

Hesperidin related apoptosis on brain glioblastoma

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Abstract

Aim: Even though the rate of brain glioblastoma tumors increases at the ages between 50 and 60, these tumors may occur at any age. Understanding of the molecular mechanisms which play role in the progress of this cancer type may lead to develop more effective strategies for a target driven therapy. Hesperidin is a herbal flavonoid which has anti-inflammatory and anti-oxidant effects that have been proven by different experiments. Also, there is no research in the literature that shows Hesperidin's anti-proliferative and anti-apoptotic effect on glioblastoma tumors.

Material and Methods: In this study, Hesperidin's potential effects on brain glioblastoma tumor treatment was studied by using U-87 cell line. Hesperidin's dosage effects on cell proliferation and vitality were measured by WST-1, and mitochondrial membrane potential was measured by JC-1. Also Caspase 3/BCA activity was measured.

Results: 10 uM and 25 uM hesperidin treatment were resulted disruption of mitochondrial membrane potential (46% with 10 uM and 28% with 25 uM) with different caspase pathway in the light of viability ratio, 10 uM hesperidin 32,6% and 25 uM hesperidin 25% alive cell on 48h incubation period.

Conclusion: Our study, which will be the first one in the literature that indicates Hesperidin's anti-apoptotic and anti-proliferative effect, has an original value. The values we will obtain will create new work fields, especially for cancer treatment and will contribute to science by becoming a new treatment option for brain glioblastoma tumors.

Keywords: Brain glioblastoma; hesperidin; apoptosis.

INTRODUCTION

The most common and aggressive tumors are brain tumors including glioblastomas. Almost 25% of all functional tissue tumors of the brain and 20% of intracranial tumors are defined as glioblastomas. Their malign characteristics properties firstly related with their location and relation with blood map and also fast grow (1).

Even though the treatment options are available, some options are successful while some of options are not because of the tumors mostly having different cell type. The other dilemma is blood-brain barrier that might be inaccessible for some of the treatment agents. The average survival rate is 4.5 months in condition of absence of any treatment. Oppositely, survival rate might be extended to 15 months by using radiation therapy, chemotherapy and temazolomide (2). That is the reason why new approaches are available to research all the time for the treatment options including herbal products.

Hesperidin is one of the most popular agents for cancer therapy. Hesperidin is a kind of flavonoids that found in citrus and have the effect of antioxidant, anti-inflammatory, analgesic, antimicrobial, anti-hypertensive and diuretic effects (3-7). On the other hand, researchers have showed the anti-lipidemic, cardioprotective, anti-hypertensive and antidiabetic effects, which are usually associated with an antioxidant defense mechanism and suppression of proinflammatory cytokine production (8). Rong et al. have worked with hesperidin by using neonatal rats. After hesperidin treatment, they have showed that the protective effect from hypoxia-ischemia and also showed the protective impact on neurons on oxygen-glucose deprivation (9). Another study was reported that use of hesperidin ended up protective impact on cisplatin treated nephrotoxicity model on rats (10). Selveraj and Pugalendi have observed the usage of hesperidin in myocardial injury help to lipid concentration on plasma, heart and liver tissue (11). In another study of hepatocellular carcinoma

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cells have showed the capability of hesperidin to inhibited invasive behavior by suppressing the activator protein-1 and NF- κ B (12).

Human breast cancer cell line MCF-7 and hesperidin relation has worked by Naratajan et al. They have reported that hesperidin induces apoptosis of cancer cells (13). In addition to these studies, hesperidin has reported synergistic effect with diazepam and also its opioid receptors are responsible for hesperidins sedative and antinociceptive effects (14-16).

In our research, we have aimed to investigate the hesperidin impact on glioblastomas as an new approach by using U-87 glioblastoma cell line and MRC-5 (as a healthy control). Since the antiproliferative and apoptotic effects of hesperidin were examined in many cancers, our study has the new value because of the answer for glioblastomas.

MATERIAL and METHODS

Cell Culture

Glioblastoma cell line U-87 and healthy cell line MRC-5 were purchased from ATCC [American Type Culture Collection, Manassas, VA]. U-87 cell line was cultured with DMEM (Gibco, 41965, containing Glucose, L-Glutamin) and MRC-5 was cultured with RPMI 1640 (Lonza BE12-918F L-Glutamine and phenol red free). The media for both cell lines were prepared with suitable concentration of fetal bovine serum and penicillin/streptomycin. Cells were incubated and cultured in a condition of 37°C in 5% CO₂.

