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Original paper

Phycocyanin Induces Apoptosis in Glioblastoma Cancer Cells *in vitro*

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Abstract

Background and aim: Glioblastoma multi pulmonary fibrosis is the most common malignant tumor of the central nervous system that occurs in the spinal cord or brain. Since Chemotherapy has many side effects in patient, todays the use of alternative natural ingredients in the direction of treatment expanded. The phycocyanin is a compound produced in cyanobacteria that has various properties, including anticancer properties. This study, in order to investigate the effects of Phycocyanin on the induction of apoptotic death on glioblastoma cell lineage .

Materials and methods: Glioblastoma brain cell line (U87) was purchased from the cell bank of Iran Pasteur Institute, were cultured in RPMI 1640 medium containing 10 to 20% of the serum of fetal bovine animals and treated by Phycocyanin. Gene expression were investigated by means of real-time polymerase chain reaction.

Results: The results of this study showed that phycocyanin has cytotoxic effects on glioblastoma cells and increases the expression level of caspase-8 gene.

Conclusion: The present study showed that the phycocyanin had cytotoxic effects on glioblastoma cells and induces apoptosis via enhancing caspase-8 gene expression level.

Keywords: *Phycocyanin, Glioblastoma, Apoptosis, Caspase3*

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Introduction

Cancer is a genetic disease of the somatic cells, which defects in normal cell division or the failure of the programmed cell pathway is its major cause and is a common name for more than a hundred diseases, and in fact, there are different types of cancer [1] – [3]. Generally, an imbalance between apoptosis and cell proliferation leads to various cancers. Genes and various factors are involved in apoptosis, including caspases such as 3 and 8 [4] – [6]. One of the most common cancers is brain glioblastoma, the definitive treatment of which is still unknown, so that people who die shortly after the disease die. Glioblastoma Multiform is the most prevalent and malignant primary brain tumor, usually occurring in the 6th to 7th decades of life [7]. This tumor was first described by Wiersch in 1963. Later, this tumor was named by Cushing and Bailey as glioblastoma or spongiablastoma. The reason for the selection of this name is the macroscopic examination of tumor coloration due to the presence of bleeding and necrosis with the tumor [8], [9].Apoptosis is a highly controlled process that is activated by various signals and destroys the target cell, and this pathway is essential for the growth and development of the organs, immune response and tumor suppression [10].Within the cells there are positive and negative regulatory paths and the balance between these two paths determines the fate of the cell. Morphologically, apoptotic cells are characterized by cellular depletion, chromatin density, and nuclei of accumulation and destruction of DNA. One of the important pathways that can destroy cancer cells is the path to apoptosis, which changes in the proteins of this pathway makes it more susceptible to cancer. Therefore, in recent years, the treatment of cancer cells by induction of apoptosis is the main target of cancer research

In humans, apoptosis has an external pathway or receptor-dependent mitochondrial receptor, each of which uses different caspases. Caspases are part of the cysteine family - aspartate proteases and an important intermediate for the process of apoptosis. The external pathway of apoptosis was initiated through death receptors and led to Caspase 8 activation. The activated caspase 8 can directly activate the active caspases or act through the Bid protein [11]- [14].

Today, one of the most extensive studies to treat cancer is related to the anticancer effects of Cyanobacteria. Cyanobacteria are gram negative photosynthesis bacteria. Cyanobacteria *Anabaena sp*. has split-free rabbits, complex colonies, and phycoblial proteins, and often have intermediate heterocysts [15].

Phycocyanin is derived from the Greek phyconic term "algae" and cyanine from the English word "cyan", which usually means the shadow of the blue green (close to "blue") and the Greek "kyanous" Different colors are "dark blue". Phycocyanin has many properties, including anti-inflammatory, anti-diabetic, anti-oxidant, antifungal, antibacterial, inhibitory free radicals [16], [17].

Since glioblastoma is one of the most common cancers in the brain, the aim of this study was to investigate the anticancer effects of phycocyanin on the expression of apoptosis pathogens including caspase 3 and 8, and the rate of expression of these genes on the U87 cell line was studied.

