



Evaluation of the effect of Chrysin and Caffeic acid phenethyl ester on eIF4E expression in AGS cell line

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ABSTRACT

Introduction: The Ras/Akt/mTORC1 signal transduction pathways play a critical role in regulating translation and converge on initiation factor eukaryotic translation initiation factor 4E (eIF4E) which is overexpressed in various malignancies. In the current study we aimed to assess the effect of chrysin and caffeic acid phenethyl ester (CAPE) on eIF4E expression level in human stomach cancer AGS cell line.

Methods: AGS cells were treated with 15, 20, 30 and 40 μ M concentration of chrysin and CAPE separately, then eIF4E expression was evaluated in treated cells using real time-PCR method.

Results: A significant decrease in eIF4E expression in the cells following 40 μ M chrysin treatment was observed ($p < 0.05$). There was a significant decrease in CAPE-treated cells in a dose dependent manner. Indeed the cells treated with 30 and 40 μ M concentrations of CAPE, showed a significant decline in eIF4E expression ($p < 0.05$).

Conclusion: Our results suggest that CAPE and chrysin may be useful as a potential therapeutic agent for treatment of gastric cancers with an elevated eIF4E expression level.

Implication for health policy/practice/research/medical education:

Chrysin and caffeic acid phenethyl ester – two components of propolis decreases eukaryotic translation initiation factor 4E (eIF4E) gene expression and might be useful in developing chemotherapeutic agents for treating human gastric cancer.

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Introduction

Gastric cancer is the cause of cancer-related mortality in the world. It is the fourth most frequent cancer while is the second most common cause of cancer death (1). However, American Cancer Society has estimated that stomach cancer affects 21,600 new cases in the USA in 2013 (2). This disease had more prevalence in Asian populations, including Korea, Japan, and China (3). Despite considerable improvements in surgical, chemo, radio and other adjuvant therapies, the five-year survival rate of patients at the advanced stage remains less than 20

to 25% (4,5). Recently, regulation of oncogenesis that is grip of eukaryotic translation initiation factor 4E (eIF4E), has been considered by many researchers. eIF4E is one of the subunits of eIF4F complex which recognizes the 5' cap structure of spectrum mRNAs involving in angiogenesis, metastasis and cell growth (6) which is activated via PI3K/AKT signaling pathway (7,8). Recently, there have been many reports on the elevated levels of eIF4E in various human cancers such as human gastric cancer cell lines (9). Hence, expression of this factor may be a potential target for therapeutic purpose for cancer.

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Chrysin and Caffeic Acid Phenyl Ester (CAPE) are two components of propolis. These components have important biological roles. It has been proposed that propolis components such as chrysin and CAPE could be potential candidate agents in treatment of cancer due to their beneficial anticancer properties (10-12). It has been demonstrated before that Chrysin and CAPE may have anti-cancer properties because of apoptotic induction and inactivation of PI3K/Akt pathway effects (13-15).

Many studies have reported an elevated activity of PI3K/Akt pathways and expression of eIF4E in cancer cells (9,16) and it has been proposed that chrysin may inactivate PI3K/Akt signaling pathway through down-regulation of NF- κ B pathway and conversely, activating of IAP. Thus, it might play an important role in apoptosis induction by promoting caspase-3 activity (15). Moreover, U937 cells treated by chrysin have shown an increased cytochrome c release from the mitochondria into cytosol (15).

CAPE and Chrysin activate several caspases, they inhibit PI3K/Akt signal pathway, IAP, c-FLIP (anti-apoptotic proteins), and lead to suppress in the activity of IKK and NF- κ B (11,12). Considering multiple beneficial effects of CAPE and Chrysin in apoptosis and down-regulating of eIF4E, we conducted an experiment to evaluate eIF4E mRNA expression with this component in gastric cancer AGS cell line.

Materials and Methods

Cell culture and treatment

In this experimental study, the human AGS cell line was obtained from Royan institute and was cultured in DMEM supplemented with 10% FBS and 1% Penicillin-streptomycin.

IC₅₀ was determined in AGS cells following incubation a serial dilutions of chrysin (Sigma Aldrich cat#:C80105) and CAPE (Sigma Aldrich cat#:C8221) separately for 48 h. Then AGS cells were treated with Chrysin and CAPE in four concentration 15, 20, 30 and 40 μ M for 48 h in triplicate.

