CROATIAN MEDICAL JOURNAL
CMJ

44(6):690-696,2003

BASIC SCIENCES

Comparison of Protective Effects of Catechin Applied *in Vitro* and *in Vivo* on Ischemia-Reperfusion Injury in the Isolated Rat Hearts

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Aim. To determine overall cardiac effects of flavonoid catechin on ischemia-reperfusion injury in isolated rat hearts *in vitro* and *in vivo*.

Methods. After perfusing hearts at the pressure of 70 mm Hg, coronary perfusion was interrupted for 30 minutes and then re-established. There were four experimental groups, with 10 rats each. The first group received 100 μmol/L of catechin added to the perfusate 10 minutes before, during, and 10 minutes after ischemia; the second group underwent *in vivo* pretreatment with catechin (250 mg/kg body weight) applied intragastrically for 10 days; the third group received it as a single dose 1 h before sacrifice; and the fourth group received saline. An additional group of 10 hearts served as untreated, nonischemic time control. The variables included heart rate, atrioventricular conduction time, cardiac rhythm, isovolumetric left ventricular pressure, coronary flow and responsiveness, oxygen consumption, relative cardiac efficiency, lactate dehydrogenase release, and myocardial lipid peroxidation.

Results. Catechin added to the perfusate increased the coronary flow and ratio of oxygen delivery to myocardial oxygen consumption before ischemia and during reperfusion. Following 30 minutes of reperfusion, isovolumetric left ventricular developed pressure recovered to 42 ± 3%, 63 ± 3%, 71 ± 2%, and 55 ± 3% of the initial control values in the control, catechin 1 h, catechin for 10 days, and catechin *in vitro* group, respectively. Cardiac efficiency and coronary responsiveness were also best preserved in the group receiving catechin for 10 days.

Conclusion. Application of catechin *in vitro* and *in vivo,* irrespective of duration of application, resulted in cardioprotection against ischemia-reperfusion injury, but long-term pretreatment provided more favorable effects. Directly applied, catechin acted as a vasodilator.

Key words: *catechin; coronary reperfusion; heart; rats; reperfusion injury*

Reperfusion following global myocardial ischemia can result in compromised cardiac function and malignant dysrhythmias. In the pathogenesis of ischemic and reperfusion injury, oxygen-derived free radicals play an important role (1). Numerous studies have investigated procedures or drugs that may be protective during ischemia and facilitate myocardial recovery upon reperfusion. The recognized beneficial effects of vegetable, fruit, and red wine consumption on cardiovascular system have been partly attributed to their flavonoid content (2). The mechanisms by which flavonoids may contribute to human health include antioxidant action, modulation of immune function, and reduction of platelet adhesion (3). Red wine is a significant dietary source of flavonoids, with catechin (cyanidol-3) as the major flavonoid (4). Several *in vitro* studies with human plasma have shown that catechin, along with vitamin C, acts as a "first line defense" free radical scavenger and protects lipid soluble antioxidants, such as vitamin E and vitamin A, from oxidation (5,6). After ingestion of red wine, plasma catechin concentration increases and reaches maximum after 1 hour (7). Accordingly, intragastric administration of epicatechin, optical stereoisomer of catechin, enhances antioxidative capacity of plasma in rats 1 hour after administration. In the isolated rat hearts subjected to ischemia-reperfusion (8) and anoxia-reoxygenation (9), catechin applied *in vitro* in the perfusate increased the recovery of contractile function and decreased the incidence of dysrhythmias. Manach et al (10) studied the differences in catechin bioavailability between rats on 14-day catechin diet and rats fed a single catechin meal before sacrifice. They found no significant difference in pharmacokinetic properties of catechin between observed groups, and assumed that catechin effects were limited to the postprandial period only.

These results suggest that catechin has potentially cardioprotective effects against ischemia and hypoxia. However, no study has examined catechin overall effects on cardiac function or compared effects of catechin applied *in vitro* and *in vivo* on the function

and recovery of isolated heart subjected to severe global ischemia and reperfusion. The aim of this study was to determine the effects of catechin in the control conditions and during the postischemic reperfusion, not only on contractile function, but also on cardiac rhythm, oxygen utilization, coronary flow, coronary responsiveness, and biochemical markers of myocardial tissue injury. Another aim was to compare the effects of catechin, applied *in vivo* intragastrically for 10 days and as a single dose, and 1 hour before sacrifice, with the effects of catechin applied directly in the perfusate of the isolated heart i*n vitro*.

