Effect of Acute and Chronic Tea Consumption on Platelet Aggregation in Patients With Coronary Artery Disease

Stephen J. Duffy, Joseph A. Vita, Monika Holbrook, Peter L. Swerdloff, John F. Keaney, Jr

Abstract—Epidemiological studies suggest that tea consumption is associated with a decreased risk of cardiovascular events, but the mechanisms of benefit remain undefined. Platelet aggregation is a precipitating event in cardiovascular disease, and tea contains antioxidant flavonoids that are known to decrease platelet aggregation in vitro. To test the effect of tea consumption on platelet aggregation, we randomized 49 patients with coronary artery disease to either 450 mL of black tea or water consumed initially, followed by 900 mL of tea or water daily for 4 weeks in a crossover design. Ex vivo platelet aggregation in platelet-rich plasma was assessed in response to ADP and thrombin receptor–activating peptide at baseline and 2 hours and 4 weeks after beverage consumption. We observed dose-dependent platelet aggregation in response to each agonist, and neither relation was altered by acute or chronic tea consumption. Plasma flavonoids increased with acute and chronic tea consumption, indicating adequate absorption of tea flavonoids. In conclusion, these results demonstrate that acute and chronic black tea consumption does not affect ex vivo platelet aggregation in patients with coronary artery disease. These findings suggest that an effect of tea flavonoids on platelet aggregation is unlikely to be the explanation for the reduction in risk of cardiovascular events noted in epidemiological studies. (Arterioscler Thromb Vasc Biol. 2001;21:1084-1089.)

Key Words: platelet aggregation ■ tea ■ flavonoids ■ coronary artery disease

Recent epidemiological studies strongly suggest an inverse relationship between tea consumption and cardiovascular disease risk.1–5 with 1 notable exception.6 There is also convincing evidence that dietary intake of antioxidant flavonoids from tea and other sources (eg, red wine, onions, apples, and broccoli) is associated with reduced cardiovascular risk.1–5,7–9 The benefit of high flavonoid intake may be greater for individuals with established coronary artery disease (CAD).1,10 although favorable effects have also been demonstrated in people without evidence of atherosclerosis.5,8,9

One proposed mechanism for the apparent benefit of tea and other sources of flavonoids is their favorable effect on platelet aggregation.11–16 These polyphenols may inhibit platelet aggregation by a number of different mechanisms, including inhibition of lipooxygenase, cyclooxygenase,13,17 cAMP phosphodiesterase,12,18 and cGMP phosphodiesterase.19 Other platelet-inhibitory effects of flavonoids include thromboxane receptor antagonism,15 scavenging of reactive oxygen species such as superoxide anion,20 decreasing phospholipase C activation by blunting hydrogen peroxide production,21 and inhibition of lipid peroxidation.14 Flavonoids also enhance nitric oxide (NO) production from the endothelium.22 NO is a potent inhibitor of platelet adhesion, aggregation,23–25 and thrombosis,26 and impaired platelet production of NO has been associated with acute coronary syndromes.27

A recent study demonstrated dose-dependent inhibition of human platelet aggregation in vitro with green tea catechins, which are important tea flavonoids.28 Therefore, we hypothesized that tea consumption would inhibit platelet aggregation in patients with CAD. To test this hypothesis, we performed a randomized, placebo-controlled, crossover study of acute and chronic black tea consumption for its effect on ex vivo platelet aggregation. We chose black tea rather than green tea because this is the most commonly consumed type of tea in the United States and has been associated with reduced risk of atherosclerotic disease in many epidemiological studies.

Methods

Study Volunteers

Patients referred to Boston University Medical Center were screened for enrollment, and those with CAD, defined as a history of percutaneous or surgical revascularization or the presence of ≥1 coronary stenosis >70% on coronary angiography, were eligible for the study. Exclusion criteria included uncontrolled hypertension, heart failure, recent myocardial infarction (<3 months), or unstable angina. Participants were also excluded if they were taking antioxidant vitamin supplements (vitamin C or E) in doses greater than the recommended daily allowance. The study was approved by the Institutional Review Board of Boston Medical Center, and all volunteers provided written, informed consent.

