CAROTENOID PIGMENTS OF TOMATOES AND THEIR ANTITUMORAL POTENTIAL

DIANA CENARIU[1], ADELA PINTEA[2], EVA FISCHER-FODOR[3], MIHAI CENARIU[2], GIANINA CRISAN[1]

[1]University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
[3]Institute of Oncology "Prof.Dr.I.Chiricuta", Cluj-Napoca, Romania
mihai.cenariu@usamvcluj.ro

Abstract: *The aim of this study was to show the influence of a tomato extract on hepatocellular carcinoma cells in vitro, proving their capacity to modulate the secretion of VEGF and ET-1 and thus demonstrating its inhibitory effect on tumor neovascularization. Human hepatocellular carcinoma cell line HepG2 was treated with a tomato extract, obtained from fresh tomato fruits. The expression of VEGF and Endothelin-1 by the treated cells, compared to an untreated control was assessed by ELISA. Our results proved that VEGF and Endothelin-1 expression was significantly diminished by the tomato extract, as compared to the untreated control. Therefore, our in vitro study showed that the carotenoid rich tomato extract is able to decrease tumoral neoangiogenesis in hepatocellular carcinoma and needs to be followed by clinical trials that could open new perspectives regarding the use of tomato carotenoids as adjuvant therapeutic agents.*

Key words: *carotenoids, tomato, antitumoral, neovascularization inhibition*

INTRODUCTION

One of the most frequent and dramatic neoplasm diagnosed in humans nowadays is hepatocellular carcinoma [1]. Various vegetables and fruits are recognized to contain antioxidants whose antitumoral potential was proven; carotenoid pigments of tomatoes, especially lycopene, have already been included in clinical studies [2]. Lycopene was shown to decrease proliferation of tumoral cells and inhibit neongiogenesis in some types of malignant or benign tumors: prostatic hyperplasia [3], prostate cancer [4] and colon adenocarcinoma [5]. Neoangiogenesis is one of the most frequent phenomena that occurs during the neoplasic disease and participates extensively to cancer proliferation and growth. Release of endothelin-1 (ET-1), a vascular growth factor, is inhibited by lycopene in normal endothelial cells [6, 7] and we also proved the same in colon adenocarcinoma [5]. Together with VEGF these growth factors are usually expressed by tumoral cells [8, 9] and are extremely important in modulating the activity of the vascular system.

Human HepG2 hepatocellular carcinoma cell line expresses ET-1 and VEGF and therefore was chosen for this study, in the attempt to prove that carotenoid pigments extracted from tomatoes are able to inhibit the secretion of such growth factors, and thus to decrease neoangiogenesis

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MATERIALS AND METHODS

The human hepatocellular carcinoma cell line (HepG2) was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK). The cells were grown in

Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS (Sigma Aldrich, St Louis, MO, USA) at 37°C, 5% CO_2 and saturated humidity.

The carotenoid rich tomato extract was obtained from fresh tomatoes, using a classical method [10] characterized by HPLC and solubilized in tetrahydrofuran (THF, Sigma Aldrich), as previously described [5]. The extract had a total carotenoid content of 19.14 mg/ml.

After the cell cultures reached sub-confluence, they were trypsinized and seeded into 6-well plates where they treated with serial dilutions of the tomato extract, 0.1- 15 mg/ml, prepared with phosphate buffered saline (PBS, Sigma Aldrich). As the final ratio extract: culture media was 1:20, the active concentration was between 50 μg/ml and 950 μg/ml.

The ELISA assay was performed after 24 hours of cultivation in the presence of the extract. The supernates were collected and analyzed using a VEGF ELISA kit (BlueGene Biotech, Shanghai, China) and an Endothelin-1 kit (Cusabio Life Science, Wuhan, China). The pure protein concentrations for the standard curve were between 0 - 1000 pg/ml for VEGF and 0 - 200 pg/ml for Endothelin-1. All methods were specific for human samples and the sensitivity was below 0.7 pg. The methodology used was the one recommended by the manufacturers. Briefly, we used the antibody-coated plates from the kits; the standards and samples were pipetted into wells in triplicate. VEGF and ET-1 from the sample was allowed to bind with the specific antibody on the plate, followed by 4 subsequent wash steps. A biotin-conjugated antibody specific against human VEGF / ET-1 was added, washed, and avidin conjugated Horseradish Peroxidase (HRP) was added. After the last washing step, the substrate for HRP was added to the wells in order to develop a quantifiable color reaction, directly related to the amount of the targeted protein. The color reaction was stopped with sulphuric acid and the plate was read with an ELISA reader (Tecan Sunrise, Tecan Group, Männedorf, Switzerland), at 450nm. The results were created using the incorporated Magellan software. Normalization of the results was performed after counting the cells in each well and expressing the protein production to $5x10^5$ cells.

