRP and UA values in neither the combined population nor among the male and female genders in both study and control groups.

The result of this study indicated that tea supplementation can significantly reduce CRP levels in both moderate risk and high risk groups of both males and females compared to controls suggesting an anti-inflammatory action. Black teas, green teas and phytochemical compounds in tea (epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), theaflavin and theaflavin-3′-3′′-digallate (TF-3)) can selectively interfere with the production and/or function of cytokines (Varilek et al., 2001; Wheeler et al., 2004; Nag-Chaudhuri et al., 2005; Huang et al., 2006; Navarro-Perán et al., 2008; Kim et al., 2008; Roy et al., 2008). The black tea infusate used in this study contained relatively large amounts of these polyphenolics: total gallic acid derivatives (50 ± 1 mg/L), total flavan-3-ols and flavonols (42 ± 2 mg/L) and (32 ± 1 mg/L, respectively), total theaflavins (89 ± 1 mg/L) and large amounts of uncharacterized thearubigins. The TEAC and FRAP values were of the order of 1055 ± 23 and 825 ± 23 μmol/g dry weight, respectively. Consistent with our results, data from a large cross-sectional study indicated that regular tea drinkers had significantly lower levels of CRP and serum amyloid A (a family of apolipoproteins found predominantly associated with high-density lipoprotein (HDL) in plasma, with different isoforms being uniquely expressed constitutionally and in response to inflammatory stimuli) levels (De Bacquer et al., 2006). In a randomized 6-week trial, treatment equivalent to four cups of black tea per day induced together with lower platelet activation, a decrease in plasma CRP levels compared to placebo (Steptoe et al., 2007). The precise role of CRP in the development or progression of atherosclerosis remains however to be defined. A study by Rufail et al. (2006) indicated that CRP inhibited in vitro oxidation of LDL, suggesting that the protein may limit the atherogenic oxidation in vivo. Consistent with an in vivo antioxidant role of CRP, the latter been reported to be localized in atheroslerotic lesions (Torzewski et al., 1998, 2000; Venugopal et al., 2005) showed that vascular endothelial cells can be induced to express CRP, indicating probably that the
protein may be produced in the environment where LDL is being oxidized.

Thus high plasma concentration of CRP may reflect a degree of protection in cardiovascular diseases. Moderate intake of black tea may improve the levels of independent predictors of the risk factors of cardiovascular events. The effects seem to be ascribed primarily to the synergistic effects of the tea polyphenols. Given that tea is the most consumed beverage in the world after water, and that inflammation plays a role in every disease process, including arthritis, diabetes, cancer, heart disease, and obesity, this finding on tea may have implications relative to the synergistic effects of the tea phenolics. Given that tea is also increasingly consumed for its possible health benefits, the finding on its anti-inflammatory effects may have important health implications for many disease processes.

Conflict of interest

The authors declared no conflict of interest.

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