Study Design

Patients were studied during 3 visits, each 4 weeks apart. Before each visit, patients fasted overnight and, if applicable, were asked not to
smoke for 24 hours. All patients were taking aspirin 325 mg/d throughout the study period. Patients were asked to maintain their usual diet but to exclude red wine and other tea consumption during the 8-week study period. Baseline dietary flavonoid intake was estimated by a 1-week food-frequency questionnaire at the first visit. The questionnaire included the major dietary sources of flavonols (quercetin, kaempferol, and myricetin) and flavanons (the various catechins), which were quantified by use of food flavonoid content charts.39

The sequence of beverage consumption is outlined in Figure 1. Briefly, patients underwent assessment of platelet function at 6 time points: (1) baseline; (2) 2 hours after consumption of 450 mL of freshly brewed black tea (acute tea); (3) after consuming 900 mL of black tea per day for 4 weeks, but none on the morning of study (chronic tea); (4) 2 hours later that same day after consuming 450 mL of freshly brewed black tea (acute on chronic tea); (5) after consuming 900 mL of water per day for 4 weeks (chronic water); and (6) 2 hours after consuming 450 mL of water (acute water). Patients were assigned to consume tea first or water first, as shown in Figure 1, on the basis of computer-generated random numbers. The acute effects of tea were examined at the 2-hour time point, coincident with maximal flavonoid bioavailability.30 For the acute studies, 9.7 g of fresh tea leaf (World Blend, provided by the Tea Trade Health Research Association, London, England) was brewed in a standard brewer (Bunn-O-Matic Corp) for 5 minutes with 1 L of fresh water. The chronic consumption studies were performed with freeze-dried tea to aid in the standardization of dosing. The freeze-dried tea was prepared by Lipton, Inc, from the same tea leaf as used in the acute studies. The compositions of the 2 tea preparations are detailed in Table 1. To increase compliance and more closely mimic usual practice, participants were permitted to add sugar, lemon, or milk to the tea, as desired.30 Compliance was confirmed by direct questioning.

### Platelet Studies
Platelet-rich and platelet-poor plasma were prepared from 20 mL of patient blood collected via a 21-gauge needle into a 30-mL syringe.

TABLE 1. Components of Prepared Tea

<table>
<thead>
<tr>
<th>Component</th>
<th>Brewed Tea, mg/dL</th>
<th>Powdered Tea, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>42</td>
<td>30</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>3.6</td>
<td>6.9</td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>3.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>1.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Epicatechin gallate</td>
<td>6.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Total catechins</td>
<td>13.3</td>
<td>12.9</td>
</tr>
<tr>
<td>Total theoflavins</td>
<td>6.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Tea solids</td>
<td>525</td>
<td>467</td>
</tr>
<tr>
<td>Total polyphenols</td>
<td>163</td>
<td>150</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>106</td>
<td>97</td>
</tr>
</tbody>
</table>

Components are in tea after preparation in water for consumption.

### Results

**Baseline Characteristics**
A total of 66 patients were enrolled. Eight patients withdrew, and 9 had platelet aggregation studies that were technically inadequate at ≥1 time points; these data were excluded before unblinding. Thus, 49 participants completed the 8-week protocol and had platelet aggregation studies at all 6 time points. Twenty-five volunteers commenced with tea first and 24 with water first; their clinical characteristics are shown in Table 2. Clinical characteristics were balanced. Concurrent cardiovascular medications among the 49 patients were aspirin in 49 (100%), β-blockers in 44 (90%), lipid-lowering therapy in 37 (76%), calcium channel blockers in 16 (33%), nitrates in 14 (29%), and ACE inhibitors in 5 (10%).

As previously described,31,32 In preparation for aggregation studies, platelet counts were standardized to 300 000/μL with autologous platelet-poor plasma. Platelet counts were determined with a Coulter Counter (model ZM, Coulter Electronics). Aggregation was induced in 0.4 mL platelet-rich plasma by the addition 20 μL of ADP (1, 2.5, 5, and 10 μmol/L; final concentration) or thrombin receptor-activating peptide (TRAP, 5, 10, 20, and 50 μmol/L). These studies were performed at 37°C at a constant stirring rate of 1200 rpm in a BioData PAP-4 aggregometer, as previously described.33

### Biochemical Analyses
Serum total cholesterol, HDL cholesterol, triglycerides, and glucose were measured with an automated analyzer (Hitachi Instruments, model 917). LDL cholesterol was calculated by use of the Friedewald formula. Plasma catechins, important tea flavonoids, were measured by high-performance liquid chromatography (HPLC) as previously described.34

Data are mean ± SD or number (%).