All experiments were performed in triplicate and the data was statistically interpreted using the Graph Pad Prism 5 biostatistics software (GraphPad Software, La Jolla, USA).

RESULTS

The amount of vascular endothelial growth factor (VEGF) was significantly lower in treated HepG2 cells, as compared to the untreated control (Fig.1) (One-way Anova test, Bonferroni post-test, in the 95% confidence interval, $p<0.01$).

The extracts also inhibited the ET-1 production in HepG2 cells treated with the carotenoid rich tomato extract, the decrease being very significant when compared to untreated control (One-way Anova test, Bonferroni post-test, in the 95% confidence interval, $p<0.01$) (Fig.2).

Antioxidants, like tomato extracted carotenoids have shown significant effects against hepatocellular carcinoma, while their great advantage when compared classical chemotherapeutic drugs is that they lack the important side effects [11]. Lycopene was shown to interfere with phosphoinositide 3-kinase (PI3K), Akt and Wnt signaling pathways in other types of tumors [12]. The tomato extract that was used in our research also had high lycopene content, as shown by our previously published data [5] and seems to act similarly against

HepG2 cells, showing a good potential to suppress neoangiogenesis by modulating vascular growth factors in vitro.

Since neoangiogenesis, the formation of new blood vessels in tumor mass is a critical step in tumor progression and metastasis; several therapeutic efforts are directed to develop new drugs which counteract this phenomenon [5]. The carotenoid rich tomato extract that was employed in the present work was able to significantly reduce VEGF and ET-1 production in HepG2 cells, therefore opening new perspectives towards its use in *in vivo* studies.

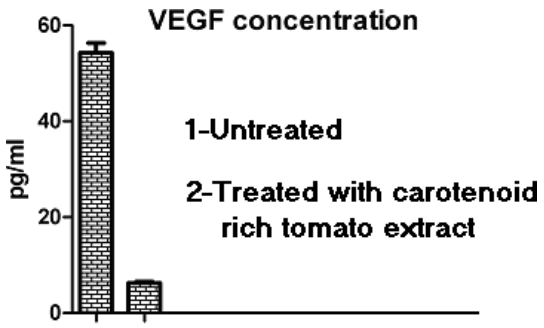

Fig. 1 VEGF concentration in untreated cells and cells treated with the carotenoid rich tomato extract; results are expressed in pg/ml and represent median values of three measurements. SEM is also shown, in the form of error bars

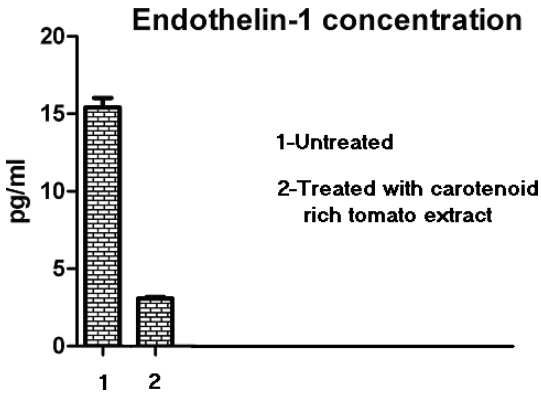

Fig. 2 Endothelin-1 concentration in untreated cells and cells treated with the carotenoid rich tomato extract; results are expressed in pg/ml and represent median values of three measurements. SEM is also shown, in the form of error bars

Endothelin-1 induces an increased DNA replication and therefore cell growth and proliferation, increasing mitosis through the activation of phosphoinositide 3-kinase signaling pathway [13]. The inhibitory effect of the carotenoid rich tomato extract shown in our study is of therefore great importance in decreasing the hepatocellular carcinoma cell growth. As our extract inhibited both VEGF and ET-1 production in HepG2 cell line *in vitro*, it is expected that PIK3 signaling pathway will be modulated as well.

CONCLUSION

Our in vitro experiments proved that the carotenoid rich tomato extract acts against hepatocellular tumor cells by reducing the tumor neoangiogenesis. It was able to significantly reduce the level of vascular growth factors VEGF and ET-1, therefore proving a significant antitumoral effect. The in vitro studies need to be followed by clinical trials that could open new perspectives regarding the use of tomato carotenoids as adjuvant therapeutic agents in hepatocellular carcinoma.

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