ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(5): 3691-3698
doi: 10.25083/rbl/27.5/3691.3698

Received for publication, August 27, 2020
Accepted, October, 20, 2020

Original article

Biphasic response of epidermoid adenocarcinoma cells to curcumin and EGCG co-treatment

OANA-ALINA HAZAPARU[1#], ALEXANDRU FILIPPI[1,2#], OANA-CEZARA VLAD[1], CARMEN-MARIANA CHIFIRIUC[3,4], CONSTANȚA GANEA[1], MARIA-MAGDALENA MOCANU[1*]

[1] Department of Biophysics, "Carol Davila" University of Medicine and Pharmacy, 050474, Bucharest, Romania

[2] Department of Pathophysiology and Pharmacology, "Nicolae Simionescu" Institute of Cellular Biology and Pathology, 030167 Bucharest, Romania

[3] Microbiology Immunology Department Faculty of Biology University of Bucharest 1-3 Portocalelor Lane, 77206 Bucharest, Romania

[4] Research Institute of University of Bucharest – ICUB, Bucharest, Romania

[#] These two authors equally contributed to this work

Abstract

We investigated the combinatorial treatment of curcumin and epigallocatechin-gallate (EGCG) in human epidermoid carcinoma cell line A-431. When combined, concentrations of EGCG and curcumin lower than 25 μM increased proliferation, but 50 μM curcumin led to near complete proliferation/viability inhibition. As reference, the compounds were also tested on normal fibroblast cells, CCD-1070Sk, where they did not show any toxicity, except in the highest concentration sample. In A-431 cell line, curcumin and EGCG combinations induced cell death by apoptosis, collapse of the mitochondrial membrane depolarization (ΔΨm) and increase the cell number in S phase of cell cycle. Nevertheless, the most powerful compound in inducing the above effects was curcumin. Next, we have shown that EGCG scavenged ROS produced by 25 μM curcumin with an EC50 of 44.34 μM EGCG. In conclusion, curcumin and EGCG combinations exert ΔΨm collapse, cell death and cell cycle arrest in A-431 cells at concentrations not affecting the viability of normal skin fibroblasts.

Keywords curcumin, EGCG, epidermoid adenocarcinoma, biphasic response, cytotoxicity

To cite this article: OANA-ALINA HAZAPARU. Biphasic response of epidermoid adenocarcinoma cells to curcumin and EGCG co-treatment. *Rom Biotechnol Lett.* 2022; 27(5): 3691-3698
DOI: 10.25083/rbl/27.5/3691.3698

*Corresponding author: Maria-Magdalena Mocanu; e-mail: magda.mocanu@umfcd.ro;
Cell phone: +40745084184

Introduction

The polyphenols are known for their ability to interfere with tumor formation, which may also reduce the risk of developing different types of cancer (LAUBACH et al. [10]). A better understanding of the mechanisms of actions of phytochemicals can help us adopt the best strategy in cancer prevention. One of the most well-known polyphenols are the catechins found in green tea, especially epigallocatechin-3-gallate (EGCG) and it has been used frequently as an anti-carcinogenic agent *in vivo* and *in vitro* studies (HU et al. [5]). Curcumin is the natural polyphenol from turmeric, found in the roots of the herbaceous perennial plant, *Curcuma longa*, which is naturally found in Asian countries (HEWLINGS et al. [4]). Recent studies revealed the useful properties of curcumin in cancer progression by inducing apoptosis and oxidative stress in tumour formation, in cooperation with modulator activity of inflammatory signaling pathways (SHABANINEJAD et al. [13]). In previous studies, our research group studied the effects of curcumin or EGCG in A-431 epidermoid cancer cell line, but as individual exposure (CIOLAC et al. [2], MOCANU et al. [12], FILIPPI et al. [3]). New studies demonstrated that EGCG may also have synergistic activity with curcumin and other drugs, in different cancer cell lines (JIN et al. [6]).

The aim of the study was to investigate the combinatorial effect of curcumin and EGCG in the hypertriploid human epidermoid carcinoma cell line, A-431. Several concentrations of curcumin and EGCG are used to study the effect of curcumin and EGCG on colony formation, viability, mitochondrial membrane depolarization and formation of reactive oxygen species (ROS). We also demonstrated that curcumin and EGCG induce apoptosis in a dose-dependent manner and this is correlated with the increase of curcumin concentration. To investigate whether EGCG and curcumin have harmful effects onto normal human cells, these two compounds were tested in experiments with CCD-1070Sk, a normal fibroblast skin cell line.

