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Cell Cycle Progression of Jurkat (Leukemia) and LA-N-5 (Neuroblastoma) Cell Lines under the Influence of Taheri Consciousness Fields

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Abstract

Mohammad Ali Taheri has introduced T-consciousness as a third element of the universe, in addition to matter and energy. There is a wide variety of Taheri Consciousness Fields (TCFs) that cannot be measured directly. However, it is possible to investigate their effects on various subjects. Previously, survival and death of different kinds of cancer cell lines have been evaluated under influence of TCFs. This experiment was designed with the aim of reproducing obtained results and investigating the effects of two types of TCFs (1 and 2) on this behavior of Jurkat cell line with lymphoblast morphology and LA-N-5 cell line with fibroblast morphology, which cause leukemia and neuroblastoma, respectively. First, the effects of TCFs on the Jurkat cell line was examined using the MTT assay at 12, 24, and 48 hours. Additionally, after 48 hours, cell cycle analysis was performed in both cell lines using flow cytometry. According to the obtained data, TCF2 increased the viability of the Jurkat cell line at 12 hours, while TCF1 caused a significant increase in the G2/M phase, which may suggest checkpoint activation and accumulation of cells in this phase under the influence of the field. For LA-N-5, the results showed that TCF2 led to a significant increase in the S phase percentage. This increase was accompanied by a decrease in the G2/M phase, which may indicate an S-phase arrest. However, TCF1 did not induce any significant changes. These observations indicate that the application of different TCFs can lead to varying outcomes. Further studies are required to clarify the effects of TCFs at the biological levels.

Keywords: Taheri Consciousness Field; Neuroblastoma; Flow cytometry, LA-N-5; Jurkat; Leukemia

Introduction

The Jurkat cell line is an immortal T lymphocyte cell line derived for the first time from the peripheral blood of a boy with T-cell leukemia. This cell line has often been used as a primary T cell line to study several events in T cell biology, including T cell signaling and molecular events in the HIV life cycle (Schneider et al., 1977). Many of the most common childhood cancers diagnosed with brain tumors such as Wilms' tumor, rhabdomyosarcoma, and high-risk neuroblastoma have very low survival rates (ACS Special Report 2014).

Neuroblastoma is the most common extracranial solid tumor in children. The prevalence of this disease is 1 in 8000 to 10,000 births and the 5-year survival rate is more than 95% for children in low-risk and moderate groups (Maris et al., 2007). These tumors are highly metastatic and resistant to conventional treatments like radiation or chemotherapy, and the LA-N-5 cell line is one of the cellular models of these tumors (Shastry et al., 2001).

According to Taheri's theory, T-Consciousness fields (TCFs) exist with a non-frequency-based nature and are a subset of the Cosmic Consciousness Network. The effects of these fields are initiated through the mind of an announcer (Faradarmangar) and occur with brief, and instantaneous attention. It is hypothesized that the information transmitted through these fields can induce changes in the subject under study. Previously, the effects of TCFs on cancer cell lines *in vitro* have been evaluated (Taheri et al., 2022 a, b). In this study, the effect of the TCFs 1 and 2 on the LA-N-5 cell line causing neuroblastoma and Jurkat cell line causing leukemia was investigated.

Materials and Methods

TCFs application

In this study, samples were under influence of TCFs for 12, 24, and 48 hours. Control in this study is as follows: negative control is the Jurkat cells which are untreated with no TCFs and drug, and positive control for Jurkat cells includes cells treated with temozolomide, and for the LA-N-5, cells treated with doxorubicin.

Cell culture

Cell lines were purchased from the Pasteur Institute of Iran and cultured in 1640 Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum (Gibco Laboratories, Grand Island, NY), 100 IU/ml penicillin, and 100 µg/ml streptomycin. Cells were maintained in an incubator at 37 °C (Memmert, Schwabach, Germany) with 5% CO_2 and a humidified atmosphere. Cells in the logarithmic growth phase were used for all experiments in this study.

MTT Assay

The MTT assay was used to evaluate cytotoxicity and cell viability following TCF treatment. A total of 3×10^3 cells were seeded in each well of a 96-well culture plate. Cell viability under these fields was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). For this purpose, MTT (Sigma, Taufkirchen, Germany) was prepared at a concentration of 0.2 mg/ml in RPMI-1640 medium. The cells were incubated at 37 °C for 4 hours. After incubation, the medium was replaced with 100 µl of dimethyl sulfoxide (DMSO) and 25 µl of Sorenson's buffer (0.1 M glycine, 0.1 M NaCl, adjusted to pH 10.5 with 0.1 M NaOH). The cells were then incubated at 37 °C for 30 minutes, and absorbance was measured at 570 nm using a microplate reader (Tecan, Sunrise, Switzerland).

