Arch Clin Exp Med 2018;3(3):123-126. e-ISSN: 2564-6567

DOI:10.25000/acem.449087 Araştırma makalesi / Research article

Hesperidin triggering apoptosis on neuroblastoma cell

Nöroblastoma hücrelerinde hesperidinin apoptozu tetiklemesi

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Abstract

Aim: Neuroblastoma accounts for approximately 10% of all pediatric cancers and approximately %15 of cancer related deaths in children. Understanding of the molecular mechanisms which play role in the progress of this cancer type may lead to develop more effective strategies for therapy. Flavanoids are popular subject for this new strategies. Hesperidin is herbal flavonoid which is found abundantly in citrus that has been studied on several cancer cell lines. For this purpose, it was aimed to investigate is the apoptotic effects of hesperidin on neuroblastoma tumors using SH-SY5Y cell line.

Methods: Hesperidin was performed on SH-SY5Y and MRC-5 cell line by WST-1, Caspase-3 and Annexin V in a time and dose dependent manner.

Results: 2.5 µM hesperidin and 5 µM hesperidin were found the most suitable dosage for neuroblastoma cell line because of the success on decreasing cell proliferation. Hesperidin has resulted with the ability for apoptotic cell death compared with control group [MRC-5 cell line, $p<0.05$ for all]. 2.5 µM and 5 µM hesperidin concentration for 48h were ended up early apoptotic results as 53.65% for 2.5 µM and 38.90% for 5 µM. There was no significant change on caspase-3 activity.

Conclusions: Our study suggests that hesperidin would be effective against neuroblastoma tumors. We believe with further investigation this study will be helpful for developing new research areas in neuroblastoma tumors.

Keywords: Hesperidin, neuroblastoma, apoptosis.

Öz

Amaç: Çocukluk çağında rastlanan pediatrik kanserlerin yaklaşık %10'luk bir kısmını oluşturan nöroblastoma, çocuklarda kansere bağlı ölümlerin yaklaşık %15'lik bir bölümünden de sorumludur. Bu kanser türünün ilerlemesi esnasında rol alan moleküler olayların anlaşılması, tedavi açısından daha etkili yöntemlerin ortaya konmasına aracılık edebilecektir. Flavanoidler kanser üzerinde yeni tedavi stratejileri geliştirilmesi açısından popülerdir. Hesperidin turunçgillerde bolca bulunan ve bir çok kanserli hücre hattında çalışılan bir flavonoiddir. Çalışmamızda hesperidinin nöroblastoma hücre hattı SH-SY5Y üzerinde apoptotik etkinliğinin araştırılması amaçlanmıştır.

Yöntem: Hesperidinin SH-SY5Y hücre proliferasyonu ve canlılığı üzerinde doz bağımlı etkisi için WST-1, kaspaz enzim aktivitesinin değerlendirilmesi için Kaspaz 3/BCA ve apoptotik değerlendirme için Annexin V analizleri yapılmıştır.

Bulgular: 2,5 µM ve 5 µM hesperidin hücre proliferasyonu üzerinde azalma etkisi yarattığından hücre hattında uygulanmak üzere seçilmiştir. Hesperidin kontrol grubuna göre nöroblastoma üzerinde apoptotik hücre ölümüne neden olmuştur [MRC-5 hücre hattı, hepsi için $p<0.05$]. 2,5 µM hesperidin ve 5 µM hesperidin 48 saatlik inkübasyon sonucu sırası ile %53,65 ve %38,90 apoptoz ile sonuçlanmıştır. Kaspaz 3 aktivitesinde ise herhangi bir değişim gözlenmemiştir.

Sonuç: Bu çalışmamızda elde ettiğimiz sonuçlardan, nöroblastoma tümörlerinde hesperidinin antikarsinojenik etki gösterdiği izlenimi edinilmiştir. Elde edilen verilerden yola çıkarak, çalışmamızın yapılacak ileri araştırmalar ile birlikte nöroblastoma tümörlerinde yeni araştırma alanlarının oluşmasında katkısı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: nöroblastoma, hesperidin, apoptoz.