Materials and Methods

Cell cultures and treatments

Experiments were performed using epidermoid carcinoma cell line A-431 and normal skin fibroblast CCD-1070Sk cells from American Type Culture Collection. The cells were grown in DMEM (Sigma, Saint Louis, MO) supplemented with 10% heat inactivated FBS (Sigma, Saint Louis, MO), 2 mM L-glutamine (Thermo Fisher Scientific/ Gibco, Carlsbad, CA) and 1% Penicillin/Streptomycin (Thermo Fisher Scientific/ Gibco, Carlsbad, CA), in a humidified incubator at 37°C, 5% CO2. Curcumin (Sigma, Saint Louis, MO) and EGCG (Sigma, Saint Louis, MO) were used from 50 mM solutions prepared in DMSO (Sigma, Saint Louis, MO) in the day of the experiment. In all control samples, DMSO was added at the same volume as in the treated samples.

Cytotoxicity assay

A-431 and CCD-1070Sk cells were seeded in 96-well flat-bottom microplates at a density of 10^4 and 2 x 10^4 cells/well, respectively 24 h prior to the experiments and incubated for 48 h with either 25 μM curcumin plus 0, 5, 10, 25, 50 μM EGCG or 25 μM EGCG plus 0, 5, 10, 25, 50, 100 μM curcumin or DMSO control. The viability assay was carried out with water soluble tetrazolium salt (Roche Diagnostics GmbH, Mannheim, Germany) to evaluate the influence of number of the cells on the viability experiments. The assay is based on the reduction of water soluble tetrazolium salt (WST-1) to formazan in viable cells due to mitochondrial dehydrogenase activity. The absorbance of formazan was measured at 450 nm and corrected at 620 nm using a 96-well spectrophotometer (Infinite 200 PRO plate reader, Tecan Life Sciences).

ROS production and viability assay

Cells plated in 6 well plates at 2 x 10^5 cells/well were let to adhere for 24 h and then treated with 25 μM curcumin plus 0, 5, 10, 25, 50,100 μM EGCG or 25 μM EGCG plus 0, 5, 10, 25, 50, 100 μM curcumin or DMSO control for 48 h. Trypsinized cells and those found in the supernatant were stained for 30 min with 5-(and-6)-carboxy-2',7'- dichlorodihydro-fluorescein diacetate (caboxy-

H_2DCFDA, Molecular Probes) and propidium iodide for 15 min and washed in PBS; the samples were measured with Gallios (Beckman Coulter, Brea, CA) flow cytometer (excitation: 488 nm, emission: 525 ± 40 nm for carboxy-H_2DCFDA and emission: 620 ± 30 nm for propidium iodide). Curcumin fluorescence was subtracted from treated, non-stained samples. EC50 was calculated by the best nonlinear fit using Equation (1):

$$Y = \frac{Top - Bottom}{1 + 10^{(LogIC50 - X) \times HillSlope}} \quad (1)$$

where, X is logarithm with base 10 of the concentration of EGCG, LogEC50 is the logarithm with base 10 of the half-maximal effective concentration, Y is carboxy-H_2DCFDA fluorescence intensity, and "top" and "bottom" are plateau values.

Mitochondrial membrane potential

A-431 cells were grown in 6 well plates, incubated for 48 h with 25 μM curcumin plus 0, 5, 10, 25, 50, 100

μM EGCG or 25 μM EGCG plus 0, 5, 10, 25, 50, 100 μM curcumin or DMSO control, stained 15 minutes with 5 μg/ml JC-1 (Thermo Fisher, Carlsbad, CA) at 37°C, trypsinized, washed in PBS and measured with Gallios (Beckman Coulter, Brea, CA) flow cytometer (excitation: 488 nm, JC1 monomer emission: 525 ± 40 nm, JC1 aggregates emission: 575 ± 30 nm). Curcumin fluorescence was subtracted from treated, non-stained samples. EC50 values were calculated from inhibition curves fitted in Prism 5 (GraphPad Software Inc., USA).