Hesperidin concentrations and WST-1 cytotoxicity assay

0,1,5,7.5,10,25 and 50 μ M hesperidin were used on U-87 and MRC-5 cell line that seeden in 96 well plate. The time depended manner was examined at 24h, 48h and 72h. At the end of the time manner for each plate WST-1 solution was added 10 μ L into the each well. After adding the solutions, the plates were incubated 2 hours cultured in a condition of 37°C in 5% CO₂. The absorbances were measured at 450 nm wavelengths by using a Multiscan ELISA reader [Thermo Fisher Scientific, Germany].

Hesperidin's Caspase-3 activity

Caspase-3 activity was determined to find any apoptotic sign for hesperidin. The activity was worked by caspase-3 colorimetric assay kit [BioVision Research Products,USA] step by step according to kit instruction. The results were measured by spectrophotometry under 405 wavelengths on ELISA reader [Thermo Electron Corporation Multiskan Spectrum, Finland]. Due to the protein characteristic of caspase, Bradford assay was used to normalize the protein concentrations as well.

Mitochondrial membrane potential of hesperidin on glioblastoma cells

Apoptotic pathway has some biochemical changes on cells includes the mitochondrial membrane potential. It is a sign to measure the apoptotic condition. JC-1 Mitochondrial membrane potential kit (Cayman Chemicals, MI, USA) was

used to determine cells conditions. The instruction was used step by step and finally measurements were done by flow cytometer.

Statistical analysis

Results are expressed as the mean standard error of the mean [SEM].

RESULTS

WHesperidin concentrations and WST-1 cytotoxicity assay

Hesperidin was used 1,5,7.5,10,25,50 μ M concentration and 24,48 and 72 h time period on U-87 and MRC-5 cell line. The results have showed there were no negative effects on healthy cell line. For U-87 cell, 10 μ M and 25 μ M concentrations were choosen the most effective dosage and 48 h for the time manner. 10 μ M hesperidin ended up 32.6% and 25 μ M hesperidin ended up 25% alive cell on 48h incubation period ($p < 0.05$)(Figure 1).

Hesperidin's Caspase-3 activity

10 μ M hesperidin and 25 μ M hesperidin were used to analyze the caspase activity in 48 h time manner. The results have showed the 13% decreasing level of caspase level by 10 μ M hesperidin. For 25 μ M hesperidin usage has ended up 16% decreasing too. Due to the both doses, hesperidin was found effective to decrease the caspase 3 activity ($p < 0.05$) (Figure 2). The healthy line MRC-5 has showed no change with hesperidin.

Mitochondrial membrane potential of hesperidin on glioblastoma cells

Decreasing the level of mitochondrial membrane potential is characterized by the release of the cytochrome c from mitochondria into the cytoplasm to activate the caspase enzymes. This important change in the membrane potential has a significant impact on the activation of the cellular apoptosis. This change was determined by JC-1 mitochondrial membrane potential kit (Cayman Chemicals, MI, USA). It has ended up promising results by reducing membrane potential for 46% with 10 μ M hesperidin and 28% with 25 μ M hesperidin in 48 h. incubation period ($p < 0.05$) (Figure 3). In addition to this there could not find any change for MRC-5 in the manner of hesperidin.

