Original Article

Novel Combination of Periodontal Ligament Stem Cells (PDLSCs)-Conditioned Medium and Taurine Increase Apoptosis in U87Glioma Tumor Cells: Possible Relevance to Glioblastoma Treatment

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Abstract

Background & Objective: Glioblastoma Multiforme is a very aggressive primary brain cancer that has a median overall survival even if the treatment period is less than 1 year. Despite progress in glioblastoma diagnosis and treatment, the prognosis of patients with glioblastoma still remains poor. Recently, it has been detected that stem cell therapies are an effective method for brain tumor cell targeting. Also, the anti-inflammatory and anti-apoptotic activity of taurine against cancerous cells has been found.

Materials & Methods: In this study, we employed taurine as an anti-cancer drug and PDLSCs as a potential agent for improving anti-cancer drug efficiency. Then, we investigated their effect on the glioma tumor cells in in-vitro 2D cell culture and in vivo.

Results: Taurine had the best apoptotic activity on the C6 glioblastoma cells at the concentration of 80 µM after 72h post-treatment. The obtained results showed that a combination of taurine/PDLSCs induced the expression of caspase-3, caspase-8, and IL-17Ra and down-regulated the expression of Bcl-2 and IL-17Ra. Uses of taurine and PDLSCs suppress the migration of the cancerous C6 cells after 48h and show good potential in the suppression of GBM metastasis.

Conclusion: The taurine at concentration of 80µM has a strong potential in decreasing the viability of cancerous C6 Glioblastoma cells. Therefore, taurine and PDLSCs combination, due its therapeutic efficacy, has a considerable potential to be a successful method to glioblastoma brain cancer treatment.

Keywords: Glioblastoma Multiform, Taurine, Periodontal Ligament Stem Cells, Apoptosis

Introduction

Glioblastoma multiform (GBM) is well-known as a highly aggressive tumor of the central nervous system (1). Even though many efforts have been devoted to suppress the progress of this aggressive malignant tumor, conventional treatments such as surgery, chemotherapy, and radiation have improved the median survival of GBM patients by an average of only 14 months (2, 3, 4). Developing methods to get over this aggressive disease is of great importance these days. Taurine (2-aminoethanesulfonic acid), with a simple chemical structure containing a thiol group, is widely found in human tissues (5). Even though taurine was well-known as the

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main nutrient for cats in 1975 (6), further studies revealed that it is significantly reduced in human bodies over time and humans suffer from taurine deficiency (7, 8). So, taurine has since become famous as an essential component in human bodies [8]. It has been shown that one way to predict the progress of several cancer types at the early stages is by measuring taurine levels in the human body (9). It has been reported that taurine level in the patient with breast and bladder level is significantly lower and higher, respectively compared to the healthy human group (9, 10). Recently, the inhibitory effect of taurine in the growth and proliferation of breast cancer cells has been reported (11, 12). Wang showed that taurine decreases and increases the expression of anti-apoptotic Bcl-2 protein and pro-apoptotic Bax protein and surprisingly induces apoptosis of S180 cell xenograft tumors in nude mice (13). The undesirable side effects of anti-cancer drugs promote scientists to look for alternatives such as combining drugs with other potential agents to minimize the drug dosage and improve its efficiency (14, 15). Stem cells (SCs)-based therapy shows promising potential for treating different human disease including cancer (16). Among various cell types which can be employed for cancer cell therapy, mesenchymal stem cells (MSCs) are introduced as an effective agent for targeting cancer cells (16). It has been reported that MSCs block various paths of growth and proliferation of cancer cells (16). Also, the tendency of MSCs in migration into the sites of injury and cancer makes them a favorable vehicle for targeting cancer (16). Although different researches suggest that MSCs have the great potential ability in cancer treatment, obstacles and risks were also described (17). Periodontal ligament stem cells (PDLSCs) are highly specialized connective tissues that surround the tooth root. PDLSCs can be considered as an anti-cancer agent, because of owning properties of MSCs (18). To the best of our knowledge, the effect of a combination of taurine and PDLSCs has not been a matter of discussion in recent years. In this study, we have hypothesized that PDLSCs can augment the effect of taurine on the suppression of brain tumor growth.

Materials & Methods

PDLSCs Isolation and culture

PDLSCs were isolated according to the instruction of the Ethics Committee of Tehran University of Medical Science, Tehran, Iran. To this end, healthy premolars were selected from the donors with orthodontic demands at the School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran. All of the donors signed informed consent forms. Periodontal ligament tissues were obtained by scraping the middle part of the root surfaces. The obtained sections were placed in Hank's medium (HBSS; Invitrogen, Carlsbad, CA) containing 1% (v/v) penicillin-/streptomycin (Sigma, USA) and 1 µg/mL amphotericin B and were transferred to the cell culture laboratory. The roots sections were digested at collagenase type I (3 mg/mL; Sigma, USA) and incubated at 37°C in 5% CO2 for 45 - 60 minutes. The tissues were cultured at the standard culture media (Gibco Dulbecco's modified eagle medium (DMEM)-F12, 15% fetal bovine serum (FBS), 1% penicillin-/streptomycin). After cell migration from the tissue into media and reaching confluence of ~ 80%, ~ 103 cells per well were seeded in petri dishes with a diameter of 10 cm and cultured in pre-warmed (37°C) cell culture media (Gibco Dulbecco's modified eagle medium (DMEM)-F12, 15% fetal bovine serum (FBS), 1% penicillin-/streptomycin). The characterization of isolated PDLSCs

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phenotype after third passage was done by flow cytometry (FACCS Calibure, BD bioscience San Jose, CA, USA) for specific surface markers with the fluorescein isothiocyanate- or phycoerythrin - or peridinin chlorophyll protein complex-conjugated anti-(CD90, CD146, CD105, CD31, CD34, and CD45) monoclonal antibodies.

