RESEARCH / INVESTIGACIÓN

Inflammatory response of stem cell secreting conditioned media in SH-SY5Y cell line

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DOI. 10.21931/RB/2020.05.03.13

Abstract: Mesenchymal stem cells (MSCs) are reported to secrete anti-inflammatory cytokines and growth factors, which makes MSCs a promising candidate in the treatment of various neurodegenerative diseases. SH-SY5Y show extreme inflammatory response under LPS and an inadequate inflammatory response when treated with Wharton's jelly, conditioned media. This study mainly focuses on the inflammatory (pro and anti-inflammatory) response of SH-SY5Y by gene expression study. SH-SY5Y cell line used for cell culture and RT- q PCR was done with 5 different primers. In this article, lipopolysaccharides (LPS) show a significant result in pro-inflammatory and pro-apoptotic. In this article, we focus on the therapeutic approach of stem cells, which reduce inflammation by secreting stem cell factors to cure various neurodegenerative diseases.

Key words: Inflammation, MSCs, SH-SY5Y, Gene expression, quantitative PCR.

Introduction

SH-SY5Y is neuroblastoma human cell lines. SH-SY5Y secrete growth factors, cytokines, micro-RNAs, proteasomes, and exosomes, which play a vital role in inflammation[1]. Inflammation includes two forms of signals, the signal that intimate and retain inflammation and signal that bar down the process Inequality between the two signals frond inflammation unhampered, which leads to cellular and tissue damage[2]. In macrophages, inflammation has three significant roles, i.e., antigen presentation, phagocytosis, and immunomodulation, through the production of various cytokines and growth factors[3]. Macrophages have a choleric role in the initiation, maintenance, and resolution of inflammation[4,11]. Macrophages get activated and get deactivated during the inflammatory process[4,11]. Activation signals include cytokines, lipopolysaccharides, and extracellular matrix proteins[2,3]. LPS increases inflammation in which there is speedy cell proliferation with fast cell death[5]. Activated macrophages get deactivated by anti-inflammatory cytokines and cytokines rival, which mostly produced by macrophages[5,11]. Macrophages have both beneficial and detrimental outcomes in inflammation and are promising therapeutic interference for inflammation[5,11]. Conditioned media from MSCs contain various cytokines; growth factors and micro RNAs play an essential role in attuning inflammatory process[8]. Cytokines are called inflammatory mediators[2,5]. IL-β is pro-inflammatory mediators, and IL10 and TGF-β are anti-inflammatory mediators[2]. When there are neuroinflammation chemokines, regulate cell migration. Chemokines act as a neuro-modulators that increase inflammation and development[4,10].

When the expression of Bcl-2 is high, it protects neurons from neuronal death (*in vivo* and *in vitro*)[13]. Bcl2 is a protein that maintains cellular homeostasis by regulating apoptosis and has both anti-apoptotic and anti- necrotic effect[15,16]. BAX is linked with Bcl-2, which is protein-encoding gene[15,16]. Our study focuses on the inflammatory response of five different genes. BAX, IL-10 and TGF- β1 has anti- an inflammatory response which protects from neural cell death by reducing inflammation. We are moving towards therapeutics, so instead of stem cells, we focus on stem cell factors[6,7,14]. Stem cell factors are considered as a promising therapeutic approach that helps in Parkinson's disease or Alzheimer's disease[6,7]. In the future, it is predicted that stem cell factors can activate death neurons and can be a novel therapeutic approach[6,7,14].

Methods

CELL CULTURE – DMEM media is used with 10% FBS, and 1% penicillin as an antibiotic which used for SHSY5Y cell culture, and the cells were incubated in T25 flask at 37°C with 5% CO2.

EXTRACTION and RT-q PCR – RNA was isolated by using Isoplus reagent. cDNA was synthesized using the Primer Script III First- Strand cDNA Synthesis kit (TAKARA BIO). Real-time RT-PCR analysis was performed using the Quanti Tect SYBR Green PCR kit (QIAGEN). All the above methods were executed according to the manufacturer's instructions. Real-time analysis of like BAX, Bcl-2, IL-1β, TGF- β1, and IL-10 was done in duplicates on a CFX384 Real-time PCR system (BIO-RAD). CFX Manager software was used to analyze the results.