Material and Methods

Fifty male Wistar rats (weight range, 290-310 g) received intraperitoneal injection of 300 mg of urethane and 1,000 units of heparin. After becoming unresponsive to noxious stimulation, they were intubated and connected to the respirator for small animals. Following thoracotomy, the inferior and superior venae cavae were cut and the aorta was cannulated distal to the aortic valve. Each heart was immediately perfused retrogradely through the aorta and excised. All hearts were perfused at control perfusion pressure of 70 mm Hg, as measured at the aortic root. The perfusate, a modified Krebs-Henseleit solution, was filtered in-line (5 μm pore size) and had the following composition (in mmol/L): NaCl 118; $NaHCO_3$ 25; sodium pyruvate 2; KH_2PO_4 1.2; KCl 4.6; $MgSO_4$ 1.2; $CaCl_3$ 1.3; glucose 11; and insulin 5 units/L. Perfusate and bath temperatures were maintained at 36.6 ± 0.1 °C with a thermostatically controlled water circulator. The solution was equilibrated with a gas mixture of 96% O_2 and 4% CO_2.

All procedures on animals were approved by the Ethical Committee of the Split University School of Medicine.

Left ventricular pressure was measured isovolumetrically with a transducer (UFI type 1050, Morro Bay, CA, USA) connected to a thin, saline-filled latex balloon (Hugo Sachs Elektronik, KG, March-Hugstetten, Germany) inserted into the left ventricle through the mitral valve from a cut in the left atrium. Balloon volume was adjusted to maintain a left ventricular diastolic pressure at 0 mm Hg during the initial control period. Two pairs of Teflon coated silver bipolar electrodes (125 μm in diameter) were placed subepicardially at the appendage of right atrium and at the right ventricular pulmonary conus, respectively, to monitor intracardiac electrograms, from which spontaneous sinoatrial rate and atrioventricular conduction time were measured. The two electrode signals were amplified and displayed continuously on an oscilloscope for monitoring. Atrial rate was determined from the right atrial beat-to-beat interval; atrioventricular conduction time was determined as the interval between the superior right atrial beat and the right ventricular pulmonary conus beat. Electrogram intervals were recorded on-line by digital timer systems that allowed instantaneous interval and rate analyses. Ventricular tachycardia (VT) was defined as 4 or more consecutive uniform or multiform ventricular waveforms and a faster ventricular than atrial rate. Ventricular fibrillation was defined by the presence of erratic activity in the ventricular electrogram and by the absence of pressure generation by the left ventricle (11).

Coronary flow was measured at a constant temperature with an electromagnetic flow probe (Biotronix BL610-2A, with series 2000C extracorporeal transducer, 1.5 mm I.D., Biotronix Laboratories Inc., Kensington, MD, USA) placed into the aortic inflow line. The flowmeter was calibrated daily by collecting timed samples into a volumetric cylinder over the 0-24 mL/min flow range. The inflow was frequently zeroed by temporarily bypassing the flow transducer.

Electrograms, heart rate, atrioventricular conduction time, coronary flow, left ventricular and perfusion pressure were digitized (PowerLab/16S, ADInstruments; Castle Hill, Australia) and recorded at 1,000 Hz (CHART version 4.2, ADInstruments) on PC for later analysis.

Coronary sinus effluent was collected by placing a cannula into the right ventricle through the pulmonary artery.

Coronary inflow and outflow (coronary sinus) O_2 tensions (mm Hg) were measured off line with an intermittently self-calibrating analyzer system (IL 1610 Instrumentation Laboratory, Milan, Italy). Because oxygen supply to the heart depends solely on the crystalloid solution, O_2 delivery (DO_2) was calculated form the inflow O_2 tension times O_2 solubility (24 μL/mL saline/760 mm Hg) times coronary flow per gram of wet heart tissue. Myocardial O_2 consumption (MVO_2) was calculated as O_2 solubility times coronary flow per gram times (inflow O_2 – outflow O_2 tension difference). The DO_2/MVO_2 ratio was calculated to asses the direct vasodilatory response to drugs apart from the response caused by metabolic factors (e.g., a decrease in coronary flow and O_2 delivery secondary to decreased contractility and O_2 consumption). The measurement is based on the assumption that local metabolites are produced in proportion to myocardial O_2 consumption and are major factors that regulate coronary flow (12). Relative cardiac efficiency was calculated as the product of heart rate (beats per minute) and developed isovolumetric left ventricular pressure (systolic minus diastolic left ventricular pressure, in mm Hg) divided by O_2 consumption (mL min^{-1} g^{-1}) and expressed as (mm Hg beat)/(0.1 μL O_2 g^{-1}). By this index, a relative decrease in the amount of oxygen consumed to perform an isovolumetric contraction indicates improved cardiac efficiency (11).

