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PROPOLIS OR CAFFEIC ACID PHENETHYL ESTER (CAPE) INHIBITS GROWTH AND VIABILITY IN MULTIPLE ORAL CANCER CELL LINES.

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Abstract

Objective: Propolis is a natural antimicrobial resin from honeybee hives that contains caffeic acid phenethyl ester (CAPE), which has anti-proliferative activity against some human cancers, including colon, liver, lung and breast – although limited evidence has evaluated this potential in oral cancers. Based upon this information, the primary objective of this study was to evaluate the anti-tumor effects of CAPE against multiple well-characterized oral cancer cell lines. Methods: Using well-characterized oral cancer cell lines (SCC15, SCC25 and CAL27), CAPE was administered at 100 ug/mL to assess any effects on cellular viability or growth over three days. A normal, non-cancerous cell line (HGF-1) was also included.

Results: The results of this pilot study demonstrated that CAPE administration significantly reduced both viability and proliferation in all three oral cancer cell lines. Viability was significantly reduced between 30.3% and 35.4% among the oral cancer cell lines ($p<0.05$), but remained unchanged in the HGF-1 normal cell control ($p=0.878$). Growth was significantly inhibited between 53.1% and 60.6% among the oral cancer cell lines ($p<0.05$) but was not affected in the HGF-1 normal cell control ($p=0.341$).

Conclusions: Although the reductions in both cellular viability and proliferation were distinct for each cell line, all exhibited a similar trend and were within a narrowly defined range. These results strongly suggest that CAPE administration had a significant and immediate effect on oral cancer growth and viability and therefore should be considered as the basis for future studies as a potential complementary and alternative therapy for oral cancer.

Key words: Caffeic Acid Phenethyl Ester (CAPE), Propolis, Oral cancer, Complementary and alternative medicine.

Introduction:

Propolis is available over-the-counter and has been designated by the Food and Drug Administration (FDA) as GRAS or generally regarded as safe [1,2]. It has been used as a traditional homeopathic remedy, which is well known among apiary workers or bee keepers [3,4]). In addition, propolis has been demonstrated to facilitate honeybee resistance to mites and other potential beehive hazards [5,6].

Propolis is a natural antimicrobial resin from honeybee hives that contains caffeic acid phenethyl ester (CAPE), the main active component [7,8]. Propolis is reported to exhibit anti-bacterial and free radical scavenging properties [9-11]. Although some evidence has found propolis and extractions containing CAPE have anti-proliferative activity against some human cancers, including colon, liver, lung and breast – limited evidence has evaluated this potential in oral cancers [12-16].

Although some studies have used oral cancer cell lines, each of these studies utilized only one cell line or cell lines derived from only one patient [17-20]. Based upon this preliminary evidence, the primary goal of this study was to evaluate the anti-tumor potential of propolis - and more specifically the primary active agent caffeic acid phenethyl ester or CAPE against multiple well-characterized oral cancer cell lines in tandem. The working hypothesis for this pilot study was that CAPE would exhibit similar effects on oral cancer viability and growth inhibition.

Methods

Cell culture

Three oral cancer cell lines were obtained from American Tissue Culture Collection (ATCC), which included SCC15 (CRL-1623), SCC25 (CRL-1628) and CAL27 (CRL2095). CAL27 cells were grown in in Dulbecco's Modified Eagle's Medium (DMEM) with the addition of 10% fetal bovine serum (FBS) obtained from Fisher Scientific (Fair Lawn, NJ). SCC15 cells were grown in in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F12 (DMEM:F12) containing 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15mM HEPES and 0.5 mM sodium pyruvate, which was also supplemented with 10% FBS. A non-cancerous, normal cell control line HGF-1 (CRL-2014) was also obtained and cultured in DMEM with 10% FBS. All cells were maintained in a humidified tissue culture incubator supplemented with 5% $C0_2$.