**Statistical Analysis**
Data are presented in the text and tables as mean ± SD. Data in the figures are presented as mean ± SEM. Baseline characteristics were compared by unpaired Student’s t test, χ² test, or Fisher’s exact test as appropriate. The effects of treatment (baseline, acute tea, chronic tea, acute on chronic tea, acute water, and chronic water) on biochemical markers and platelet aggregation were compared by repeated-measures ANOVA or repeated-measures MANOVA, with post hoc Student-Newman-Keuls comparison, as appropriate. Univariate clinical and biochemical predictors of platelet aggregation were determined by linear regression. Analyses were performed with SPSS for Windows version 10.0 (SPSS Inc). Statistical significance was accepted at a value of P<0.05.

**Data**

**TABLE 2. Clinical Characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water-First Group</th>
<th>Tea-First Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Age, y</td>
<td>55.6±9.2</td>
<td>54.6±9.5</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>19 (79)</td>
<td>19 (76)</td>
</tr>
<tr>
<td>Diagnosis of hypercholesterolemia, n (%)</td>
<td>19 (79)</td>
<td>20 (80)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>196±30</td>
<td>190±37</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>12 (50)</td>
<td>16 (64)</td>
</tr>
<tr>
<td>Family history of CAD, n (%)</td>
<td>11 (46)</td>
<td>11 (44)</td>
</tr>
<tr>
<td>Smoking history, n (%)</td>
<td>19 (79)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>32±25</td>
<td>30±21</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>5 (21)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.8±5.8</td>
<td>30.6±6.3</td>
</tr>
</tbody>
</table>

Data are mean ± SD or number (%).
The estimated mean dietary flavonoid intake at baseline was 62.1 ± 59.5 mg/d (median 44.2 mg/d), and the mean baseline total plasma catechin levels were 26.4 ± 15.2 ng/mL. Baseline flavonoid intake correlated with baseline total plasma catechin levels (r = 0.35, P = 0.015).

Platelet Aggregation
Addition of both ADP and TRAP to platelet-rich plasma resulted in dose-dependent platelet aggregation (Figures 2 and 3). Platelet aggregation in response to either agonist did not correlate with baseline flavonoid intake or total plasma catechin levels. Acute or chronic tea consumption had no effect on the extent or rapidity of ADP- or TRAP-induced platelet aggregation (Figure 2). In addition, acute or chronic tea consumption had no effect on either the extent or rapidity of TRAP-induced platelet aggregation (Figure 3). These responses were highly reproducible, because acute and chronic water consumption also had no effect. By post hoc analysis, total plasma catechin concentrations were significantly greater after acute, chronic, and acute on chronic tea consumption compared with baseline and with water consumption (P < 0.05).

Biochemical Parameters
As shown in Table 3, chronic water or tea consumption had no effect on fasting lipid or glucose levels. Plasma catechin levels were available at all 6 time points in 40 patients. Acute and chronic (with overnight abstinence) consumption of tea increased plasma catechin levels (Figure 5, P = 0.001 by repeated-measures ANOVA), whereas water consumption had no effect. By post hoc analysis, total plasma catechin concentrations were significantly greater after acute, chronic, and acute on chronic tea consumption compared with baseline and with water consumption (P < 0.05).

Figure 2. Effect of tea consumption on ex vivo platelet aggregation in response to ADP. Platelet aggregation was assessed in platelet-rich plasma in response to 1, 2.5, 5, and 10 μmol/L ADP before and 2 hours and 4 weeks after water or tea consumption in 47 patients with CAD, as described in “Methods.” Acute (A), chronic (B), and acute on chronic (C) tea consumption did not affect the extent of ADP-induced platelet aggregation (P = 0.91 by MANOVA), nor did acute (D), chronic (E), or acute on chronic (F) tea consumption affect the rapidity of ADP-induced platelet aggregation (P = 0.79 by MANOVA).