Cell cycle evaluation

A number of 2 x 10^5 A-431 cells were seeded in 6 well plates and, after being left to adhere for 24 h, the cells were synchronized in the G_0/G_1 phase of the cell cycle by 24 h incubation in serum free media. At this point, the cells were treated for 48 h with 25 μM curcumin plus 0, 5, 10, 25, 50 μM EGCG or 25 μM EGCG plus 0, 5, 10, 50, 100 μM curcumin or DMSO control, fixed for 3 h in 70% ethanol at -20°C, stained with propidium iodide in RNase staining buffer (BD Bioscience, San Jose, CA). Measurements are carried out with Gallios (Beckman Coulter, Brea, CA) flow cytometer (excitation: 488 nm, emission: 620 ± 30 nm). Deconvolution and gating of the populations in different cell cycle phases was done automatically in Flowing Software 2.5.1 (University of Turku, Finland).

Apoptosis assay

A-431 cells plated in 6 well plates at 2 x 10^5 cells/well were let to adhere for 24 h and then treated with 25 μM curcumin plus 0, 5, 10, 25, 50, 100 μM EGCG or 25 μM EGCG plus 0, 5, 10, 25, 50, 100 μM curcumin or DMSO control for 48 h. The supernatant was collected, added to the trypsinized cells, washed in PBS and stained with propidium iodide and Annexin V-APC. The samples were measured with Gallios (Beckman Coulter, Brea, CA) flow cytometer (excitation: 488 nm, emission: 620 ± 30 nm for propidium iodide and excitation: 635 nm, emission: 660 BP for Annexin V-APC). Curcumin fluorescence was subtracted from treated, non-stained samples.

Data analysis

Data are represented as mean ± standard error of the mean (s.e.m.). Significance was assayed ANOVA with Dunnett post-test for multiple column analysis. A value of $p < 0.05$ was set as level of significance. Raw data were analyzed using Flowing Software 2.5.1 (University of Turku, Finland) and ImageJ v.1.48 (NIH, USA) and for the statistical analysis Microsoft Excel 2010 (Microsoft Inc., USA) and Prism 5 (GraphPad Software Inc., USA) were used.

Results and Discussions

Small EGCG and curcumin concentrations increase cell proliferation, high curcumin concentrations lead to cytotoxicity in the A-431 cell line. Normal fibroblasts are unaffected

In our experiments (Fig. 1A, 1B) with A-431 cell line, small concentrations of EGCG and curcumin led to a moderate increase in proliferation (a maximum of 167.8 ± 26.22 % for the 25 μM EGCG, 10 μM curcumin sample compared to the 100 ± 35.4 % in the control), while the higher curcumin concentrations of 50 and 100 μM highly reduced the number of metabolically active cells (21.6 ± 5.2% and 14.26 ± 4.2%, respectively). In normal fibroblasts cell line, CCD-1070Sk small compound concentrations also led to an increase in proliferation (160.2 ± 30.3% in the 25 μM curcumin plus 5 μM EGCG sample), while the highest concentrations tested, 25 μM plus 100 μM curcumin led to a moderate drop in viable cells (60.2 ± 21.7%).

EGCG and curcumin combinations induce cell death in the A-431 cell line

To confirm the results obtained in WST-1 tests, we assayed cell death by flow cytometric measurement of propidium iodide stained A-431 cells (Fig. 1C, 1D). The viability of 25 μM EGCG plus up to 10 μM curcumin treated samples was not affected while higher curcumin concentrations were highly cytotoxic. 25 μM EGCG plus 25 μM curcumin reduced viability to 42.7 ± 12.7% (**$p<0.01$, one way ANOVA, Dunnett post-test), 25 μM EGCG plus 50 μM curcumin to 36.0 ± 4.6% (***$p<0.001$) and 25 μM EGCG plus 100 μM curcumin to 7.0 ± 0.9% (***$p<0.001$). This effect is caused mainly by curcumin as 25 μM curcumin alone reduced viability to 47.0 ± 4.3% and the addition of increasing EGCG concentrations up to 100 μM did not substantially change this proportion.

