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Green tea (*Cammellia sinensis*) attenuates ventricular remodeling after experimental myocardial infarction

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ABSTRACT

Background: Considering the high morbidity and mortality after myocardial infarction (MI), the study of compounds with potential benefits for cardiac remodeling is reasonable. Green tea (GT) (*Cammellia sinensis*) is the most consumed beverage in the world. The potential action mechanisms of GT include anti-inflammatory, anti-apoptotic, antioxidant, and lipid-lowering properties.
Objective: This study analyzed the effects of GT on cardiac remodeling following coronary occlusion in rats.
Methods: Male Wistar rats were divided into four groups: control (C), control green tea (GT), myocardial infarction (MI), and myocardial infarction and green tea (MI–GT). GT and MI–GT were fed with standard chow with 0.25% Polyphenon 60 (Sigma-Aldrich Canada, Oakville, ON, Canada). After 3 months of observation, echocardiographic and isolated heart study, oxidative stress, energy metabolism, serum lipids, extracellular matrix, and apoptosis were evaluated.
Results: GT reduced cardiac hypertrophy and improved systolic and diastolic dysfunction. Concerning oxidative stress, GT reduced protein carbonyl, increased Nrf-2, and restored antioxidant enzyme activity to the control pattern. Energy metabolism was affected by MI that presented with lower fatty acid oxidation and accumulation of triacylglycerol, increased serum lipids, impairment of the citric acid cycle, and oxidative phosphorylation. GT stimulated the glucose pathway and mitochondrial function after MI by increasing pyruvate dehydrogenase, Complex I, ATP synthase, and glycogen storage. In addition, MI changed the extracellular matrix including MMP-2 and TIMP-1 activity and increased apoptosis by 3-caspase, all of which were attenuated by GT.
Conclusion: GT attenuated cardiac remodeling after MI, associated with improvement in systolic and diastolic dysfunction. Oxidative stress, energy metabolism, apoptosis, and extracellular matrix alterations are all potential mechanisms by which GT may take part.

1. Introduction

Acute myocardial infarction (MI) can induce molecular, biochemical, cellular, and interstitial alterations that change the structure and geometry of the left ventricle (LV) in a process known as cardiac remodeling, which is associated with ventricular dysfunction [1,2].

Ischemic insult exerts a great impact on cardiac remodeling because of the lack of oxygen that causes neurohormonal response, phagocyte recruitment, mitochondrial dysfunction, and reactive oxygen species (ROS) formation [3]. These alterations are involved in myocyte necrosis followed by lengthening and slippage of the remaining myocytes that occur early after acute MI.

Furthermore, over time, remote sites, distant from the infarcted areas, are also affected by oxidative stress, energy metabolism abnormalities, myocyte hypertrophy and apoptosis, extracellular matrix (ECM) alterations, and others. In this setting, the abnormalities of energy metabolism exert a crucial role. Under normal conditions, fatty acids (FAs) are the main substrates utilized by mitochondrial respiration [4,5]. Within heart remodeling, the fuel preference switches to glucose. This change protects the heart, in part, because FAs waste more oxygen to be oxidized. However, over time, while not oxidized, FAs accumulate inside myocytes, leading to cardiac lipotoxicity [6,7]. Lipotoxicity is a potential inductor of apoptosis and dysfunction. Changes in fuel oxidation and mitochondrial dysfunction lead to low energy for myocyte contraction and increased oxidative stress. High levels of ROS directly damage and destruct cellular structures such as membranes, DNA, and

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proteins. Moreover, ROS activate pathways that induce myocyte hypertrophy, apoptosis, and changes of ECM compounds [8,9].

At present, the chronic treatment of MI has been done with drugs that modulate some of the pathways involved in the remodeling process. However, the mortality associated with cardiac remodeling/dysfunction remains unacceptably high [2].

Considering that the remodeling process after MI is a broad complex with high morbidity and mortality, the study of drugs, foods, and nutrients with potential benefits in cardiac remodeling is reasonable.

Green tea (GT) (*Cammellia sinensis*) is the most consumed beverage in the world. It has been suggested that drinking GT brings health benefits. The potential action mechanisms of GT include anti-inflammatory, anti-apoptotic, antioxidant, and lipid-lowering properties. Experimental studies have shown the role of GT in cardiac oxidative stress and metabolic pathways [10–12]. However, the role of GT in the remodeling process after MI remains to be elucidated. Therefore, the objective of this study was to analyze the effects of GT on cardiac remodeling following coronary occlusion in rats.

2. Methodology

All animals received care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85–23, revised 1996). The study was approved by The Ethics Committee on Animal Experiments of our institution. Male Wistar rats, from the Central Animal Facility of our institution, weighing between 200 and 250 g were used. The animals were initially divided into two groups: the infarction group (I), which was submitted to experimental infarction, and the control group (C), which underwent the same procedure of experimental infarction, but without coronary artery occlusion.

2.1. Coronary artery ligation

The MI was produced as previously described [13,14]. Briefly, the rats were anesthetized with an intramuscular injection of ketamine (70 mg/kg) and xylazine (5 mg/kg), and after a left thoracotomy, the heart was exteriorized by lateral compression of the thorax and connected to the descending artery approximately 2 mm from the origin with wired polyvinyl (5–0 Ethicon) between the border of the left atrium and pulmonary artery. The heart was then replaced in the thorax, the lungs were expanded with positive pressure, and the pneumothorax was aspirated.

