Dietary green tea extract increases phase 2 enzyme activities in protecting against myocardial ischemia-reperfusion

Masoumeh Akhlaghia, Brian Bandy

Abstract

Green tea catechins are dietary antioxidant compounds that have been shown to protect against myocardial ischemia-reperfusion (IR) injury. Considering reports that catechins can induce phase 2 enzymes in cultured cells and some organs, we hypothesized that part of the protection to heart against IR injury may involve elevation of phase 2 enzyme activities. Rats were fed for 10 days with either control diet (sham and control groups) or the diet mixed with 0.25% green tea extract. At the end of 10 days, hearts were excised and subjected to global ischemia for 20 min followed by reperfusion for 2 hours. The hearts were compared for indices of cell death, oxidative stress, and phase 2 enzyme activities. Hearts from the green tea group had a 65% to 85% decrease in markers of apoptosis, a tendency to higher total glutathione, and higher activities of the phase 2 enzymes glutamate cysteine ligase and quinone reductase. The results support a possible involvement of phase 2 enzymes in the protection by green tea catechins against myocardial IR injury.

Keywords: Cell death; Glutamate cysteine ligase; Green tea; Heart; Ischemia-reperfusion; Phase 2 enzymes; Quinone reductase; Rat

Abbreviations: DCIP, 2,6-dichloroindophenol; GCL, glutamate cysteine ligase; GSH, reduced glutathione; GSSG, oxidized glutathione; IR, ischemia-reperfusion; LDH, lactate dehydrogenase; PMSF, phenylmethylsulfonyl fluoride; QR, quinone reductase; TBARS, thiobarbituric acid reactive substances.

1. Introduction

Flavonoids, a group of plant antioxidants, have been the subject of investigations over decades due to their preventive and therapeutic effects especially in terms of cardiovascular diseases and cancer [1]. Ischemia-reperfusion injury is one of the situations where flavonoids have shown benefits. Protective effects of flavonoids against ischemia-reperfusion (IR) injury have been reported in various organs including heart [2-4].

Catechins are an important group of flavonoids with unique biological activities. Catechins are particularly known for their helpful properties on the cardiovascular system [5,6]. Green tea, a traditional drink used over thousands of years, is a prominent source of catechins especially epigallocatechin gallate. Administration of green tea extract or various forms of catechins before or during IR in heart has been associated with better cardiac performance, limited infarct size, and reduced apoptosis [7-13]. However, the mechanisms of their protection have not yet been elucidated in complete detail.

Reports that green tea catechins can elevate phase 2 enzymes in cultured cells and certain organs (reviewed recently by Na and Surh [14]) led us to hypothesize that part of the beneficial effects of catechins in heart IR could be rendered through an increase in the activities of these detoxification enzymes. Phase 2 enzymes are a group of proteins that defend against pro-oxidant electrophiles and xenobiotics, and whose expression is regulated by the Nrf2/antioxidant response element pathway [15]. Elevation of phase 2 enzymes may protect hearts in states of IR by
diminishing oxidative stress and suppressing inflammatory responses. Our objective was to determine whether protection against IR by dietary catechins was accompanied by an increase in phase 2 enzyme activities. We therefore investigated the effect of feeding green tea extract on the activities of two representative phase 2 enzymes, glutamate cysteine ligase (GCL), and quinone reductase (QR), in ischemic-reperfused heart and in liver. Glutamate cysteine ligase is the rate-limiting enzyme for glutathione synthesis and so by increasing production of glutathione may help defend the heart against oxidative stress [16,17]. Quinone reductase is a commonly measured phase 2 enzyme under control of the Nrf2 signaling pathway [18,19] that has previously been shown in vitro to be induced by green tea catechins [20].

The majority of studies showing elevation of phase 2 enzyme expression/activity by green tea catechins have been conducted in vitro with individual catechins such as epigallocatechin gallate. The current study investigates whether dietary delivery of a mixture of green tea catechins can influence activities of phase 2 enzymes, which may help to protect the heart against IR injury. To the extent that this occurs, it suggests that green tea may provide a nutritional benefit for people with ischemic heart disease or in acute situations of IR such as in myocardial infarction or coronary bypass surgery.