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Ethics Committee Approval: Due to the fact that the study was no human or animal study, no ethical approval has been taken.
Etik Kurul Onayı: Etik Kurul: Çalışma insan ve hayvan çalışması olmadığı için etik kurul onayı alınmamıştır.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: This study was supported by Istanbul University Scientific Research Projects with a number of 51219.
Finansal Destek: Bu çalışma İstanbul Üniversitesl Bilimsel Araştırma Projeleri tarafından 51219 numaralı proje ile desteklenmiştir.

Geliş Tarihi / Received: 30.07.2018
Kabul Tarihi / Accepted: 14.09.2018
Yayın Tarihi / Published: 30.11.2018

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Introduction

Neuroblastoma is a rare type of cancer even though it is very common extracranial tumor in childhood. The tumors are generally composed of sympathetic nervous system and originated from the adrenal glands; however, the abdomen, the chest, the spinal cord and the paraspinal ganglia might be the origin [1].

Approximately 650 new cases of neuroblastoma are diagnosed each year in United States. Almost 90% of these cases are up to 5 years old and also diagnosed age average is 19 months old [2]. Patients are divided to low, medium and high-risk groups according to their clinical, pathological conditions and genetic factors. Survival rates of the low risk group are up to 90%. Avoidance of aggressive treatment and minimization of chemotherapy-induced toxicity is the main goal for all cases except in the high-risk group [3]. In almost 70% of the patients, metastasis can be seen after that they diagnosed. In this way, these groups of the patients are changing into high-risk group. The predicted 5-year survival rate of high-risk group of neuroblastoma patients is between 40%-50%. However, almost half of these patients are relapsing, by that way it makes the 5-year survival rate decrease to the range of 20% [4, 5].

Aggressive approaches such as chemotherapy, surgical intervention, autologous hematopoietic stem cell [autologous-HSCN] and radiation therapy are followed in the treatment of neuroblastoma for the high-risk group. After success of the treatment, the aim is to prevent the recurrence of the disease [4, 6].

Even though the choices of treatment options are still going on, the new approaches should be improved for high-risk neuroblastoma patients. The main reason of the treatments are not sufficient is because of the resistance to chemotherapy [7]. That is why new, comprehensive and effective treatment options should be investigated [8].

Hesperidin is a flavan-on glycoside that founds in citrus such as oranges, lemon [9]. Some studies have shown that hesperidin has the ability to protect system from oxidative damage and free radicals [10]. In addition to this, hesperidin has also the several properties about anti-inflammatory, analgesic, antifungal, antiviral, and anticancer effects [11, 12].

In this research, we aimed to investigate of hesperidin role on SH-SY5Y neuroblastoma cell line due to its possible capability of additional cancer treatment.

Material and methods

Cell culture method

Neuroblastoma cell line SH-SY5Y and MRC-5 cell line as a healthy control was obtained from ATCC [American Type Culture Collection, Manassas, VA]. SH-SY5Y neuroblastoma cell line was cultured in a mixture media containing EMEM [ATCC, 30-2003, L-Glutamin] and Ham's F-12 [Lonza, BE12-615F, L-Glutamin]. MRC-5 cultured with RPMI 1640 [Lonza, BE12-918F, L-Glutamin]. All media was prepared with 1% penicillin/streptomycin and 10% fetal bovine serum. Cells were cultured in a condition of 37°C in 5% CO_2.

Level of cytotoxicity

WST-1 cell proliferation assay was used to determine the cytotoxicity level of hesperidin on SH-SY5Y and MRC-5 cell lines. $1x10^4$ cells/well was seeded for treatment. 0, 2.5, 5, 10, 25 and 50 µM concentrations of hesperidin were treated and incubated for 24, 48 and 72h 37ºC in 5% CO_2 in time and dose depended manner on cells including MRC-5 cell line. After the incubation period, 10 µl of WST-1 were added all wells for 2h. Color development was measured at 450 nm using a Multiscan ELISA reader [Thermo Fisher Scientific, Germany].

Detection of caspase-3 enzyme activity

One of the important sign for apoptosis is cellular caspase-3 enzyme activity. Caspase-3 activity was determined by caspase-3 colorimetric assay kit [BioVision Research Products,USA]. The protocol was used step by step according to kit instruction. The aim of the procedure is to measure chromophore p-nitroanilide [pNA] after cleavage from the labeled substrate DEVD-pNA by spectrophotometry under 405 wavelengths on ELISA reader [Thermo Electron Corporation Multiskan Spectrum, Finland]. In addition, Bradford assay was used to normalize the protein concentrations.