Lactate dehydrogenase (LDH) concentration was measured with a Boehring commercial kit (Boehring Mannheim, Mannheim, Germany) by continuous collection of the effluent on reperfusion and by spot sampling of the collected effluent at 10 minutes. Lactate dehydrogenase release was expressed as mL collected/minute times wet heart weight (mU g^{-1} min^{-1}).

At the end of the experiment, the atria were removed and ventricles weighted. The formation of lipid oxidation products in the heart tissue was evaluated as thiobarbituric acid reactive substances (TBARS) and expressed as malondialdehyde equivalents per wet heart weight (13).

Catechin and adenosine diphosphate (ADP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Solutions containing catechin were protected from light.

Protocol

Forty male Wistar rats were randomly assigned to one of the four groups, each consisting of 10 rats. The first group received 3 mL of saline intragastrically by direct stomach intubation for 10 days, after which they were sacrificed. Their hearts were isolated and subjected to 30 minutes of no-flow normothermic global ischemia followed by 30 minutes of reperfusion. The second group received catechin for 1 hour according to the following protocol: 3 mL of saline were administered intragastrically to animals for 10 days; catechin (250 mg/kg body weight) dissolved in 3 mL of saline was administered intragastrically 60 minutes before the sacrifice, after which the animals' hearts were subjected to ischemia and reperfusion. The third group of rats received catechin for 10 days as follows: catechin (250 mg/kg body weight) dissolved in 3 mL of saline was administered intragastrically for 10 days, after which the animals were sacrificed and their hearts subjected to ischemia and reperfusion. In the fourth group of rats, catechin was applied *in vitro*. The fourth group received 3 mL of saline intragastrically for 10 days, after which they were sacrificed and their hearts subjected to ischemia and reperfusion, with catechin (100 μmol/L) present in the perfusate for 10 minutes before, during, and for 10 minutes after ischemia. An additional group of 10 hearts served as untreated, nonischemic time control to determine time dependent deterioration of heart function. Initial control measurements were recorded after 30-minute stabilization period. To determine endothelium-dependent vasodilatation, 0.1 mL of ADP (600 μmol/L) was given at the beginning and at the end of each experiment (14). Increase in the coronary flow was expressed as a percentage of values measured before the application of ADP. Measurements were made during the last minute of the initial stabilization period, after a 10-minute exposure to perfusate (with or without catechin) just before initiating ischemia, at 10, 20, and 30 minutes of ischemia, and at 10, 20, and 30 minutes of reperfusion following ischemia.

Statistical Analysis

All data were expressed as means ± standard error of the means (SEM). Kruskal-Wallis test followed by Dunn's test was performed to assess intergroup statistical differences in cardiac functions, whereas statistical differences within each group for values obtained over the time course were determined by Friedman test followed by Dunn's test. Kruskal-Wallis test followed by the same post-hoc test was used to assess the differences in biochemical markers and duration of dysrhythmias. The incidence of dysrhythmias was evaluated by chi-square test with 4x2 contingency table. Difference in vasodilatation induced by ADP, at the beginning and at the end of each experiment, was determined by Wilcoxon test. Nonparametric tests were used due to a small sample size (10 rats in each group). Mean values were considered significant at $p < 0.05$.

Results

Effects on Heart Rate and Atrioventricular Conduction Time

The initial values for heart rate and atrioventricular conduction time did not differ among the groups (Table 1). After 10 minutes of perfusion with catechin, there were no changes in the above parameters in catechin *in vitro* group. With the onset of ischemia, atrioventricular time was sharply prolonged and atrioventricular block occurred in all hearts. Atrial rate decreased gradually over the 30-minute period of ischemia in all hearts. Ventricular electrical activity rapidly decreased with the onset of ischemia and ceased by the end of the ischemic period in all hearts. During the first 10 minutes of reperfusion, the atrial rate returned to the control levels in all three groups treated with catechin but remained slightly lower in the control group in comparison with the initial values. These values did not change to the end of reperfusion. Atrioventricular conduction time returned to the control levels in all groups after 10 minutes of reperfusion.

