The effects of Caffeic Acid Phenethyl Ester (CAPE) on oxidative stress and hypoxia-induced cell damage

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Introduction

Myocardial Infarction causes irreversible cell death in heart. Reperfusion to ischemic area can salvage dying heart tissue. However, reperfusion also leads to additional cell damage (1). Ischemia/Reperfusion (I/R) injury is caused by massive release of reactive oxygen species from intracellular (e.g., uncoupled electron transport chain) or extracellular sources (e.g., infiltrated neutrophils), which further leads to cell damage and cell death. To date, there is no clinical treatment available to mitigate reperfusion injury.
Caffeic Acid Phenethyl Ester (CAPE, Fig1), a natural component of propolis from honeybee hives, exhibits anti-oxidant and anti-inflammatory effects (2). Our lab has shown that CAPE given during reperfusion exerted cardioprotection in an isolated rat heart I (30 min)/R (60 min) injury model (3). However, the other effects and underlying mechanisms of CAPE under I/R conditions needs to be further determined. In this study, we evaluated the effects of CAPE on oxidative stress caused by activated rat neutrophils or hind limb I/R. We also determined the effects of CAPE on a hypoxia mimetic, cobalt chloride ($CoCl_2$), induced H9C2 cell death.

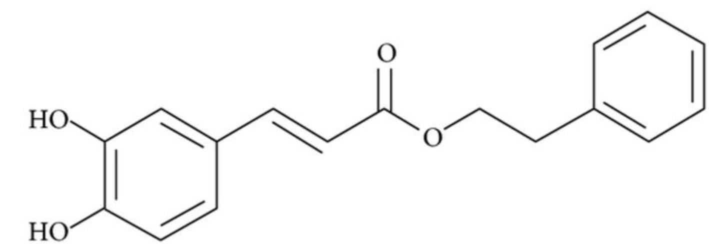

Figure 1. CAPE chemical structure.

Hypothesis

We hypothesized that CAPE would reduce superoxide production in isolated rat neutrophils. We also hypothesized that CAPE would reduce blood hydrogen peroxide levels in a hind limb I/R rat model. Lastly, given reduction in oxidative stress, CAPE would be cardioprotective against $CoCl_2$-induced cell death in H9C2 cardiac myoblasts.

Methods

Measurement of superoxide (SO) Release from Rat Neutrophils: Rat neutrophils were collected by intraperitoneal lavage after intraperitoneal injection of 0.5% glycogen. The SO release from neutrophils was measured spectrophotometrically (model 260 Gilford; Nova Biotech, El Cajon, CA) by the reduction of ferricytochrome c (Sigma Chemical Co.) as previously described. The effects of CAPE (0.5-40 μM) on phorbol-12-myristate-13-acetate (PMA, 30 nM) stimulated SO release from neutrophils (5×10^6) were calculated by the peak change (360 s) of absorbance at 550 nm from time 0.

Real-time Measurement of blood H_2O_2 levels in a hind limb I/R rat model: We have developed an innovative technique to real-time measure blood H_2O_2 levels from femoral veins: one subjected to I/R while the other is used as a non-ischemic sham control (4). The calibrated H_2O_2 microsensors (100μm, WPI inc.) connected to a free radical analyzer (Apollo 4000, WPI inc.) are inserted into a catheter placed inside each femoral vein. Ischemia of femoral circulation in one limb was induced by clamping the femoral artery/vein for 30 minutes, subsequently reperfusion was introduced for 60 min by removing the clamp. Drugs (CAPE, 0.95 mg/Kg, equivalent to 40 μM in blood) or saline (for non-drug control group) was applied through jugular vein catheter at the beginning of reperfusion. We continuously recorded blood H_2O_2 levels in pA and collect the data at 5 min. intervals during a 15 min. baseline period, 30 min. ischemia and 60 min. reperfusion. After experiments, the changes in blood H_2O_2 levels were calculated as relative H_2O_2 blood levels (μM) between I/R limb and sham limb after correction to the calibration curve of H_2O_2 microsensors.