RNA extraction and cDNA synthesis

Total RNA was extracted using Biozol (cat# BSC51M1) according Kit protocol. RNA concentration was measured by NanoDrop1000 UV-Vis spectrophotometer (Thermo Scientific Inc., USA). Then, cDNA was synthesized according to the manufacturer's instructions (Thermo Scientific Inc., USA).

Real time PCR assay

Real time-PCR was performed using Rotor-Gene 3000 (Corbett Robotics, Australia), Maxima SYBR Green/Rox qPCR Master Mix (Thermo Scientific Inc., USA) and specific primers. Primer sequences for eIF4E were: sense 5'-CCTACAGAAC AGATGGGCACTC-3', antisense 5'-GCCCAAAGTCTTCAACAGTATCA-3' (6) and for GAPDH was sense: 5'-TTCACCACCATGGAGA-

AGGC-3' RW: 5'-CCCTTTTGGCTC CACCCT-3'.

Each reaction was performed in a final 25 μ l mixture containing 12.5 μ l of Maxima SYBR Green/Rox qPCR Master Mix, 2 μ l of each primers, 11.1 μ l of DEPC Treated Water and 1 μ l cDNA. Thermal cycling was performed as described herein: initial denaturation for 10 min at 95 $^{\circ}$ C; followed by 40 cycles of 95 $^{\circ}$ C for 30 s, 57 $^{\circ}$ C for 30 s and 72 $^{\circ}$ C for 30 s.

The melting curve plot profile of real time PCR product was: Ramp from 72 degrees to 95 degrees, Rising by 1 degree(s) each step, Wait for 45 s on first step, then Wait for 5 s on for each step after wards Acquire to Melt A on FAM/ SYBR. eIF4E expression was normalized with GAPDH mRNA and relative expression analyzed using $\Delta\Delta$ Ct method.

Statistics analysis

The results were expressed as mean \pm SD and statistical analyses were done using GraphPad Prism software (v5.01, USA) and Kruskal-Wallis tests followed by post hoc comparisons, Dunn's multiple comparison.

Results

Effect of Chrysin on eIF4E

Different chrysin concentrations ranging around IC₅₀ dose were used to evaluate the effect of chrysin on expression of eIF4E.

As it is shown in Figure 1 expression of eIF4E was not significantly altered by 15, 20 and 30 μ M concentrations of chrysin, whereas concentration of 40 μ M markedly decreased eIF4E expression in AGS cells (3.84-fold, $p \leq 0.05$).

Effect of CAPE on eIF4E

The effect of CAPE on AGS cells is shown in Figure 2, expression of eIF4E was not significantly altered by 10, 15, and 20 concentrations of CAPE, whereas concentration of 30 and 40 μ M noticeable decreased eIF4E expression in CAPE treated AGS cells (3.03-fold, $p \leq 0.05$ for

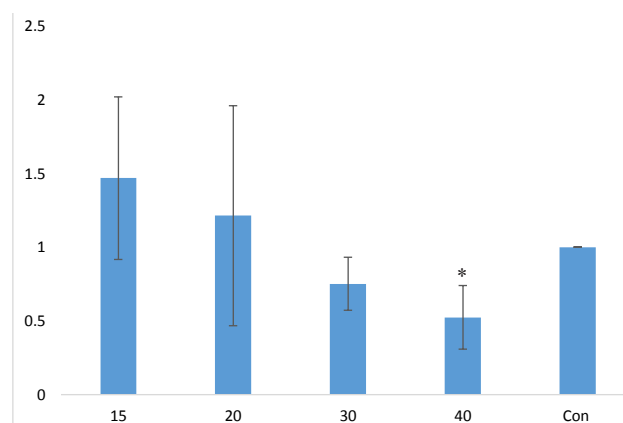


Figure 1. The effect of chrysin on eIF4E expression in AGS cells. The data are expressed as means \pm SD and (*) $p \leq 0.05$ compared with the corresponding value of control group.

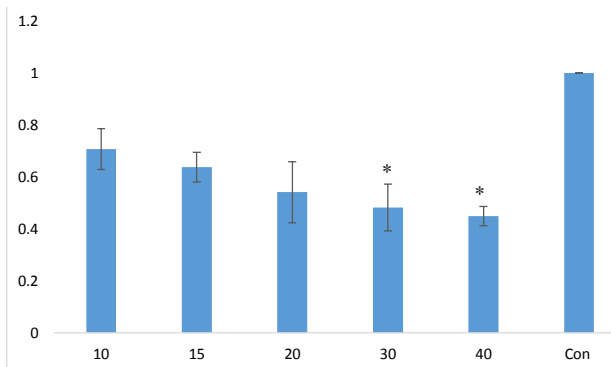


Figure 2. The effect of CAPE on eIF4E expression in AGS cells. The data are expressed as means \pm SD and (*) $p \leq 0.05$ compared with the corresponding value of control group.