Preparation of conditioned medium

Conditioned medium (CM) was obtained by collecting the medium above the PDLSCs when the confluence of cells reached ~ 80%. The collected medium was filtered through a 0.22 µm membrane and mixed with fresh media of C6 glioblastoma cells.

C6 Glioblastoma cells culture

The C6 glioblastoma cells were purchased from Cell Bank of the Pasteur Institute, Iran and cultured in Dulbecco's modified Eagle's medium (DMEM, high glucose, Gibco, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Atlanta Biologicals) and penicillin/streptomycin (100 U mL21, 0.1 mg mL21). Cells were incubated at the temperature of 37 º C with an atmosphere containing 5 % CO2. The culture media was refreshed every 3 days.

Synergy experiments

To evaluate the effect of PDLSCs and taurine on the behavior of C6 glioblastoma cells growth, viability, and migration, we followed the protocol as described in Ref (17). Briefly, after reaching the confluence of C6 glioblastoma cells to ~ 80%, the synergy between taurine and PDLSCs-CM was evaluated in C6 cell lines in vitro. To this end, C6 cells were incubated with each agent (different concentrations of taurine and PDLSCs-CM) separately and in combination for 1, 3, and 7 days.

Cell viability

The C6 glioblastoma cells viability at different time points under the effect of PDLSCs-CM and different concentrations of taurine was evaluated using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay kits according to the manufacturer's instructions. The MTT viability assay was carried out after 7 days C6 glioblastoma cell seeding in the presence of different concentration of taurine. After 7 days, the culture media was removed and 100 µL of MTT solution (1 mg/mL in PBS) was added to each well. After that, well plates containing cells and MTT solution were incubated for 4 h. After adding 100 µL of DMSO and incubation for 10 minutes on a rotary shaker in a dark place at room temperature, the UV absorbance of samples was read at 570 nm using an ELIZA reader (Expert 96, Asys Hitch, Ec Austria).

Real-Time Polymerase Chain Reaction (PCR)

Total RNA expressed from C6 glioblastoma cells alone or in the presence of taurine, PDLSCs, and taurine-PDLSCs-CM were evaluated by real-time reverse transcription (RT) polymerase chain reaction (RT-PCR) using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and reverse transcription was carried out with a Sensiscript Reverse Transcriptase Kit (Qiagen). The used primer sequences (IL17-Ra, TRAF31P2, BCL2, Caspase8, and Caspase3) are presented in table 1.

Table 1. PCR primers sequences

Gene	Reverse (5'-3')	Forward (5'-3')
GAPDH	CCTTGACGGTGCCATGGAAT	TCGCCAGCCGAGCCA
IL17-Ra	TCCACAGGGTGAAGCTCACAC	AATTTCCTTGTGCCTGACTGTGAG

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Gene	Reverse (5'-3')	Forward (5'-3')
TRAF31P2	CTTAGGGATACCGTGATGATAATCG	GAACTCAATCTGCATCATTCGATG
BCL2	AAAATACAACATCACAGAGGAAGTAGACTG	TCAATCACGCGGAACACTTG
Caspase8	ACTGGATGATGACATGAACCTGC	CCTCCCCTTTGCTGAATTCTTC
Caspase3	AAAAGCACTGGAATGACATCTCG	GAAACATCACGCATCAATTCCAC

A 7300 RT-PCR machine (Applied Biosystems, Carlsbad, CA, USA) was employed according to special cytokine primers and general SYBR green fluorescence finding for 10 min at 94 °C followed by 45 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. Isolation of total RNA was performed from C6 glioblastoma cells and treating cells using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Reverse transcribing to cDNA was done by the ExScript RT Reagent Kit (Takara Bio Inc., Otsu, Japan), according to the manufacturers' instructions.

Cell cycle experiments

Cell cycle experiments were performed according to the protocol described in Ref. (19) using Propidium Iodide Flow Cytometry Kit (Abcam). Briefly, ~ 105 C6 glioblastoma cells were cultured at a 6-well plate for 24 h. Then, the media was taken out and the cells were treated by taurine, PDLSCs-CM or combination of taurine/PDLSCs-CM. Then, treated cells were kept at the atmosphere with a temperature of 37 °C containing 5% CO2 for 72 h. After that, obtained C6 cells were harvested, washed with PBS, fixed with cold 70% ethanol, and kept at -20 °C for 24 h. After washing with PBS, the achieved cells were stained using 50 mg/mL propidium iodide (PI, Sigma Aldrich), and dissolved in 100 mg/L RNase A (Sigma Aldrich). Cell cycle experiments were carried out and evaluated using flow cytometry (FCM, Becton Dickinson, and San Jose, CA) and analyzed using Modifit LT software, respectively.