2.2. Experimental groups

After the first echocardiogram, animals were divided into four groups. Control animals were divided into two groups: control (C) fed with standard chow, and control green tea (GT) fed with standard chow with 0.25% Polyphenon 60 (Sigma-Aldrich Canada, Oakville, ON, Canada) composed of 65.4% catechins, which represent 29.2% epigallocatechin gallate, 21% epigallocatechin, 7.9% epicatechin gallate, and 7.3% epicatechin. Based on food intake of about 30 g/d per rat, green tea extract intake was adjusted to approximately 200 mg/kg body weight per day [15].

Infarcted animals, with MI larger than 35% [16], were also divided into two groups: myocardial infarction (MI) fed with standard chow, and myocardial infarction and green tea (MI–GT) fed with standard chow with 0.25% Polyphenon 60 (Sigma-Aldrich Canada, Oakville, ON, Canada). The animals were maintained in individual cages with free access to water and controlled feed intake. The temperature and humidity were controlled. The light cycles (light/dark) were 12 h. After 3 months, the animals were submitted to echocardiographic study.

2.3. Echocardiographic analysis

Forty-eight hours after the surgical procedure, we performed the first echocardiographic measurement of infarct size, and confirmed there were no differences between the infarcted groups. At the end of the three-month follow-up, echocardiography was performed again. Rats were anesthetized by intramuscular injection of a mixture of ketamine (50 mg/kg) and xylazine (0.5 mg/kg). The cardiac structures were measured according to previous methods in an infarcted rat model [16–18]. Left ventricle end-diastolic dimension (LVDD), left ventricle end-systolic dimension (LVSD) and posterior wall thickness (LVWT) were measured at the maximal diastolic dimension, taken at the maximal anterior motion of the posterior wall. The diastolic area (DA) and systolic area (SA) were measured by planimetry in two-dimensional echocardiography.

LV systolic function was assessed by the following parameters: fractional shortening (FS) [(LVDD − LVSD) / LVDD] × 100, fractional area change (FAC) (DA − SA / DA × 100) and tissue Doppler imaging (TDI) of mitral annulus systolic velocity (S′). Diastolic function was evaluated by E wave deceleration time (EDT) assessed by pulsed wave Doppler and early diastolic mitral inflow velocities (E) and TDI of early mitral annulus diastolic velocity (E′) ratio (E/E′) [18].

2.4. In vitro left ventricular function analysis

The procedures and measurements for assessing left ventricular function were performed following a previously described method [19]. The main pulmonary artery was cut to vent the right ventricle, and a latex balloon was placed into the LV via the mitral valve orifice. In the isovolumetrically beating ventricle, paced at 240 beats/min with an artificial pacer, the balloon volume was increased over a diastolic range of 0–25 mmHg. The maximum systolic pressure, maximum rate of ventricular pressure rise (+dP/dt), and decreased maximum rate of ventricular pressure rise (−dP/dt) were recorded.

2.5. Morphometric analysis

At the completion of the functional study, morphometric analysis of the myocardium was performed as previously described. The lengths of the infarcted and viable muscle for both the endocardial and epicardial circumferences were determined by planimetry. Infarct size was calculated by dividing the endocardial and epicardial circumferences of the infarcted area by total epicardial and endocardial ventricular circumferences. Measurements were performed on midventricular slices (5–6 mm from the apex), under the assumption that the left midventricular slice showed a close linear relation with the sum of the area measurements from all heart slices [20].

Collagen fractional area was analyzed in tissues stained with Picro Sirius red as described previously, in remote areas free from MI [21]. Immunofluorescence staining was used to evaluate myocyte cross sectional area (CSA) and capillary-to-myocytes ratio, as described previously [7,22,23]. Images were acquired using Olympus Fluorescence microscope BX51 (Olympus Latin America Inc.). Wheat germ agglutinin fluorescein isothiocyanate (FITC) labeled (WGA-FITC — Sigma-Aldrich Co. LLC. L4895) was used to stain cardiac myocytes [7]. Image-Pro Plus 3.0 (Media Cybernetics, Silver Spring, MD) was used to measure myocyte area. Double staining with WGA-FITC and Lectin from *Bandeiraea simplicifolia* (*Griffonia simplicifolia*) (IB-4 — Sigma-Aldrich Co. LLC. L2140), 1:100/ Streptavidin — Cy3™ from *Streptomyces avidinii* (labeled donkey anti-sheep IgG, A21436; Invitrogen) 1:200 were used to evaluate the capillary-to-myocytes ratio in the same area.

2.6. Tissue metalloproteinase inhibitor-1 (TIMP-1)

TIMP-1 production was measured by ELISA according to the manufacturer's instructions (R&D Systems), as previously described [24]. Cardiac homogenates were coated with capture antibodies to TIMP-1 in 96-well plates (Nunc). Standard rat cytokine and biotinylated anti-TIMP-1 were added. Plates were incubated at room temperature for 30 min with streptavidin and then revealed by adding H_2O_2 + OPD (Sigma). Color development was stopped with H_2SO_4, and optical density was measured at 492 nm.