Measurement of H9C2 cell viability after cobalt chloride incubation: H9C2 rat myoblasts were incubated in different doses of $CoCl_2$ for 24 hours to evaluate the dose-response effects of cobalt chloride on cell viability. Another set of experiments were further determine cell viability when H9C2 cells were pretreated with CAPE (0.5-20 μM) for 24 hr, then being incubated in $CoCl_2$ (800 μM) for an additional 24 hours. Cell viability was determined by measuring absorbance at 450 nm after adding tetrazolium as instructed by CCK8 kit (Dojindo Molecular Technologies, Inc).

Statistical Analysis: All data in the figures are presented as means ± S.E.M. The data of two groups was analyzed by Student t-test. $p<0.05$ are considered to be statistically significant.

Results

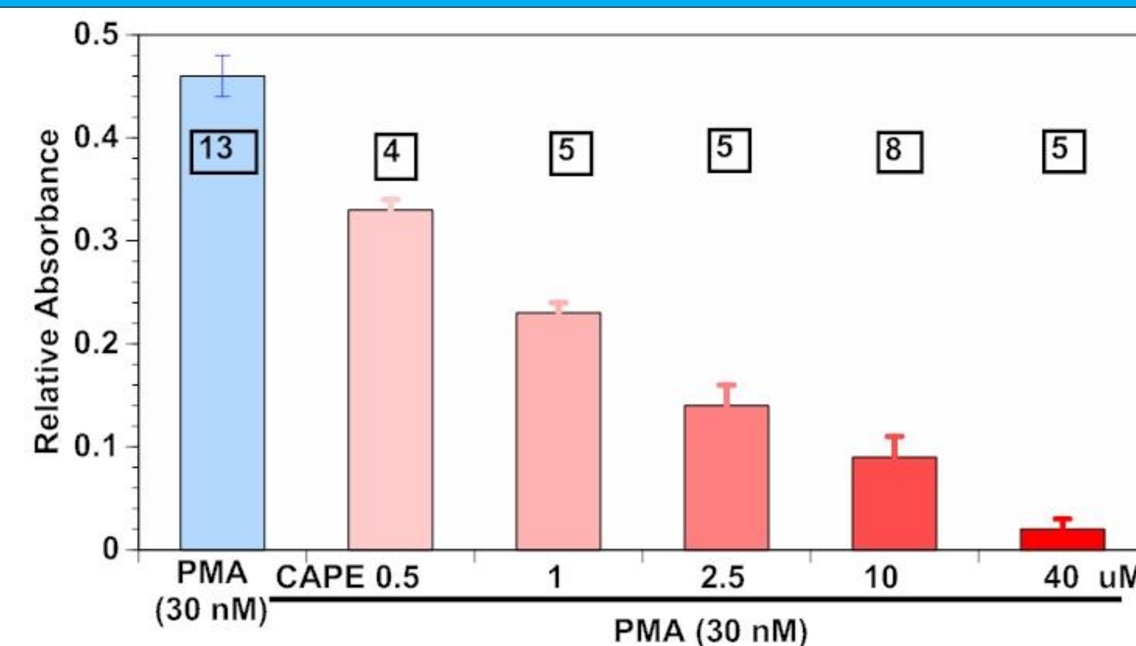

Figure 2. Dose dependent effects of CAPE (0.5- 40 μM) on neutrophil SO release stimulated by PMA (30 nM).

