IJBPAS, August, 2023, 12(8): 3781-3787

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

CELL LINE STUDIES OF RGD-FBG BASED NANOSCAFFOLDS OF EVEROLIMUS DRUG FOR GLIOBLASTOMAS

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Received 15th Sept. 2022; Revised 15th Oct. 2022; Accepted 4th Dec. 2022; Available online 1st Aug. 2023

https://doi.org/10.31032/IJBPAS/2023/12.8.7363

ABSTRACT

Aims/Objective: To evaluate and characterized RGD sequenced fibrin/fibrinogen based nanoscaffolds (acid-labile linker) for the treatment of brain tumor targeting using u-87MG cell lines.

Place and Duration of Study: Department of Pharmaceutics, Parul Institute of Pharmacy and Research, Parul University, Vadodara, between 2017 to 2021.

Methodology: Nanoscaffolds was prepared with Drug–linker–Fbg conjugate solution, using modified water-in-oil (W/O) emulsification/solvent extraction method. The conjugate solution was evaluated with the molecularly targeted compound and assessed the effect of Everolimus on the cell lines; "gene or protein measurement" referred to studies that measured levels of molecular markers in response to Everolimus. Cell viability was the outcome of interest of the carried-out research work. Data on cell viability included the assay, quantification technique used and cell viability measurement.

Results: MTT assay has been performed on formulation for assessing the % viability of the drug on U-87 MG cell line. Comparison of API with Formulation revealed that the formulation produces less cell viability as compared to individual API. The absorbance of the produced formazan is proportional to the number damaged or dying cells.

Conclusion: As per the study design the formulated nanoscaffolds cell viability for

formulation (50µg/ml) was studied which refers to the ability of a cell to perform its biochemical and physiological processes, particularly in regards to its metabolism and ability to divide that shows the lower effect of the IC50, which suggests the more cytotoxic the drug is to that specific cancer cell type.

Keywords*: Everolimus, Nanoscaffolds, U-87MG*

1. INTRODUCTION:

In 1968, Pontén and Macixtyre initiated the Uppsala (U) series of malignant glioma (MG) cell lines by establishing four cell lines derived from human malignant gliomas, still widely used by researchers in the glioma field **[1]**. Researchers from the Uppsala University have found also that one of the most used GBM cell lines, U87MG, obtained from American Type Culture Collection (ATCC) was quite different from that collected from the original tumor.

Cell culture and cell lines have assumed an important role in studying physiological, pathophysiological and the differentiation processes of specific cells. It allows the examination of stepwise alterations in the structure, biology, and genetic makeup of the cell under controlled environments.

This is a hypodiploid human cell line with the modal chromosome number of 44 occurring in 48% of cells. The rate of higher ploidy was 5.9%., Twelve markers were common to all cells, including der(1)t(1;3) (p22;q21), der(16)t(1;16) (p22;p12), del(9) (p13) and nine others **[2]**.

2. CELL LINE STUDY: MTT ASSAY

Gliomas are a heterogenous group of neoplasms derived from various glial cells and account for 40-50% of all intracranial tumors. Glioblastoma multiforme (GBM), the most malignant type of glioma (WHO grade IV), is highly invasive and tend to diffusely infiltrate into the surrounding normal brain parenchyma. These characteristics prevent the tumor cells to reside within the margins of the therapeutic resection.

The accelerated proliferation of GBM cells also drives the pronounced complexity of these tumors that typically encompasses a huge genetic diversity along with a heterogeneous microenvironment. The presence of hypoxic and hyper-perfused regions, redox gradients and different pro-inflammatory cytokines sustain a dynamic environment, promoting both the proliferative and infiltrative phenotypes.

Primary aim of study' had three categories: "therapeutic evaluation" referred to studies comparing the effects on cell lines of another therapeutic agent; "pathway modification" referred to studies that altered cellular pathways with a molecularly targeted compound and assessed the effect of Everolimus on the

cell lines; "gene or protein measurement" referred to studies that measured levels of molecular markers in response to Everolimus **[3]**.

Cell viability was the outcome of interest of the carried-out research work. Data on cell viability included the assay, quantification technique used and cell viability measurement.