2.7. Myocardial metalloproteinase-2 (MMP-2)

MMP-2 activity was determined in samples by the non-infarcted area, as previously described [25]. Briefly, samples were loaded into wells of 8% SDS-polyacrylamide containing 1% gelatin. The gel was incubated at 37 °C overnight in an activation solution consisting of 50 mM Tris pH 8.4 and 5 mM $CaCl_2$. Staining was performed for 1 h with 2.5% Coomassie Blue, and destaining in 30% methanol and 10% acetic acid until clear bands over a dark background were observed. The gels were photographed and analyzed in a Carestream Molecular Imaging image analyzer (Carestream Health, Inc., USA).

2.8. Cardiac energy metabolism and oxidative stress

Samples of about 200 mg of the LV were used to determine the concentration of total protein, lipid hydroperoxide (LH), antioxidant enzymes, and energy metabolism, as previously described [7]. The activity of the enzymatic complexes of the mitochondrial respiratory chain was determined after resuspension of the pellet. The readings were performed in a μQuant microplate reader (Bio-Tec Instruments, Winooski, VT, USA) controlled by Gen5 2.0 software. All reagents used were from Sigma (St. Louis, USA).

2.8.1. Cardiac triacylglycerol determination

The concentration of cardiac triacylglycerols was determined using the enzymatic method with formation of quinone imine, as previously described [7].

2.8.2. Glycogen determination

For myocardial glycogen determination of the LV, samples of 200 mg were homogenized in 0.1 mol/L perchloric acid and centrifuged (1400g for 10 min) The supernatant was used for initial glucose determination and the remainder (10 μL) was assayed in a reaction medium containing 50 mM acetate buffer pH 4.5 and amyloglucosidase at 55 °C for 10 min to determine the final glucose concentration. The glycogen concentration was calculated by the difference between final and initial glucose [26].

2.8.3. Protein carbonyl determination

Carbonyl protein was determined according to the method of Reznick and Packer [23]. Cardiac samples were centrifuged in a medium containing 10 mM dinitrophenylhydrazine (DNPH) and 50% trichloroacetic acid (w/v). The resultant pellet was washed three times in a mixture of ethanol and ethyl acetate (1:1; v/v) and resuspended with guanidine hydrochloride (6 M). The concentration of carbonyl protein was quantified at 360 nm using an extinction coefficient of 22,000 $M^{-1} \cdot cm^{-1}$ [27,28].

2.8.4. Serum lipids

Blood samples were collected and serum lipid triacylglycerols (TAGs), total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and high density lipoprotein (HDL) were assessed as previously described [7].

2.9. Western blot for evaluation of caspase 3, BCL-2, PPARα, and PGC-1α expression

Briefly, protein extraction was performed with RIPA buffer, diluted in Laemmli buffer, separated by electrophoresis using a Mini Protean system 3 Electrophoresis Cell (Bio-Rad, Hercules, CA, USA) [7]. The membrane was incubated with the primary antibody caspase-3 antibody (Cell Signaling Technology®), B-cell lymphoma 2 (BCL-2), peroxisome proliferator-activated receptors (PPARα) and Peroxisome Proliferator-Activated Receptor Gamma, Coactivator 1 (PGC-1α) antibodies (Santa Cruz Biotechnology, Inc.) overnight. After that, the membrane was washed and incubated with the secondary antibody anti-IgG-HRP (Cell Signaling Technology®). Finally, immunodetection was performed using chemiluminescence according to the manufacturer's instructions (SuperSignal West Pico Chemiluminescent Substrate, Thermo Scientific, USA). The nitrocellulose membranes were exposed to X-Omat AR X-ray film (Eastman Kodak Co., USA) for predetermined time periods for each protein studied. The antibody used was the normalization of GAPDH (6C5), rabbit IgG (Santa Cruz Biotechnology, Inc., Europe), at 1:5000 dilution. Quantitative analysis of the blots was performed by Scion Image software (Scion Corporation, Frederick, Maryland, USA, available at http://www.scioncorp.com/) as previously described [7,24].

2.10. Myocardial nuclear factor erythroid-derived 2 (Nrf-2)

Briefly, nuclear protein extraction was performed with buffer (10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.05% NP40). The supernatant was discarded (cytoplasmic fraction) and the pellet resuspended with buffer (5 mM HEPES, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 26% glycerol (v/v)); NaCl was added, then the result was homogenized. After waiting 30 min on ice, samples were centrifuged again at 15,000 rpm for 20 min at 4 °C and the supernatant (nuclear fraction) was collected and used for the quantification of Nrf-2. The electrophoretic run and next steps were the same as used for Western blot previously described (primary antibody Nrf-2, rabbit polyclonal IgG, Santa Cruz Biotechnology, Inc., Europe, sc-722; 1:400. Secondary antibody goat anti-rabbit IgG-HRP, Santa Cruz Biotechnology, Inc., Europe, sc 2004; 1:8000) [24].