C6 Glioblastoma apoptosis experiments

Likewise, the cell cycle evaluation, ~ 105 C6 glioblastoma cells were cultured in a 6-well plate.

After 24 h incubation, the media was taken out and the cells were treated by taurine, PDLSCs-CM or combination of taurine/ PDLSCs-CM for 72 h. Then, the cells were washed using PBS and stained with 5 µL of Annexin V-FITC in binding buffer for 15 min in the dark. Cell apoptosis was quantified by flow cytometry (FCM, Becton Dickinson, and San Jose, CA).

C6 Glioblastoma Scratch assay

To evaluate our new combination of PDLSCs derived CM and taurine on invasion of tumor cells, ~ 105 C6 glioblastoma cells were cultured in a 6-well plate and kept overnight to reach confluent monolayer. A 1 mm wide scratch was made onto the monolayer using a 200 µL pipette tip and the cells were washed twice with PBS to remove all floating cells. Then, the cells were treated by taurine, PDLSCs-CM or a combination of taurine/PDLSCs-CM. To evaluate the efficiency of the treatments on C6 cell migration, samples were continually imaged for 0, 24, and 48 h by phase-contrast microscope. C6 cell migration ability was reported as migration distance from the edges of the scratch toward the center of it using Image J software (NIH, Bethesda, MD, USA).

In vivo experiments

Twenty adult male Wistar rats (4 months old, weighing 240-280 g) were purchased from Tehran University of Medical Sciences,

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Tehran, Iran. Rats were housed in cages under a controlled environment (23 ± 2 °C, 55% relative humidity and a 12-h light/ dark cycle under specific-pathogen-free conditions), and treated according to the guidelines of the Animal House of Tehran University of Medical Sciences, Iran, where standard commercial pellets for feeding (Behparvar CO.) and water and other animal health conditions during all-time course of the experiment were performed.

The rats were randomly divided into four groups (five rats per group): (I) Control sample with C6 glioblastoma cell injection and without any following treatment, (II) The rats with C6 glioblastoma cell injection and treated by injection of ~ 105 PDLSCs into tumor site, (III) The rats with C6 glioblastoma cell injection and treated by injection of optimized amount of taurine drug into tumor site, and (IV) The rats with C6 glioblastoma cell injection and treated by injection of combination of optimized amount of taurine + ~ 105 PDLSCs into tumor site. The animals were euthanized 21 days post-treatment with an intraperitoneal injection of 70 mg Ketamine 5% + 6 mg Xylazine 2% per Kg body weight (injection volume: 0.5 mL). Afterwards, the harvested tissues (tumor mass) were fixed in the 10% neutral buffered formalin (NBF, PH = 7.26) for 48 h, then processed and embedded in paraffin. The 5µm thick sections were prepared and stained with hematoxylin and eosin (H&E). The histological slides were evaluated by the independent reviewer, using light microscopy (Olympus, Japan). Mitotic index (10 high power field (HPF), randomly selected) were counted for each sample. Any changes including inflammatory response and necrosis were assessed in the tumor section of different groups, comparatively.

Statistical Analysis

Results are reported as the mean ± standard deviation (SD). One-way analysis of variance (One-way ANOVA) was used to compare the means from multiple experimental groups, followed by post-hoc Tukey test. Significant differences were shown as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Results

Taurine induces death in C6 Glioblastoma cells in a dose-dependent manner

To reach the optimized concentration of taurine, C6 glioblastoma cancer cells were treated using 40, 80, and 160 µM of taurine for 72 h. The viability of C6 glioblastoma cells was quantified by MTT assay in an addictive dose of taurine (40, 80, and 160 µM) after 72 h. The results showed that the viability of C6 cells was decreased by increasing the dose of taurine up to 80 µM in a dose-dependent manner. The MTT analysis showed no significant difference in the viability of C6 cells by increasing the concentration of taurine from 80 µM to 160 µM (Chart 1a). Therefore, the optimized concentration of taurine for the best effect in decreasing the viability of C6 cells was chosen to be 80 µM.

Taurine and PDLSCs-CM Leads to Expression of Related Apoptotic Genes in C6 Glioblastoma cells

To evaluate the effect of taurine and PDLSCs on the expression of apoptotic genes such as caspase-3 and caspase-8 and anti-apoptotic factors expression such as BCL-2 on C6 glioblastoma cancer cells, real-time PCR assays were performed (Chart 1b). The results showed that the expression of caspase-3 and caspase-8 genes from C6 cancerous cells at the mRNA level was significantly increased when C6 glioblastoma cancer cells were treated by 80 µM taurine along with PDLSCs-CM as compared to control samples (untreated C6 cells) ($p < 0.001$). Furthermore, the BCL-2 gene expression is significantly lower

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to other examined groups ($p < 0.001$). These results strongly confirm the superior effect of the combination of taurine and PDLSCs on the cell apoptosis by down-regulating the expression of Bcl-2 and up-regulating the expression of caspase-3 and caspase-8 compared to either taurine or PDLSCs.

Combination of Taurine and PDLSCs-CM decreases the Expression of Related Regulatory or Proinflammatory Factors in C6 Glioblastoma cells

TRAF3IP2 is a gene that presents the regulation responses to cytokines by members of the Rel/NF-kappaB transcription factor family. This gene family exhibits a significant function in inherent immunity in the face of pathogens and stress. To evaluate the level of inflammation in C6 glioblastoma cancer cells after treatment with taurine and PDLSCs, the expression of TRAF3IP2 mRNA was assessed. Our results showed that the expression of the TRAF3IP2 gene in treated C6 cancer cells with taurine, PDLSCs, or combination of taurine/PDLSCs was increased compared to untreated C6 cancer cells (Chart 1b).