Cell lines are created in two main ways:

They can be developed directly from a living organism (e.g., a patient or an animal); in this case, a single cell may be stimulated to allow it to expand into multiple cells. This is sometimes called "clonal expansion," although no cloning is used. Here, "clonal" refers to the fact that the cells are all clones of each other (i.e., identical).

They can originate from a living organism but be maintained over many years (e.g., a commercially obtained cell line). These cells are produced similarly to the method described above, but they go through more rounds of cell division to create a stable profile for each cell line.

U-87 MG cell line has been used to study the effects of the lysosomal destabilizing drug Siramesine on glioblastoma **[4]**.

3. MATERIAL AND METHODS

The general investigational aim of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is to measure viable cells in relatively high throughput (96-well plates).

Cells & Controls:

Incubator with 5% CO_2 at 37°C. Microplates – 96 well plates were used.

Solutions & Solvents:

Dissolve 500 mg MTT powder (Sigma, St. Louis, USA) in 10 mL PBS. Stirred with a magnetic stirrer for approximately 1 h in the dark. Filter sterilized the solution with a 0.22 mm filter (Millipore) and stored in 10-mL aliquots at −20°C.

Acidified Ethanol:

Formazan crystals were dissolved in acidified ethanol solution. To make this solution, 50 mL 2M HCl was added to 2.5L ethanol. The solution was stored at least a month at room temperature before use.

Media Preparation & Estimation of cell viability:

Culture media: -

Step 1:

- Eagle's minimum essential medium (EMEM) + 10 % Fetal Bovine Serum (FBS) + 100U/ml penicillin + 100µg/ml streptomycin
- Media was propagated at 37°C in 5% CO_2 atmosphere

Step 2:

- EMEM + 2mM Glutamine + 1% Non-essential amino acid + 1mM Sodium pyruvate + % FBS
- Split sub-cuture (70-80%), and cells

were seeded at 5×10^3 cells/cm^2

- Cells were seeded using 0.25% trypsin, 5% CO_2 at 37°C.

Method and Incubation:

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

- U-87 MG ($5x10^3$) were plated in 96 well plates in 200 µL of EMEM medium per well and incubated for 24 hrs.
- Cells were incubated with different conc. of test solution for 48 hrs. Medium was removed from all the wells and wells were fed with 200 µL of fresh complete medium.
- Cells were incubated with different conc. of test solution for 48 hrs. Medium was removed from all the wells and wells were fed with 200 µL of fresh complete medium.
- 100 µL of MTT solution was added to each well plate and incubated for 4 hrs. Cell plates were centrifuged at 3000 rpm, for 10 min & culture media was discarded.
- Each cells were treated with 200 µL of DMSO and the solution was added to dissolve MTT formazan crystals.
- DMSO solution was measured at 540 nm with micro plate reader immediately. Cell viability was calculated
- Viability plots were plotted by plotting % viable cells (y-axis) against the treatment**.**
- Half-maximal inhibitory concentration (IC_{50}) is informative measure of a drug's efficacy. It indicates how much drug is needed to inhibit a biological process by half, thus providing a measure of potency of an antagonist drug in pharmacological research **[5-11]**.
- %Cell viability was calculated by treating cells and assessing against U-87 MG cell line. Cells with fresh media, Blank media, Drug, Formulation and triton were treated and absorbance was measured. The media was supplemented with growth factors.

4. RESULTS AND DISCUSSION

- Glioblastoma (GBM) is the most malignant and highly aggressive brain tumor. In this study, four types of typical GBM cell lines (LN229, SNB19, U87, U251) were cultured in a microfabricated 3-D model to study there in vitro behaviours.
- MTT assay has been performed on formulation for assessing the % viability of the drug on U-87 MG cell line.
- Comparison of API with Formulation revealed that the formulation

produces less cell viability as compared to individual API.