Analysis of phosphatidylserine exposure by Annexin V

When apoptosis occurs in the cell, phosphatidylserine [PS] components start to move from cell through the cell surface. This make PS work as an apoptotic marker. Due to the PS role as a marker, the level of PS was examined by staining with the green fluorescent Annexing V-FITC [BD Pharmingen, Germany]. First of all, cells were suspended with dyes in 250µl buffer. After suspension cell mixture were analyzed immediately by flow cytometry.

Statistical analysis

Results are expressed as the mean standard error of the mean [SEM]. The data were analyzed using one-way ANOVA.

Results

Hesperidin inhibiton of SH-SY5Y cell line in a time and dose manner

0, 2.5, 5, 10, 25, 50 µM concentrations of hesperidin were used to determine of cytotoxicity level for 24, 48 and 72h. Results are given on Figure 1. 2.5 µM and 5 µM hesperidin concentrations were the most effective for decreasing the proliferation with the time of 48h (p=0.008) for SH-SY5Y cell line. Moreover, there were no important changes in the healthy cell line (p=0.014). The only loss of viability values were 3.97% for 2.5 µM hesperidin, and 2.93% for 5 µM hesperidin were found for MRC-5 cell line as healthy control.

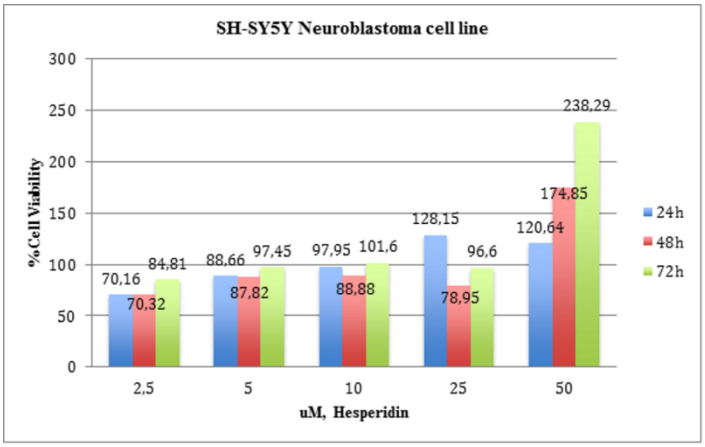

Figure 1. Cell viability after hesperidin treatment.

Detection of caspase-3 enzyme activity

SH-SY5Y and MRC-5 cell lines were incubated with 2.5 µM and 5 µM hesperidin concentrations for 48h according to

the cytotoxicity test. Compared with untreated cells, there was almost no change in any manner (Figure 2). There was also no change in caspase3 activity of MRC-5 cells under the same conditions.

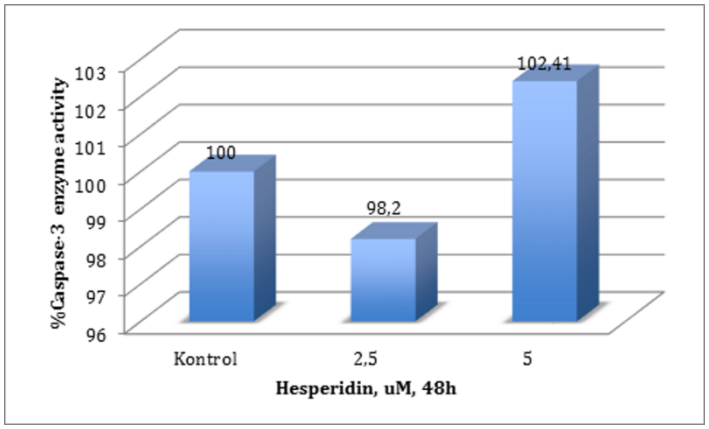

Figure 2. Caspase-3 enzyme activity for SH-SY5Y.

Phosphatidylserine exposure

Annexing V was performed SH-SY5Y and MRC-5 cell lines that both were exposed with 2.5 µM and 5 µM hesperidin concentration for 48h. According to results, hesperidin has the ability for apoptotic cell death compared with control group (MRC-5 cell line, $p<0.05$ for all). Furthermore, there were no important changes in MRC-5 cell line (Figure 3).