Combination of Taurine and PDLSCs-CM decreases the Expression of Related Tumorigenic Factors in C6 Glioblastoma Cells

IL-17RA gene presents the proliferation of tumorigenic factors (20). To evaluate whether taurine and PDLSCs modulate IL-17RA in glioblastoma cancer cells, C6 glioblastoma cancer cells were employed and were stimulated with 80 µM of taurine and specified concentration of PDLSCs-CM alone or in combination (Chart 1b). As shown in Figure 1c, the results of qRT-PCR revealed that 72-h stimulation of C6 glioblastoma cancer cells with either PDLSCs-CM or taurine/PDLSCs-CM significantly reduced the expression of IL-17RA. Stronger inhibition of IL-17RA was achieved with the simultaneous use of optimized concentration of taurine and PDLSCs-CM.

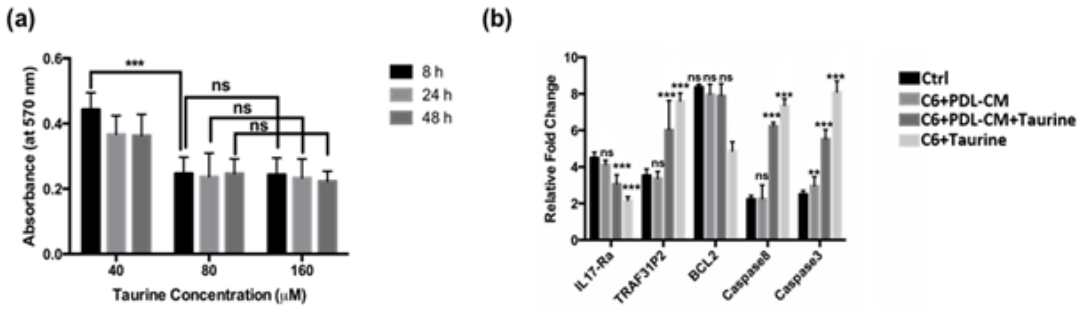


Chart 1. The effect of taurine and PDLSCs alone and in combination on the (a) cell viability and (b) expression of IL17-Ra, TRAF31P2, BCL-2, caspase-8, and caspase-3 in cancerous C6 Glioblastoma cells in different groups including control, C6 treated with PDLSCs-CM, C6 cells treated with 80µM Taurine and C6 cell treated with PDLSCs+80µM Taurine. Significant differences are shown as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. ns stands for not statistically significant

Taurine and PDLSCs-CM induce apoptosis in C6 Glioblastoma Cells

It is well-known that the loss of mitochondrial membrane significantly results in cell apoptosis. Destruction of the mitochondrial membrane causes a release of apoptogenic factors that results in cell death (21). In this study, we compare C6 glioblastoma cells apoptosis and necrosis that are induced by 80 µM of taurine and specified concentration of PDLSCs-CM alone or in combination. induced by 80 µM of taurine and

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specified concentration of PDLSCs-CM alone or in combination. As can be seen in Figure 1a, the total apoptosis rate of C6 glioblastoma cancer cells without any treatment by either taurine or PDLSCs is 0.46% including early and late apoptosis of 0.19 % and 0.27 %, respectively. Figures 1b, c, and d show the C6 glioblastoma cancer cells treated by PDLSCs-CM and a combination of taurine/PDLSCs-CM suffer significant higher apoptosis level and lower necrosis level compared to cells treated by taurine drug alone. Late apoptosis occurs in 5.27% and 7.5% of C6 glioblastoma cancer cells after treatment by taurine and the combination of taurine/PDLSCs-CM, respectively. Treatment of cancerous C6 cells with PDLSCs-CM and taurine/PDLSCs-CM induces necrosis in 3.94 % and 3.74 % of the cells, respectively. The significant higher late apoptosis of cancerous C6 cells treated by a combination of taurine and PDSCs-CM can be described by the benefits of simultaneous use of anti-cancer drug release and stem cell therapy methods.