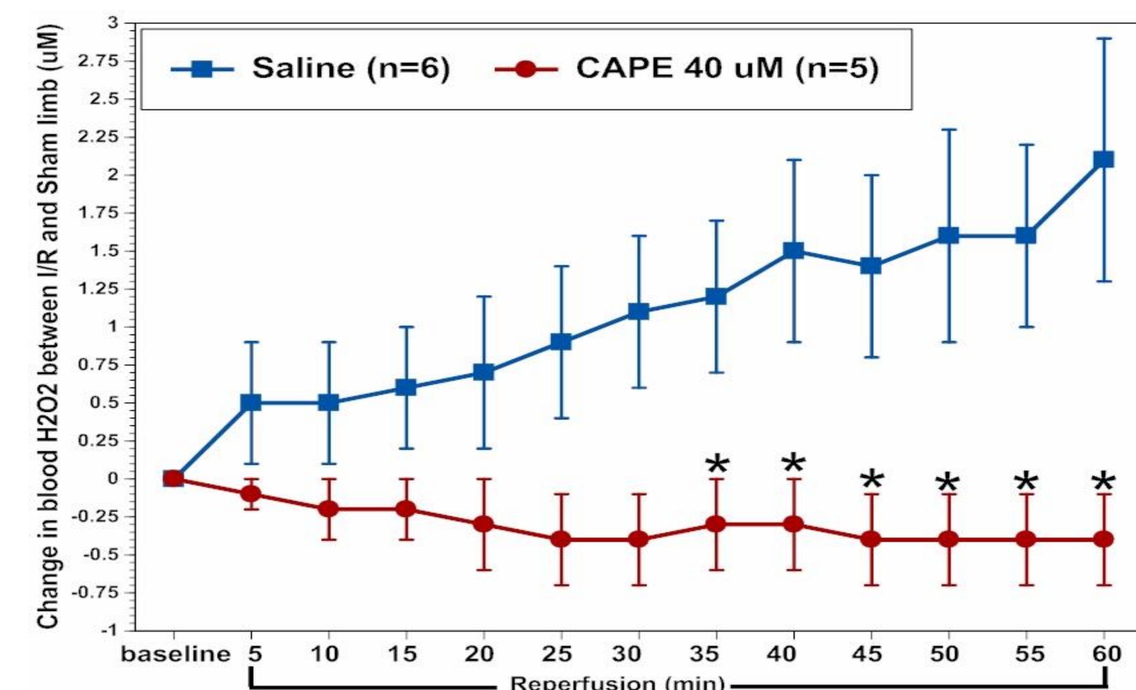

Figure 3. Effects of CAPE (40 μM) on blood H_2O_2 levels during reperfusion compared to saline control. *p<0.05 vs saline control.