Materials and Methods

This experiment was conducted at the Faculty of Pharmacy and Pharmaceutical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. In this study, Glioblastoma brain cell line (U87) was purchased from the cell bank of Iran Pasteur Institute, were cultured in RPMI 1640 medium containing 10 to 20% of the serum of fetal bovine animals and treated by Phycocyanin. RPMI 1640 medium was used to cultivate the tumor cell. The cells were transferred to 6 well plate

and incubated for 24 hours. After incubation, the cells were exposed to 25,50,100,150, 200, and 250 µg/ml of phycocyanin and cell viability was measured using MTT assay method. Real time-PCR was used to evaluate caspase-3 gene expression level. Data were analyzed using SPSS20 software and ANOVA.

Results

The results of this study showed that higher doses of phycocyanin (more than 10µg/ml) has cytotoxic effects on glioblastoma 24 hours after treatment (Figure 1).

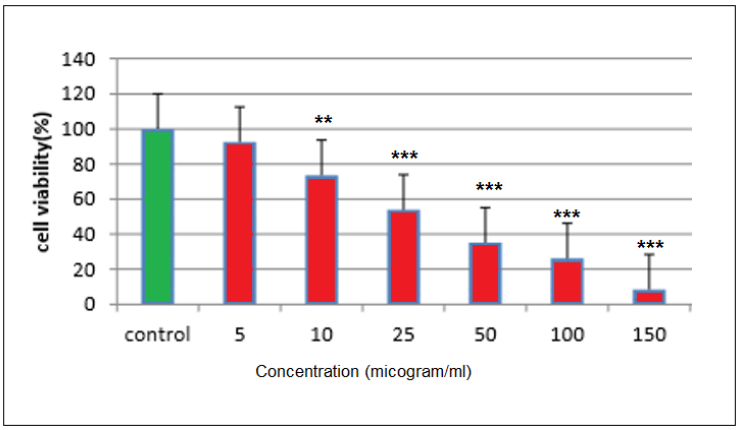

Fig. 1. Viability of glioblastoma cells in control (untreated) group and groups exposed to different doses of phycocyanin. * indicates significant difference compared with control group (**: $p<0.01$ and ***: $p<0.001$).

Caspase-3 expression level also significantly increased in glioblastoma cell exposed to IC50 dose of phycocyanin.

Discussion

Our findings confirmed the cytotoxic effects of phycocyanin on glioblastoma cancer cells in vitro which is mediated by caspase-3 dependent apoptosis. It has been reported that phycocyanin has anti-oxidative function, anti-inflammatory activity, anti-cancer function, immune enhancement function, liver and kidney protection pharmacological effects. Thus, phycocyanin has an important development and utilization as a potential drug, and phycocyanin has become a new hot spot in the field of drug research [18]. The studies also demonstrate that phycocyanin exerts anti-pancreatic cancer activity by inducing apoptotic and autophagic cell death, thereby identifying phycocyanin as a promising anti-pancreatic cancer agent [19]. It has also been shown that under 625-nm laser irradiation, c-phycocyanin generated cytotoxic stress through ROS induction, which killed MDA-MB-231 breast cancer cells depending on concentrations [20]. Mounting evidence has demonstrated that C-Phycocyanin (C-PC) exhibits marked antitumor activity in a wide type of tumors, such as pancreas cancer, breast carcinoma, lung cancer, and colon cancer [21]. A study on the effects of phycocyanin on non-small-cell lung cancer (NSCLC) cells proposes a mechanism of action for phycocyanin involving both NSCLC apoptosis and down regulation of NSCLC genes [22]. Phycocyanin also exerts its anti-cancer activity on colon cancer cells by blocking the cell cycle at the G0/G1 phase and inducing cell apoptosis involving the decrease of Bcl-2/Bax, activation of caspase 3 and release of cytochrome c [23].

Conclusion

The present study showed that the phycocyanin had cytotoxic effects on glioblastoma cells and induces apoptosis via enhancing caspase-8 gene expression level.

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Conflict of interests

The authors declare that there is no conflict of interests.

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