2. Methods and materials

2.1. Diets

The feed was AIN-93G diet (Dyets Inc, Bethlehem, Pa) without tert-butylhydroquinone (an antioxidant and potential phase 2 enzyme inducer). For the green tea group (IR/Tea), the diets were mixed with green tea extract to 0.25% (wt/wt) using a V-shell blender under 100% nitrogen. Green tea extract was the standardized product Polyphenon 60 (Sigma-Aldrich Canada, Oakville, ON, Canada) containing 65.4% total catechins, including 29.2% epigallocatechin gallate, 21.0% epigallocatechin, 7.9% epicatechin gallate, and 7.3% epicatechin [21]. The feeds were stored at −20°C until serving to the animals.

2.2. Experimental groups

Male Wistar rats weighing 350 to 400 g (12 weeks of age) were maintained at 2 per cage in a controlled standard environment at 22°C with a 12-hour dark/light cycle and handled under humane care in compliance with the guidelines of the Canadian Council on Animal Care and the University Committee on Animal Care and Supply. The animals were acclimated to the conditions and the control AIN-93G diet (free of catechins) for 3 days before commencing the intervention. The animals were assigned to one of the Sham (n = 5), IR/Control (n = 8), or IR/Tea (n = 6) groups. The first two groups were fed AIN-93G diet alone while the IR/Tea group received AIN-93G diet supplemented with 0.25% green tea extract, a dose of Polyphenon 60 that has previously shown protection against chemical carcinogenesis in rats [22,23]. Based on food intake of about 30 g/d per rat, this amounts to a green tea extract intake of approximately 200 mg/kg body weight per day (providing about 130 mg/kg body weight of catechins). This dose compares to those used previously where protection against heart IR injury was observed with oral delivery of 200 mg/day per kilogram of body weight of epigallocatechin gallate [8] or 250 mg/kg body weight per day of catechin [10]. Based on a catechin content of about 200 mg for a 200-mL cup of green tea [24], and converting the dose of 130 mg/kg body weight based on body surface area [25], this would amount to about 6 to 7 cups/day for a 60-kg human. All animals received the corresponding diet and water ad libitum for 10 days until the time of the surgery. This length of feeding is similar to previous studies that delivered green tea extract or catechins orally for 7 [7], 10 [9,10], 14 [13], or 21 [8] days. The animals gained an average of 47.0 ± 5.4 g over the 10 days and there was no difference in weight gain between the groups (Table 1).

2.3. Ischemia-reperfusion

After anesthetizing rats with isoflurane (2% in oxygen delivered at 1 L/min) and heparinization, livers were excised and snap-frozen in liquid nitrogen. Hearts were then excised, immersed in cold perfusion medium and cannulated through the aorta on a Langendorff apparatus. The time under isoflurane anesthesia was 7 to 8 minutes, which should not be enough to provide significant preconditioning against IR injury [26,27]. Ischemia and reperfusion of the isolated hearts was conducted as described previously [7,8,10,11,28]. Perfusion was performed with a modified Krebs-Henseleit buffer containing 118 mmol/L NaCl, 1.2 mmol/L KH2PO4, 4.7 mmol/L KCl, 1.7 mmol/L CaCl2, 1.2 mmol/L MgSO4, 20 mmol/L sodium acetate, and 10 mmol/L glucose, pH 7.4 at the constant pressure of 110 cm H2O. The buffer was bubbled with 100% oxygen and warmed to 37°C. A small water-jacketed chamber was also used to keep the hearts at 37°C. After 15 minutes stabilization, global ischemia and reperfusion were established for 20 minutes and 2 hours, respectively. In pilot experiments, we found that this protocol produced significant apoptotic cell death compared to sham, as observed previously [28]. The sham hearts were perfused for the 15-minute stabilization period only. At the end of the experiment, the hearts were harvested and

