

Anti-Melanoma Effect of Caffeic acid and Caffeic Acid Phenethyl Ester through the alleviation of Oxidative Stress

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Introduction. According to American Academy of Dermatology (2013), one person dies every hour in the U.S. due to melanoma while incidence rates are higher in Australia. Oxidative stress activates transcription factors that lead to inflammatory pathways and in the long run, can play an important role in carcinogenesis mechanism i.e. melanoma development. Proliferation at high rates in cancer cells is closely associated to high concentrations of reactive oxygen species (ROS) [1]. Caffeic acid (CA), a polyphenolic phytochemical present in coffee beans and most vegetables, has numerous beneficial properties i.e. its anti-thrombotic effect [2]. Caffeic acid phenethyl ester (CAPE), an ester derivative of CA found in Propolis, is another ideal example exhibiting diverse biological activities such as anti-inflammatory, neoplastic and anti-cancer activities as tested on a variety of cell lines [3,4]. Both compounds inhibit NF-κB and certain enzyme activities such as lipoxygenases, cyclooxygenase, GST & xanthine oxidase and have been reported to have anti-metastatic and anti-tumor effects via selective MMP-9 enzyme activity suppression [5]. There are anti-cancer drugs available but are highly cytotoxic and have side effects. CA and CAPE meet the criteria of a desirable drug in terms of their bioavailabilities [6,7] and bioactivities having dual inhibitory activities for specific enzyme activities and melanoma-related proteins at the molecular level.

Materials and Methods. DPPH assay was performed on pure compounds of CA (C0625) and CAPE (C8221) to measure their radical scavenging activities. MTT assay was performed on B16 mouse melanoma and human epidermal melanocyte (HEM) to determine the effect of CA and CAPE on cell viability. Intracellular hydrogen peroxide produced by cells was measured using the OxiSelect fluorometric assay carried out on B16 and SK-MEL-28 human melanoma. Extracted proteins and RNAs were subjected to western blotting and real-time PCR, respectively, to quantify the expressions of the proteins associated with oxidative stress inhibition as well as to elucidate the pathways involved. Cell cycle assay was done to determine which phase the cells were arrested.

Results and Discussion. Various concentrations of CA and CAPE were tested and 15 μm and 20 μm were found to be the optimum concentrations . The effect of CA and CAPE were observed to be dose-dependent and their IC_{50}values for DPPH radical scavenging activity were comparable to that of the standard, Trolox. Our results agree with previous studies [5] that structural factors contribute to apparent active scavenging radical activity of both test compounds specifically the catechol moiety with a 3,4-dihydroxyl configuration which heavily influences their degrees of radical scavenging activity. These results suggest that these two compounds may be beneficial antioxidants for counteracting various free radicals under intracellular conditions. Differentiating the two, CA has one phenolic ring while CAPE have two phenolic rings linked by an esteric bond, as well as, a higher lipid solubility.

A sensitive quantitative fluorometric assay was also carried out on untreated (control), pre- or post-treated with CA or CAPE to measure the hydrogen peroxide concentrations present in the cells. Interestingly, although CA was able to lower the level of oxidative stress brought by treatment with hydrogen peroxide. Given either as pre- or post-treatment, α-MSH (positive control) and CAPE were limited to inhibiting oxidative stress when given as a pre-treatment. This phenomenon is suggested to have been affected by the loss of α-MSH in the cell lines in the study which was naturally found in normal cells as its defense mechanism against ROS. The inhibitory activities can be explained by the regulation of oxidant and antioxidant balance in the cells and that non-enzymatic antioxidants like CA and CAPE react with oxygen free radicals to suppress the need for endogenous O_2-scavenging antioxidants and enhance the cellular antioxidant activity. The extent of inhibition of melanoma-associated proteins distinguishes the biological activities of the two compounds apart. There appears to be a structure-activity relationship responsible for the compound differences. Regulation of proteins p-38, p-21, AKT, phospho-p53, and genes *Mitf* and *Atm* revealed its action mechanism to be related to DNA damage response and repair. The cell cycle results indicated that the specific inhibition of *Mitf* in cells treated with CA and CAPE were associated with G0/G1 arrest. Both have potential anti-aging effects and possibly prevent melanoma owing to their anti-oxidant properties. Further studies will be performed to elucidate the mechanism of action of CA and CAPE and their possible therapeutic effects against melanoma *in vivo.*

References

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