Epigallocatechin-3-gallate Moderates Nitric Oxide Production during Ischemia and Reperfusion of Cardiomyocytes

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Abstract

Background: Epigallocatechin-3-gallate (EGCG) is a major green tea polyphenol that attenuates myocardial reperfusion injury, but the underlying mechanisms are not clear. Endothelin-1 (ET-1) and nitric oxide (NO) are mediators of reperfusion injury in the heart. We hypothesize that ET-1 and NO are myocardial targets of EGCG that may modulate reperfusion injury.

Methods: Neonatal rat cardiomyocytes were isolated by enzymatic dissociation and subjected to simulated ischemia and reperfusion. Cardiomyocytes were cultured in media with 4.5 g/L glucose at normoxic conditions (21% O2) for 24 hours or without glucose at hypoxic conditions (0.5% O2) for 18 hours. Ischemic cells were then harvested or reperfused for 6 additional hours in glucose-containing media at normoxic conditions. EGCG at 0.1, 1, or 10 μM was added at time = 0. ET-1 was measured in culture media by ELISA and total NO production was monitored with a gas-permeable probe.

Results: Ischemia increased NO production by cardiomyocytes compared with normoxic cells and 6 hr reperfusion of ischemic cells further increased NO levels. EGCG at 1 and 10 μM attenuated the ischemia-induced rise in NO after 18 hr. EGCG at 10 μM also alleviated the reperfusion-triggered elevation in NO after 18 hr of ischemia and 6 hr reperfusion. Cardiomyocyte ET-1 production increased with simulated ischemia and reperfusion (5.3 ± 2.7 pg/mL) compared with cells cultured under normal conditions for 24 hr (4.1 ± 3 pg/mL, p=.05), but EGCG treatment did not affect ET-1 production.

NO Production by Cardiomyocytes (nM)				
Treatment EGCG (μM)	18 hr normoxia	24 hr normoxia	18 hr ischemia	18 hr ischemia/6 hr reperfusion
untreated	171±20	173±28	225±27[a]	247±33[a]
0.1	161±26	175±72	229±44	209±52
1	152±20	148±48	179±9[b]	184±37
10	153±13	144±36	162±17[b]	173±25[b]

Note: a = p<0.05 versus normoxia at same time, b = p<0.05 versus untreated at same time. Means ± SD of three experiments.

Conclusions: Moderation of NO may be one mechanism by which EGCG provides myocardial protection. A reduction of NO-generated mediators may be beneficial for minimizing inflammation and oxidative stress during ischemia and reperfusion.

Introduction

Epigallocatechin-3-gallate (EGCG) is a major green tea polyphenol that attenuates myocardial reperfusion injury, but the underlying mechanisms are not clear. As with the setting of inflammation and cancer, EGCG has been shown to regulate several molecular targets in the setting of reperfusion injury as well.[1] Endothelin-1 (ET-1) and nitric oxide (NO) are mediators of reperfusion injury in the heart. Both ET-1 and NO form a feedback loop with ET-1 leading to vasoconstriction and NO causing vasodilation. Our laboratory was interested in further characterizing cardiomyocyte response to ischemia and reperfusion with the administration of EGCG. We hypothesize that ET-1 and NO are myocardial targets of EGCG that may modulate reperfusion injury.

Introduction (continued)

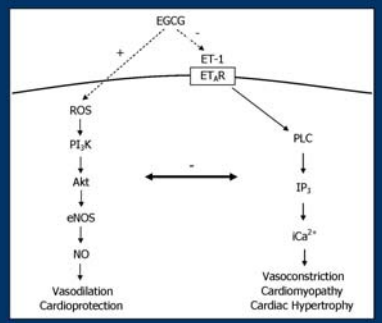

Figure 1. Simplified EGCG Pathway. *The abbreviations used are: EGCG, epigallocatechin-3-gallate; ROS, reactive oxygen species; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; ET-1, endothelin-1; ETAR, endothelin-A receptor; PLC, phospholipase C; IP3, inositol trisphosphate; iCa²⁺, intracellular calcium.*

Aim

- To characterize changes in ET-1 and NO production using an ischemia/reperfusion model.
- To correlate these changes with different concentrations of EGCG.
- To study the cardio-protective effects of EGCG during reperfusion.

