

Bee Venom Induces Unfolded Protein Response in A172 Glioblastoma Cell Line

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Background: Glioblastoma is a type of brain tumor with poor response to available therapies, and shows high rate of mortality. Despite remarkable advancements in our knowledge about cytogenetic and pathophysiologic features of glioblastoma, current treatment strategies are mainly based on cytotoxic drugs; however, these therapeutic approaches are facing progressive failure because of the resistant nature of glioblastomas. In the recent years, however, promising results have emerged owing to targeted therapies toward molecular pathways within cancerous cells. Unfolded Protein Response (UPR) is a remarkable signaling pathway that triggers both apoptosis and survival pathways within cells, and therefore induces UPR-related apoptotic pathways in cancer cells by ER stress inducers.

Objectives: Recently, the role of Bee venom (Bv), which contains powerful bioactive peptides, in inducing UPR-related apoptosis was revealed in cancer cell lines. Nevertheless, currently there are no reports of Bv potential ability in induction of UPR apoptotic routes in glioblastoma. The aim of current study was to evaluate possible role of Bee venom in inducing of UPR pathway within A172 glioblastoma cell line.

Materials and Methods: We treated the A172 glioblastoma cell line with different Bv doses, and assessed UPR-related genes expression by real-time Polymerase Chain Reaction (PCR).

Results: The IC50 of Bv for the studied cell line was 28 µg/mL. Furthermore, we observed that Bv can induce UPR target genes (Grp94 and Gadd153) over-expression through a dose-dependent mechanism.

Conclusions: Our results suggest the potential role of Bv as a therapeutic agent for glioblastomas.

Keywords: Glioblastoma; A172 Cell Line; Unfolded Protein Response; Bee Venom

1. Background

Glioblastoma is a deadly brain malignancy with progressive resistance to available therapies. Despite significant molecular advancements in clinical research, our therapies against cancer still largely depend on general cytotoxic effects of drugs (1). As a result, many neoplastic conditions, which work in an invasive manner, will eventually acquire resistant against conventional therapies (2, 3). Recently, targeted therapies considering molecular adaptors of tumor cells have created promising opportunities to fight progression of multiple tumors (3-5).

Unfolded Protein Response (UPR) signaling pathway is a survival/apoptotic pathway, which is activated upon Endoplasmic Reticulum (ER) stress. Unfolded Protein Response has been demonstrated to be able to induce powerful apoptotic routes in different cancerous cells including glioblastomas. Although multiple agents have been reported as UPR inducers within neoplastic glioblastoma cell lines (6-9), because of limitations in their toxicity level and effectiveness, their clinical use have had restrictions in many cases. Considering these

issues, more investigations are required to identify new potential and applicable ER stress inducer agents. These potential agents would subsequently provide us with wider resources for developing new drugs in different forms of cancer.

Lately, anti-cancerous and anti-proliferative properties of Bee venom (Bv) have been disclosed by multiple investigations. These reports represent the great therapeutic potential of Bv as an effective agent in many cancers. Bee venom consists of powerful active peptides such as melittin and phospholipase A2 (PLA2). These molecules target multiple cancerous cell lines including renal, lung, liver, prostate and bladder (10-15). Nevertheless, the potential effects of Bv on glioblastoma have not been studied.

2. Objectives

Our aim was to evaluate the possible role of Bv in activation of UPR in the A172 glioblastoma cell line.

3. Materials and Methods

This study was carried out at the Avicenna Research Institute of Mashhad University of Medical Sciences in 2014.

3.1. Cell Culture

A172 glioblastoma cell line (Pastor Institute, Iran) was cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% Fetal Bovine Serum (FBS) (Gibco) and 1% pen-strep (Biosera). Cells were grown at 37°C and 5% CO₂.

3.2. Bee Venom Toxicity Determination

In order to determine Bv cytotoxicity, IC₅₀ of the agent was determined through the methylthiazol tetrazolium (MTT) assay. Cells were seeded in a 96-plate and after 12 hours, previously prepared Bv concentrations of 0, 5, 10, 20, 40, 80, and 160 µg/mL were added to the plates. As a positive control, IC₅₀ concentration of Cisplatin was determined for the A172 cell line. Treated cells were incubated in 5% CO₂ and 37°C for 24 and 48 hours. Next, 10 µL of MTT (5 mg/mL in PBS) was added to plates and incubation was continued for four hours in the dark. After this step, the supernatant of the cell culture was replaced with dimethyl sulfoxide (DMSO), and light absorption was determined at 540 nm using an Enzyme Linked Immunosorbent Assay (ELISA) reader. The experiment was carried out in triplicates and IC₅₀ concentration was calculated by the Graph pad prism v5 software.

3.3. RNA Extraction

We used the total RNA extraction kit (Parstous, Iran) to obtain cellular RNA. The experiment was performed according to the manufacturer's instructions, and RNA quality was assessed by running the product on 1% agarose gel.

3.4. cDNA Synthesis

cDNA was synthesized using a purchased kit (Parstous, Iran) according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) on housekeeping GAPDH gene was performed to confirm cDNA synthesis.