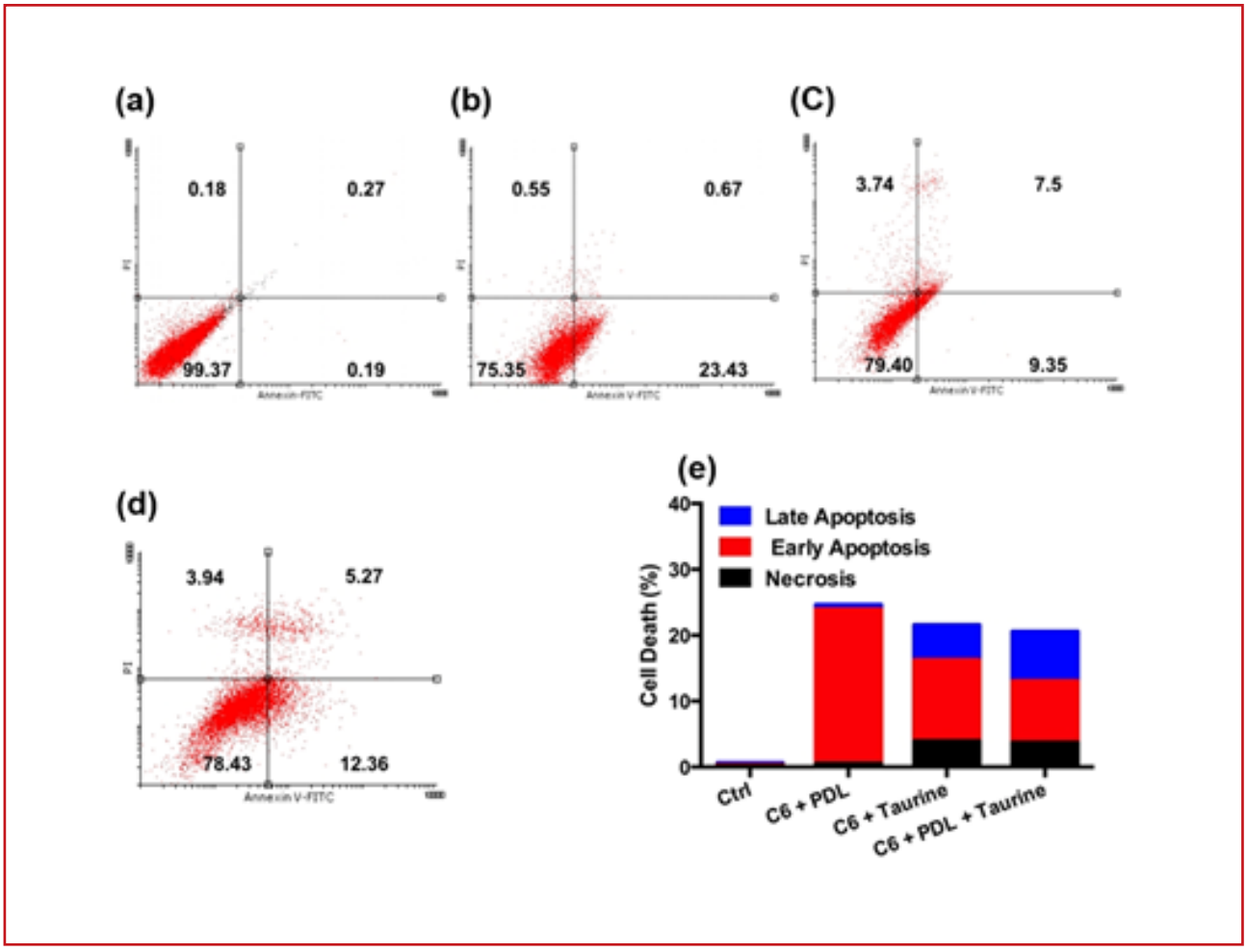


Figure 1. Evaluation of the apoptosis mechanism of C6 glioblastoma cells (a) untreated and treated by (b) PDLSCs-CM, (c) taurine, and (d) combination of taurine/PDLSCs-CM. Untreated and treated Saos-2 cells were stained with FITC-conjugated annexin V and propidium iodide and exposed to flow cytometry technique. (d) Comparison of cancerous C6 glioblastoma cells death rates exposed to taurine and PDLSCs-CM alone and in combination after 72 h. The samples containing taurine have the same concentration of 80 µM taurine

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Taurine and PDLSCs-CM induce C6 Glioblastoma Cells Cycle arrest

Cancer cell proliferation can be evaluated by cell cycle technique (22). Here, we study the effect of taurine and PDLSCs alone or in combination on C6 glioblastoma cancer cells cycle by flow cytometry. As can be seen in Figure 2, all treated samples, with/without the taurine drug, results in an enrichment of C6 glioblastoma cancer cells in G0/G1 phase with a decrease in the number of cells in G2/M phase. Furthermore, cancer cell therapy using PDLSCs-CM causes C6 cells cycle alterations with G0/G1 arrest.