Curcumin induces reactive oxygen species formation, EGCG scavenges ROS

The concentration of 25 μM curcumin, found to induce cell death in about 50% of cells, induced massive ROS production (MFIR of 7.7). EGCG alone did not generate ROS as it does when used alone (FILIPPI et al. [3]) and increasing concentrations of EGCG scavenged ROS efficiently (with a MFIR of 5.03 in the 25 μM curcumin and 50 μM EGCG sample, **$p<0.01$, and a MFIR of 3.81 in the 25 μM curcumin and 100 μM EGCG sample, ***$p<0.001$, one way ANOVA, Dunnett post-test) (Fig. 2). EGCG mediated the reduction of curcumin-induced ROS production with an EC50 of 43.3 μM. Increasing curcumin concentrations could not be tested as carboxy-H_2DCFDA bleached curcumin fluores-

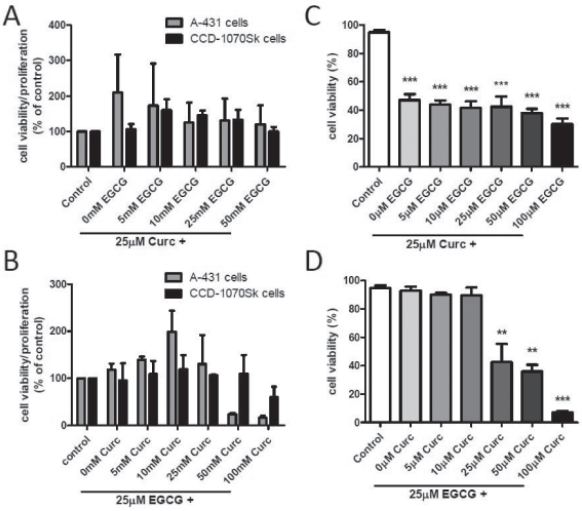

Figure 1. EGCG – curcumin co-treatment induces tumor cell death. (A) The total number of viable cells as influenced by cell death and cell proliferation is mainly unaffected by 25 μM curcumin plus varying EGCG concentrations in both cancer and normal cells. (B) 25 μM EGCG plus higher than 50 μM curcumin drastically reduces the viability/proliferation of cancer cells, with no or little effect on normal, not transformed, cells. (C) 25 μM curcumin reduces by itself the viability to about 50% and the addition of up to 100 μM EGCG contributes to an additional drop in viability to about 30% in A-431 cells. (D) By itself, 25 μM EGCG does not influence tumor cell viability and the addition of curcumin reduces the viability in a dose-dependent manner, with 100 μM curcumin being toxic to almost all A-431 cells. Each column represents mean and s.e.m. from three repetitions and significance was assessed with ANOVA / Dunnett post-test *p<0.05, **p<0.01, ***p<0.001.

cence possibly by FRET and curcumin only treated cells had higher fluorescence than the corresponding curcumin plus carboxy-H_2DCFDA samples.

Mitochondrial membrane potential collapse after exposure to treatment

When cells were treated with a fixed 25 μM EGCG concentration and increasing curcumin concentrations, curcumin showed an EC50 value 23.5 μM in mitochondrial membrane depolarization, with 50 and 100 μM curcumin inducing $\Delta\Psi m$ collapse in most of the cells (85.1 ± 8.3% cells and 83.6 ± 15.7, respectively, **p<0.01, way ANOVA, Dunnett post-test) (Fig. 3B). This effect was almost entirely caused by curcumin and not EGCG as 25 μM curcumin alone induced mitochondrial potential collapse in about half the cells (48.9 ± 18.7%) (Fig. 3E). The lower $\Delta\Psi m$ reduction action of EGCG was also shown in experiments where the curcumin concentration was fixed at 25 μM and no substantial added drop in $\Delta\Psi m$ was observed

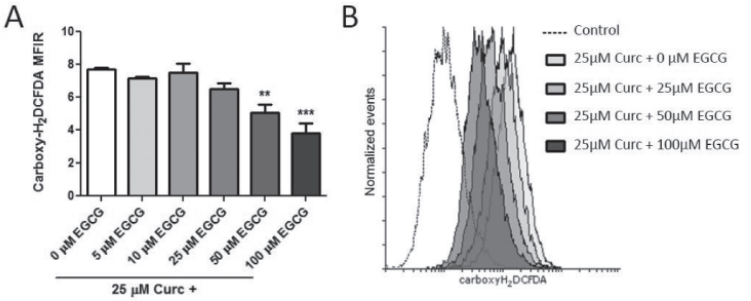

Figure 2. EGCG scavenges the Reactive Oxygen Species (ROS) produced by curcumin. (A) EGCG shows a dose dependent decrease in the ROS generated after 25 μM curcumin treatment, the highest concentration tested, 100 μM EGCG, halving the fluorescence associated with the ROS intracellular concentration. (B) Also shown are representative histograms for 25 μM curcumin plus varying EGCG concentrations treated samples versus control. **p<0.01, ***p<0.001 by ANOVA with the Dunnett post-test.