Cell Cycle Analysis

Cell cycle progression analysis was performed by staining with propidium iodide. The cells were cultured in 6-well plates (1×10^5 cells per well) and kept overnight in a standard incubator. The cells in the experimental group were washed, separated, and harvested, then resuspended, fixed in 70% ethanol, and kept for another 72

hours at 4 °C. Cells were stained at 37 °C for one hour using 50 µg/ml PI. The proportion of cells at different stages of the cell cycle was assessed using a flow cytometer in the FACSCalibur system (Miltenyi Biotec FACSQuant 10).

Flow cytometry

Possible changes in apoptosis were measured after TCF1 treatment using the flow cytometric Annexin V/Propidium Iodide (PI) method. A total of 1 × 10^5 cells were cultured in a 6-well plate. Cells were then exposed to TCF1 treatment once per day throughout the study period. After 24 hours, the cells were trypsinized and centrifuged at 1,500 rpm for 5 minutes. The cells were stained with Annexin V and PI according to the manufacturer's instructions. For staining, 2 µl of Annexin V, 1 µl of PI, and 100 µl of binding buffer were added to each sample. The cells were incubated for 15 minutes at room temperature in the dark. Samples were analyzed by flow cytometry (Macs Quant Analyzer 10, Miltenyi Biotech, Germany), and the rate of apoptosis was assessed using FlowJo software (Tree Star, San Carlos, CA).

Statistical Analysis

Data were analyzed using GraphPad Prism software, version 6.0 (San Diego, CA). All values are presented as mean ± standard error. All analyses were repeated at least three times. To determine the significance of differences, t-tests and analysis of variance (ANOVA) were used, and p-values < 0.05 were considered statistically significant.

Results and Discussion

The MTT assay results for the Jurkat cell line are shown in Figure 1. As observed, TCF2 led to increased viability at 12 hours, while at other time points, the TCFs did not show a significant effect.

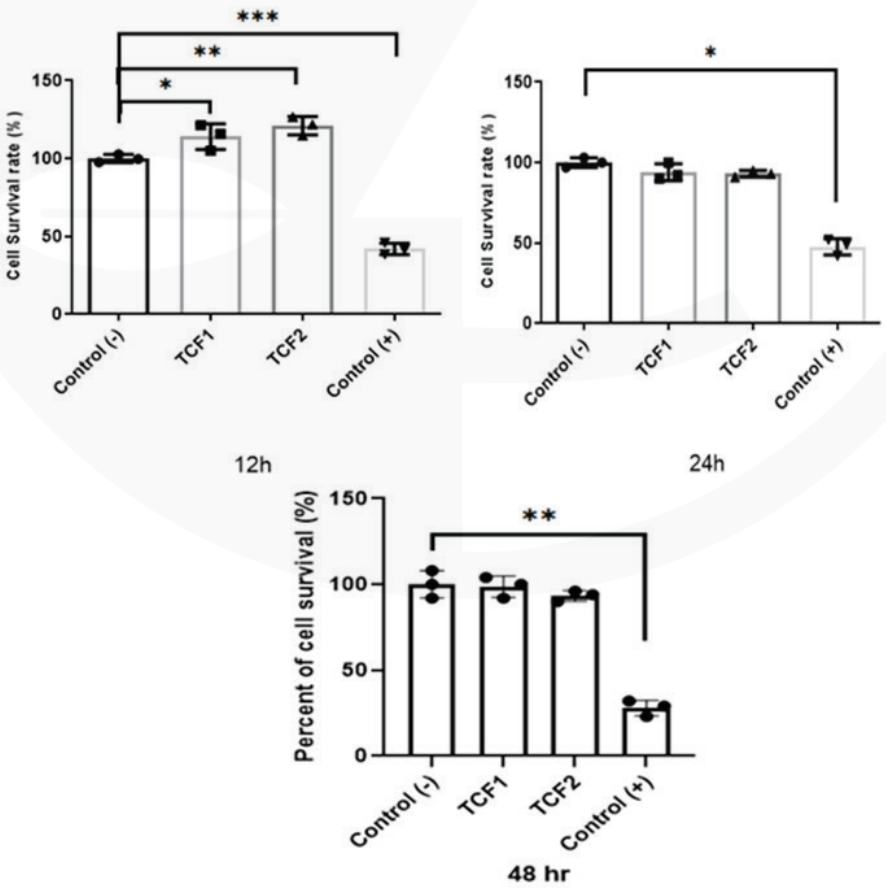

Figure 1. MTT assay results for the Jurkat cell line at 12, 24, and 48 hours (under the influence of TCF1 and TCF2). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