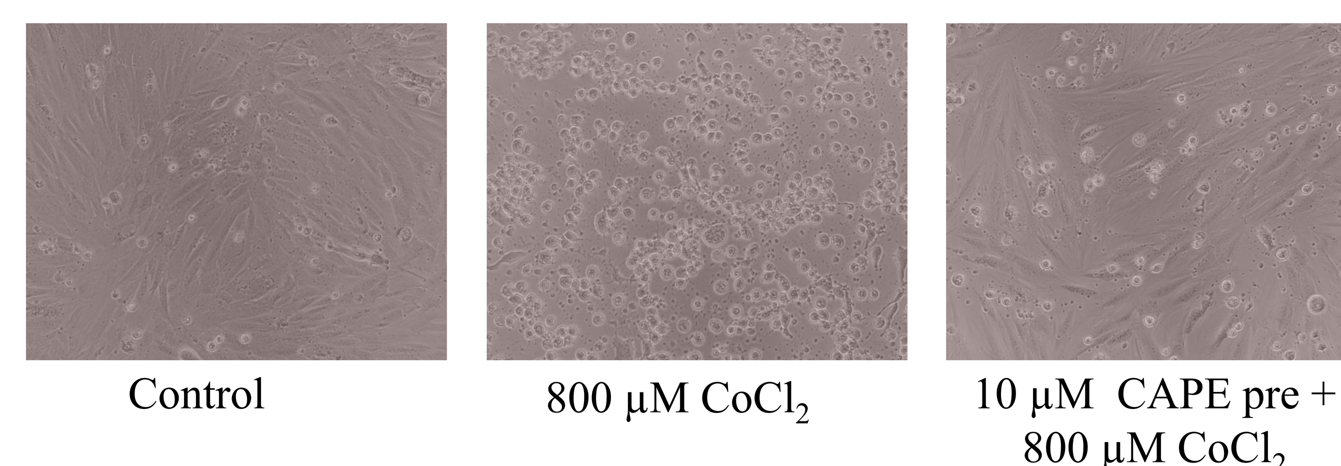

Figure 4. Representative pictures of H9C2 cells (10X) under control, 800 μM $CoCl_2$, and 10 μM CAPE pretreatment with 800 μM $CoCl_2$.

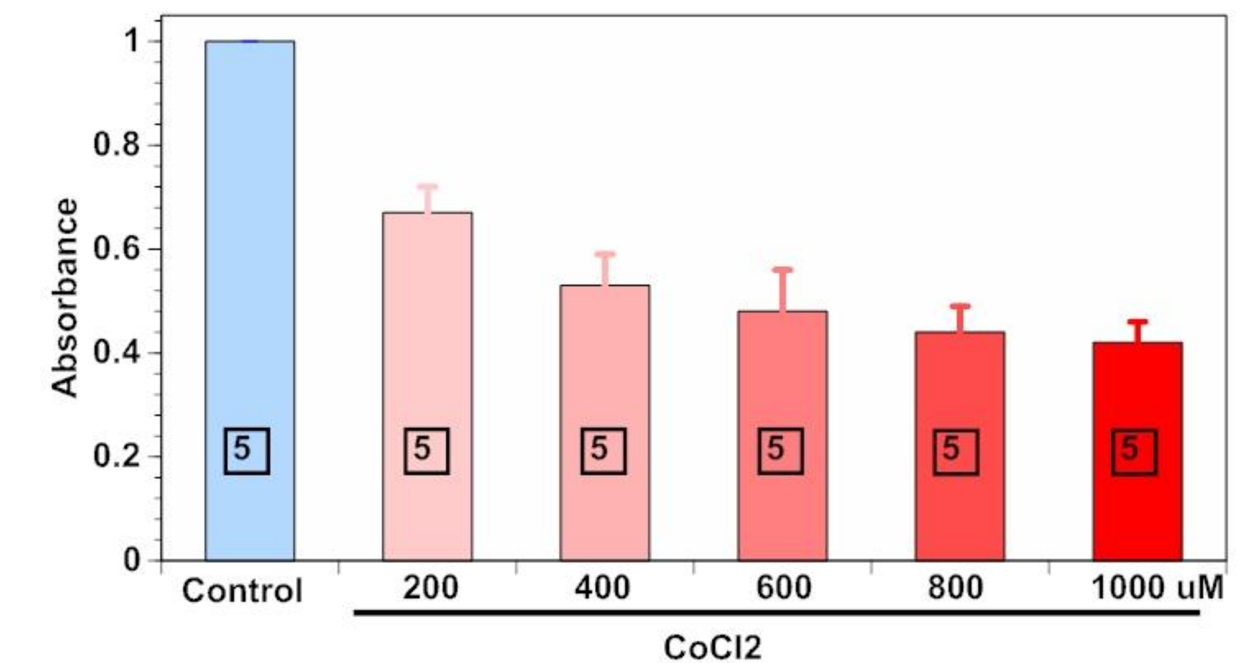

Figure 5. Dose-dependent effects of $CoCl_2$ (200 to 1000 μM) on H9C2 cell viability compared to control.