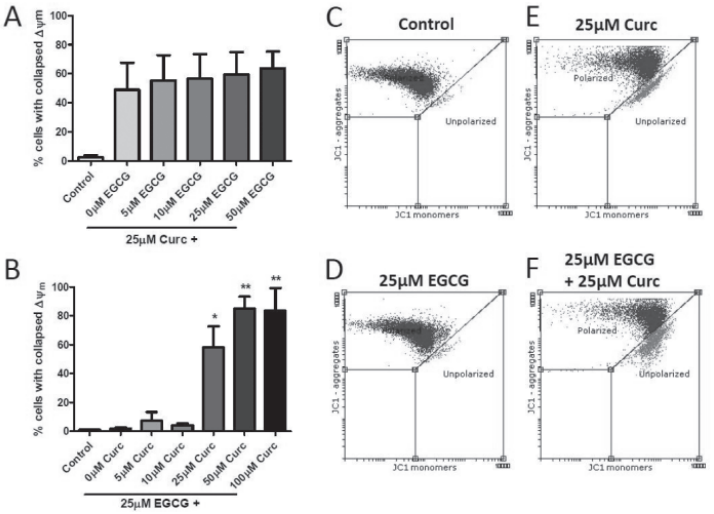

Figure 3. Curcumin leads to mitochondrial membrane depolarization. (A) 25 μM curcumin treatment results in $\Delta\Psi m$ collapse in more than half the cancer cells, while the co-administered EGCG adds little to this effect. (B) 25 μM EGCG alone does not alter the $\Delta\Psi m$, but the co-administered curcumin shows a dose dependent collapse of the $\Delta\Psi m$. The dot plots show in blue cells with normal $\Delta\Psi m$, as indicated by the predominance of JC-1 aggregates fluorescence and in red cells with collapsed $\Delta\Psi m$ having more JC-1 in monomeric form for the (C) control cells, (D) 25 μM EGCG, (E) 25 μM curcumin and co-treatment with (F) 25 μM EGCG and 25 μM curcumin. *p<0.05, **p<0.01 by ANOVA with the Dunnett post-test.

with an increase in EGCG concentration of up to 50 μM (Fig. 3A).

Flow cytometric measurement of cell cycle phase after exposure to treatment

Incubation with 25 μM curcumin (9.6 ± 2.5 compared to 5.8 ± 3.5% in control) nearly doubled the number of A-431 cells in the S phase of the cell cycle and this level maintained in plateau with the addition of up to 100 μM EGCG (with the sole exception of an even higher increase to 13.7 ± 3.5% in the case of 50 μM EGCG) (Fig. 4). The percent of cells in the G_2/M phase also rose moderately (41.1 ± 9.0 compared with 31.8 ± 2.7% in control) and these changes were associated with lower cell numbers in the G_0/G_1 phase. EGCG treatment does not influence the cell cycle progression of A-431 cells.

Effect of the treatment on apoptosis

To test whether the induced cell death was by means of apoptosis, we assessed the Annexin V-APC/ propidium iodide staining of EGCG/curcumin co-treated cells (Fig. 5). In these experiments, 25 μM curcumin alone induced early apoptosis in 11.1 ± 1.8 % cells (compared with the 1.1 ± 0.4% in the control cells, ***p<0.001) and late apoptosis in 23.2 ± 2.5 % (compared with the 0.8 ± 0.6% in the control cells, ***p<0.001). Addition of up to 100 μM EGCG did not significantly change the apoptosis inducement profile of 25 μM curcumin alone. However, when cells were treated with a fixed 25 μM EGCG concentration plus varying amounts of curcumin, a dose-dependent response was obtained for both early and late apoptosis inducement.

The main results of this study are related to biphasic effect of EGCG and curcumin, the lack of toxicity or reduced toxicity in non-transformed cells and the ability of EGCG to scavenge ROS after their induction by curcumin. The anti-proliferative effect of EGCG-curcumin or curcumin-EGCG was associated with mitochondrial membrane collapse and apoptosis. Nevertheless, CCD-1070Sk non-transformed fibroblasts are not sensitive to concentration of EGCG or curcumin used in this study, with only one exception in case of the highest dose of combined treatment, respectively 100 μM curcumin and 25 μM EGCG.