<table>
<thead>
<tr>
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<th>Before</th>
<th>After</th>
<th>Difference</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>384.0 ± 5.6</td>
<td>428.2 ± 10.0</td>
<td>44.2 ± 4.7</td>
</tr>
<tr>
<td>IR/Control</td>
<td>368.5 ± 5.3</td>
<td>418.0 ± 9.5</td>
<td>49.5 ± 7.0</td>
</tr>
<tr>
<td>IR/Tea</td>
<td>371.0 ± 2.9</td>
<td>418.2 ± 6.7</td>
<td>47.2 ± 4.5</td>
</tr>
</tbody>
</table>

Data are the means ± SEM. There was no significant difference between groups either before or after 10 days feeding, or in the weight gained.
weighed (whole), and the ventricles were snap-frozen in liquid nitrogen and kept at −70°C for later analysis. Frozen ventricles were crushed to a powder using a tissue pulverizer prechilled in liquid nitrogen. For tissue analyses, small amounts of the frozen tissues were homogenized in the appropriate buffer using a microcentrifuge tube homogenizer. Where needed, the protein content of the homogenates was determined by the Bradford technique [29].

2.4. Lactate dehydrogenase

Heart effluents were collected for 1 min at the beginning and end of the stabilization period and at minutes 1, 3, 5, 7, 10, 15, 20, 30, 60, 90, and 120 of the reperfusion period. Coronary flow rate was calculated as milliliters of effluent per minute per gram of wet weight of heart. To measure lactate dehydrogenase (LDH) [30], 200 μL of the effluent was mixed with 800 μL of pyruvate solution containing 50 mmol/L potassium phosphate, pH 7.4, 2.5 mmol/L pyruvate, and 180 μM NADH and kinetic of the NADH consumption was recorded for 30 seconds at 340 nm. Lactate dehydrogenase activity was expressed as units of LDH released into the perfusate per milliliter of coronary flow per wet weight of the heart.

2.5. Caspase-3 activity

Caspase-3 activity was assayed according to a method described by Masini et al [31] with some modifications. Briefly, 30 mg of the frozen tissue was homogenized in 500 μL cold lysis buffer containing 10 mmol/L HEPES, 50 mmol/L KCl, 5 mmol/L MgCl₂, 0.5% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate, 10 mmol/L dithiothreitol, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 2 μg/mL leupeptin, 2 μg/mL aprotinin, pH 7.4. The homogenates were incubated on ice for 15 min, centrifuged at 10 000 g for 10 minutes at 4°C, and a 170-g supernatant of the supernatant was used for analyses. The homogenates were incubated for 10 minutes at 4°C, and a 170-μL sample of the supernatant was incubated with 340-μmol/L final concentration of caspase-3 substrate Ac-DEVD-p-nitroanilide (Sigma-Aldrich Canada, Oakville, ON, Canada) for 1 hour at 37°C. The absorbance of the cleaved substrate, p-nitroanilide, was measured at 405 nm. The extinction coefficient of 8300 M⁻¹ cm⁻¹ was used for determination of p-nitroanilide concentration.

2.6. DNA fragmentation

DNA was extracted from the tissues and subjected to agarose gel electrophoresis according to a method described by Fliss and Gattinger [32]. The gels were then imaged with an Alpha Innotech digital imaging system, and the bands were densitometrically analyzed using the AlphaEase software. The intensity of the fluorescence was measured in the center of the non-fragmented DNA band and of fragmented DNA at a fixed distance from the first measurement, and the data were expressed as the ratio of fragmented to non-fragmented DNA.

2.7. Thiobarbituric acid reactive substances

The thiobarbituric acid reactive substances (TBARS) assay was performed according to Ohkawa et al [33] with some modifications. Fifty milligrams of the frozen tissues was homogenized with 200 μL of lysis buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1 mmol/L EDTA, 10 mmol/L EGTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mmol/L dithiothreitol, pH 7.4), containing 0.0002% butylated hydroxytoluene. The homogenates were incubated for 30 minutes at room temperature and centrifuged at 15 000g for 10 minutes at 4°C. Then, 100 μL of the supernatants was added to 350 μL of thiobarbituric acid solution containing 12% acetic acid, pH 3.5, 0.6% sodium dodecyl sulfate, 0.45% thioctic acid, and 0.0002% butylated hydroxytoluene, and heated for 1 h at 95°C. The heated samples were cooled and centrifuged at 4000g for 10 minutes, and the absorbance of the supernatants was measured at 532 nm. Malondialdehyde prepared from tetraethoxypropane by acid hydrolysis was used as a standard [34].