Effects on Cardiac Rhythm

All hearts exhibited normal sinus rhythm during the initial control period. During the period of ischemia, the most frequent dysrhythmias were second- and third-degree atrioventricular block with or without atrial or ventricular arrest. During the period of reperfusion, duration of ventricular tachycardia and ventricular fibrillation significantly decreased in all three groups treated with catechin, compared with the control group (Table 2). The incidence of ventricular fibrillation was similarly decreased in all catechin groups, whereas the incidence of ventricular tachycardia was not affected by any treatment and did not differ among the groups (Table 3). Within five minutes of reperfusion, all hearts spontaneously converted to the sinus rhythm.

Effects on Coronary Flow, Endothelium Function, and Oxygen Supply/Demand Ratio

Coronary flow did not differ among the experimental groups during the initial control period (Fig. 1). Before the ischemic period, catechin in the perfusate caused an increase in coronary flow. With the onset of reperfusion, coronary flow transiently increased in all groups, and then returned to the preischemic levels. Throughout the reperfusion period, coronary flow remained highest in the catechin *in vitro* group.

ADP was given at the beginning and at the end of each experiment to determine endothelium-dependent vasodilatation. At the beginning of each experiment, ADP increased coronary flow similarly in all groups (Table 4). Coronary responsiveness to ADP at the end of the experiment was diminished in all groups, except in the group that received catechin for 10 days. In the time control group, which had a basal coronary flow of 12.0 ± 1.7mL/min/g, coronary responsiveness remained similar throughout 70 minutes of perfusion (152 ± 11% vs 147 ± 9%).

Table 2. Effects of catechin (CTCH), applied intragastrically for 10 days, 1 h before sacrifice, or added to the perfusate, on duration of cardiac dysrhythmias during 30 minutes of reperfusion following 30 minutes of global ischemia

Variable	Duration (s)				
		CTCH			
(mean ± SEM)	control	1 h	10 days	*in vitro*	p
Ventricular fibrillation	38.6 ± 7	11 ± 5*	8 ± 0*	5 ± 2*	< 0.0001
Ventricular tachycardia	191.8 ± 20.6	105 ± 19.4*	102 ± 29.3*	110 ± 5.5*	0.0096

*$p < 0.05$ vs control (Kruskal-Wallis and Dunn's tests). Ten animals per group.

Table 3. Effects of catechin (CTCH), applied intragastrically for 10 days, 1 h before sacrifice, or added to the perfusate, on incidence of cardiac dysrhythmias during 30 minutes of reperfusion following 30 minutes of global ischemia

	Incidence of dysrhythmias (No. of rats)			
		CTCH		
Variable	control	1 h	10 days	*in vitro*
Ventricular fibrillation*	10	2	4	3
Ventricular tachycardia†	10	10	10	10

*Chi-sqare = 15.539, d.f. = 3, p = 0.0014. Ten animals per group.
†Statistical analysis non applicable.

Table 1. Effects of catechin (CTCH), applied to rats intragastrically for 10 days, 1 h before sacrifice, or added to the perfusate, on heart rate and atrioventricular (AV) conduction time before, during, and after global ischemia*

Variable	Heart rate (beats/min)				AV time (ms)			
		CTCH				CTCH		
(mean ± SEM)	control	1 h	10 days	*in vitro*	control	1 h	10 days	*in vitro*
1 min of perfusion	279 ± 10	274 ± 14	280 ± 13	288 ± 14	48 ± 4	45 ± 3	46 ± 4	45 ± 5
10 min of perfusion	283 ± 12	269 ± 13	284 ± 16	285 ± 11	46 ± 4	46 ± 3	47 ± 3	47 ± 4
10 min of ischemia	166 ± 7†	180 ± 9†	199 ± 11†*	191 ± 10†*	AVB	AVB	AVB	AVB
30 min of ischemia	0†	0†	0†	0†	AVB	AVB	AVB	AVB
10 min of reperfusion	239 ± 8†	247 ± 11	277 ± 14‡	275 ± 11‡	49 ± 2	48 ± 4	49 ± 5	49 ± 3
30 min of reperfusion	240 ± 9†	248 ± 11	282 ± 10‡	279 ± 8‡	48 ± 3	49 ± 4	47 ± 3	47 ± 3