Figure 3. Effect of tea consumption on ex vivo platelet aggregation in response to TRAP. Platelet aggregation was assessed in platelet-rich plasma in response to 5, 10, 20, and 50 μmol/L TRAP before and 2 hours and 4 weeks after water or tea consumption in 45 patients with CAD, as described in “Methods.” Acute (A), chronic (B), and acute on chronic (C) tea consumption did not affect the extent of TRAP-induced platelet aggregation (P = 0.21 by MANOVA), nor did acute (D), chronic (E), or acute on chronic (F) tea consumption affect the rapidity of TRAP-induced platelet aggregation (P = 0.42 by MANOVA).

Figure 4. Effect of water consumption on ex vivo platelet aggregation in response to ADP and TRAP. Platelet aggregation was assessed in platelet-rich plasma in response to 1, 2.5, 5, and 10 μmol/L ADP or 5, 10, 20, and 50 μmol/L TRAP before and 2 hours and 4 weeks after water or tea consumption, as described in “Methods.” The extent (A and C) and rapidity (B and D) of platelet aggregation were not affected by water consumption.
catechins,38 and has been shown by Rein and colleagues to function analyzer. Cocoa is high in flavonoids, especially like effect on primary hemostasis as measured with a platelet activation acutely and had an aspirin-

TABLE 3. Biochemical Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Chronic Water</th>
<th>Chronic Tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>193±34</td>
<td>190±36</td>
<td>189±30</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>112±28</td>
<td>106±32</td>
<td>105±30</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>46±12</td>
<td>46±12</td>
<td>47±15</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>188±104</td>
<td>189±106</td>
<td>198±167</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>117±37</td>
<td>122±51</td>
<td>119±40</td>
</tr>
</tbody>
</table>

Data (mean±SD) are from a fasting blood sample taken on arrival on each of the 3 days of attendance.

Discussion

The results of the present study demonstrate that ex vivo platelet aggregation in response to ADP and thrombin receptor–activating peptide is not inhibited in patients with CAD by acute or chronic consumption of tea, despite adequate absorption of tea flavonoids, as evidenced by increased total plasma catechin levels. These findings suggest that inhibition of platelet aggregation by tea flavonoids is unlikely to explain the apparent inverse association between tea consumption and atherosclerotic cardiovascular events.

Previous studies of inhibition of platelet aggregation in humans by use of flavonoid-containing foodstuffs have yielded conflicting data. In vitro studies have consistently shown that flavonoid-rich foods, such as red wine, and flavonoid extracts inhibit platelet aggregation11; the concentrations tested, however, have generally been higher than those that can be attained in vivo with nutritional supplements.35 Nevertheless, several recent reports have suggested that increased dietary flavonoid consumption may inhibit platelet activation and function in humans.36,37 Rein and colleagues28 demonstrated that green tea catechins and epicatechin gallate, a major compound in green tea catechins, inhibited ADP-, collagen-, epinephrine-, and calcium-stimulated platelet activation acutely and had an aspirin-like effect on primary hemostasis as measured with a platelet function analyzer. Cocoa is high in flavonoids, especially catechins,38 and has been shown by Rein and colleagues to decrease plasma markers of oxidation.39 Keevil and coworkers37 randomized 10 healthy volunteers to consume purple grape juice, orange juice, and grapefruit juice for 1 week each in a crossover design and found that purple grape juice consumption decreased whole-blood platelet aggregation as measured by impedance aggregometry, whereas the other juices did not. Purple grape juice had a total polyphenolic concentration nearly 3 times higher than that of the other juices.