2.8. Glutathione measurements

The amount of the reduced glutathione (GSH) and oxidized glutathione (GSSG) was determined from the extent of 5′-dithiobis(2-nitrobenzoic acid) reduction for GSH and from consumption of reduced nicotinamide dinucleotide phosphate (NADPH) in the presence of glutathione reductase for GSSG [35]. Briefly, 30 mg of the frozen tissue was mixed with 300 μL meta-phosphoric acid solution containing 1% meta-phosphoric acid. The samples were left on ice for 15 minutes and centrifuged at 10 000g for 15 min, and the supernatants were used for analyses.

2.9. Glutamate cysteine ligase activity

The activity of GCL in the heart and liver tissues was determined by detecting the fluorescent product of reaction between 2,3-naphthalenedicarboxyaldehyde and γ-glutamylcysteine, the product of GCL activity [36].

2.10. Quinone reductase activity

Activity of QR in the heart and liver tissues was determined by assessing the ability of tissues to reduce 2,6-dichloroindophenol (DCIP) with NADPH as electron donor, in the presence and absence of the QR inhibitor dicoumarol, as described by Spencer and Rifkind [37]. For homogenization, 30 mg of frozen tissue was lysed in 200 μL of 50 mmol/L potassium phosphate buffer, pH 7.6, containing 1 mmol/L PMSF, 5 μg/mL aprotinin, and 5 μg/mL leupeptin.

2.11. Adenosine triphosphate measurement

Tissue adenosine triphosphate (ATP) content of the heart homogenates in lysis buffer (described above for TBARS) containing 1 mmol/L PMSF, 5 μg/mL aprotinin, and 5 μg/mL leupeptin was determined by a bioluminescence kit (Sigma, Saint Louis, Mo).
2.12. Statistical analyses

The data presented are means ± SEM. A power analysis indicated that a sample size of 5 or more rats per group would be suitable for detecting a difference between two groups with a 20% difference in means, 20% relative SD for each group, \( \alpha \) level of 5% and \( \beta \) level of 50%. Statistical analyses were performed with 1-way analysis of variance. When the F-test detected a significant between groups difference (\( P < .05 \)), pairwise comparisons were performed using Fisher’s least significant difference (LSD) post-hoc test. Lactate dehydrogenase and liver data were assessed by 1-tailed Student t-test. A \( P \) value of less than .05 was considered significant.

3. Results

Feeding of green tea extract did not affect necrotic cell death, as measured by LDH release, or influence a decrease in coronary flow during reperfusion (Fig. 1). Lactate dehydrogenase, an intracellular enzyme which is released from cells during plasma membrane disruption, increased exponentially from a baseline of 0.001 U/mL per gram of wet weight at the end of the stabilization period (average of all three groups) to 0.012 U/mL per gram of wet weight at 120 minutes of reperfusion (IR/Control group). Treatment with green tea extract failed to prevent this elevation. Feeding green tea extract also had no effect on coronary flow, which decreased from 4.8 mL/min per gram of wet weight at the end of the stabilization period to 1.1 mL/min per gram of wet weight after 120 min of reperfusion. A severe depletion of ATP after IR was also unaffected by feeding green tea extract (Table 2).

However, as indicated by markers of apoptosis, feeding green tea extract showed good protection to myocardial cells from apoptotic cell death. Increases in caspase-3 activity and DNA fragmentation after IR were inhibited by 85 and 65%, respectively, in the animals of the IR/Tea group (Table 2).