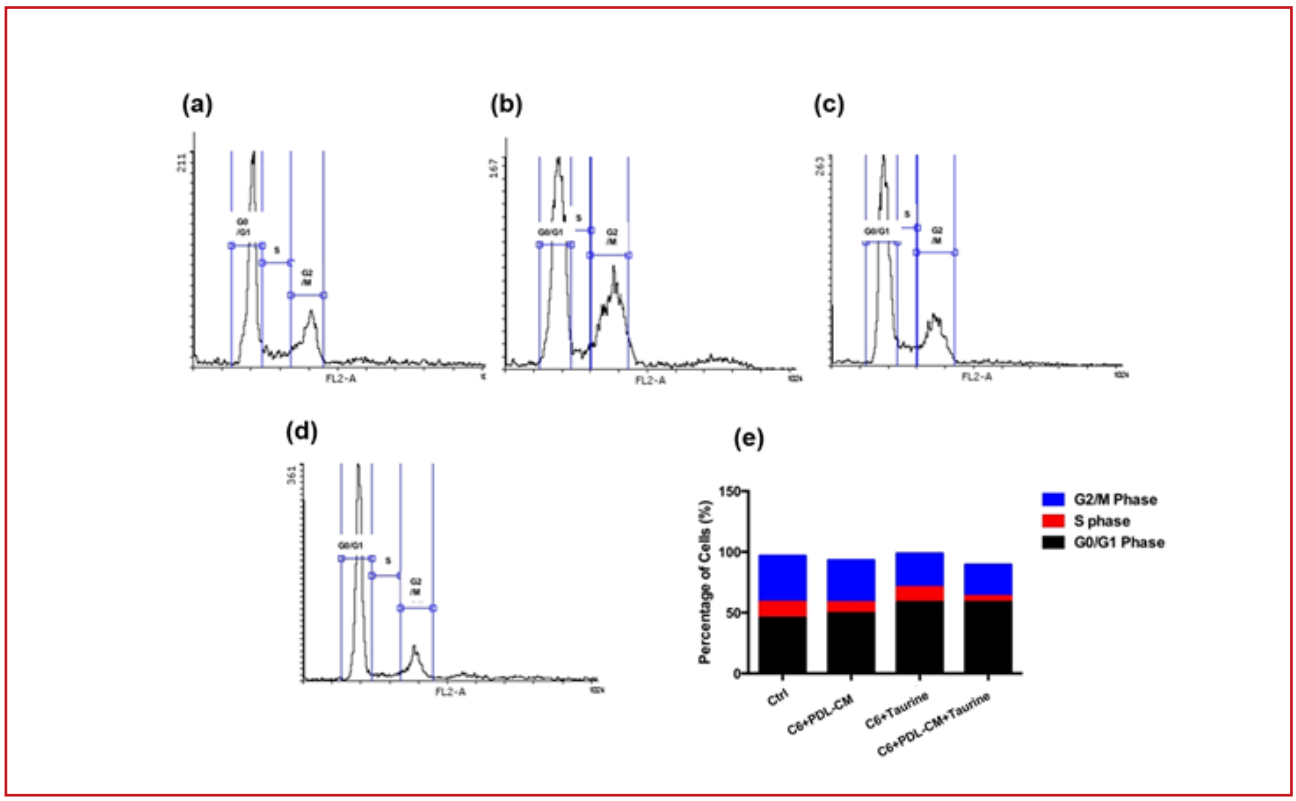


Figure 2. Cell cycle distribution of C6 glioblastoma cells (a) untreated and treated by (b) PDLSCs-CM, (c) taurine, and (d) combination of taurine/PDLSCs-CM. The samples containing taurine have the same concentration of 80 µM taurine. (e) Quantitative analysis of different cell phase populations

Taurine and PDLSCs-CM arrest C6 Glioblastoma Cells Migration

The effect of taurine and PDLSCs alone or in combination on the migration ability of C6 Glioblastoma cells was evaluated using scratch assay (Figure 3). Initial captured pictures showed a similar distance gap for different samples. As can be seen in Figures 3a and b, cells in the control sample (without using taurine or PDLSCs) have fast migration toward opening area (There is a significant increase in migration distance over time). Furthermore, the whole distance gap was covered with migrated cells eventually for control samples. Even though either taurine or PDLSCs-CM has no significant effect on the migration ability of cancerous C6 cells, when a combination of taurine/PDLSCs-CM was employed, no efficient migration was observed (Figures 3a and b).

Figure 3: (a) Scratch test assay test for the evaluation of cell migration for C6 (untreated C6 glioblastoma cells) and cells treated by taurine and PDLSCs-CM alone and in combination. b) Quantitative analysis of the cell migration shows the gap size on the

scratch wound assay. Significant differences are shown as *p < 0.05, **p < 0.01, and ***p < 0.001. ns stands for not statistically significant.

Figure 3: (a) Scratch test assay test for the evaluation of cell migration for C6 (untreated C6 glioblastoma cells) and cells treated by taurine and PDLSCs-CM alone and in combination. b) Quantitative analysis of the cell migration shows the gap size on the scratch wound assay. Significant differences are shown as *p < 0.05, **p < 0.01, and ***p < 0.001. ns stands for not statistically significant.