3.5. Real-Time Polymerase Chain Reaction

We measured the expression of the two major UPR target genes, Grp94 and Gadd153, via quantitative real time PCR. The employed primers had the following sequences: forward: 5'-TCGCCTCAGTTGAACATTGAC-3' and reverse: 5'-CTTCGTCTCTTCAGGTTCTTC-3' for Grp94 and forward: 5'-TGAAATGAAGAGGAAGAATCAAAA-3' and reverse: 5'-CAGC-CAAGCCAGAGAAGCA-3' for Gadd153. The reaction mixture to run the experiment included: 10 µL SYBR Green dye (Parstous), 1 µL mixed primer, 1 µL cDNA, 0.7 µL ROX dye, and 7.6 µL diluted water. The reaction was performed for 40 cycles on Stratagene Mx 3000 instrument at the denaturation phase (95, 30 seconds), annealing phase (60,

30 seconds) and extension phase (72, 45 seconds). Housekeeping GAPDH gene was applied as the normalizer.

4. Results

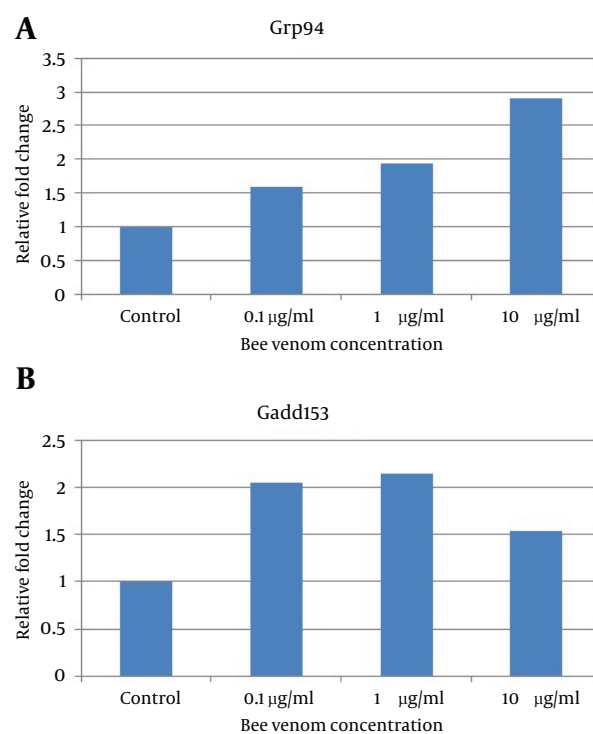
4.1. IC₅₀ of Bee Venome in A172 Cell Line

After 24 and 48-hour periods, 50% viability of cells in exposure to Bv was obtained as 28.53 and 28.30 µg/mL, respectively.

4.2. Bee Venom Induces Dose-Dependent Unfolded Protein Response Target Genes Expression

Expression of the two UPR target genes, Grp94 and Gadd153, was assessed at 0.1, 1 and 10 µg/mL Bv concentrations. Figure 1 shows the respective alternations in expression of Grp94 and Gadd153 genes in response to utilized Bv concentrations.

Figure 1. Unfolded Protein Response Target Genes Expression in Control (Untreated) and Bee Venom-Treated A172 Glioblastoma Cells



A, Grp94; B, Gadd153. Fold changes have been demonstrated in various states. Both genes were overexpressed in Bv treated cells showing UPR induction. Increased fold changes at 0.1, 1, and 10 µg/mL Bv concentrations were 1.5, 1.9, and 2.9 for Grp94 and 2, 2.1, and 1.5 for Gadd153 respectively. P for difference of two genes expression at different concentrations and control state was less than 0.05 (t-student test). However, difference between exposed groups was not significant.

5. Discussion

Poor responsiveness to therapy and low overall survival rate of patients suffering from glioblastoma necessitates more extensive researches to provide new and targeted therapeutic protocols (3, 5). The resistant nature of this tumor against many conventional therapies demonstrates the dynamic course of disease progression, which requires appropriate growth rate of related medications (4). In recent studies, molecular targets involved in apoptotic processes have created an opportunity to develop more effective drugs for cancer. In this regard, UPR-signaling pathway is an interesting area of investigations (6, 9, 16). Although relationships of Bv and different cellular apoptotic pathways have been described, UPR activation has been uncovered as an important mechanism of Bv impact on various tumor cell lines (13). In the present research, we also observed that UPR target genes, Grp94 and Gadd153, were overexpressed in a dose dependent manner by Bv treatment of the glioblastoma cell line. Endoplasmic Reticulum stress and UPR pathway participate in apoptotic death of glioblastoma cell lines exposed to anti-tumor agents such as berberine (16) and cannabinoid (17). Interestingly, ER stress has sensitized several drug-resistant glioblastoma cell lines to radio (18) and chemo (19) based therapeutic approaches. Therefore, UPR activation in A172 glioblastoma cell lines by Bv can also result in apoptosis initiation in cancerous cells. As we found in the current study, elevated doses of Bv caused stronger target genes expression indicating the potential capacity of Bv to be used in dose-coordinated trials.

Regarding difficulties in managing of glioblastoma and its current poor prognosis, development of new generation-targeted therapies seems to be urgent. In the current study, we found that Bv can induce dose-dependent UPR signaling activation in A172 glioblastoma cell line. Therefore, we suggest that Bv and its derivative bioactive peptides could play important roles in future UPR-based therapeutic approaches for glioblastoma.

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Authors' Contributions

Study concept and design: Ali Bazi. Acquisition of data: Ali Bazi, Mohsen Sisakht and Mehran Gholamin. Analysis and interpretation of data: Ali Bazi, Mohsen Sisakht and Mehran Gholamin. Drafting of the manuscript: Ali Bazi. Critical revision of the manuscript for important intellectual content: Mohammad Reza Keramati. Statistical analysis: Ali Bazi, Mohsen Sisakht. Administrative, technical, and material support: Mehran Gholamin, Mohammad Reza Keramati.

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