Cell viability and proliferation

All assays were done in triplicate with each experimental condition containing eight wells, resulting in a combined data set of n=24 for each cell line and variable. Cell viability was measured at baseline (starting time point) and all subsequent time points using the Trypan Blue exclusion assay and the TC20 Cell Counter from Bio-Rad (Hercules, CA). Proliferation data was also obtained from the Trypan Blue exclusion assay, but was then subsequently quantified and confirmed using a BioTek ELx808 Absorbance Microplate Reader from Fisher Scientific (Fair Lawn, NJ).

Caffeic Acid Phenethyl Ester (CAPE)

Experimental assays were conducted using CAPE obtained through Fisher Scientific (Tocris Bioscience, Minneapolis, MN), Formula Weight 284.31- also known as 3-(3,4- Dihydroxyphenyl)-2-propenoic acid 2-phenylethyl ester. Assays were conducted using 100 ug/mL, which is within the concentration range 50 – 200 ug/mL utilized in other studies involving CAPE [17-20].

Statistical analysis

Differences in viability and proliferation between the starting time point and all subsequent time points were measured using two-tailed Students t-tests with a significance value of α=0.05. Because multiple two-tailed t-tests may have a higher possibility of Type I error, analysis of variance (ANOVA) was also performed to verify each result.

Results

Baseline measurements of cell viability revealed similar, non-significant differences among each

cell line, which ranged from 79.3% to 84.2% (Figure 1). More specifically, SCC15 (81.3%), SCC25 (79.3%), CAL27 (81.1%) and HGF-1 (84.2%) exhibited comparable levels of viability that were not statistically significant (p>0.3). The addition of CAPE at a concentration of 100 ug/ML significantly reduced cellular viability by the first time point, day one (d1) in all oral cancer cell lines; SCC15 -17.4% (63.9% viability), SCC25 -22.2% (57.1% viability), CAL27 -21.3% (59.8%), which was significantly lower than the initial baseline measurements, p<0.05. In addition, these effects were also observed at two additional time points (day two, d2 and day three, d3), which were also significantly lower than the baseline measurements and negative controls (without the addition of CAPE), p<0.05. However, the addition of CAPE did not exhibit any significant effect on the normal, non-cancerous oral cell line HGF-1 at any time point of this assay, p=0.878.

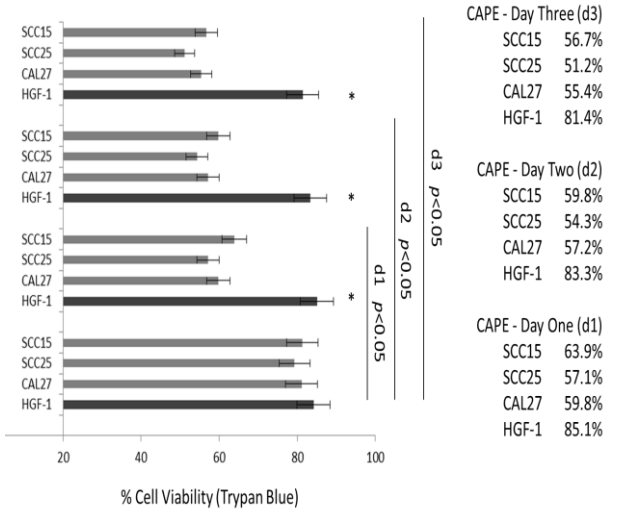

Figure 1: Effects of CAPE (100 ug/mL) on cellular viability. The addition of CAPE (100 ug/mL) significantly reduced viability significantly among the oral cancer cell lines SCC15, SCC25 and CAL27 at each time point (days one, two and three: d1, d2, d3), compared with baseline measurements and parallel non-CAPE (negative) controls, p<0.05. The normal gingival fibroblast cell line HGF-1 did not exhibit any significant changes to viability under CAPE administration, p=0.878*.

To determine if the significant reductions in cellular viability induced by CAPE administration among the oral cancer cell lines also affected cell growth, 96-well proliferation assays were also conducted using CAPE at these concentrations (Figure 2). The results of this assay revealed that HGF-1 proliferation was similar under both the negative control and experimental (CAPE) assay conditions, p=0.341. However, these results also clearly demonstrated that CAPE administration was sufficient to significantly reduce oral cancer growth among all three cell lines tested.

