The cardioprotective effects of caffeic acid phenethyl ester (CAPE) on myocardial ischemia/reperfusion (I/R) injury

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INTRODUCTION

Reperfusion injury is the acceleration of heart damage which occurs during the reintroduction of coronary blood flow to a prolonged ischemic area [1]. Oxidative stress is a major cause of reperfusion injury by reducing the bioavailability of nitric oxide (NO), damaging cellular function leading to cell death/apoptosis. To date, there is no effective clinical treatment for reperfusion injury. CAPE is an active component of propolis collected from honeybee hives that exhibits both anti-oxidant and anti-inflammatory effects [2]. Recently, CAPE when given prior to ischemia was found to be cardioprotective against I/R injury [3,4]. The beneficial effects of CAPE are possibly mediated by upregulating heme oxygenase-1 and/or increased bioavailability of NO. However, the effects of CAPE when given at reperfusion in myocardial I/R (MI/R) injury has not been evaluated. This study tested the effects of CAPE on postreperfused cardiac function, infarct size, and putative mechanisms in an isolated rat MI/R model when given at reperfusion.

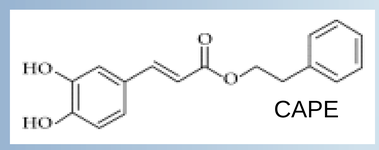

Figure 1. Chemical structure of CAPE. Adapted from Murtaza et al, 2014.

HYPOTHESIS

In this study, we hypothesized that CAPE when given at reperfusion would attenuate I/R induced cardiac contractile dysfunction and infarct size. Moreover, the cardioprotective effects of CAPE would be inhibited by a non-selective NO synthase inhibitor (N^G-nitro-L-arginine methyl ester (L-NAME)) or a heme oxygenase-1 inhibitor (tin protoporphyrin (SnPP)).

METHODS

Isolated Rat Heart MI/R Experiments: Hearts were isolated from male Sprague Dawley rats (275-325g, Charles River, Springfield, MA) via Langendorff heart preparation as previously described [5]. Experimental protocol is shown in Figure 2.

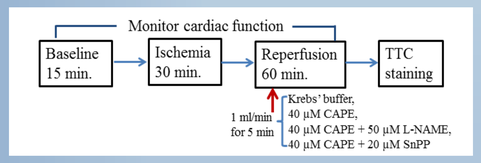

Figure 2. Flow diagram of experimental protocol.

A pressure transducer (SPR-524, Millar Instruments, Inc., Houston, TX) was inserted into the left ventricle to record cardiac function (e.g. left ventricular end systolic and diastolic pressure (LVESP & LVEDP, respectively). Coronary flow was measured by a flow probe (T106, Transonic Systems, Inc., Ithaca, NY) which was placed in the inflow perfusion line. Data was recorded using a Powerlab Station acquisition system (ADInstruments, Grand Junction, CO) every 5 min during baseline and reperfusion.

Determination of Infarct Size: At the end of the experiment, the left ventricle of the heart was sectioned into 2 mm thick slices that were subjected to 1% triphenyltetrazolium chloride (TTC) staining to detect infarcted (unstained) and viable (stained brick red) areas. Infarct size was expressed as the percentage of infarcted tissue weight to the total tissue weight as previously described [5].

Statistical Analysis: All data in the figures are presented as means ± S.E.M. The data was analyzed by ANOVA using post hoc analysis with Student-Newman-Keuls test. $p<0.05$ are considered to be statistically significant.

RESULTS & DISCUSSION

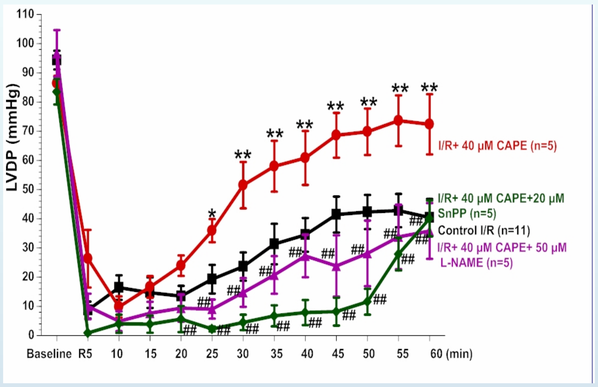

Figure 3. Time course of LVDP (Difference between LVESP and LVEDP) for Control I/R, I/R + 40 μM CAPE, I/R + 40 μM CAPE + 50 μM L-NAME, and I/R + 40 μM CAPE + 20 μM SnPP groups. (* p< 0.05, ** p< 0.01 vs. I/R; ##p< 0.01 vs. I/R + 40 μM CAPE)