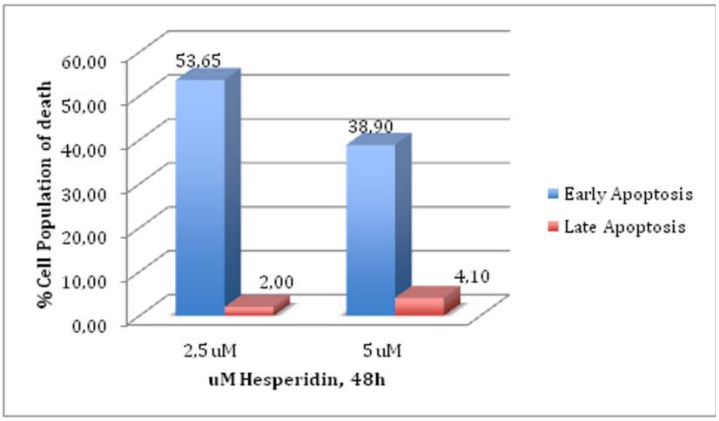

Figure 3. Cell population of death for SH-SY5Y

Discussion

Neuroblastoma treatment has some difficulties with resistance to chemotherapeutic agents especially if the patients are in high-risk group. The survival rate, resistance capacity and relapsing the disease are important points for improving new options or additional treatments [7, 8]. Flavonoids that found in citrus are secondary metabolites with several anti-oxidative, anti-inflammatory, anti-carcinogenic and neuroprotective capacity [13]. Due to the previous studies, even high concentration of hesperidin could not be a reason for any serious side effects [14]. According to literature, we could not be able to find any study about hesperidin and neuroblastoma relation. We have worked with SH-SY5Y cell line for neuroblastoma and MRC-5 as a healthy cell line. Hesperidin works as an anti-carcinogenic effect by stopping the cell cycle [15] and stimulating the apoptotic pathways [16]. Ghorbani et al. [17] have worked with hesperidin activity on NALM-6 leukemia cell line and their study has resulted with apoptosis and also stimulation to increased level of p53 and down regulation of NF-kB. Febriansah et al. [18] have investigated to time and dose depended hesperidin concentration on human breast cancer cell line. Not only they have come up with hesperidin's apoptotic potential, but also hesperidin has the antagonistic effect with doxorubicin. Yumnam et al. [19] have showed the inhibition of proliferation on hepatocellular carcinoma cell line by using hesperidin however they also have resulted that there was no change on healthy cell line. Tamilselvam et al. [20] have pointed out that only hesperidin could not have any change on proliferation of SK-N-SH neuroblastoma cell line; however, hesperidin and rotenone had the effective results by decreasing the proliferation by using at the same time. The study has showed that rotenone has the effective role by increasing the expression of caspase-3 and caspase-9. They also claim that only hesperidin has not been effective on any caspase activity. In addition, the research has mentioned about neuroprotective activity of hesperidin on neuroblastoma cell line [20]. In our study, we have the similar caspase activity results on SH-SY5Y neuroblastoma cell line by using only hesperidin. Dourado et al. [21] have showed the apoptotic activity of hesperidin on Loucy leukemia cell line by using Annexin V-FITC method. Their results have showed that 80% survival cell, 14% early apoptotic cell and 5% late apoptotic cell by using 10 uM hesperidin. Not only our results have the similar apoptotic effect with Dourado et al. [21] on SH-SY5Y cell line but also we have seen no change on MRC-5 cell line that was our healthy control. Hesperidin might be effective for apoptosis with another caspase enzyme activity except caspase-3.

In conclusion, hesperidin has shown anticarcinogenic activity on neuroblastoma cells consistent with other investigations of the mechanism of inducing hesperidin in our study.

We think that it will be an important step in eliciting details of the induction mechanism of hesperidin and in terms of target-oriented therapy for neuroblastoma treatment with further studies and additional methods.

