Antiproliferative Effects of Carvacrol on Neuroblastoma Cells

Karvakrolün Nöroblastom Hücreleri Üzerindeki Antiproliferatif Etkisi

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ABSTRACT

Objective: Neuroblastoma is a pediatric solid tumor that originates from the neural crest. The survival rate of these malignancies is below 50 %. The most common treatments for the disease have several adverse effects which make them invasive for patients and limits the applicability as well as continuity of the treatment. Therefore, researchers seek new agents to reduce adverse effects in order to improve the treatment. In this wise, plant-derived phytochemicals are promising drug candidates, such as carvacrol which has anti-cancer features in some cancers.
Method: Human neuroblastoma cell lines, KELLY (N-MYC positive) and SH-SY5Y (N-MYC negative) were grown in RPMI 1640 (KELLY) or DMEM (SH-SY5Y). Cells were treated with a range of concentration of carvacrol. Cellular proliferation was monitored by using xCELLigence Real-Time Cell Analyzer (RTCA) (ACEA Biosciences, Inc.) device to evaluate the antiproliferative effect of carvacrol on the neuroblastoma cells.
Results: Considering the proliferation curve of SH-SY5Y, results indicate that carvacrol has antiproliferative effects on N-MYC negative neuroblastoma cells, SH-SY5Y cells at all concentrations.
Conclusion: The data illuminate that the carvacrol has an antiproliferative role on neuroblastoma cells. In this context, this phyto-compound might be a promising agent for the treatment of this malignancy.

Keywords: Carvacrol, neuroblastoma, proliferation, pediatric cancers

ÖZ

Amaç: Nöroblastom, nöral krest kaynaklı pediyatrik bir solid tümördür. Bu malignitelerin hayatta kalma oranı %50'nin altındadır. Hastalık için en yaygın tedavilerin, hastalar için invaziv olmasını sağlayan ve tedavinin uygulanabilirliğini ve sürekliliğini sınırlayan çeşitli olumsuz etkileri vardır. Bu nedenle, araştırmacılar tedaviyi iyileştirmek ve olumsuz etkileri azaltmak için yeni ajanlar aramaktalar. Bu açıdan bitki kaynaklı fitokimyasallar, bazı kanserlerde anti-kanser özelliklerine sahip olan karvakrol gibi umut verici ilaç adaylarıdır.
Yöntem: İnsan nöroblastom hücre dizileri, KELLY (N-MYC pozitif) ve SH-SY5Y (N-MYC negatif) RPMI 1640 (KELLY) veya DMEM'de (SH-SY5Y) büyütüldü. Hücreler bir dizi karvakrol konsantrasyonu ile muamele edildi. Hücresel proliferasyon, karvakrolün nöroblastom hücreleri üzerindeki anti-proliferatif etkisini değerlendirmek için xCELLigence Gerçek Zamanlı Hücre Analiz Cihazı (RTCA) (ACEA Biosciences, Inc.) cihazı kullanıldı.
Bulgular: SH-SY5Y'nin proliferasyon eğrisi göz önüne alındığında, sonuçlar karvakrolün tüm konsantrasyonlarda N-MYC negatif nöroblastom hücreleri SH-SY5Y hücreleri üzerinde anti-proliferatif etkilere sahip olduğunu göstermektedir.
Sonuç: Veriler, karvakrolün nöroblastom hücreleri üzerinde anti-proliferatif bir role sahip olduğunu aydınlatmaktadır. Bu bağlamda, bu fitokimyasal malignite için umut verici bir ajan olabilir.

Anahtar kelimeler: Karvakrol, nöroblastoma, proliferasyon, çocukluk çağı kanserleri

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INTRODUCTION

Data acquired since 1975 indicate an increase in cases, especially in developed countries [4,5]. Childhood cancers take the second place after child accidents in developed countries, although they are rare diseases when all cancer cases are evaluated [1,2]. According to the September 2018 data of the World Health Organization, 300.000 children are diagnosed with cancer every year from birth to the age of 19 [3]. Neuroblastomas are pediatric solid tumors originating from neural crest, which are mostly seen in children aged 0-5 years. The survival rate of these malignancies is below 40% and differs in subtypes [6]. This disease is rarely hereditary and only 1% of all cases have familial origin. Treatment strategies of neuroblastoma generally depend on risk groups of disease. Generally older children with metastatic tumors with extra MYCN copies are accepted as high stage tumors; and infants with even metastatic tumors without MYCN copies are considered as low risk. Chemotherapy of neuroblastoma generally includes combination of chemotherapeutic agents such as cyclophosphamide, cisplatin, carboplatin, vincristine, doxorubicin (Adriamycin), etoposide, topotecan, and busulfan [7,8]. While all of these treatment strategies have their own adverse effects, the adverse effects related to systemic chemotherapy is much restrictive due to their higher mortality rates.