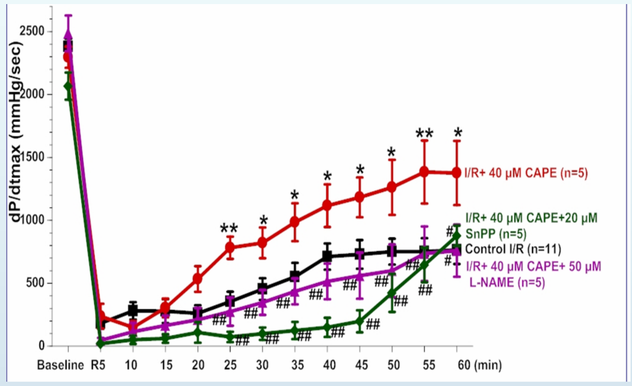

Figure 4. Time course of dP/dtmax (maximal rate of left ventricular systolic pressure over time) for Control I/R, I/R + 40 μM CAPE, I/R + 40 μM CAPE + 50 μM L-NAME, and I/R + 40 μM CAPE + 20 μM SnPP groups. (* p< 0.05, ** p< 0.01 vs. I/R; # p<0.05, ##p< 0.01 vs. I/R + 40 μM CAPE)

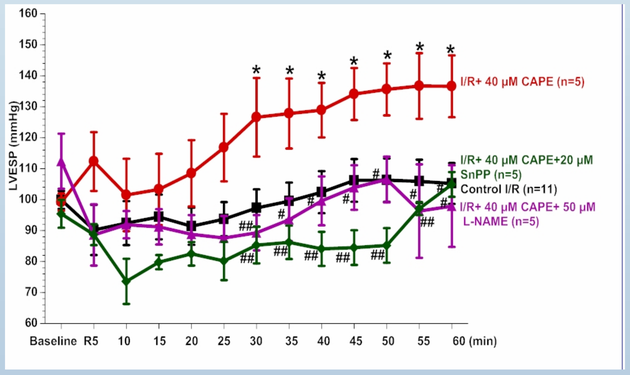

Figure 5. Time course of LVESP for Control I/R, I/R + 40 μM CAPE, I/R + 40 μM CAPE + 50 μM L-NAME, and I/R + 40 μM CAPE + 20 μM SnPP groups. (* p< 0.05 vs. I/R; # p<0.05, ##p< 0.01 vs. I/R + 40 μM CAPE)

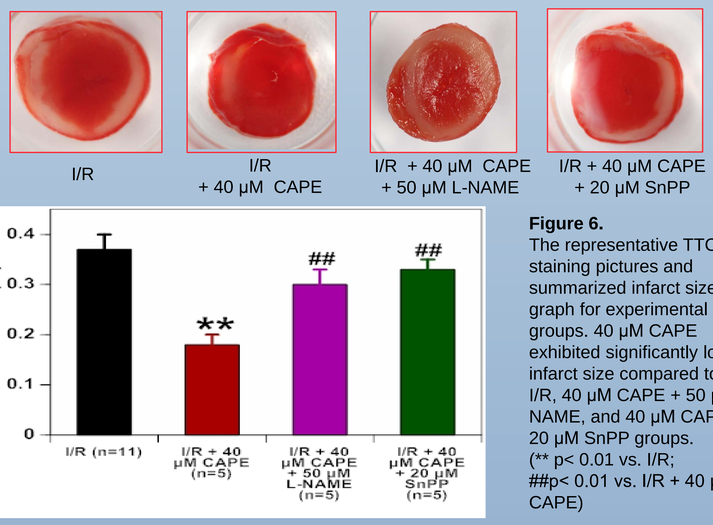

Figure 6. The representative TTC staining pictures and summarized infarct size graph for experimental groups. 40 μM CAPE exhibited significantly lower infarct size compared to the I/R, 40 μM CAPE + 50 μM L-NAME, and 40 μM CAPE + 20 μM SnPP groups. (** p< 0.01 vs. I/R; ##p< 0.01 vs. I/R + 40 μM CAPE)

CAPE (n=5) restored LVDP to 85 ± 14% of baseline value at 60 min post-reperfusion compared to untreated control I/R hearts (n=11) that only recovered to 45 ± 8% of baseline value ($p<0.01$).

CAPE (n=5) restored LVESP to 139 ± 14% of baseline value at 60 min post-reperfusion compared to untreated control I/R hearts (n=11) that only recovered to 106 ± 7% of baseline value ($p<0.05$).

CAPE (n=5) restored dP/dt_{max} to 60 ± 11% of baseline value at 60 min post-reperfusion compared to untreated control I/R hearts (n=11) that only recovered to 33 ± 5% of baseline value ($p<0.05$).

CAPE (n=5) also significantly reduced infarct size to 19 ± 2% compared to 37 ± 3% in untreated control I/R hearts (n=11) ($p<0.05$).

A non-selective nitric oxide synthase inhibitor, L-NAME (50 μM, n=5); or a heme oxygenase-1 inhibitor, SnPP (20 μM, n=5), significantly abolished the cardioprotective effects of CAPE (all $p<0.05$).

CONCLUSION

Our results suggest that CAPE when given at reperfusion improves post-reperfused contractile function and reduces infarct size. The cardioprotective effects of CAPE may be mediated by increasing NO bioavailability and/or heme oxygenase-1 activity. The effects of CAPE on mitochondrial function during I/R will be determined in future experiments.

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