Interestingly, the biphasic effect of EGCG and curcumin was identified in both transformed and non-transformed cell lines. Lower concentrations of EGCG and curcumin increased cell proliferation, while higher concentrations induced cytotoxic effects. These data are in line with previous reported results, since EGCG and curcumin have been shown to display both anti-oxidative effect due the phenol rings (TIPOE et al. [15], ABRAHAMS et al. [1]), and pro-oxidative function through increased production of ROS (LI et al. [11], KOCYIGIT et al. [9]). For instance, the anti-oxidant effect of EGCG has further applications in cardio-vascular protection, where catechin demonstrated the ability to decrease lipid peroxidation or to scavenge free radicals

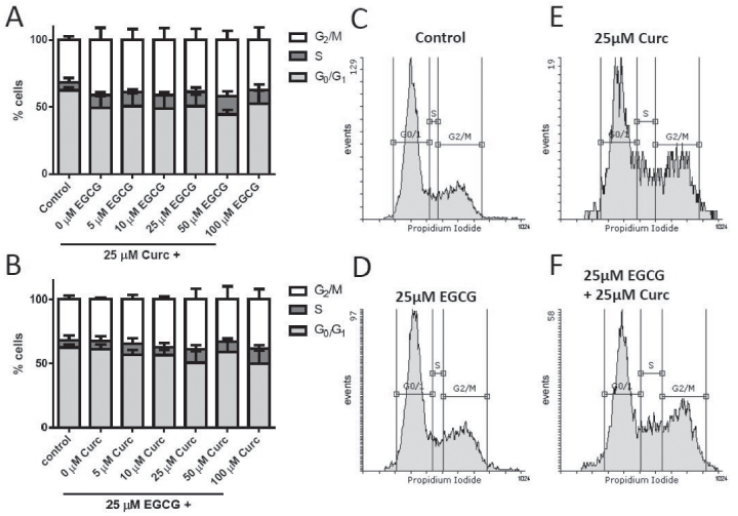

Figure 4. Curcumin alters the cell cycle progression. (A, B) 25 µM curcumin reduces the number of cells found in the G_0/G_1 phase of the cell cycle with a concomitant doubling of the cell numbers in the S phase and a slight increase in the number of cells in the G_2/M phase. Increasing EGCG concentrations of up to 100 µM do not seem to alter the cell cycle progression. Also shown are representative histograms showing gated cells in each cell cycle phase for (C) control cells, (D) 25 µM EGCG treated cells, (E) 25 µM curcumin treated cells and (F) co-treatment with 25 µM EGCG and 25 µM curcumin. Gates were automatically set in Flowing Software 2.5.1 using the built in deconvolution for cell cycle analysis function.

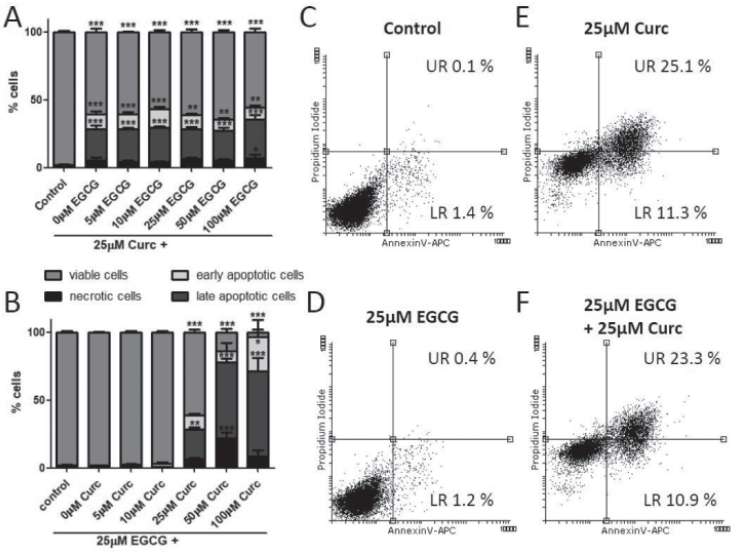

Figure 5. Curcumin induces tumor cell death by apoptosis. (A) 25 µM curcumin led to apoptosis as shown by the Annexin V-APC binding to one third of the cells. Adding EGCG to this concentration did not significantly change the viability or percent late (UR) versus early (LR) apoptotic cells. (B) Also, 25 µM EGCG by itself does not influence apoptosis induction but the addition of curcumin induces apoptosis in a dose-dependent manner for concentration larger than 10 µM curcumin, with 100 µM curcumin killing almost all cells through apoptosis. The dot plots show Annexin V-APC and Propidium Iodide binding in (C) control cells, (D) 25 µM EGCG, (E) 25 µM curcumin and (F) co-treatment with 25 µM EGCG and 25 µM curcumin. At the tested time of 72 h most apoptotic cells were late apoptotic cells. *p<0.05, **p<0.01, ***p<0.001 by ANOVA with the Dunnett post-test.