*Abbreviations: AVB – atrioventricular block; 1 min of perfusion – initial values before exposure to catechin for group CTCH *in vitro*; 10 min of perfusion – values after exposure to catechin for group CTCH *in vitro*. Ten animals per group.
†$p < 0.05$ vs 1 min of perfusion (Friedman and Dunn's tests – rows).
‡$p < 0.05$ vs control (Kruskal-Wallis and Dunn's tests – columns).

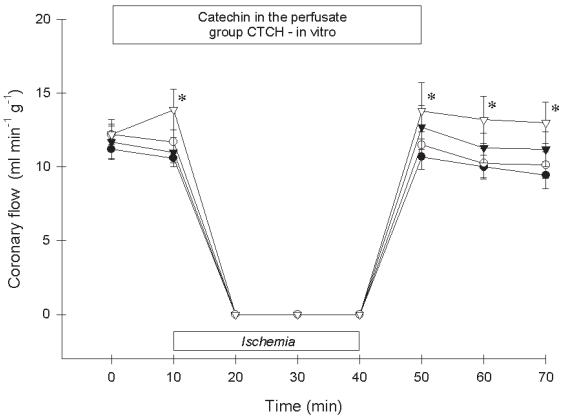

Figure 1. Effects of catechin (CTCH), applied to rats intragastrically for 10 days, 1 h before sacrifice, or added directly to the perfusate *in vitro*, on coronary flow before and after 30 minutes of ischemia. All data are means ± standard error of mean. *p<0.05 vs control (Kruskal-Wallis and Dunn's tests). Each group consisted of 10 rats. Closed circles – control; open circles – catechin for 1 h; closed triangles – catechin for 10 days; open triangles – catechin *in vitro*.

Table 4. Effects of catechin (CTCH), applied intragastrically for 10 days, 1 h before sacrifice, or added to the perfusate, on coronary responsiveness to adenosine diphosphate (ADP) before and after global ischemia

Variable (mean ± SEM)	Increase of coronary flow after ADP (%)*			
		CTCH		
	control	1 h	10 days	*in vitro*
Before ischemia	144.5±7	147.6±5.1	145.8±9.1	139±3.2
After ischemia	120.1±2.9	126.7±4.6	139±4	123.7±2.7
p†	<0.0001	<0.0001	0.1117	<0.0001

*Expressed as a percentage of values before the application of ADP.
†Wilcoxon test. Ten animals per group.

Initial myocardial oxygen consumption values did not significantly differ among the groups and these values did not change during the entire experiment.

Initial values of oxygen delivery/consumption ratio did not significantly differ among the groups (Fig. 2). Changes in DO_2/MVO_2 reflected the changes in the coronary flow. Before the ischemic period, catechin in the perfusate caused an increase in oxygen delivery/consumption ratio, in catechin *in vitro* group. With the onset of reperfusion, DO_2/MVO_2 returned to the preischemic levels. Throughout the reperfusion period DO_2/MVO_2 was the highest in the catechin *in vitro* group.

Effects on Left Ventricular Pressure and Cardiac Efficiency

Developed left ventricular pressure (systolic minus diastolic) did not differ among the experimental groups during the initial control period (Fig. 3). Exposure to catechin for 10 minutes before ischemia did not affect developed left ventricular pressure. During ischemia, contractile activity ceased so that systolic and diastolic left ventricular pressure equalized. Diastolic left ventricular pressure was 0 mm Hg in all hearts before ischemia and remained at that level during first 20 minutes of ischemia (Fig. 4). At the end of ischemia, diastolic left ventricular pressure increased in all experimental groups and it was highest in the control group. With the onset of reperfusion, developed left ventricular pressure increased in all hearts, but remained lowest in the control group. After 30 minutes of reperfusion, developed left ventricular pressure recovered to 42±3%, 63±3%, 71±2%, and 55±3% of the initial control values in the control, catechin 1 hour, catechin for 10 days, and catechin *in vitro* group, respectively. With the onset of reperfusion, diastolic left ventricular pressure increased further in all hearts, but remained highest in the control group. The values of diastolic left ventricu-

