The effects of Caffeic Acid Phenethyl Ester (CAPE) on hydrogen peroxide-induced oxidative stress in rat H9c2 myoblasts compared to common antioxidants

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Introduction

Cellular injury from oxidative stress, the result of increased reactive oxygen species (ROS) formation and/or decreased antioxidant reserves, is a common feature in many cardiovascular diseases. High levels of ROS have a harmful effect on the functional and structural integrity of the tissue, damaging proteins and lipids leading to loss of organelle function and cell death/apoptosis. However, common antioxidants (e.g. vitamin E) fail to show any benefits for cardiovascular disease patients in clinical studies. Therefore, development of a novel compound to mitigate oxidative stress is clinically necessary. Caffeic Acid Phenethyl Ester (CAPE, Figure 1), a natural compound derived from honeybee propolis, exhibits not only anti-oxidant but also anti-proliferative and anti-inflammatory effects. Our lab has shown that CAPE given during reperfusion significantly reduces hydrogen peroxide (H₂O₂) levels in an I/R model. We have also reported that the cardioprotective effects of CAPE may be mediated by increasing the bioavailability of heme oxygenase-1 (HO-1), the rate-limiting enzyme in heme degradation. HO-1 has shown upregulation in treatment with CAPE under oxidative stress in HUVEC cells. However, no studies have explored if CAPE pretreatment in H9c2 myoblasts following H₂O₂ injury can be cardioprotective by inducing HO-1. In this study, we compared the effects of CAPE to several known antioxidants (caffeic acid, vitamin C, and a vitamin E analog trolox) in H9c2 cells under oxidative stress induced by H₂O₂. We also investigated CAPE’s effects on intracellular reactive oxygen species (ROS) and heme oxygenase-1 (HO-1) induction in H₂O₂-induced H9c2 cells.

Hypothesis

We hypothesized that H9c2 rat myoblasts pretreated with CAPE would provide cardioprotection compared to common antioxidants under H₂O₂-induced oxidative stress. Given CAPE’s ability to limit cell death, we also hypothesized that CAPE pretreatment would reduce intracellular ROS production in H₂O₂-induced H9c2 cells. Further, we hypothesized that increasing CAPE dosage would lead to increased HO-1 induction.

Methods

Measurement of H9c2 cell viability after hydrogen peroxide and test compound incubation: H9c2 rat myoblasts (American Type Culture Collection) were cultured, maintained, and seeded 24 hours prior to experiments. Cells were treated with various doses of H₂O₂ (100-700 µM) for 24 hours to evaluate H₂O₂ dose effects on cell viability. To determine each test compound’s effect on H9c2 cells under oxidative stress, cells were pretreated with CAPE (1-40 µM), trolox (50-400 µM), caffeic acid (1-40 µM), or vitamin C (100-5000 µM) for 24 hours, then incubated with H₂O₂ (500 µM) for an additional 24 hours. Cell viability was determined by measuring absorbance at 450 nm using a CK-K assay (Dojindo Molecular Technologies). Cellular morphology was observed using microscopy.

Measurement of H9c2 intracellular ROS production following hydrogen peroxide and CAPE pretreatment incubation: H9c2 rat myoblasts were incubated with 25 µM non-fluorescent and cell-permeable dichlorofluorescein diacetate (DCFDA, Abcam) for 45 minutes. After washing of DCFDA with incubation buffer, cells were treated with various doses of CAPE (1-40µM) and H₂O₂, 500 µM. After incubation, the fluorescence, excited at 480 nm, was recorded at 520 nm using a fluoroscan Ascent FT scanner (Thermo Scientific) at 1 hour and 24 hours post-treatment. The fluorescence signals were normalized to the initial control at time zero. The relative fluorescence served as an index to the production of reactive oxygen species.

Results

We found that H₂O₂ dose-dependently reduces H9c2 cell viability. CAPE and Trolox dose-dependently rescue cell viability, while caffeic acid and vitamin C do not protect against H₂O₂-induced cell damage. We demonstrated that CAPE dose-dependently reduces intracellular ROS and reduces HO-1 expression. In summary, CAPE cardioprotective and antioxidant effects in H9c2 myoblasts are mediated by HO-1 induction. Future studies will test if CAPE’s effects are reduced by using a HO-1 inhibitor in H9c2 myoblasts under oxidative stress.

Conclusions

We validated that H₂O₂ dose-dependently reduces H9c2 cell viability. CAPE and Trolox dose-dependently rescue cell viability, while caffeic acid and vitamin C do not protect against H₂O₂-induced cell damage. We demonstrated that CAPE dose-dependently reduces intracellular ROS and reduces HO-1 expression. In summary, CAPE cardioprotective and antioxidant effects in H9c2 myoblasts are mediated by HO-1 induction. Future studies will test if CAPE’s effects are reduced by using a HO-1 inhibitor in H9c2 myoblasts under oxidative stress.

References


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