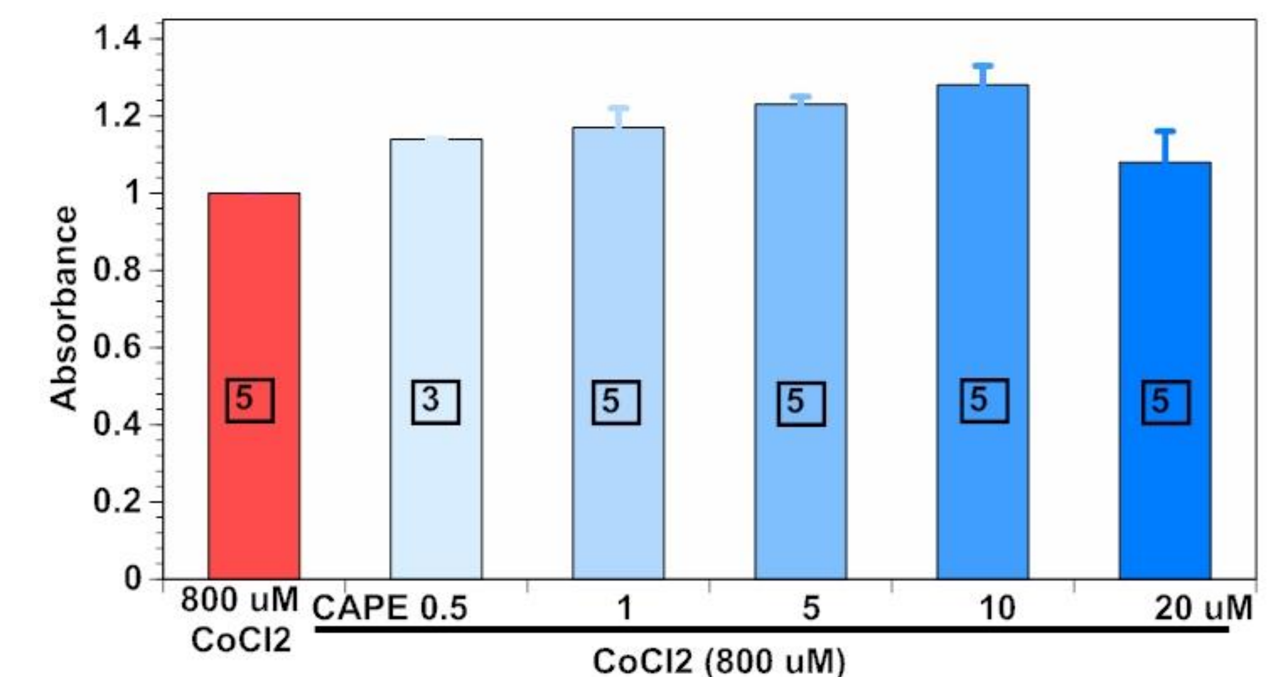

Figure 6. Dose-dependent effects of CAPE pretreatment (0.5 to 20 μM) on $CoCl_2$ (800 μM)-induced cell death.

Conclusions

We found that CAPE dose-dependently reduced SO release from PMA activated neutrophils. We also demonstrated that CAPE given during reperfusion significantly reduced blood H_2O_2 levels in hind limb I/R model. Lastly, CAPE pretreatment mitigated $CoCl_2$ induced H9C2 cell death. In summary, CAPE exerted anti-oxidant effects to reduce oxidative stress, which may protect cardiomyocytes from a hypoxia insult. The underlying mechanisms will be determined in future experiment.

References

1. Hausenloy, D. J., et al. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. Journal of Clinical Investigation. (2013); 123.1: 92-100.
2. Murtaza, G., et al. Caffeic acid phenethyl ester and therapeutic potentials. BioMed Research International. (2014): 1-9.
3. Castellano, A. J., et. al. The Cardioprotective Effects of Caffeic Acid Phenethyl Ester (CAPE) on Myocardial Ischemia/Reperfusion (I/R) Injury. The FASEB Journal. (2016); 30 (1): Supplement1207.2
4. Lindon H. Young, et. al. Direct measurement of hydrogen peroxide (H2O2) or nitric oxide (NO) release: A powerful tool to assess real-time free radical production in biological models. American Journal of Biomedical Sciences. (2011); 3(1):40-49.

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