The reasons for the apparent discrepancies with the present study may be related to the study designs or a true difference between the sources of flavonoids. The first study36 assessed markers of platelet activation and function, and the second study37 measured whole-blood aggregation. In the present study, we assessed ex vivo platelet aggregation in platelet-rich plasma, a method that has been shown to relate to CAD mortality40 and previous myocardial infarction.41 In addition, we studied greater numbers of volunteers than the previous investigations. Importantly, our volunteers had known CAD and were taking aspirin and other cardiac medications that may affect platelet function, in contrast to the healthy volunteers in these previous reports. Finally, the content and type of flavonoid vary between different foodstuffs,1,2,3,9 and this may affect absorption, distribution, and effect of the different flavonoids. We demonstrated adequate absorption of ingested flavonoids by an increase in total plasma catechin levels, however, and catechins are thought to be an important part of the beneficial effect of tea.28 Further to this point, in a study of 18 healthy volunteers randomized to 2 weeks of onions, dried parsley (both rich in flavonoids), or placebo in a crossover design, Janssen and coworkers39 recently found that the flavonoid supplements did not affect ex vivo platelet aggregation, findings consistent with those of the present study. The purified flavonoids found in onions and parsley, however, were able to inhibit ADP- and collagen-induced platelet aggregation in vitro, but only at high concentrations. The authors concluded that the antiaggregatory effects of flavonoids demonstrated in vitro are due to concentrations that cannot be attained in vivo. A recent study by Kang and colleagues28 demonstrated that green tea catechins and epigallocatechin gallate, a major compound in green tea catechins, inhibited ADP-, collagen-, epinephrine-, and calcium ionophore A23187–induced human platelet aggregation in vitro dose-dependently and had significant effects in vivo in a mouse model of pulmonary thrombosis. We are not aware, however, of any other published studies of the effect of tea consumption on platelet function in humans.

There has been recent speculation that the aspirin-like effect of alcohol on platelet aggregation, and possibly an explanation of the favorable effect of alcohol on cardiovascular morbidity and mortality,42 relates to the flavonoid content of the beverage.43,44 This is particularly so for red wine,7,44 which, unlike white wine, has been shown in several studies34,44 to be an effective inhibitor of platelet aggregation. The epidemiological evidence that red wine is more beneficial than other alcoholic beverages, however, is not conclusive.45 Moreover, there is recent evidence that much of the salutary effect of regular, moderate consumption of red wine on platelet function is due to its alcohol content rather than flavonoids, because white wine, or alcohol in clear fruit juice, had similar effects.35,46

Enhanced platelet aggregation in patients with CAD has been associated with reduced platelet antioxidant defenses,47
and platelet aggregation may be augmented by oxidant stress. Several studies have shown that the antioxidant α-tocopherol inhibits platelet aggregation. Nitrosothiols have also been shown to inhibit platelet aggregation. Antioxidant flavonoids in tea may also inhibit platelet aggregation by a number of mechanisms. "Tea also contains caffeine, and caffeine consumption has been shown in recent studies to inhibit platelet aggregation, possibly by upregulation of adenosine A_2A receptors. We did not, however, find any effect of acute or regular tea consumption for 4 weeks on platelet aggregation. Thus, other mechanisms need to be invoked to explain the findings of the beneficial effect of tea shown in epidemiological studies.

An important limitation of the present study is that all patients were taking daily antiplatelet doses of aspirin. Thus, one might argue that tea may have had a favorable effect on platelet aggregation in healthy volunteers or in patients with CAD not on aspirin. Any recommendation involving second-order prevention of CAD with tea, however, would necessarily include patients on aspirin, because it is a proven prevention strategy. Therefore, any putative benefit of tea in this population in the absence of aspirin would be meaningless. In addition, aspirin is a relatively weak inhibitor of platelet aggregation, and the dose-response to each agonist was robust. Nevertheless, a platelet inhibitor that acted by a different mechanism than aspirin might have potential therapeutic benefit. A second limitation of the study is the use of water as placebo, which prevented us from blinding subjects to treatment. Previous experience with clinical studies of tea consumption indicates that it is not possible to produce a convincing placebo beverage that looks and tastes like tea but lacks tea flavonoids (personal communication, Douglas Balentine, Lipton, Inc.). Third, this study used caffeine-free tea in an attempt to examine the effects of tea as it is usually consumed. Caffeine, or other components of tea, may have abrogated any beneficial effect of tea on platelet aggregation. Caffeine, however, has been reported to inhibit platelet aggregation ex vivo. Finally, although we tested the effect of tea on platelet aggregation with 2 agonists, our results do not exclude the possibility that tea may inhibit platelet aggregation to other stimuli.

Apart from water, tea is currently the most widely consumed beverage worldwide. Therefore, any health effects of tea may have important public health implications. Certainly, epidemiological data suggest that tea consumption is associated with decreased risk of cardiovascular disease.

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