2.11. Statistical analysis

Data normally distributed were expressed as mean ± standard error. Data with non-normal distribution were transformed before comparison and were also expressed as mean ± standard error. For comparison of infarct size, Student's t-test was used. Comparison among the four groups was performed by two-way ANOVA and Holm–Sidak post hoc test. We analyzed the effect of MI, GT, and the interaction between the factors (MI × GT). Data analysis was performed with SigmaPlot for Windows version 12.0 (Systat Software, Inc.). In all cases, the significance level was 5%. P values of interaction, GT and MI are denoted by P int, P GT and P MI, respectively.

3. Results

Forty-eight hours after the coronary ligation, echocardiographic study was performed to identify myocardial infarct size larger than 35% of the whole heart mass. Therefore, the MI group was composed with animals with infarct size 41.2% ± 6.40 and MI–GT 42.8 ± 4.12 (P = 0.20). After 3 month of observation, the infarct size of MI group was 42.9% ± 4.40 and MI–GT 42.0 ± 4.13 (P = 0.71).

The echocardiographic study results are shown in Table 1. The morphological evaluation showed that GT attenuated the LV enlargement and decreased left ventricle mass index (LVMI) induced by coronary occlusion. The systolic function assessment evidenced an increase in FS and S′ in MI–GT group compared to MI. Finally, considering diastolic function, it was observed decrease EDT after myocardial infarction, that was attenuated by GT.

The isolated heart study data are shown in Table 2. The coronary occlusion induced decreased values of systolic pressure (SP), + dP/dt, and − dP/dt. GT increased the values of SP independently of MI, suggesting improved systolic function with treatment.

Morphometric analyses revealed that myocardial infarction increased myocytes CSA (μm [2]) and GT attenuated this damage (C = 171 ± 19.1, GT = 219 ± 19.1, MI = 313 ± 19.1, MI–GT = 229 ± 19.1; P int = 0.20, P GT = 0.34, P MI < 0.001) (Fig. 1). Collagen fractional area (%) (C = 2.94 ± 0.10, GT = 3.00 ± 0.01, MI = 3.1 ± 0.01, MI–GT = 3.00 ± 0.01; P int = 0.33, P GT = 0.72, P MI = 0.74) and capillary-to-myocytes ratio (C = 1.1 ± 0.13, GT = 1.0 ± 0.13, MI = 1.3 ± 0.13, MI–GT = 1.1 ± 0.13; P int = 0.53, P GT = 0.25, P MI = 0.24) were similar among the groups.

The cardiac oxidative stress results are shown in Table 3. MI decreased Nrf-2 (Fig. 2) and catalase, and increased LH and carbonyl protein. On the other hand, infarcted animals treated with GT presented higher levels of Nrf-2 and catalase, associated with decreased values of carbonyl protein and LH, at the limit of statistical significance.

The energy metabolism data are shown in Table 4. Coronary occlusion increased phosphofructokinase (PFK) and cardiac TAG, associated with decreased values of pyruvate dehydrogenase (PDH), ATP synthase, β-HAD, citrate synthase (CS), Complex I, Complex II, and cardiac glycogen. GT supplementation attenuated alterations in PDH, PFK, ATP synthase, Complex I, and cardiac glycogen.

Considering the regulators of cardiac energy metabolism, the coronary occlusion reduced expression of PPAR-α (C = 1.46 ± 0.21, GT = 1.09 ± 0.18, MI = 0.79 ± 0.18, MI–GT = 0.92 ± 0.18; P int = 0.22, P GT = 0.83, P MI = 0.03), but not PGC-1 (C = 1.04 ± 0.16, GT = 1.16 ± 0.15, MI = 1.33 ± 0.15, MI–GT = 1.34 ± 0.14; P int = 0.69, P GT = 0.68, P MI = 0.13). On the other hand, GT did not change the variables (Fig. 3).

The serum lipid results are shown in Table 5. MI induced increased levels of cholesterol, LDL, and TAG, associated with decreased values of HDL. In infarcted animals, GT attenuated these alterations.

Considering MMP activity and inhibitors, GT increased TIMP-1 levels (C = 42.61 ± 2.98, GT = 44.73 ± 3.26, MI = 37.63 ± 2.76, MI–GT = 54.74 ± 2.43[a,c]; P int = 0.016, P GT = 0.003, P MI = 0.390; [a]different from MI; [c]different from GT). Likewise, MMP-2

Table 1
Echocardiographic data.

	C (21)	GT (20)	MI (9)	MI–GT (10)	P int	P GT	P I
HR (bpm)[d]	270 ± 5.63	265 ± 8.60	266 ± 5.77	264 ± 7,78	0.863	0.955	0.662
LVDD/BW (mm/kg)	17.14 ± 1.28	17.59 ± 1.95	25.16 ± 2.28[b]	22.67 ± 1.67[a,c]	0.003	0.033	<0.001
LVMI (g/kg)	1.71 ± 0.83	1.72 ± 0.82	3.35 ± 0.12[b]	2.87 ± 0.11[a,c]	0.023	0.022	<0.001
LVSD (mm)[d]	3.89 ± 0.15	3.69 ± 0.15	8.47 ± 0.22	7.64 ± 0.20	0.383	0.009	<0.001
FAC	73.33 ± 1.13	73.19 ± 1.16	32.59 ± 1.73	33.50 ± 1.64	0.712	0.789	<0.001
FS (%)	52.33 ± 1.1	53.59 ± 1.1	20.66 ± 1.68	28.43 ± 1.59	0.024	0.002	<0.001
S′	1.95 ± 0.04	1.87 ± 0.04	1.28 ± 0.07[b]	1.49 ± 0.06[a,c]	0.001	0.26	<0.001
EDT (ms)	47.05 ± 1.86	46.85 ± 1.91	34.35 ± 3.02[b]	44.22 ± 2.85[a]	0.046	0.055	0.003
E/E′	18.3 ± 1.00	20.0 ± 1.00	28.0 ± 1.53	25.2 ± 1.38	0.077	<0.001	0.958