References

1. Ploessl C, Pan A, Maples KT, Lowe DK. Dinutuximab An Anti-GD2 Monoclonal Antibody for High-Risk Neuroblastoma. Ann Pharmacother. 2016;50:416-22.
2. London W, Castleberry RP, Matthay KK, Look AT, Seeger RC, Shimada H, et al. Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. J Clin Oncol. 2005;23:6459-65.
3. Irwin MS, Park JR. Neuroblastoma: paradigm for precision medicine. Pediatr Clin North Am. 2015;62:225-56.
4. Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol. 2015;33:3008-17.
5. Schrey D, Vaidya SJ, Levine D, Pearson AD, Moreno L. Additional Therapies to Improve Metastatic Response to Induction Therapy in Children With High-risk Neuroblastoma. J Pediatr Hematol Oncol. 2015;37:e150-e153.
6. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. New Engl J Med. 1999;341:1165-73.
7. Keshelava N, Seeger RC, Groshen S, Reynolds CP. Drug resistance patterns of human neuroblastoma cell lines derived from patients at different phases of therapy. Cancer Res. 1998;58:5396-405.
8. Xiu LJ, Sun DZ, Jiao JP, Yan B, Qin ZF, Liu X et al. Anticancer effects of traditional Chinese herbs with phlegm-eliminating properties–An overview. J Ethnopharmacol. 2015;172:155-61.
9. Roohbakhsh A, Parhiz H, Soltani F, Rezaee R, Iranshahi M. Neuropharmacological properties and pharmacokinetics of the citrus flavonoids hesperidin and hesperetin—a mini-review. Life Sci. 2014;113:1-6.
10. Agrawal YO, Sharma PK, Shrivastava B, Ojha S, Upadhya HM, Arya DS et al. Hesperidin produces cardioprotective activity via PPAR-γ pathway in ischemic heart disease model in diabetic rats. PloS One 2014;9:e111212.

11. Chen MC, Ye YY, Ji G, Liu JW. Hesperidin upregulates heme oxygenase-1 to attenuate hydrogen peroxide-induced cell damage in hepatic L02 cells. J Agric Food Chem. 2010;58:3330-5.
12. Meiyanto E, Hermawan A, Anindyajati A. Natural products for cancer-targeted therapy: citrus flavonoids as potent chemopreventive agents. Asian Pac J Cancer Prev. 2012;13:427-36.
13. Lv X, Zhao S, Ning Z, Zeng H, Shu Y, Tao O et al. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chemistry Central Journal. 2015;9:1-14.
14. Scalbert A. Zamora-Ros R. Bridging evidence from observational and intervention studies to identify flavonoids most protective for human health. Am J Clin Nutr. 2015;101:897-8.
15. Choi EJ, Hesperetin induced G1-phase cell cycle arrest in human breast cancer MCF-7 cells: involvement of CDK4 and p21. Nutr Cancer. 2007;59:115-9.
16. Nazari M, Ghorbani A, Hekmat-Doost A, Jeddi-Tehrani M, Zand H. Inactivation of nuclear factor-κB by citrus flavanone hesperidin contributes to apoptosis and chemo-sensitizing effect in Ramos cells. Eur J Pharmacol. 2011;650:526-33.
17. Ghorbani A, Nazari M, Jeddi-Tehrani M, Zand H. The citrus flavonoid hesperidin induces p53 and inhibits NF-κB activation in order to trigger apoptosis in NALM-6 cells: involvement of PPARγ-dependent mechanism. Eur J Nutr. 2012;51:39-46.
18. Febriansah R, Putri DD, Sarmoko, Nurulita NA, Meiyanto E, Nugroho AE. Hesperidin as a preventive resistance agent in MCF–7 breast cancer cells line resistance to doxorubicin. Asian Pac J Trop Biomed. 2014;4(3):228-233.
19. Yumnam S, Park HS, Kim MK, Nagappan A, Hong GE, Lee HJ, et al. Hesperidin induces paraptosis like cell death in hepatoblatoma, HepG2 cells: Involvement of ERK1/2 MAPK. PloS One. 2014;9:e101321.
20. Tamilselvam K, Braidy N, Manivasagam T, Essa MM, Prasad NR, Karthikeyan S, et al. Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. Oxidative Medicine and Cellular Longevity. 2013;2013.
21. Dourado GK, Stanilka JM, Percival SS, Cesar TB. Chemopreventive Actions of Blond and Red-Fleshed Sweet Orange Juice on the Loucy Leukemia Cell Line. Asian Pac J Cancer Prev. 2015;16:6491-9.