More specifically, CAPE administration inhibited SCC15 proliferation by 24.4% over twenty four hours (d1), by 37.1% by the second day (d2), and by 60.6% on day three (d3), which was statistically significant at all three time points, p<0.05. Similarly, SCC25 proliferation was also significantly inhibited by CAPE administration - by 29.7% at d1, by 36.4% at d2, and by 56.6% at d3, p<0.05. Finally, CAPE administration was sufficient to inhibit CAL27 proliferation by 36.4% at d1, 37.2% at d2, and by 53.1% at d3, p<0.05.

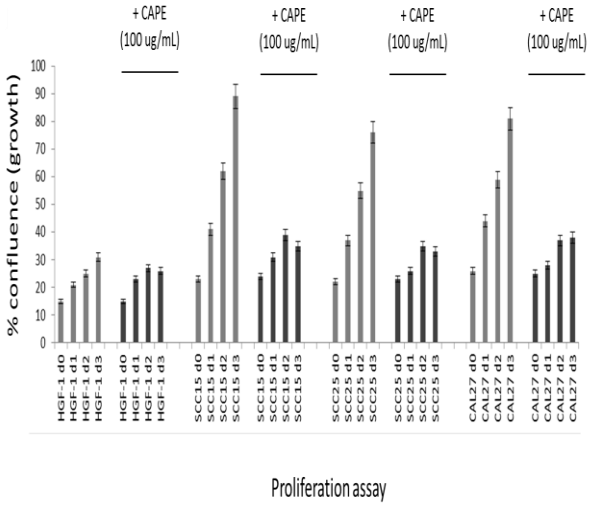

Figure 2: Effects of CAPE (100 ug/mL) on cellular proliferation. Oral cancer growth was significantly inhibited by CAPE administration at each time point of the three day assay, with the most significant reduction observed at day three (d3) for CAL27 (-53.1%), SCC25 (-56.6%), and SCC15 (-60.6%), p<0.05. Non-cancerous, normal cell control HGF-1 was not significantly inhibited by CAPE administration, p=0.341.

Discussion

The working hypothesis for this pilot study was that CAPE would exhibit similar effects on oral cancer viability and growth inhibition. The results of this pilot study demonstrated that CAPE administration significantly reduced both viability and proliferation in three, well-characterized oral squamous cell carcinoma cell lines. Although the reductions in both cellular viability and proliferation were distinct for each cell line, all exhibited a similar trend and were within a narrowly defined range, which provides support for the initial hypothesis.

Due to the similar response from all three oral cancer cell lines, an investigation into the mechanisms that may be responsible for these observations is warranted. Although this initial pilot study did not seek to determine the mechanisms of action, previous reports of the anti-oxidant, anti-mitogenic, and anti-carcinogenic properties of CAPE suggest that some of the mechanisms responsible for these effects include the inhibition of NFkB, lipid peroxidation, protein tyrosine kinase, ornithine decarboxylase, and matrix metalloproteinase (MMP)-9 catalytic activity.[21-23] Other studies have revealed that enhanced expression and activation of the tumor suppressor p53 and activation of pro-apoptotic Bax and caspase-3 may also be induced by CAPE administration.[24,25] These results must also be viewed in context of the limitations that were intrinsic to this type of pilot study. First, and most importantly, this study was limited to only three commercially available oral cancer cell lines. Future studies should include additional oral cancer cell lines, as well as other normal oral cell lines, which should provide additional insights and information about the potential anti-oral cancer properties of CAPE administration. In addition, this study involved a relatively short time interval (three days), although the significant reductions in cell proliferation over this short time period may suggest CAPE administration may provide a promising complementary and alternative therapy to traditional oral cancer treatments.

Conclusion

Although these data are preliminary, the results clearly demonstrated CAPE administration had a significant and immediate effect on oral cancer growth and viability and therefore should be considered as the basis for future studies as a potential therapeutic agent for oral cancer.