Several studies indicate some phytochemicals have promising favorable effects on diseases with lower adverse effects as an alternative way to these therapies [9]. Phytochemicals are plant-derived secondary metabolites having organic contents as alkaloids, terpenes, and phenolic compounds. A submember of the terpene family, monoterpenes, are biologically active substances present in aromatic plants like thyme, pepperwort and wild bergamot. Researchers have revealed that these compounds like menthol, geraniol, linalool have anti-oxidant, anti-obesity, anti-cancer, as well as many more biological activities [10,11]. Likewise, another phenolic monoterpene, carvacrol is found in oregano and thymus derived essential oils. It is a good candidate due to its several anti-cancer effects (Figure 1) [12,13]. Therefore, this study was designed to understand the effect of carvacrol on neuroblastoma cell lines, KELLY and SH-SY5Y [14].
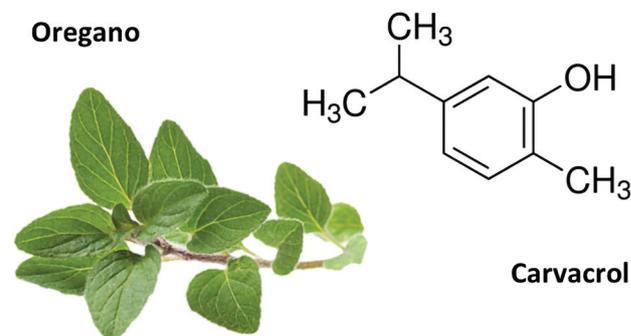

Figure 1. Structure of carvacrol as a phenolic monoterpene.

MATERIAL and METHODS

Cell Culture

Human neuroblastoma cell lines, KELLY (N-MYC positive) and SH-SY5Y (N-MYC negative) were grown in RPMI 1640 (KELLY) or DMEM (SH-SY5Y) supplemented with 10% fetal bovine serum, 1% (v/v) penicillin/streptomycin and 1% L-glutamine in a 5% CO_2 incubator at 37°C.

Real-Time Cell Proliferation Assay

The principle of xCELLigence Real-Time Cell Analyzer (RTCA) Single-Plate (SP) system (ACEA Biosciences, Inc.) is to reveal the impedance changes by the interaction of adherent cells to the gold microelectrodes. Cells (10^4 cells/well) cultured in 96-well plate for 24 hours. Test compounds were added to the growth medium after 24 hours of seeding and scanned for 48 hours to see the effect of chemotherapeutics.

RESULTS

The effects of carvacrol on the proliferation of neuroblastoma cancer cell line KELLY and SHSY5Y, were investigated by real time cell analysis system. Three different doses, as 12.5 μM, 25 μM and 50 μM, were applied to see the effects of carvacrol on KELLY and SH-SY5Y. According to the results, carvac-

rol showed antiproliferative effects on both KELLY and SH-SY5Y cell. On SH-SY5Y cells, antiproliferative effect of carvacrol was seen on 25 µM and 50 µM doses. The application of 12.5 µM dose carvacrol, did not show an anti-proliferative or proliferative effect (Figure 2). All carvacrol doses (12.5 µM, 25 µM and 50 µM) applied on KELLY cells showed antiproliferative effect (Figure 3).
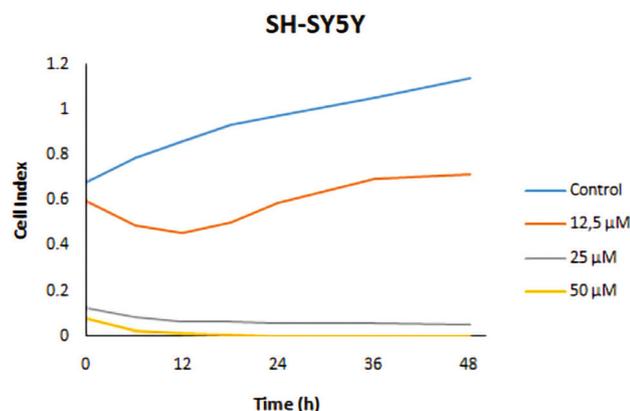

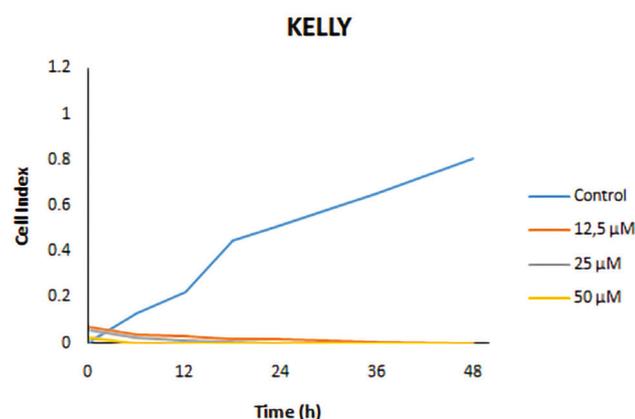**Figure 2. Anti-proliferative effect of carvacrol on SH-SY5Y cell line.**

Figure 3. Anti-proliferative effect of carvacrol on KELLY cell line.

DISCUSSION

Plant-derived compounds attract many researchers for their desire to discover new therapeutic approaches in order to reach more applicable and sustainable therapies, in parallel with their day by day increasing interest in use of phytochemicals also in cancer research. This study illuminates the role of a phenolic compound named carvacrol on a well-known pediatric malignancy, neuroblastoma for its antiproliferative properties. Several researches illuminate antiproliferative role along with some other anti-cancer features of carvacrol on some adulthood cancers in the past. This phyto-compound envokes apoptosis and cell cycle arrest in human prostate, liver, lung, colorectal and cervial cancers [1-5]. This study has focused on the antiproliferative effect of this substance on human neuroblastoma cell lines KELLY and SH-SY5Y. The data of this study illuminate that the carvacrol has antiproliferative effect on both KELLY and SH-SY5Y cell lines. The application of 25 µM or 50 µM carvacrol, shows antiproliferative effect on both KELLY and SH-SY5Y cells. But 12.5 µM dose of carvacrol shows different results according to the cell type. Lower doses of carvacrol may have different effects on different neuroblastoma cells. In summary, this study has suggested that a plant-based phyto-compound, carvacrol can be used to inhibit proliferative feature of neuroblastoma cells, KELLY and SH-SY5Y.

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