(TIPOE et al. [15]). At the same time, the anti-oxidant activity of curcumin was investigated in relationship of its ability to protect nervous system (ABRAHAMS et al. [1]). On the other hand, the pro-oxidative activity of both natural compounds, EGCG and curcumin, was frequently associated with inhibition of highly proliferative cancer cells (LI et al. [11], KOCYIGIT et al. [9]).

Combined treatment of curcumin and EGCG resulted in induction of cell death, demonstrated by spectrophotometric and flow cytometric measurements, collapse of mitochondrial membrane potential, cell cycle arrest and apoptosis in A-431 epidermoid carcinoma cells. Nevertheless, most of the effects have been due to curcumin administration and to a lower extent due to EGCG. Curcumin is known to have anti-cancer effects such as autophagy, apoptosis and cell cycle arrest (ZHU et al. [17]), increased expression of death receptor 5 (JUNG et al. [7]), and altered expression of a large number of kinases, enzymes and anti-apoptotic proteins such as Bcl-2 (SHANMUGAM et al. [14]).

Moreover, curcumin is a potent inducer of ROS production in cancer cells is known from studies that use a fixed curcumin concentration and follow the kinetics of ROS production (KHAN et al. [8]). Interestingly, our results indicated a reduction in ROS induced by curcumin after combined treatment with EGCG. This might be seen as a caution when discussing pro-oxidative effect, in case of combined administration of higher doses of curcumin and low doses of EGCG. Nevertheless, this effect might represent an asset in case of EGCG, since this compound might display protective effect in normal cells after exposure to cancer therapy, such as ionizing radiation (ZHU et al. [16]).

Conclusions

In conclusion, our experiments showing the effect of EGCG and curcumin co-treatments, indicate both anti- and pro-oxdative effects, the later ones demonstrating induction of cell death, cell cycle arrest and $\Delta\Psi m$ collapse in A-431 epidermoid cancer cells. These treatment combinations induced a greater drop in viability/proliferation of the cancer cells compared to normal skin fibroblasts. However, caution should be taken in case of utilizing higher concentrations of curcumin as pro-oxidative agent in combination with EGCG, since the later one might be responsible for ROS scavenging.

References

1. ABRAHAMS, S., HAYLETT, W. L., JOHNSON, G., CARR, J. A. & BARDIEN, S. Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: A review. *Neuroscience* 2019; 406(-): 1-21, doi: 10.1016/j.neuroscience.2019.02.020.
2. CIOLAC, O. A., FILIPPI, A., MARU, N., POPA, M., CHIFIRIUC, C., GANEA, C. & MOCANU, M. M. Reduction of the clonogenic potential and collapse of the mitocondrial membrane potential in A-431 epidermoid carcinoma cell line induced by curcumin *Rom Biotech Lett* 2017; 22(6): 13068-13077, doi: 10.26327/RBL2017.18.
3. FILIPPI, A., PICOT, T., AANEI, C. M., NAGY, P., SZOLLOSI, J., CAMPOS, L., GANEA, C. & MOCANU, M. M. Epigallocatechin-3-O-gallate alleviates the malignant phenotype in A-431 epidermoid and SK-BR-3 breast cancer cell lines. *Int J Food Sci Nutr* 2018; 69(5): 584-597, doi: 10.1080/09637486.2017.1401980.
4. HEWLINGS, S. J. & KALMAN, D. S. Curcumin: A Review of Its' Effects on Human Health. *Foods* 2017; 6(10): 92, doi: 10.3390/foods6100092.
5. HU, G., ZHANG, L., RONG, Y., NI, X. & SUN, Y. Downstream carcinogenesis signaling pathways by green tea polyphenols: a translational perspective of chemoprevention and treatment for cancers. *Curr Drug Metab* 2014; 15(1): 14-22, doi: 10.2174/1389200214666131211155613.
6. JIN, G., YANG, Y., LIU, K., ZHAO, J., CHEN, X., LIU, H., BAI, R., LI, X., JIANG, Y., ZHANG, X., LU, J. & DONG, Z. Combination curcumin and (-)-epigallocatechin-3-gallate inhibits colorectal carcinoma microenvironment-induced angiogenesis by JAK/STAT3/IL-8 pathway. *Oncogenesis* 2017; 6(10): e384, doi: 10.1038/oncsis.2017.84.
7. JUNG, E. M., LIM, J. H., LEE, T. J., PARK, J. W., CHOI, K. S. & KWON, T. K. Curcumin sensitizes tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through reactive oxygen species-mediated upregulation of death receptor 5 (DR5). *Carcinogenesis* 2005; 26(11): 1905-13, doi: 10.1093/carcin/bgi167.
8. KHAN, M. A., GAHLOT, S. & MAJUMDAR, S. Oxidative stress induced by curcumin promotes the death of cutaneous T-cell lymphoma (HuT-78) by disrupting the function of several molecular targets. *Mol Cancer Ther* 2012; 11(9): 1873-83, doi: 10.1158/1535-7163.MCT-12-0141.
9. KOCYIGIT, A. & GULER, E. M. Curcumin induce DNA damage and apoptosis through generation of reactive oxygen species and reducing mitochondrial membrane potential in melanoma cancer cells. *Cell Mol Biol (Noisy-le-grand)* 2017; 63(11): 97-105, doi: 10.14715/cmb/2017.63.11.17.
10. LAUBACH, V., KAUFMANN, R., BERND, A., KIPPENBERGER, S. & ZOLLER, N. Extrinsic or