Methods

1. Neonatal rat cardiomyocytes were isolated by enzymatic dissociation and subjected to simulated ischemia and reperfusion.
2. Cardiomyocytes were cultured in media with 4.5 g/L glucose at normoxic conditions (21% O2) for 24 hours or without glucose at hypoxic conditions (0.5% O2) for 18 hours.
3. Ischemic cells were then harvested or reperfused for 6 additional hours in glucose-containing media at normoxic conditions.

Methods (continued)

4. EGCG at 0.1, 1, or 10 μM was added at time = 0.
5. Total NO production was monitored with a gas-permeable probe (Harvard Apparatus). NO levels were measured at two areas in each cell culture plate immediately before harvesting. The mean was calculated and used for analysis.
6. ET-1 was measured in culture media by ELISA.
7. All assay results were read on a Multiskan EX microplate reader (Thermo EC) using Ascent software for data handling and analysis.
8. Statistical analysis determined by student's t-test with significance at p<0.05.

Results

- Ischemia increased NO production by cardiomyocytes compared with normoxic cells and 6 hr reperfusion of ischemic cells further increased NO levels.
- EGCG at 1 and 10 μM attenuated the ischemia-induced rise in NO after 18 hr.
- EGCG at 10 μM also alleviated the reperfusion-triggered elevation in NO after 18 hr of ischemia and 6 hr reperfusion.

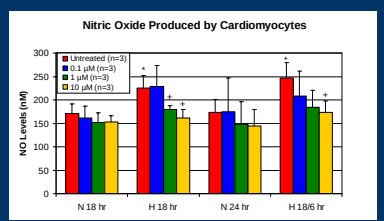

Figure 2. Nitric Oxide Produced by Cardiomyocytes. Ischemia increased NO production by cardiomyocytes compared with normoxic cells and 6 hr reperfusion of ischemic cells further increased NO levels. EGCG at 1 and 10 μM attenuated the ischemia-induced rise in NO after 18 hr. EGCG at 10 μM also alleviated the reperfusion-triggered elevation in NO after 18 hr of ischemia and 6 hr reperfusion. *N = normoxia, H = hypoxia, * = p<0.05 versus normoxia at same time, + = p<0.05 versus untreated at same time point.*

Results (continued)

- Cardiomyocyte ET-1 production increased with simulated ischemia and reperfusion (5.3 ± 2.7 pg/mL) compared with cells cultured under normal conditions for 24 hr (4.1 ± 3 pg/mL, p=.05), but EGCG treatment did not affect ET-1 production.

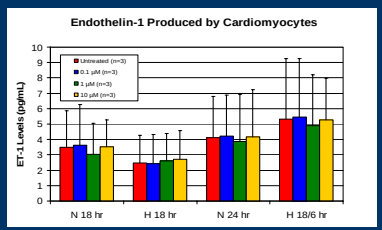

Figure 3. Endothelin-1 Produced by Cardiomyocytes. Cardiomyocyte ET-1 production increased with simulated ischemia and reperfusion (5.3 ± .2.7 pg/mL) compared with cells cultured under normal conditions for 24 hr (4.1 ± 3 pg/mL, p=.05), but EGCG treatment did not affect ET-1 production . *N = normoxia, H = hypoxia.*

Conclusion

1. Moderation of NO may be one mechanism by which EGCG provides myocardial protection.
2. A reduction of NO-generated mediators may be beneficial for minimizing inflammation and oxidative stress during ischemia and reperfusion.
3. EGCG treatment did not affect ET-1 production.

References:

[1]Aneja R, Hake PW, Burroughs TJ, Denenberg AG, Wong HR, Zingarelli BR. (2004) Epigallocatechin, a green tea polyphenol, attenuates myocardial ischemia reperfusion injury in rats. *Molecular Medicine.* 10:55-62.

Financial & Regulatory Disclosures:

None