The obtained data from cell cycle analysis can be observed in Table 1. Analysis of cell cycle distribution showed that Jurkat cells responded differently to TCF1 and TCF2. In the control group without field exposure, most cells were in the G1 phase (75.11%). Exposure to TCF1 led to a significant increase in the G2/M population (from 3.73% in the control to 8.99%), accompanied by a slight decrease in the S and G1 phases. This increase in the G2/M fraction indicates a delay or checkpoint activation at the G2/M transition and likely reflects an accumulation of cells before entering mitosis under the influence of TCF1. Notably, the S phase did not increase, further confirming that the main effect is limited to the G2/M boundary and not DNA synthesis. In contrast, TCF2 induced smaller changes in the cell cycle profile, with G2/M only slightly increased compared to control (5.24%). The percentages of S and G1 phases remained almost unchanged. This minor change suggests that TCF2 had no significant effect on cell cycle progression in this cell line. TCF1 treatment thus led to an increase in the G2/M phase in these cells.

Moreover, apoptosis assessment showed no significant changes in this cell line under the influence of the consciousness fields (Table 2). The population of viable cells (Q4) remained above 97% under all conditions, and no significant increase was observed in apoptotic (Q3/Q2) or necrotic (Q1) fractions. These data indicate that neither TCF1 nor TCF2 induced detectable cytotoxicity or apoptosis at the time of the experiment, which is consistent with the MTT assay results.

It is noteworthy that the slight increase in G2/M under TCF1 was not accompanied by increased apoptosis, suggesting a cell cycle delay. However, a modest increase in necrosis was observed in cells exposed to TCF2. Overall, TCF1 appears to affect regulatory mechanisms related to the G2/M transition, whereas TCF2 maintains a cell cycle profile close to the control, consistent with its previously observed effect in enhancing cell viability. The combination of maintained viability and altered G2/M distribution under TCF1 highlights the potential regulatory, rather than cytotoxic, effect of this field on Jurkat cell proliferation.

Table 1. The Cell cycle analysis of the Jurkat cell line under the influence of Taheri Consciousness Fields (TCFs).

Samples	Cell cycle percentage		
	G1	S	G2/M
Control (-)	75.11	21.16	3.73
TCF1	72.78	18.23	8.99
TCF2	73.15	21.61	5.24

Table 2. Flow cytometry analysis of the Jurkat cell line compared with the control.

% of each cell state				Sample
Q4	Q3	Q2	Q1	
97.8	1.25	0.461	0.538	Control (-)
97.39	1.09	0.604	0.518	TCF1
97.49	1.3	0.679	0.621	TCF2

TCF: T-Consciousness field; Q1: percentage of necrotic cells; Q2: percentage of late apoptotic cells; Q3: percentage of early apoptotic cells; Q4: percentage of viable cells.

Moreover, TCFs affected cell cycle progression of LA-N-5 (Table 3). Particularly, TCF2 treatment led to a significant increase in S phase (around 16%) and a notable decrease in G2/M phase cells by about 60%. This observation indicates that the effects of TCFs can vary depending on the cell line type.

Table 3. Cell cycle analysis of LA-N-5 cell line under Taheri Consciousness Fields (TCFs) compared to control.

Sample	Cell cycle percentage		
	G1	S	G2/M
Control (-)	71.32	22.61	6.7
TCF1	68.71	24.17	7.12
TCF2	71.11	26.18*	2.71*

*: p-value<0.05

As it has been mentioned in the introduction section, there are a wide variety of TCFs with specific functions introduced by Taheri. In prior studies, their influences have been demonstrated frequently (Taheri et al., 2022c). According to this theory, the subjects under study, such as cell lines in the current experiment, receive information upon exposure to the TCFs. Based on Taheri's theory, in addition to the physical body, considered as hardware, the cells possess software to manage and guide hardware.

Changing the behavior of the cell lines in this research suggests that they have received information from TCFs. It is also worth mentioning that the effects of TCFs were investigated in double-blind way and without any kinds of physical intervention.This methodology makes results of the study less likely to be biased and with adequate repetitions exhibits the influence of TCFs. In this study, before describing the mechanism of TCFs, the observed results have been reported. Further research is necessary to be designed for gaining a better insight into how these fields affect cell behavior.

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