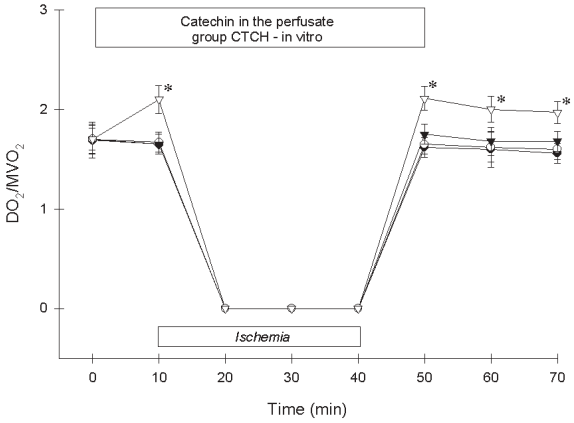

Figure 2. Effects of catechin (CTCH), applied to rats intragastrically for 10 days, 1 h before sacrifice, or added directly to the perfusate *in vitro*, on the ratio of oxygen delivery to oxygen consumption (DO_2/MVO_2) before and after 30 minutes of ischemia. All data are means ± standard error of mean. *p<0.05 vs all other groups (Kruskal-Wallis and Dunn's tests). Each group consisted of 10 rats. Closed circles – control; open circles – catechin for 1 h; closed triangles – catechin for 10 days; open triangles – catechin *in vitro*.

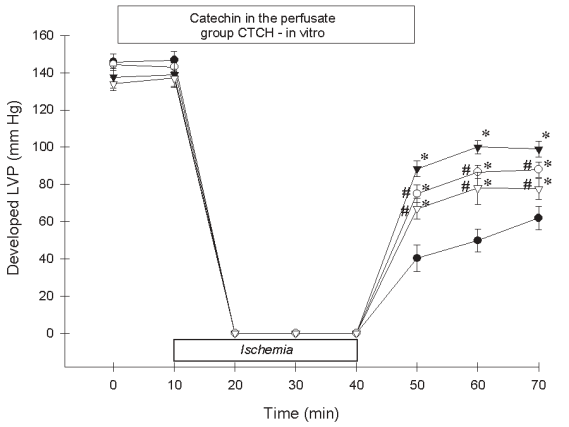

Figure 3. Effects of catechin (CTCH), applied to rats intragastrically for 10 days, 1 h before sacrifice, or added directly to the perfusate *in vitro*, on developed (systolic – diastolic) left ventricular pressure (LVP) before and after 30 minutes of ischemia. All data are means ± standard error of mean. *p<0.05 vs control. *p<0.05 vs catechin for 10 days (Kruskal-Wallis and Dunn's tests). Each group consisted of 10 rats. Closed circles – control; open circles – catechin for 1 h; closed triangles – catechin for 10 days; open triangles – catechin *in vitro*.

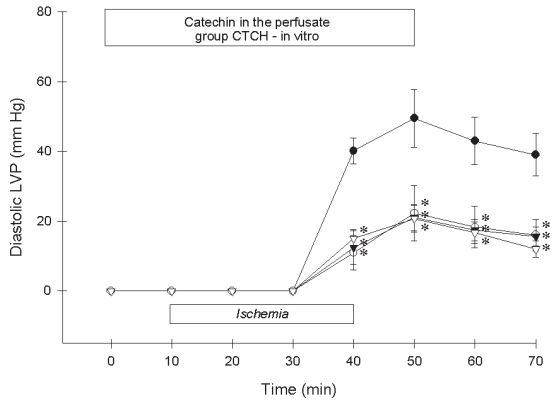

Figure 4. Effects of catechin (CTCH), applied to rats intragastrically for 10 days, 1 h before sacrifice, or added directly to the perfusate *in vitro*, on diastolic left ventricular pressure (LVP) before and after 30 minutes of ischemia. All data are means ± standard error of mean. *$p < 0.05$ vs control (Kruskal-Wallis and Dunn's tests). Each group consisted of 10 rats. Closed circles – control; open circles – catechin for 1 h; closed triangles – catechin for 10 days; open triangles – catechin *in vitro*.