Oxidative stress–induced damage to heart tissue was determined by measuring reactive aldehydes (TBARS) generated as a result of lipid peroxidation. Feeding green tea extract produced an intermediate level of TBARS following IR that was not significantly different from IR/Control or from Sham (Table 2), suggesting partial protection. Similarly, a decrease in total glutathione after IR compared to sham appeared to be partially ameliorated in animals fed green tea extract (Table 3). The content of total glutathione in hearts from animals fed green tea extract (IR/Tea) was intermediate and not significantly different from IR/Control or Sham. GSH, GSSG and GSH/GSSG were not significantly affected by feeding green tea extract.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Caspase-3 activity (( \mu )mol p-nitroanilide/min per mg protein)</th>
<th>DNA fragmentation (fragmented/non-fragmented)</th>
<th>TBARS (nmol/mg protein)</th>
<th>ATP (pg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.007 ± 0.0003( ^a )</td>
<td>0.30 ± 0.02( ^a )</td>
<td>8.89 ± 1.38( ^a )</td>
<td>810 ± 395( ^a )</td>
</tr>
<tr>
<td>IR/Control</td>
<td>0.019 ± 0.002( ^b )</td>
<td>0.58 ± 0.06( ^b )</td>
<td>14.22 ± 0.86( ^b )</td>
<td>4.92 ± 2.04( ^b )</td>
</tr>
<tr>
<td>IR/Tea</td>
<td>0.009 ± 0.001( ^a )</td>
<td>0.40 ± 0.05( ^a )</td>
<td>11.86 ± 0.52( ^a )</td>
<td>3.40 ± 1.79( ^a )</td>
</tr>
</tbody>
</table>

Male Wistar rats were fed either control AIN-93G diet (Sham and IR/Control groups) or the diet supplemented with 0.25% green tea extract (IR/Tea group). After 10 days the hearts were excised and mounted on a Langendorff apparatus. After stabilization, hearts from the IR/Control and IR/Tea groups were subjected to 20 min ischemia and 2 h reperfusion. The hearts were flash frozen in liquid nitrogen and later analyzed for caspase-3 activity, DNA fragmentation, TBARS, and ATP levels, as described in Methods and materials.

Different letters indicate significantly different at \( P < .05 \). The values are the means ± SEM.
A notable finding was that while IR of heart caused a reduction in activities of GCL and QR, these decreases were significantly ameliorated (78% and 147% protection respectively) by feeding green tea extract (Fig. 2, Table 4). The effect of feeding green tea extract on activities of GCL and QR was also measured in liver to examine whether it induced the enzymes in this organ. Liver would encounter the highest dose of postprandial and non-metabolized catechins, is rich in inducible drug-metabolizing enzymes such as QR [37] and is the major site of glutathione synthesis [38] regulated in part by GCL activity [39]. In liver (absence of IR) feeding green tea extract also elevated the activity of QR and gave a tendency (0.1 \( < P < 0.05 \)) toward an increase in GCL (Fig. 3).

### Table 3
Glutathione levels in heart tissue

<table>
<thead>
<tr>
<th></th>
<th>GSH (nmol/g tissue)</th>
<th>GSSG (nmol/g tissue)</th>
<th>GSH/GSSG</th>
<th>GSH+2GSSG (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>126.5 ± 15.2 (^a) (65%)</td>
<td>34.6 ± 5.6 (^a) (35%)</td>
<td>4.3 ± 1.1 (^a)</td>
<td>195.8 ± 14.4 (^a)</td>
</tr>
<tr>
<td>IR/Control</td>
<td>79.7 ± 3.9 (^b) (56%)</td>
<td>31.3 ± 2.0 (^a) (44%)</td>
<td>2.6 ± 0.2 (^b)</td>
<td>142.2 ± 5.1 (^b)</td>
</tr>
<tr>
<td>IR/Tea</td>
<td>95.2 ± 11.4 (^b) (56%)</td>
<td>37.1 ± 5.7 (^a) (44%)</td>
<td>2.8 ± 0.4 (^a)</td>
<td>169.3 ± 18.0 (^a)</td>
</tr>
</tbody>
</table>

The hearts described in Table 2 were analyzed for GSH and GSSG as described in Methods and materials, and the reduced/oxidized glutathione ratio (GSH/GSSG) and total glutathione (GSH + 2GSSG) were calculated. Different letters indicate significantly different at \( P < .05 \). The values are the means ± SEM. The values in parentheses show the % of glutathione in the GSH vs GSSG form.