C: control group; GT: green tea group; MI: myocardial infarction group; MI–GT: myocardial infarction + green tea group; HR: heart rate; bpm: beats per minute; BW: body weight; LVDD: left ventricle diastolic diameter; LVSD: left ventricle systolic diameter; FAC: fractional area change; FS: endocardial fractional shortening; EDT: mitral E wave deceleration time; LVMI: left ventricle mass index (LVM/BW).

[a] Different from MI.
[b] Different from C.
[c] Different from GT.
[d] Data was transformed to normal distribution, before comparison.

Table 2
Isolated heart study.

	C (6)	GT (6)	MI (7)	MI–GT (7)	P int	P GT	P MI
SP (mmHg)	135 ± 7.72	150 ± 7.72	93.5 ± 7.70	115 ± 7.70	0.68	0.03	<0.001
+dP/dt	2449 ± 279	2737 ± 243	1499 ± 279	2037 ± 279	0.16	0.12	0.001
−dP/dt	1525 ± 323	1549 ± 184	550 ± 209	812 ± 348	0.35	0.26	<0.001

C: control group; GT: green tea group; MI: myocardial infarction group; MI–GT: myocardial infarction + green tea group; SP: maximum systolic pressure; +dP/dt: maximum positive derivate; −dP/dt: maximum negative derivate.

activity (Fig. 4) was decreased by GT (C = 5.07 ± 1.01, GT = 4.83 ± 1.09, MI = 8.95 ± 1.09[b], MI–GT = 4.25 ± 1.09[a]; P int = 0.04, P GT = 0.03, P MI = 0.14; [a]different from MI; [b]different from C; [c]different from GT) (Fig. 4).

Considering the cellular death variables (Fig. 5), in infarcted animals GT decreased the protein expression of caspase-3 (17 kDa) (C = 0.97 ± 0.46, GT = 0.83 ± 0.46, MI = 2.55 ± 0.46, MI–GT = 1.13 ± 0.39; P int = 0.27, P GT = 0.04, P MI = 0.09). On the other hand, there were no differences in Bcl-2 expression (C = 1.07 ± 0.31, GT = 1.03 ± 0.29, MI = 1.00 ± 0.32, MI–GT = 1.64 ± 0.27; P int = 0.055, P GT = 0.62, P MI = 0.26).

4. Discussion

The purpose of this investigation was to analyze the effects of GT on cardiac remodeling following MI in the rat model. The mechanisms involved in the remodeling process after MI include a huge range of complex changes that may interfere with one another. In this setting, it has been described that mitochondrial dysfunction may promote oxidative stress that further leads to MMP activation and apoptosis. Poor energy metabolism capacity and apoptosis are listed as potential factors that influence or occur in parallel with ventricle dilation and dysfunction [2,5].

Our data showed that GT attenuated cardiac remodeling, associated with improvement in the systolic and diastolic dysfunction induced by coronary occlusion. In addition, these beneficial effects of GT were associated with corrections in cardiac oxidative stress, energy metabolism, lipid profile, ECM, and apoptosis.

The cardiovascular effects of GT have been studied in both experimental and clinical investigations. Indeed, GT is associated with decreased obesity [29], blood pressure [30,31], stroke incidence [32], and cardiovascular risk [33,34]. In addition, GT extract attenuates muscle loss and improves muscle function during disuse [35], and presented a cardioprotective effect on doxorubicin-induced cardiotoxicity in rats [36]. In a model of experimental myocardial infarction, the authors observed that supplementation of 400 mg/kg/day of green tea reduced the infarct size, prevented LV enlargement, and improved LV performance in post-MI rats, 7 weeks after the procedures. However, the study evaluated infarct size less than 20% of the whole cardiac mass [37].

Therefore, the beneficial effects of supplementation are consistent in different models. Importantly, the effects of GT on cardiac remodeling after MI remain to be elucidated, especially in the large myocardial infarct size. In addition, the mechanisms involved in this phenomenon are unknown.