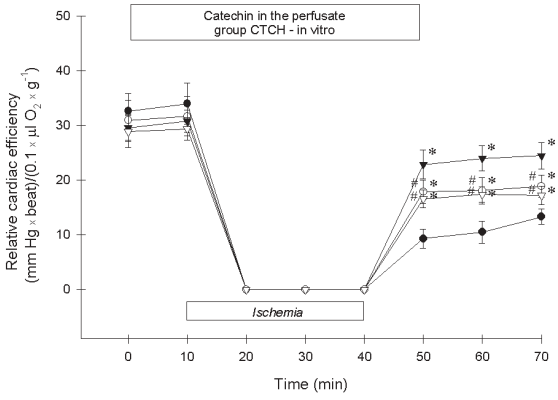

Figure 5. Effects of catechin (CTCH), applied intragastrically for 10 days, 1 h before sacrifice, or added directly to the perfusate *in vitro*, on relative cardiac efficiency before and after 30 minutes of ischemia. All data are means ± standard error of mean. *$p < 0.05$ vs control, #$p < 0.05$ vs catechin for 10 days (Kruskal-Wallis and Dunn's tests). Each group consisted of 10 rats. Closed circles – control; open circles – catechin for 1 h; closed triangles – catechin for 10 days; open triangles – catechin *in vitro*.

lar pressure at the end of reperfusion were: 39.1 ± 6.0 mm Hg, 16.0 ± 4.5 mm Hg, 15.6 ± 2.7 mm Hg, and 12.0 ± 2.3 mm Hg for the control, catechin 1 hour, catechin for 10 days, and catechin *in vitro* group, respectively.

In the time control group (ie, in the absence of ischemia-reperfusion protocol), left ventricular systolic pressure decreased to 93.0 ± 2.0% of the initial control values after 70 minutes of perfusion and left ventricular diastolic pressure remained unchanged at 0 mm Hg (data not shown).

Relative cardiac efficiency (Fig. 5) was similar for all groups during the initial control period. Before the ischemic period, catechin in the perfusate did not cause change in cardiac efficiency in the catechin *in vitro* group. With the onset of reperfusion, cardiac efficiency deteriorated in all hearts and was lowest in the control group and highest in the catechin for 10 days group.

Effects on LDH Release and Formation of Lipid Peroxides

LDH release in the effluent at 10 minute of reperfusion was significantly lower in all catechin treated groups than in the control group (Table 5). Similarly, level of myocardial lipid peroxides at the end of the experiment was highest in the control group in comparison with the catechin groups (Table 5).

Discussion

Our study showed that both *in vitro* and *in vivo* administration of catechin protects hearts subjected to global ischemia and reperfusion, as demonstrated by improved electrophysiological stability, coronary flow, coronary reserve, contractile function, cardiac efficiency, and diminished LDH release and formation of lipid peroxides. Most studies using catechin concentrated on mechanism of protection and did not monitor such a broad spectrum of cardiac functions or compare cardiac effects of catechin applied *in vitro* and *in vivo*. Moreover, in this study we compared effects of catechin applied *in vivo* for 10 days with effects of catechin applied as a single dose 1 hour before sacrifice to test the suggestion that catechin acts only for a few hours after meal (10).

One of the main results of this study is that catechin applied *in vitro* increased coronary flow without changing heart rate and contractility – the main determinants of oxygen consumption. Therefore, autoregulatory mechanism of coronary flow was blunted, as shown by the increased oxygen delivery/consumption ratio. This indicates that catechin acts as a direct vasodilatory agent. The fact that coronary flow and DO_2/MVO_2 remained increased after removal of catechin from the perfusate indicates prolonged vasodilatory effect. Although mechanisms of catechin effects on coronary vasculature have not been examined, there are several somewhat confronting results from

Table 5. Effects of catechin (CTCH), applied intragastrically for 10 days, 1 h before sacrifice, or added to the perfusate, on lactate dehydrogenase (LDH) release at 10 minutes of reperfusion and on level of lipid peroxides in heart tissue

	LDH release (U g^{-1} min^{-1}) and lipid peroxides (nmol/L g^{-1} MDA)				
		CTCH			
Variable (mean ± SEM)	control	1 h	10 days	*in vitro*	p
LDH release	39.7 ± 84.4	16.3 ± 37.1*	148.2 ± 13.4*	123.4 ± 18.2*	0.0006
Lipid peroxides†	197.7 ± 11.9	141.7 ± 8.6‡	152.4 ± 9.3‡	158.0 ± 9.5‡	0.0018