References

1. Varoni EM, Lodi G, Sardella A, Carrassi A, Iriti M. Plant polyphenols and oral health: old phytochemicals for new fields. Curr Med Chem. 2012;19(11):1706-20. Review. PMID: 22376030
2. Chan GC, Cheung KW, Sze DM. The immunomodulatory and anticancer properties of propolis. Clin Rev Allergy Immunol. 2013 Jun;44(3):262-73. Review. PMID: 22707327
3. Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. J Ethnopharmacol. 2005 May 13;99(1):69-73. PMID: 15848022
4. Tran VH, Duke RK, Abu-Mellal A, Duke CC. Propolis with high flavonoid content collected by honey bees from Acacia paradoxa. Phytochemistry. 2012 Sep;81:126-32. Epub 2012 Jul 9. PMID: 22784552
5. Popova M, Reyes M, Le Conte Y, Bankova V. Propolis chemical composition and honeybee resistance against Varroa destructor. Nat Prod Res. 2014;28(11):788-94. Epub 2014 Jan 31. PMID: 24483289
6. Antúnez K, Harriet J, Gende L, Maggi M, Eguaras M, Zunino P. Efficacy of natural propolis extract in the control of American Foulbrood. Vet Microbiol. 2008 Oct 15;131(3-4):324-31. Epub 2008 May 27. PMID: 18508208
7. Zhang P, Tang Y, Li NG, Zhu Y, Duan JA. Bioactivity and chemical synthesis of caffeic acid phenethyl ester and its derivatives. Molecules. 2014 Oct 13;19(10):16458-76. Review. PMID: 25314606
8. Cornara L, Biagi M, Xiao J, Burlando B. Therapeutic Properties of Bioactive

Compounds from Different Honeybee Products. Front Pharmacol. 2017 Jun 28;8:412. eCollection 2017. Review. PMID: 28701955