### 4. Discussion

Since the heart has low postnatal cell proliferation capacity [40], attempts directed to prevent death of myocardial cells are of therapeutic value. Several previous studies have shown protection to the heart against IR injury by oral delivery of green tea extract [7,13] or of the green tea polyphenols catechin [10], epicatechin [9], or epigallocatechin gallate [8]. Other studies have shown protective effects of green tea catechins when delivered to the perfusate [7,11]. The current study confirmed protective effects of green tea extract supplied in the diet against heart IR injury, and examined potential mechanisms. The results show that the protection is through inhibition of apoptosis rather than necrosis and support the hypothesis that part of the protection may be through elevation of phase 2 enzyme activities.

Ischemia-reperfusion injury of heart occurs through a combination of necrosis and apoptosis, depending on the severity of the insult (for reviews, see Refs. 41-43). As previously shown with oral delivery of green tea extract for 7 days [7], we found that short-term dietary administration of green tea extract inhibits IR-induced apoptosis in isolated heart. Two indicators of apoptosis (myocardial caspase-3 activity and DNA fragmentation) showed substantial (65%-85%) protection by feeding green tea extract. Of note in both of these studies is that the protection persists even after removal of the heart, showing that the protection is not due to circulating catechins in the blood.

The release of LDH to the medium is a commonly used indicator of myocardial necrosis in the isolated heart model (eg, Refs. 10,44,45) and has been used clinically as an indicator of myocardial infarction [46,47]. Assessment of necrosis by measuring LDH release into the heart effluents did not show protection by dietary green tea extract in the current protocol against this form of cell death. These results are consistent with a similar study where intragastric delivery of catechins for 10 days did not improve coronary flow or LDH release of isolated hearts subjected to IR [10]. Depletion of ATP is one of the reasons that
cells are executed by necrosis even where the process of apoptosis has been activated [48]. Accordingly, in the current study, the inability of green tea extract to prevent necrosis was associated with a lack of ability to preserve tissue ATP. These results suggest that this green tea extract treatment impeded signaling pathways upstream of caspase activation but did not block processes which lead to cellular ATP depletion.

The results showed some indications of protection against oxidative stress. Although the results for TBARS and total glutathione were not significantly different between the IR/Tea group and the IR/Control group, they were also not different between the IR/Tea group and Sham, indicating a tendency toward protection. A protective effect on TBARS formation was previously observed with oral delivery by gavage of 250 mg pure catechin for 10 days before subjecting the isolated hearts to ischemia for 30 min and reperfusion for 30 min [10]. In addition, in streptozotocin-induced diabetic rats, delivery of 300 mg/kg body weight per day of green tea extract for 4 weeks was able to decrease the heart TBARS produced by exposure to high glucose [49]. With a larger dose of green tea extract the difference between the IR/Tea group and IR/Control in markers of oxidative stress in the current protocol may have reached significance, in accordance with these previous studies that showed protection of heart against oxidative stress with higher doses [10,49]. In addition, the more prolonged reperfusion in the current study than that used previously [10] may have depleted intracellular catechins to limit their activity as direct antioxidants.

Although there was an indication of an effect on total glutathione, the current study had no significant effects of green tea extract feeding on GSH, GSSG, or GSH/GSSG. In the only previous study of the effects of catechins on glutathione in heart IR [9], it was found that oral gavage of 1 mg/kg body weight per day of epicatechin for 10 days prior, plus 2 days subsequent to 45 minutes of coronary artery occlusion gave significant protection against an increase in the GSSG/GSH ratio in the infarcted region of the heart. One difference of this previous study was that fresh epicatechin was delivered during the 2-day reperfusion period, whereas in the current study, no green tea extract was provided during reperfusion. The continued delivery of epicatechin may have been better able to influence cellular redox state during reperfusion, whereas in the current study the endogenous catechins may have been oxidized or lost during the IR protocol.