30 μ l concentration and 2.85-fold, $p \leq 0.05$ for 40 μ l concentration).

Discussion

Here we showed that eIF4E mRNA was down-regulated in AGS cells after chrysin or caffeic acid treatment. Many studies have shown the eIF4E up-regulation in various cancers such as gastric, prostate, breast, colon, lung and skin cancers (9,17-24). However, eIF4E regulates a set of oncogenes which control proliferation including CMYC, CDK2, Cyclin D1 or oncogenes that involved in metastasis such as MMP9 and Heparanase or oncogenes that play roles in invasion and angiogenesis like Mcl-1, Bcl-2, Survivin, VEGF and FGF2 (7,8).

A previous study by Liang *et al* has shown that eIF4E was increased in different gastric cancer cells including AGS cell line which may involve in vascular invasion (9).

Furthermore, recent studies have provided evidence that chrysin activates Bax, p53, p21 caspases and p38 MAPK and conversely deactivates NF- κ B pathway. Therefore, inhibition of IAP, c-FLIP and Akt activity derived by chrysin and caffeic acid may induce extrinsic pathway of apoptosis (11,12).

Targeting of eIF4E by ribavirin which compete with eIF4E for coupling to the m7G cap and therefore blocks eIF4E activity, led to a relative improvement in patients with acute myeloid leukemia (25). Considering the fact that Bcl2 and several caspases are downstream targets of eIF4E and suppressing effects of chrysin and caffeic acid on eIF4E expression, our results support the notion that chrysin and caffeic acid may have anti-cancer effects by inducing apoptosis in cancer cells.

It has been previously shown that chrysin significantly suppressed migration and invasion of triple-negative breast cancer cells (26). Moreover, it was previously demonstrated by Samarghandian *et al* that chrysin restrain the development of lung cancer cells. They notify that chrysin induces apoptosis in cancer cells via regulation of the Bcl-2 family, caspase-3 and -9 (27).

A previous report by Lirdprapamongkol *et al* indicated a

remarked over expression of VEGF in mouse T14 breast cancer cells led to resistance of these cells to apoptosis. This study showed that antimetastatic activity of DR5mAb was increased when it used with chrysin (28).

In one study that was performed by Chung *et al*, they suggest that the antimetastatic and anti-tumor effects of CAPE are mediated through the selective suppression of MMP-9 enzyme activity and transcriptional down-regulation by the dual inhibition of NF- κ B as well as MMP-9 catalytic activity (29). The MMP-9 is one of eIF4E targets therefore our results that show decline of eIF4E expression in CAPE treatment cells, confirm the antimetastatic role of this compound.

Two other studies was shown that CAPE inhibits the NF- κ B factor which Augments the susceptibility of intestinal epithelial cells to anticancer drugs. So, these studies present CAPE to be an effective assistant of chemotherapy and augment therapeutic efficacy therefore it can decline chemotherapy-induced toxicities (30,31).

Murtaza *et al* suggest that CAPE is an effective inhibitor of NF- κ B and a stimulator of the functions of glutathione S-transferase, it drains GSH levels. Subsequently, tumor cells are radiosensitized due to this drainage (32).

With regards the co-relation of eIF4E expression with cancer incidence and our observations suggest that chrysin and caffeic acid might be promising treatments for cancer which may improve many aspects of cancer such as angiogenesis and metastasis derived by down-regulating of eIF4E expression. Moreover, since eIF4E as a key components of AKT pathway, was down-regulated by both chrysin and caffeic acid, this suggests that chrysin and caffeic acid could be useful in cancer treatment due to their suppressive effect on AKT signaling. The exact mechanism underlying the down-regulating effects of chrysin and caffeic acid on eIF4E expression remained to be elucidated. To the best of our knowledge, there are no previous report about the down-regulating effects of chrysin or caffeic acid on eIF4E.

Authors' contributions

MA and NAS performed the project. HT was the supervisor and MHC and AD were the Advisors. NAS and MGA analyzed the data and MA wrote the article.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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