The critical infarct size to induce long term ventricular remodeling is above 36%, as previously described [38]. Additionally, this data has an

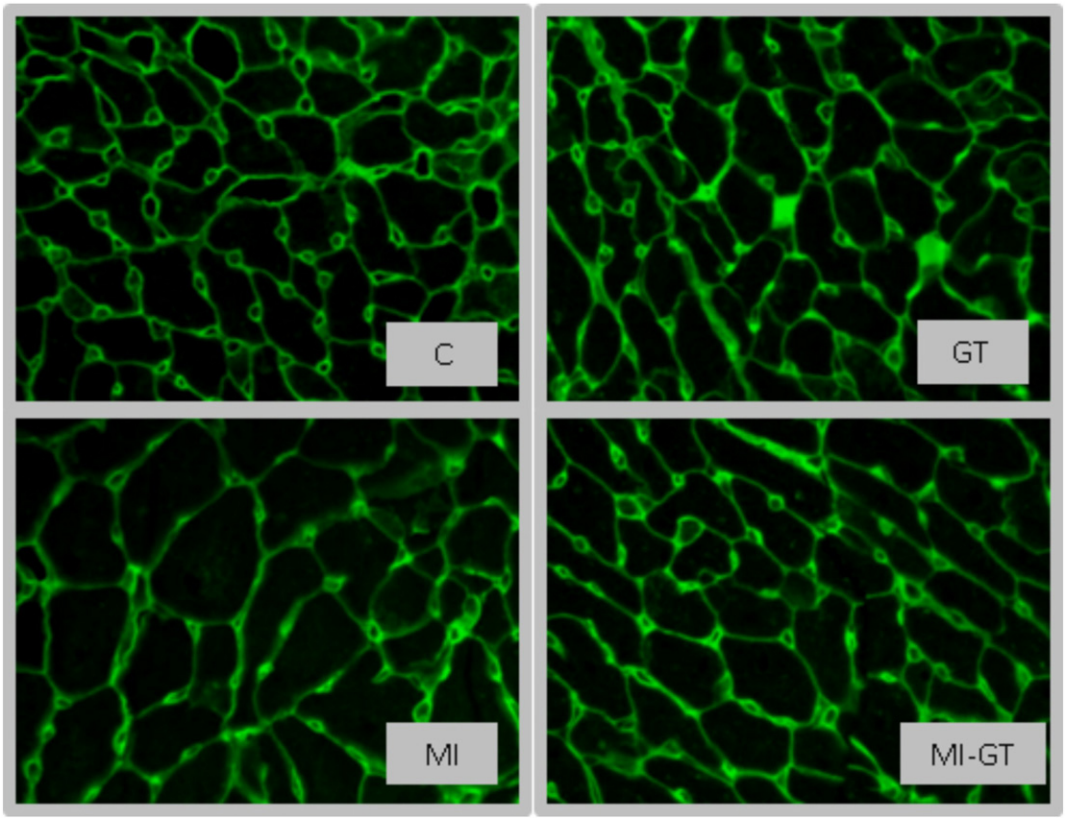

Fig. 1. Immunofluorescence to evaluate myocytes cross sectional area.

Table 3
Cardiac oxidative stress.

	C (6)	GT (6)	MI (6)	MI–GT (6)	P int	P GT	P MI
LH (nmol/g tissue)	169 ± 24.3	161 ± 20.9	220 ± 29.4[b,c]	187 ± 20.1	0.277	0.068	0.002
Carbonyl protein (nmol/mg protein)	1.04 ± 0.37	0.93 ± 0.20	2.02 ± 0.42[b,c]	1.03 ± 0.23[a]	0.002	<0.001	<0.001
SOD (nmol/mg protein)	4.36 ± 0.39	5.86 ± 0.74	11.3 ± 2.96[b,c]	7.83 ± 1.96[a]	0.002	0.672	<0.001
Catalase (μmol/g tissue)	67.9 ± 11.4	45.8 ± 6.83	35.1 ± 6.72[b]	52.3 ± 3.56[a]	<0.001	0.478	<0.001
GSH-Px (nmol/mg tissue)	34.4 ± 6.17	35.1 ± 2.94	32.2 ± 5.48	41.4 ± 7.23	0.107	0.065	0.433
Nrf-2 (AU)	0.66 ± 0.11	0.27 ± 0.12	0.33 ± 0.12[b]	0.71 ± 0.11[a,c]	<0.001	0.960	0.710

C: control group; GT: green tea group; MI: myocardial infarction group; MI–GT: myocardial infarction + green tea group; LH: lipid hydroperoxide; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; Nrf-2: nuclear receptor factor-2. AU: arbitrary unit.
[a] Different from MI.
[b] Different from C.
[c] Different from GT.

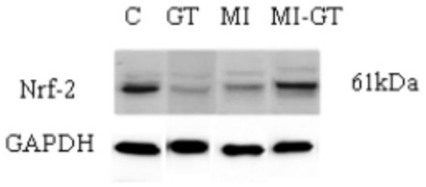

Fig. 2. Western Blot for nuclear factor erythroid-derived 2 (Nrf-2) adjusted by glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

important correlation with myocardial function [39]. Thereby, we set up the groups MI and MI–GT with similar infarct size, larger than 35%.

The main finding of the present study was that GT supplementation attenuated the LV morphological changes induced by MI. This phenomenon was characterized mainly by a decrease in hypertrophy, assessed by diastolic LV diameter, LVMI and myocytes CSA, compared to infarcted animals without supplementation. In addition, the effects of GT were associated with improvement in the systolic and diastolic dysfunction induced by coronary occlusion. It is well accepted that cardiac dysfunction after MI is associated with poor outcomes [40]. Alterations in ventricular mass, volume, geometry, and function after cardiac injury can be interpreted as an expression of the remodeling process [1,2]. Therefore, our data would indicate that GT supplementation attenuated the remodeling process after MI, in rats with large infarct size.