- The absorbance of the produced formazan is proportional to the number damaged or dying cells as shown in **Figure 1**.
- The viability of the treated cells depends on the cytotoxicity of the drugs as represented in **Table 1**.
- Cells with fresh media, Blank media, Drug, Formulation and triton were treated and absorbance was measured. The media was supplemented with growth factors where %cell viability and IC_{50} index was comparatively less cytotoxic in nature than API.
- Cell viability for formulation (50µg/ml) refers to the ability of a cell to perform its biochemical and physiological processes, particularly in regards to its metabolism and ability to divide.

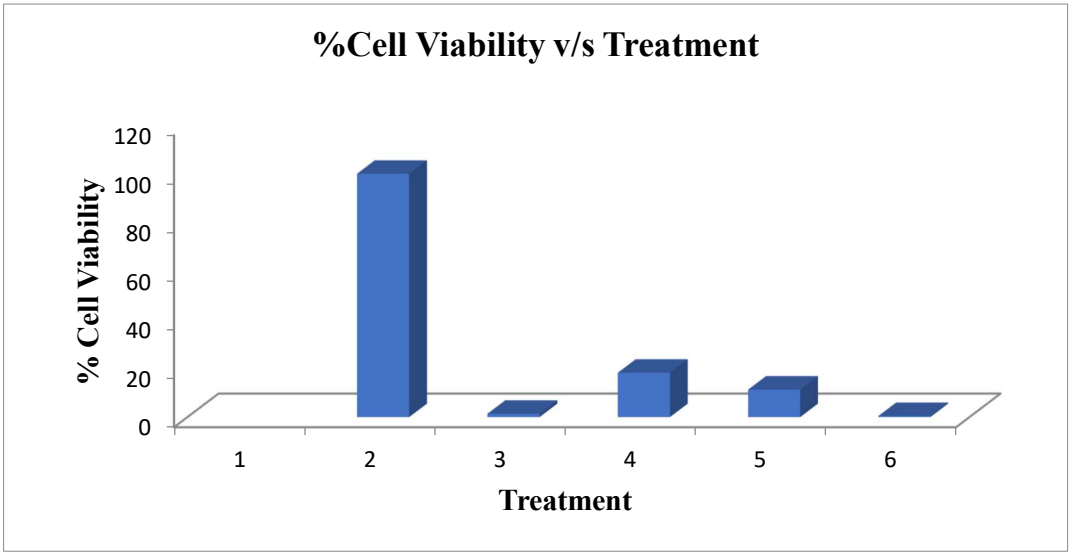

Figure 1: % Cell viability v/s Treatment

Table 1: Results of Incubation studies

Sr. no.	Treatment	48 hr Incubation Period		
		Mean Absorbance*	% Cell Viability*	IC_{50} (m/mL) 95% Confidence Interval
1	Control (Only cells with fresh media)	0.752	95	11.6
2	Only media with no cells	0.194	98	1.75
3	Everolimus (100µg/ml)	0.078	11.32	5
4	Formulation (50µg/ml)	0.094	18.67	1.79
5	Triton X100	0.007	0.43	0.09

*The lower the IC50, the more cytotoxic the drug is to that specific cancer cell type

5. CONCLUSION

The current research investigation uses RGD-FBG nanoscaffolds for brain tumor targeting particularly glioblastomas. $\alpha_v\beta_3$ is an essential survival factor for many glioblastomas including U87MG cell line. Glioblastomas responded to $\alpha_v\beta_3$ inhibition by reducing the growth rate and induction of apoptosis. $\alpha_v\beta_3$ is anchored in the cytoplasm in its inactive form by coupling to the inhibitory protein of nuclear factor type B. MTT assay has been performed on formulation for assessing the % viability of the drug on U-87 MG cell line. MTT data revealed IC_{50} patterns for the U87MG cell lines tested, and the IC_{50} values were positively correlated with the seeding densities. Therefore, MTT measures cell respiration and the amount of formazan produced is proportional to the number of living cells present in culture. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the drug. IC_{50} is the concentration of the tested drug able to cause the death of 50% of the cells and can be predictive of the degree of cytotoxic effect. The lower the value, the more cytotoxic is the substance. The formulation cell viability was found out to be 18.67% with 1.79 IC50 interval which is observed to be cytotoxic for cancer cells as related to drug.

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