*$p < 0.01$ vs control.
†Expressed as malondialdehyde (MDA) equivalents.
‡$p < 0.05$ vs control (Kruskal-Wallis and Dunn's tests). Ten animals per group.

different experimental models applicable to observations in our study. Andriambelson (15) and Duarte (16) showed on the isolated rat aorta that dilatory effects of catechin were the result of protein kinase C inhibition and not endothelium-dependent. On the other hand, Fitzpatrick et al (17) showed that flavonoid quercetin, wine, grape juices, and grape skin extracts prevented contractions of the rat aortic rings with intact endothelium (but not in the endothelium denuded rings), indicating involvement of endothelium-derived relaxing factors, such as nitric oxide (18). An additional biological effect of catechin that could interfere with vascular tonus is the ability to inhibit cAMP and cGMP phosphodiesterases (19,20).

Except for the coronary flow, all other observed parameters and indices of cardiac postischemic recovery were similar or better in the group that received catechin for 10 days in comparison with other groups receiving catechin. This was especially noticeable in the recovery of contractile function, cardiac efficiency, and preservation of endothelium dependent coronary responsiveness to ADP. Effects on electrophysiological parameters and biochemical indicators of cardiac damage were similar for all catechin treated hearts. Based on the results of this study and previous research, we can assume that free radical scavenging and chelating properties of catechin represent the key mechanism for the observed cardioprotective effects during ischemia and reperfusion. The potential role of catechin metabolites, when applied *in vivo*, could not be excluded in this study, since we did not study catechin pharmacokinetics or determine catechin metabolites.

Oxygen-derived free radicals significantly contribute to the genesis of reperfusion-induced dysrhythmias, contractile malfunction and vascular endothelium damage (1,21). The sources of free radicals generated in reperfusion include xanthine oxidase, mitochondria, polymorphonuclear cells, and auto-oxidation of catecholamines (1,21). Radicals, superoxide and hydrogen peroxide, can be further transformed into highly reactive hydroxyl radical in the presence of iron (Haber-Weiss and Fenton reactions; ref. 22). During reperfusion, calcium and free radicals damage mitochondria, which impairs the production of ATP (23).

Catechin protects lipid soluble antioxidants, such as vitamins E and A, from oxidation (6). Van der Kraaij et al (9) reported that catechin in the perfusate showed antiarrhythmic effects after anoxia-reoxygenation and reduced LDH release in the effluent, suggesting smaller extent of necrosis (8,9). Voogd et al (24) showed that catechin, applied in the perfusate, attenuated the increase in free iron in isolated rat hearts after anoxia and reoxygenation and, consequently, decreased hydroxyl radical formation through Haber-Weiss and Fenton reactions. Van Jaarsveld et al (25) found that catechin application preserved postischemic mitochondrial function and increased myocardial vitamin C content after ischemia-reperfusion, explaining it by iron-chelating and free radical scavenging activities.

The findings that long-term pretreatment with catechin provides more favorable effects after ischemia-reperfusion injury indicate involvement of mechanisms other than direct antioxidant activity. In 1986, Edes et al (26) reported protective effects of catechin applied to rats for 6 weeks in reducing myocardial lipid peroxidation and increasing glutathione content after chronic alcohol ingestion. Facino et al (27) showed that application of oligomers of catechin for 3 weeks protected against ischemia-reperfusion injury and was positively associated with an increase in plasma antioxidant activity. Another possible explanation for better protection with long-term application of catechin could be the effect on gene expression. Sato et al (28) administered orally grape seed extracts, containing mostly oligomers of catechin, to rats for 3 weeks, and found that grape seed extracts functioned as an *in vivo* antioxidant, whose cardioprotective properties might at least partially be attributed to its ability to block antideath signal through the inhibition of proapoptotic transcription factor and gene, JNK-1 and c-Jun.

In conclusion, catechin, applied both *in vitro* and *in vivo*, seems to improve cardiac recovery during reperfusion after ischemia in isolated rat heart, and that the best protection is achieved by long-term application of catechin. This is in contrast with the idea that catechin effects are limited to the short postprandial period (10).

Acknowledgment

This research was supported in part by grants TP-01/0216-05 and 0216012, from the Ministry of Science and Technology of Republic of Croatia.

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Received: March 4, 2003
Accepted: September 9, 2003

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