Another important point is the potential mechanisms involved in the beneficial effects of GT. Indeed, GT presents antioxidant properties. Importantly, oxidative stress is a critical modulator of cardiac remodeling by inducing different actions including lipid peroxidation, protein oxidation, DNA damage, cellular dysfunction, proliferation of fibroblasts, activation of metalloproteinases, induction of apoptosis, changes in calcium handling, and activation of hypertrophy signaling pathways [3,8]. In our study, as expected, MI induced oxidative stress. This fact was observed by the increase in LH and carbonyl protein, which are markers of lipid and protein damage induced by ROS. This adverse effect was mediated by decreased expression of Nrf-2, which is a critical modulator of antioxidant defenses [41]. Importantly, GT ingestion could restore Nrf-2 and antioxidant enzymes to levels near the control ones. Therefore, we assume that modulation of oxidative stress is a potential mechanism of the beneficial effects of GT in infarcted rats.

Altered energy metabolism has been also reported in cardiac remodeling. In normal conditions, free FAs are the major energy substrate for the heart, accounting for 60%–90% of energy supply. FA and glucose metabolites enter the citric acid cycle by β-oxidation and glycolysis, respectively, to generate FADH2 and NADH which, in turn, participate in the electron transport chain, with the contribution of Complex I and II, and ATP synthase. In the remodeled heart, the preference for fuel sources switches from FA β-oxidation to glycolysis [4,6]. In our study, MI induced altered energy metabolism, which was attenuated by GT. MI reduced the FA oxidation pathway that is associated with increases in serum lipids and cardiac TAG, suggesting that lipotoxicity occurred. Moreover, MI reduced mitochondrial function, impairing citric acid and oxidative phosphorylation performance. Otherwise, GT increased the level of PDH, Complex I, and ATP synthase, and restored myocardial glycogen storage. These findings suggest better use of glucose metabolism and improvement in mitochondrial function in GT groups. However, lipotoxicity was not affected by GT.

Taking into account regulation of cardiac energy metabolism, under normal conditions, PGC-1 activates nuclear respiratory factors that stimulate PPAR-α. PPARα modulates the transcription of proteins necessary for FA uptake and oxidation. In cardiac remodeling and heart failure, there is inhibition of PGC-1 and PPAR-α expression [5,6]. This study confirms this concept, since MI decreased the expression of PPAR-α. However, GT did not interfere with these variables. Thus, energy

Table 4
Cardiac energy metabolism.

	C (12)	GT (11)	MI (13)	MI–GT (12)	P int	P GT	P MI
PFK (nmol/g tissue)	105 ± 7.31	117 ± 7.64	162 ± 7.32[b]	125 ± 7.31[a]	0.001	0.13	<0.001
LDH (nmol/mg tissue)	152 ± 7.52	145 ± 7.85	186 ± 7.22	165 ± 7.52	0.38	0.05	<0.001
PDH (nmol/g tissue)	245 ± 8.96	263 ± 9.82	208 ± 9.81[b]	271 ± 8.97[a]	0.008	<0.001	0.07
β-OHADH (nmol/mg tissue)	42.4 ± 3.39	35.8 ± 3.54	26.0 ± 3.25	33.0 ± 3.38	0.063	0.84	0.002
ATP synthase (nmol/mg tissue)	83.1 ± 3.32	83.0 ± 3.46	53.5 ± 3.18[b]	75.5 ± 3.32[a]	0.002	<0.001	0.002
CS (nmol/mg tissue)	34.8 ± 1.36	29.7 ± 1.42[b]	18.3 ± 1.36[b]	21.9 ± 1.36[c]	0.003	0.18	<0.001
Complex I (nmol/mg tissue)	6.77 ± 0.23	6.59 ± 0.24	5.26 ± 0.22[b]	6.20 ± 0.23[a]	0.019	0.11	<0.001
Complex II (nmol/mg tissue)	6.48 ± 0.31	6.19 ± 0.33	4.68 ± 0.30[b]	5.82 ± 0.32	0.03	0.18	0.001
Cardiac TAG (mg/mg)	3.24 ± 0.18	5.36 ± 0.18[b]	11.9 ± 0.18[b]	11.2 ± 0.18[c]	0.01	0.02	<0.001
Cardiac glycogen (mg/mg)	36.2 ± 1.80	27.0 ± 1.80[b]	14.7 ± 1.80[b]	24.7 ± 1.80[a]	<0.001	0.83	<0.001

C: control group; GT: green tea group; MI: myocardial infarction group; MI–GT: myocardial infarction + green tea group; PFK: phosphofructokinase; LDH: lactate dehydrogenase; PDH: pyruvate dehydrogenase; β-OHADH: L-3-hydroxyacyl CoA dehydrogenase; CS: citrate synthase; ATP synthase: adenosine triphosphate synthase; Complex I (NADH dehydrogenase): nicotinamide adenine nucleotide dehydrogenase; Complex II: succinate oxide reductase; TAG: triacylglycerol.
[a] Different from MI.
[b] Different from C.
[c] Different from GT.