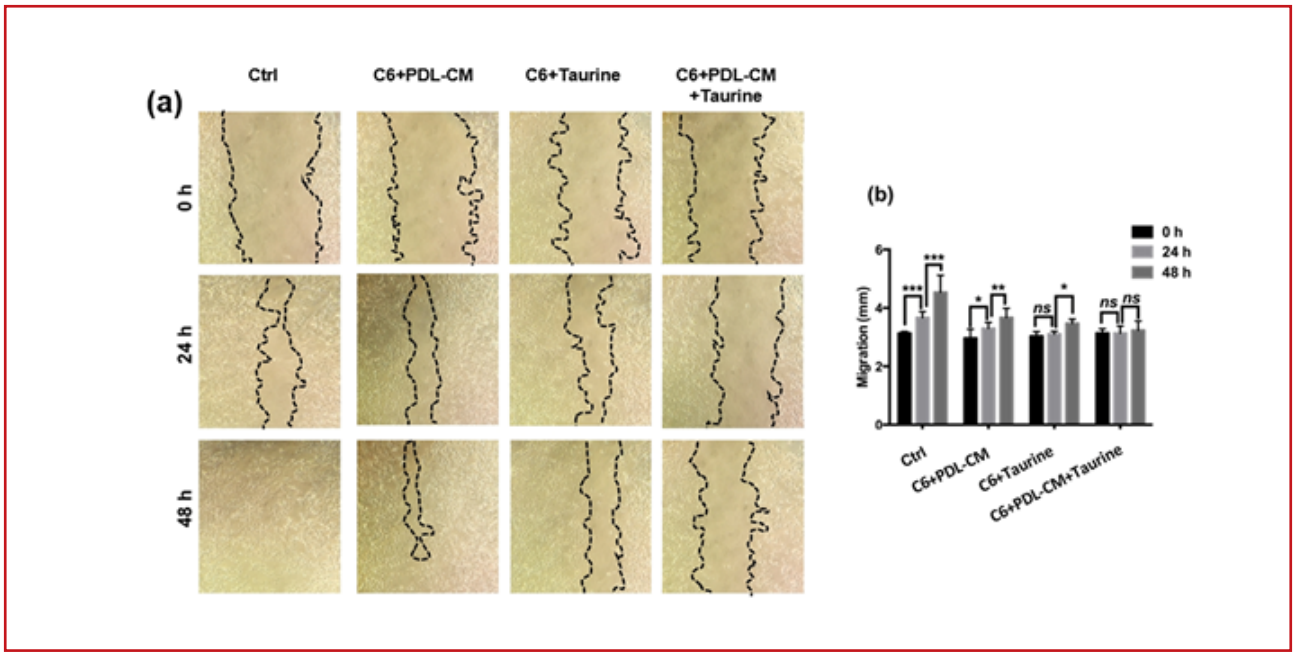


Figure 3. (a) Scratch test assay test for the evaluation of cell migration for C6 (untreated C6 glioblastoma cells) and cells treated by taurine and PDLSCs-CM alone and in combination. b) Quantitative analysis of the cell migration showing the gap size on the scratch wound assay. Significant differences are shown as *p < 0.05, **p < 0.01, and ***p < 0.001. ns stands for not statistically significant

Histopathological Study

All H&E-stained tumor sections from different experimental groups were evaluated histologically. The histopathological evaluation of the primary tumor showed a compact or solid pattern composed of thick trabeculae compressed into a compact mass. The cells were poorly differentiated in control groups which were different from sample to sample in every group. Moreover, mitotic figures are high and tumor cells displayed severe anisocytosis and anisokaryosis in the control group (Figure 4). Different degrees of cell necrosis were seen in each group of experimental animals (Figure 4). The scores of tumor tissue necrosis were highest in the taurine+PDLSCs treated rat followed by drug-treated animals and shows a significant difference between taurine+PDLSCs or drug-treated groups and others. The results have shown that taurine was able to induce tumor cell necrosis, and concurrent therapy with taurine and PDLSCs can successfully improve the percentage of treatment-related tumor cell necrosis. Hyperemia and hemorrhage were seen in drug and PDLSCs treated tumors. The histomorphometric analysis of tumor mass in different groups showed a significant increase in necrotic area and a decrease in the number of inflammatory cells, respectively for all treated samples compared to the control group.

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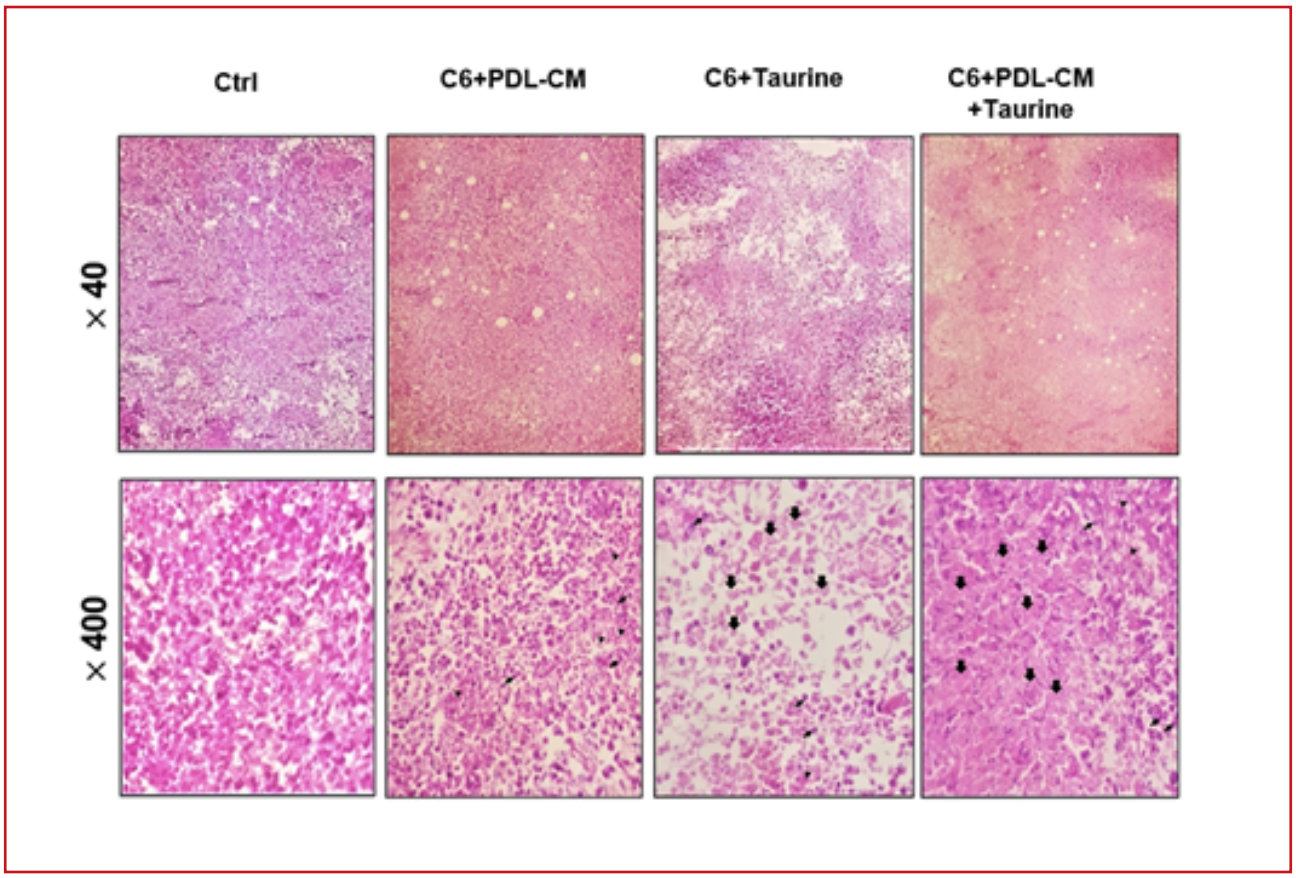