Results

IL-1β is pro-inflammatory, IL -10, and TGF –β1 are anti-inflammatory genes. When we see our result under three different concentrations i.e. LPS, Wharton's jelly conditioned media, and cord line conditioned media, we find that LPS under IL-1β has a fold change of 1.4. IL-10 and TGF-β have a fold change of 0.4 and 0.05, respectively. Cord line conditioned media in IL-10 and TGF-β1 has a fold change of 3.0 and 0.44, respectively. LPS is least in both IL-10 and TGF-β1 with 0.25and 0.005fold change, respectively. Wharton's jelly in all the three genes IL-1β, IL-10, and TGF-β1, show moderate fold change with 0.8, 1.7, and 0.34, respectively. Figure 1

BAX is pro-apoptotic, and Bcl2 is anti-apoptotic. In our result in BAX and Bcl2, there is a fold change of 7.2and 1.15 under LPS. BAX and Bcl2, when treated with Wharton's jelly conditioned media and cord line conditioned media, there is a fold change of 0.5,1.5,0.9 and 1.2, respectively.

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GENE	Tm value in CELCIUS	SEQUENCE (5'-3')
hGADPH-F	52.6	CACCACCATGGAGAAGGC
hGADPH-R	51.1	CAGGAGGCATTGCTGATGA
IL-1β -F	48	TGAAGCTGATGGCCCTAA
IL-1β -R	50.3	CTTGTCCATGGCCACAAC
IL -10-F	54.4	CCTGCCTAACATGCTTCGAGA
IL -10-R	53.2	GCTTGGCAACCCAGGTAAC
TGF-β1-F	50.3	GAGACTTTTCCGTTGCCG
TGF-β1-R	54.4	AGGAACAGACGTGTTAGTGC
BAX -F	53.2	TCATCCAGGATCGAGCAGG
BAX -R	53.2	GGCAATCATCCTCTGCAGC
Bcl2-F	55.9	GGTCATGTGTGTGGAGAGCG
Bcl2-R	53.8	CCGTACAGTTCCACAAAGGC

Table 1. AList of primer sequences used for real-time RT-PCR analysis.