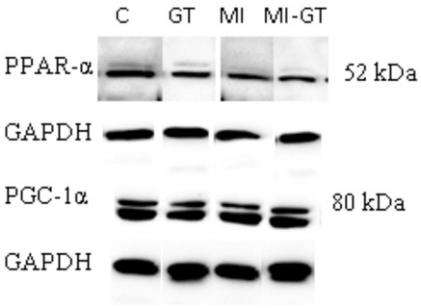

Fig. 3. Western Blot for peroxisome proliferator-activated receptors (PPARα) and Peroxisome Proliferator-Activated Receptor Gamma, Coactivator 1 (PGC-1α) antibodies adjusted by glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

metabolism, but not PGC-1 or PPAR-α, probably participates in the beneficial effects of GT in this model.

In fact, the current literature which suggests the role of PGC-1 or PPAR-α in cardiac failure remains unclear and is dependent on the experimental model and the phase of cardiac remodeling [5,42]. In addition, while the substrate switch from FA to glucose is not able to fully compensate myocardial ATP generation, it may protect the heart from more severe dysfunction [42].

Collagen fibers play a critical role in the normal heart. Therefore, rupture of the collagen network could lead to several consequences for ventricular architecture and function. In the infarcted rat model, increased metalloproteinase activity was associated with progressive ventricular dilation and ventricular dysfunction. Likewise, pharmacological inhibition of metalloproteinases in MI animals attenuated the remodeling process [43,44]. In our study, the attenuation of cardiac dilation and dysfunction induced by GT was associated with decreased activity of MMP-2 and an increase in TIMP-1, suggesting the participation of metalloproteinases in the effects of GT. Furthermore, TIMPs may exert anti-apoptotic and anti-inflammatory actions, regardless of their contribution in the activity of MMPs [45].

In different heart failure models, apoptosis is a critical modulator of left ventricular remodeling [46–48]. Two apoptosis pathways are known. In the extrinsic pathway, death ligands recruit caspase-8 or -10. The intrinsic pathway includes both pro- (BAX and BAK) and anti-apoptotic molecules (Bcl-2 and Bcl-XL). Importantly, all pathways of apoptosis converge on the activation of caspase-3, which is the effector of cellular death [47,48]. In our study, GT supplementation decreased caspase-3 levels, without changes in Bcl-2. Therefore, our data suggest that the beneficial effects of GT after MI might be explained, at least in part, by decreased apoptosis.

The results should be analyzed considering some limitation. The main limitation is that the study is experimental, allowing us to make hypothesis but we cannot extrapolate the conclusion to humans. In addition, we used one injury model and one dose of green tea.

In conclusion, GT attenuated cardiac remodeling after MI, associated with improvement in systolic and diastolic dysfunction. In addition,

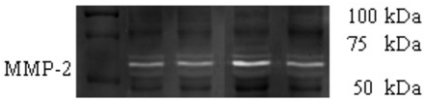

Fig. 4. Metalloproteinase 2.

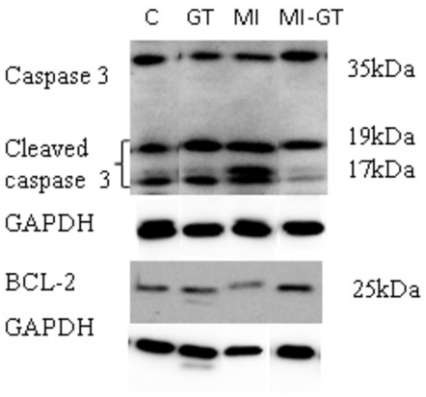

Fig. 5. Caspase 3 and B-cell lymphoma 2 (BCL-2) adjusted by glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

these beneficial effects of GT were associated with corrections of cardiac oxidative stress, energy metabolism, lipid profile, ECM, and apoptosis, without participation of PPAR-α, PGC-1, and Bcl-2.

Conflict of interest

All authors declare there are no conflicts of interest regarding the publication of this paper.

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Table 5
Serum lipids.

	C (6)	GT (6)	MI (6)	MI–GT (6)	P int	P GT	P MI
Total cholesterol (mg/dL)	96.1 ± 4.27	89.7 ± 2.83	158 ± 5.97[b]	112 ± 4.85[c,a]	<0.001	<0.001	<0.001
HDL (mg/dL)	56.54 ± 1.88	50.11 ± 2.06[b]	29.74 ± 1.22[b]	38.11 ± 2.72[c,a]	0.002	0.640	<0.001
LDL (mg/dL)	17.1 ± 2.09	16.4 ± 2.85	100 ± 6.35[b]	50.2 ± 4.41[c,a]	<0.001	<0.001	<0.001
TAGs (mg/dL)	112 ± 4.99	116 ± 5.37	144 ± 5.68[b]	120 ± 3.88[a]	0.013	0.056	0.002

C: control group; GT: green tea group; MI: myocardial infarction group; MI–GT: myocardial infarction + green tea group; HDL: high density lipoprotein; LDL: low density lipoprotein; TAGs: serum triacylglycerol.
[a] Different from MI.
[b] Different from C.
[c] Different from GT.

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