Figure 4. Histopathology of tumor mass in different treatment groups, Arrowheads: hemorrhage. Thin arrows: inflammatory cells, thick arrows: necrotic cells. H&E staining

Altogether, we can observe some interesting effects caused by taurine on glioma cells by providing some information on the molecular mechanisms of taurine inhibitory effects through increasing apoptotic genes such as caspase-3 and caspase-8.

Discussion

In this study, we found that taurine has the best effect on reducing the viability of the cancerous C6 glioblastoma cells at the concentration of 80 µM. by considering results of gene expressions and study effect of taurine or PDLSCs and combination of taurine/PDLSCs on expression of caspase-8, caspase-3, IL 17-Ra and BCL-2 from the cancerous C6 cells, it was found that, Even though either taurine or PDLSCs increases the apoptosis of the C6 glioblastoma cancer cells, a combination of taurine/PDLSCs significantly increases the late apoptosis of cancerous C6 cells. The results of this study also showed the enrichment of C6 glioblastoma cancer cells in G0/G1 phase with a decrease in the number of cells in G2/M phase by the simultaneous use of optimized concentration of taurine and PDLSCs. Tumor cell migration and invasion contribute to tumor metastasis and cause severe hurt [23]. Therefore, the nature of cancer cells in fast migration makes them an invasive agent. The regulation of tumor cell invasion and metastasis is of great importance in cancer treatments. In this study, we showed that taurine as an anti-cancer drug has no significant effect on the C6 glioblastoma cancer cells alone. However, the combined

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uses of taurine and PDLSCs suppress the migration of the cancerous C6 cells. Even though the results of this study confirmed the usefulness of taurine in control of glioma in-vitro, future studies investigated the effects of taurine in glioma in-vivo. The histopathological evaluation of primary tumor tissues in the rats showed a dramatic effect of taurine and PDLSCs on the suppressing growth of tumor tissue over time. The histomorphometric analysis of tumor mass in different groups showed a significant increase in necrosis area by simultaneous uses of taurine and PDLSCs. However, the inflammatory cells were dramatically increased by the use of taurine and PDLSCs alone or in combination.

Conclusion

In this study, we have shown that an optimized concentration of 80 µM taurine has a strong potential in decreasing the viability of cancerous C6 Glioblastoma cells. Our study also demonstrated that combined uses of taurine and PDLSCs have a potent apoptosis effect on cancerous C6 glioblastoma cells. Here, we showed that taurine/PDLSCs increased the expression of caspase-8 and caspase-3 and decreased the expression of IL 17-Ra and BCL-2 from the cancerous C6 cells possibly suggesting apoptosis of the treated cells. The results of this study confirmed the usefulness of taurine in control of migration, proliferation, and growth of glioma. In vivo experiments also showed a significant increase in the necrotic area of glioblastoma tumors through using taurine/PDLSCs. Even though the results of qRT-PCR and in-vivo experiments of this study indicated increasing the inflammatory through using taurine/PDLSCs, to the best of our knowledge, several anti-inflammatory drugs are effective and further in vitro and in vivo research is required to evaluate them.

Data Availability

All data are available upon request from the corresponding authors by email.

Author Contribution

Masoumeh Einabadi, Jafar Ai contributed to all experimental work and drafted the manuscript. Masoumeh Einabadi participated in isolation of PDLSCs and data analysis. Masoumeh Einabadi, Jafar Ai, Mohammad Kargar and Farshid Kafilzadeh contributed to all in vitro experiments evaluation and analysis. Masoumeh Einabadi Participated to the establishing Glioblastoma model and Histological study. Masoumeh Einabadi and Jafar Ai Contributed to the molecular analysis. Masoumeh Einabadi, Jafar Ai and Farshid Kafilzadeh revised the manuscript and contributed to the interpretation of data and the edition of the manuscript. Jafar Ai was responsible for the overall supervision, contributed to design research, data interpretation and manuscript revision.

Conflict of Interest

Authors have no conflict of interest.

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