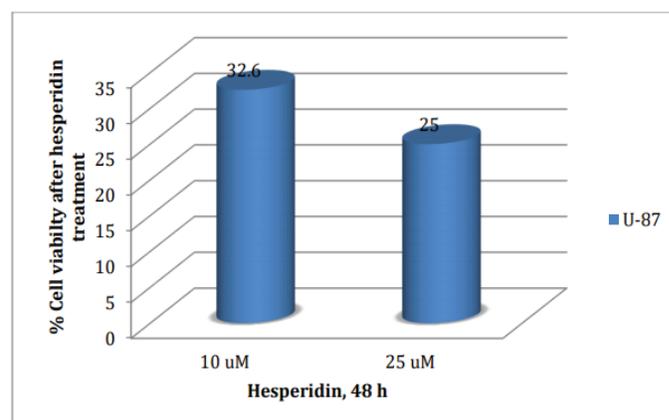


Figure 1. Cell viability after hesperidin treatment and 48 h incubation period

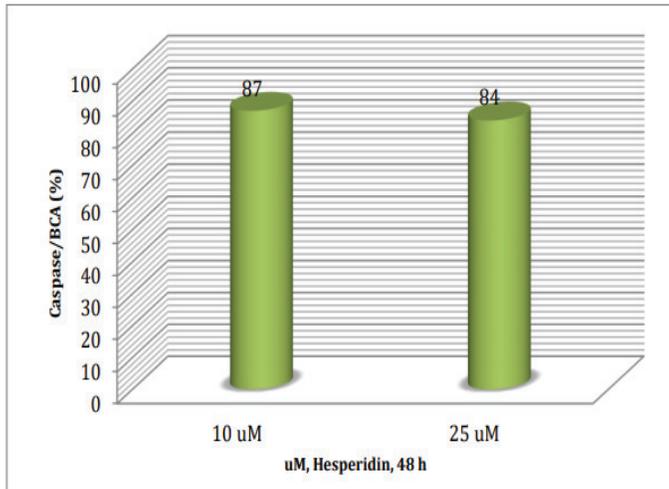


Figure 2. Caspase activity of hesperidin treatment on U-87

DISCUSSION

Malignant gliomas are common brain tumors in all brain tumors. Despite advances in chemotherapy, radiotherapy and surgical intervention, the resistance of the drugs and cytotoxic effects on healthy cells make still less successful and insufficient for the treatment. That is the reason why natural products, which are no side effects, are popular. In this research we have wanted to get a new answer for brain glioblastomas with a popular citrus product, hesperidin. There are several studies that can be found in the literature about hesperidin and its anticancer activity except brain glioblastoma (17-24). Chen et al. have worked to find out hesperidin's role on cell damage and antioxidants mechanism by using L02 hepatic cell line. They have showed the protective capacity and effective for oxidative stress of hesperidin by increasing the heme-oxygenase-1 expression level (17). In addition to this, they have also found the activation of protein kinase ERK 1/2 and nuclear translocation factor NRF 2. They have claimed that hesperidin is a potential therapeutic agent for hepatocyte damage related by oxidative stress and dysfunction of liver cell (17). Tamilselvam et al. have reported a study about a pesticide called rotenone, which is known to target the mitochondrial complex, related damage and its repair with hesperidin on neuroblastoma cells (18). They have found the loss of membrane potential of the mitochondria and increased reactive oxygen species first by using rotenone. After the damage, hesperidin usage was ended up repaired membrane and stimulates the antioxidant system (18). Despite these molecular based studies, our study has also showed that hesperidin makes the disruption of membrane potential in U-87 brain glioblastoma cells and let them through the apoptosis. Same as our results, Park et al. have reported the hesperidin's impact on decreasing the cell viability on colon cancer cells (19). In addition, similar apoptotic results with us have claimed by Saiprasad et al. (20) on colon cancer cells and Nazari et al. on lymphoma cells (21). The obtained data from our research indicate

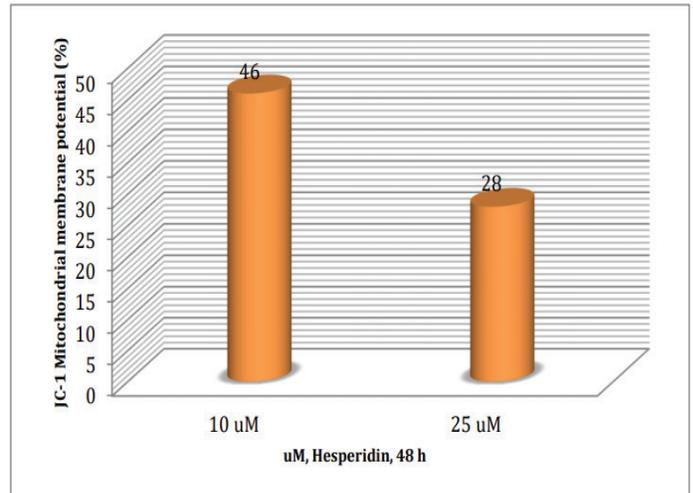


Figure 3. Change in the membrane potential after hesperidin treatment on U-87

that hesperidin has an antitumor activity on brain glioblastoma cells and it has a potential to be used as a treatment option in accordance with the other studies that hesperidin induced the apoptotic mechanisms.

CONCLUSION

Even though the promising results and hesperidin capability, the further analyses need to be clarified hesperidin induced mechanism for brain glioblastoma by molecular analyses. However, our results have a new perspective and outcome for glioblastoma, there are a few limitations for the research such as molecular expressions of related pathways, additional apoptotic analysis. Hesperidin might be one of the agents for targeted therapy not only brain glioblastoma but also other cancers by its potential.

Competing interests: The authors declare that they have no competing interest.

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