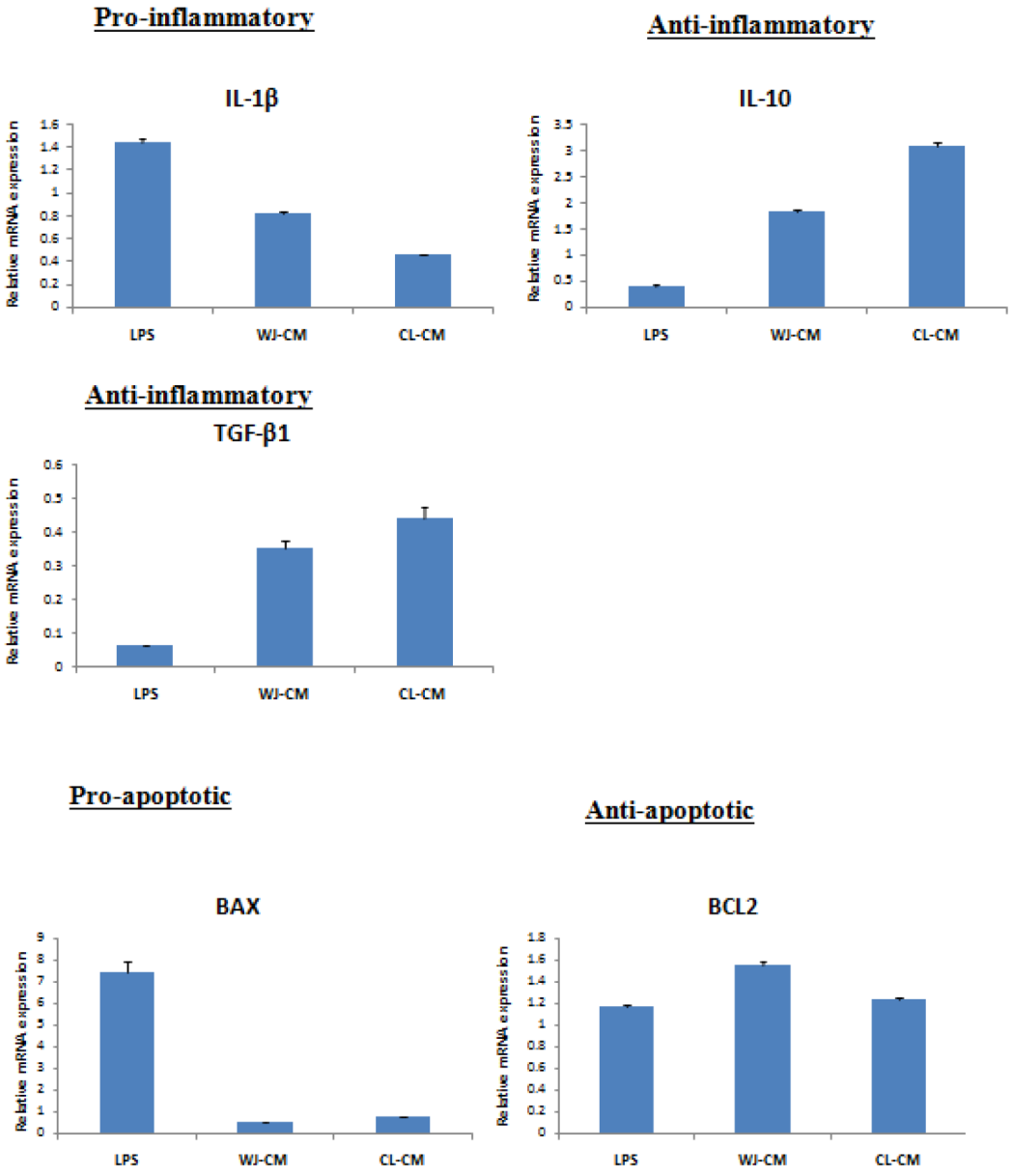

Figure 1. Pro and anti-inflammatory, pro and anti-apoptotic patterns, under three different concentrations i.e. LPS, Wharton's jelly conditioned media, and cord line conditioned media.

Discussion

Cheng Chen *et al.*, 2018 observed an anti-inflammatory effect under lipopolysaccharide[12]. Cheng Chen *et. al.*, 2018 explains that when SH-SY5Yis treated with SG-Tang, then SH-SY5Y inflammation is reduced under retinoic acid added conditioned media[12]. When we compare our result, we observe that our results are entirely contradictory in which LPS show inflammation and CM reduces inflammation. Hong Na Yang *et al.*, 2018 result explains that NSC-CM improves the expression of Bcl-2 to inhibit cell apoptosis[13]. Our result also gives similar result in which Bcl2 under conditioned media inhibit apoptosis. Zhongyang Xu *et al.* .2019 paper explains that miR-124 suppresses cell apoptosis by inhibiting Bax in H_2O_2-treated BV-2 cells[9]. When we observe our result in BAX treated with LPS and LPS is very high, so speedy cell proliferation and so fast neuronal cell death.

Conclusions

Our main aim of this research study is to check the inflammatory response. Due to inflammation, there is a death of neuronal cells, and cell proliferation occurs. In our research instead of stem cells, we are using stem cell factors which act as therapeutic agent produced by genes likes IL-10, TGF-β1, and Bcl2 which can protect the neurons from death like in case of Parkinson's disease, Alzheimer disease, etc. In the future, stem cell factors can become a promising therapeutic approach for various neurodegenerative diseases.

Abbreviation:

LPS- Lipopolysaccharide
WJ-CM- Wharton' Jelly Conditioned Media
CL-CM- Cord Line Conditioned Media

Acknowledgments

I would like to thanks Dr. Durai for technical support during this project.

Competing interests and Funding

The authors declare that there are no conflicts of interest, and the entire project was performed in CBCMT, VIT UNIVERSITY, VELLORE, INDIA.

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Received: 28 June 2020
Accepted: 15 July 2020