Intrinsic Apoptosis by Curcumin and Light: Still a Mystery. *Int J Mol Sci* 2019; 20(4): 905, doi: 10.3390/ijms20040905.

11. LI, G. X., CHEN, Y. K., HOU, Z., XIAO, H., JIN, H., LU, G., LEE, M. J., LIU, B., GUAN, F., YANG, Z., YU, A. & YANG, C. S. Pro-oxidative activities and dose-response relationship of (-)-epigallocatechin-3-gallate in the inhibition of lung cancer cell growth: a comparative study in vivo and in vitro. *Carcinogenesis* 2010; 31(5): 902-10, doi: 10.1093/carcin/bgq039.
12. MOCANU, M. M., NAGY, P. & SZOLLOSI, J. Chemoprevention of Breast Cancer by Dietary Polyphenols. *Molecules* 2015; 20(12): 22578-620, doi: 10.3390/molecules201219864.
13. SHABANINEJAD, Z., POURHANIFEH, M. H., MOVAHEDPOUR, A., MOTTAGHI, R., NICKDASTI, A., MORTEZAPOUR, E., SHAFIEE, A., HAJIGHADIMI, S., MORADIZARMEHRI, S., SADEGHIAN, M., MOUSAVI, S. M. & MIRZAEI, H. Therapeutic potentials of curcumin in the treatment of glioblstoma. *Eur J Med Chem* 2020; 188(Article ID: 112040): 1-13, doi: 10.1016/j.ejmech.2020.112040.
14. SHANMUGAM, M. K., RANE, G., KANCHI, M. M., ARFUSO, F., CHINNATHAMBI, A., ZAYED, M. E., ALHARBI, S. A., TAN, B. K., KUMAR, A. P. & SETHI, G. The multifaceted role of curcumin in cancer prevention and treatment. *Molecules* 2015; 20(2): 2728-69, doi: 10.3390/molecules20022728.
15. TIPOE, G. L., LEUNG, T. M., HUNG, M. W. & FUNG, M. L. Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. *Cardiovasc Hematol Disord Drug Targets* 2007; 7(2): 135-44, doi: 10.2174/187152907780830905.
16. ZHU, W., XU, J., GE, Y., CAO, H., GE, X., LUO, J., XUE, J., YANG, H., ZHANG, S. & CAO, J. Epigallocatechin-3-gallate (EGCG) protects skin cells from ionizing radiation via heme oxygenase-1 (HO-1) overexpression. *J Radiat Res* 2014; 55(6): 1056-65, doi: 10.1093/jrr/rru047.
17. ZHU, Y. & BU, S. Curcumin Induces Autophagy, Apoptosis, and Cell Cycle Arrest in Human Pancreatic Cancer Cells. *Evid Based Complement Alternat Med* 2017; 2017(Article ID: 5787218): 1-13, doi: 10.1155/2017/5787218.