9. Tolba MF, Azab SS, Khalifa AE, Abdel-Rahman SZ, Abdel-Naim AB. Caffeic acid phenethyl ester, a promising component of propolis with a plethora of biological activities: a review on its anti-inflammatory, neuroprotective, hepatoprotective, and cardioprotective effects. IUBMB Life. 2013 Aug;65(8):699-709. Epub 2013 Jul 11. Review. PMID: 23847089
10. Murtaza G, Karim S, Akram MR, Khan SA, Azhar S, Mumtaz A, Bin Asad MH. Caffeic acid phenethyl ester and therapeutic potentials. Biomed Res Int. 2014;2014:145342. Epub 2014 May 29. Review. PMID: 24971312
11. Akyol S, Ugurcu V, Balci M, Gurel A, Erden G, Cakmak O, Akyol O. Caffeic acid phenethyl ester: its protective role against certain major eye diseases. J Ocul Pharmacol Ther. 2014 Nov;30(9):700-8. Epub 2014 Aug 6. Review. PMID: 25100535
12. Watanabe MA, Amarante MK, Conti BJ, Sforcin JM. Cytotoxic constituents of propolis inducing anticancer effects: a review. J Pharm Pharmacol. 2011 Nov;63(11):1378-86. Epub 2011 Sep 27. Review. PMID: 21988419
13. Ozturk G, Ginis Z, Akyol S, Erden G, Gurel A, Akyol O. The anticancer mechanism of caffeic acid phenethyl ester (CAPE): review of melanomas, lung and prostate cancers. Eur Rev Med Pharmacol Sci. 2012 Dec;16(15):2064-8. Review. PMID: 23280020
14. Akyol S, Ozturk G, Ginis Z, Armutcu F, Yigitoglu MR, Akyol O. In vivo and in vitro antıneoplastic actions of caffeic acid phenethyl ester (CAPE): therapeutic perspectives. Nutr Cancer. 2013;65(4):515-26. Review. PMID: 23659443
15. Patel S. Emerging Adjuvant Therapy for Cancer: Propolis and its Constituents. J Diet Suppl. 2016;13(3):245-68. Epub 2015 Feb 27. Review. PMID: 25723108
16. Kuo YY, Jim WT, Su LC, Chung CJ, Lin CY, Huo C, Tseng JC, Huang SH, Lai CJ, Chen BC, Wang BJ, Chan TM, Lin HP, Chang WS, Chang CR, Chuu CP. Caffeic Acid phenethyl ester is a potential therapeutic agent for oral cancer. Int J Mol Sci. 2015 May 12;16(5):10748-66. Review. PMID: 25984601
17. Chung LC, Chiang KC, Feng TH, Chang KS, Chuang ST, Chen YJ, Tsui KH, Lee JC, Juang HH. Caffeic acid phenethyl ester upregulates N-myc downstream regulated gene 1 via ERK pathway to inhibit human oral cancer cell growth in vitro and in vivo. Mol Nutr Food Res. 2017 Sep;61(9). Epub 2017 Mar 20. PMID: 28181403
18. Utispan K, Chitkul B, Koontongkaew S. Cytotoxic Activity of Propolis Extracts from the Stingless Bee Trigona Sirindhornae Against Primary and Metastatic Head and Neck Cancer Cell Lines. Asian Pac J Cancer Prev. 2017 Apr 1;18(4):1051-1055. PMID: 28547940
19. Czyżewska U, Siemionow K, Zaręba I, Miltyk W. Proapoptotic Activity of Propolis and Their Components on Human Tongue Squamous Cell Carcinoma Cell Line (CAL-27). PLoS One. 2016 Jun 9;11(6):e0157091. eCollection 2016. PMID: 27281369
20. Kuo YY, Lin HP, Huo C, Su LC, Yang J, Hsiao PH, Chiang HC, Chung CJ, Wang HD, Chang JY, Chen YW, Chuu CP. Caffeic acid phenethyl ester suppresses proliferation and survival of TW2.6 human oral cancer cells via inhibition of Akt signaling. Int J Mol Sci. 2013 Apr 24;14(5):8801-17. PMID: 23615471
21. Chung TW, Moon SK, Chang YC, Ko JH, Lee YC, Cho G, Kim SH, Kim JG, Kim CH. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J. 2004 Nov;18(14):1670-81. PMID: 15522912
22. Peng CY, Yang HW, Chu YH, Chang YC, Hsieh MJ, Chou MY, Yeh KT, Lin YM, Yang SF, Lin CW. Caffeic Acid phenethyl ester inhibits oral cancer cell metastasis by regulating matrix

metalloproteinase-2 and the mitogen-activated protein kinase pathway. Evid Based Complement Alternat Med. 2012;2012:732578. Epub 2012 Dec 18. Erratum in: Evid Based Complement Alternat Med. 2016;2016:6728642. PMID: 23320037

23. Wadhwa R, Nigam N, Bhargava P, Dhanjal JK, Goyal S, Grover A, Sundar D, Ishida Y, Terao K, Kaul SC. Molecular Characterization and Enhancement of Anticancer Activity of Caffeic Acid Phenethyl Ester by γ Cyclodextrin. J Cancer. 2016 Aug 11;7(13):1755-1771. eCollection 2016. PMID: 27698914

24. Yu HJ, Shin JA, Yang IH, Won DH, Ahn CH, Kwon HJ, Lee JS, Cho NP, Kim EC, Yoon HJ, Lee JI, Hong SD, Cho SD. Apoptosis induced by caffeic acid phenethyl ester in human oral cancer cell lines: Involvement of Puma and Bax activation. Arch Oral Biol. 2017 Dec;84:94-99. Epub 2017 Sep 25. PMID: 28965045

25. Lee YJ, Kuo HC, Chu CY, Wang CJ, Lin WC, Tseng TH. Involvement of tumor suppressor protein p53 and p38 MAPK in caffeic acid phenethyl ester-induced apoptosis of C6 glioma cells. Biochem Pharmacol. 2003 Dec 15;66(12):2281-9. PMID: 14637186