In the current study, the attenuated apoptosis was accompanied by increased activities of the phase 2 enzymes, GCL and QR in heart. We also confirmed an influence of feeding green tea extract on these enzyme activities in liver, an organ which would expectedly be exposed to higher

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**Table 4**

<table>
<thead>
<tr>
<th></th>
<th>Without dicoumarol</th>
<th>With dicoumarol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DCIP Reduction</td>
<td>DCIP Reduction</td>
</tr>
<tr>
<td></td>
<td>(nmol/min per mg protein)</td>
<td>(nmol/min per mg protein)</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>88.43 ± 5.93</td>
<td>19.08 ± 1.35</td>
</tr>
<tr>
<td>IR/Control</td>
<td>75.25 ± 3.31</td>
<td>17.05 ± 1.37</td>
</tr>
<tr>
<td>IR/Tea</td>
<td>97.15 ± 4.83</td>
<td>22.49 ± 2.26</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>163.61 ± 12.57</td>
<td>64.34 ± 4.19</td>
</tr>
<tr>
<td>Tea</td>
<td>178.10 ± 10.93</td>
<td>50.32 ± 3.65</td>
</tr>
</tbody>
</table>

The specific activity of quinone reductase was assayed from the difference in the rates of reduction of DCIP in the presence and absence of the quinone reductase inhibitor dicoumarol. This difference was used to calculate the quinone reductase activities shown in Figs. 2 and 3. The values are the means ± SEM.

Fig. 3. Effect of green tea feeding on glutamate cysteine ligase and quinone reductase activities in liver. Glutamate cysteine ligase activity in liver homogenates was measured fluorometrically after reacting γ-glutamylcysteine, the product of GCL activity, with 2,3-naphthalenedicarboxyaldehyde. Quinone reductase activity in liver homogenates was measured spectrophotometrically from the rate of reduction of dichloroindophenol by NADPH in the presence and absence of dicoumarol. Control, livers from animals fed control diet; Tea, livers from animals fed green tea extract. The bars and error bars represent the means ± SEM. Different letters indicate significantly different at \( P < .05 \).
postprandial levels of catechins than heart. Although induction of phase 2 enzymes by green tea catechins has been reported in cultured cells and some other tissues [14], an effect on phase 2 enzyme activities in heart has not previously been reported. These results raise the possibility that an increase in the activities of phase 2 enzymes in heart may contribute to the cardioprotection by green tea extract. The observed tendency for an increase in total glutathione fits with the increase in heart activity of GCL, the rate-limiting enzyme in glutathione synthesis.

The dose of green tea used herein (equivalent to 6-7 cups per day for humans) is at the high end of what might be taken by regular human consumption. However this was a very short-term study, and benefits would likely be seen with lower doses over the long term, especially through indirect mechanisms such as stimulating phase 2 enzyme activities that persist after elimination of the catechins.

This study had several limitations and strengths. One limitation was in its short-term nature and relatively high dose. However, such a short-term intervention could be of use prior to an acute event such as cardiac surgery, and as noted above, a lower dose may be effective over the long term. The somewhat small n was also a limitation. Some of the marginally significant results may have been significant with a higher number of animals, although the main results were significant even at this n. Another limitation was in not being able to distinguish how much of the protection is direct or indirect. However since the heart was subjected to IR ex vivo, it would not be affected by circulating catechins. This is a strength of the study, showing that the dietary intervention produced intrinsic changes to the heart. Further experiments will be needed to determine the extent to which protection from green tea catechins is direct or indirect. Possible experiments include removing the dietary catechins for a longer time prior to IR or conducting experiments with Nrf2-knockout mice that would be unable to induce phase 2 enzymes. In addition, although we chose a quantitative measure of representative phase 2 enzyme activities, it will be important to confirm effects on gene expression in heart using molecular biology techniques such as Western blotting or polymerase chain reaction.

In conclusion, the results raise the possibility that a part of the protection by green tea extract to hearts challenged with IR may be delivered through increased activity of phase 2 enzymes. Further studies will be needed to determine the extent to which cardioprotection by green tea extract occurs directly by antioxidant activity of catechins or indirectly by increasing antioxidant and phase 2 enzyme activities.

Acknowledgment

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References


