Original Article

The Effect of Tideglusib Application on Type 1 and Type 3 Collagen Expressions by Human Dental-Pulp Derived Stem Cells: A Preliminary Study

C Güler, AM Yilmaz[1,2], L Kuru, B Ozen, OB Agrali

Department of Periodontology, Faculty of Dentistry, [1]Department of Biochemistry, Faculty of Medicine, [2]Genetic and Metabolic Diseases Research and Investigation Center, Marmara University, Istanbul, Turkey

ABSTRACT

Background: Although Tideglusib cytotoxicity studies and its effects on human dental pulp-derived stem cells (DPSCs) have been examined in previous studies, there is no study investigating the expression of type 1 collagen and type 3 collagen by Tideglusib. **Aim:** The purpose of this study is to examine the effect of Wnt signaling activation using Tideglusib execution on human DPSC to determine its potential efficacy in collagen expression. **Methods:** Stem cell isolation was performed from five human third molar wisdom tooth pulps. DPSCs identified in only one sample were treated with 50 nM Tideglusib for 24 h and 1 week. Axin-2, type 1 and type 3 collagen expressions were evaluated by Western blot analysis. DPSCs without treatment served as a negative control. The Mann-Whitney U test was used for statistical analysis. **Results:** The levels of type 1 collagen and Axin-2 in the test group were significantly higher than those in the control group at 24 h (P = 0.000, P = 0.001, respectively). Compared to the control group, a slight increase in type 3 collagen expression was observed in the test group at 24 h (P value = 0.063). Application of 50 nM Tideglusib for 1 week revealed marked decreases in type 1 and type 3 collagen expressions (P = 0.029, P = 0.038, respectively). In contrast, there was a significant increase in the level of Axin-2 (P = 0.000) compared to the control group. **Conclusion:** The fact that Wnt signaling pathway activation obtained by Tideglusib application on DPSCs confirmed by the finding in the increase of Axin-2 at short and long-term evaluation periods which is resulted in the increase in the type 1 collagen expression at 24 h and decrease at 1 week together with the decrease in type 3 collagen expression at 1 week warrants further studies to evaluate the effect of Tideglusib on extracellular matrix expression.

Keywords: *Dental pulp, GSK-3 inhibitor, periodontal regeneration, stem cell, Tideglusib, Wnt signaling pathway*

Received: 20-Dec-2023;
Revision: 12-Jul-2024;
Accepted: 29-Jul-2024;
Published: 30-Sep-2024

Introduction

Dental pulp-derived stem cell (DPSC) s are cells with high clonogenic potential,[1] which have not only the capacity to form mesenchymal cells such as adipocytes, osteoblasts, odontoblasts, and chondrocytes but also can differentiate into cells of ectodermal origin, such as neurons, Schwann cells,[2,3] flat muscle and vascular endothelial cells.[4] DPSCs are recommended to be used in bone regeneration owing to their potential to create mineralized tissue in proper settings.[5,6] It has been suggested that DPSCs can be considered as a source of cells that can replace autologous bone graft, which is one of the most important materials in tissue regeneration procedures.[7]

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_866_23

Address for correspondence: Dr. OB Agrali, Department of Periodontology, Faculty of Dentistry, Marmara University, Başıbüyük Yolu, 9/3, Başıbüyük/Maltepe 34854, İstanbul, Turkey.
E-mail: omer.agrali@marmara.edu.tr

How to cite this article: Güler C, Yilmaz AM, Kuru L, Ozen B, Agrali OB. The effect of Tideglusib application on Type 1 and Type 3 collagen expressions by human dental-pulp derived stem cells: A preliminary study. Niger J Clin Pract 2024;27:1065-72.

Canonical Wnt/β-catenin signaling pathway has different regulatory functions in myogenesis, chondrogenesis, adipogenesis, and osteogenesis.[8] Uribe-Etxebarria *et al*.[9] showed that DPSCs can be effectively reprogrammed into inducible pluripotent stem cells by the modulation of the Wnt and Notch signaling pathways. However, by reducing the Wnt signaling pathway activation, a decrease in regenerative capacity can be observed.[10] However, by activating the Wnt signaling pathway; bone gain,[11] increase in proliferation and differentiation of periodontal ligament cells, cementoblast differentiation, and cementum regeneration may be identified.[12] In light of this information, the use of Wnt signaling pathway activation in DPSC-dependent regenerative response including different types of collagen expression may be discussed.

Glycogen synthase kinase-3 (GSK-3) is an enzyme involved in the degradation complex and a component of the Wnt signaling pathway. Tideglusib is a small molecule GSK-3 antagonist that has been clinically tested for systemic use in the treatment of neurodegenerative diseases.[13] It is successful in reproductive dentin formation and is known to be beneficial in osteoblast proliferation and tissue regeneration.[14-16] Direct delivery of Tideglusib leads to promoted Wnt activity in pulp stem cells.[15,17,18] Many previous studies in the literature have been conducted on the cytotoxicity[17,18] or osteogenic, odontogenic differentiation[18] of Tideglusib on DPSCs. However, there is limited information about the effects of Tideglusib on collagen expression by DPSCs.

The study aims to investigate the potential efficacy of Tideglusib in the regenerative healing response via evaluating the effect of Wnt signaling activation on type 1 and type 3 collagen expression by DPSCs. The expression of Axin-2 was also examined to confirm that this impact emerges as a result of Wnt signaling pathway activation.

Materials and Methods

Patient selection

The inclusion criteria were as follows: systemically healthy subjects indicating wisdom teeth extraction for orthodontic reasons; extracted teeth are free of decay, restoration, and root canal treatment. The subjects were informed about the study and provided full consent to participate the study.

Study plan

Stem cells isolated from the human third molar wisdom tooth pulp samples were obtained from 5 patients [Table 1]. The cell group with the highest stem cell markers belonged to a 19- year-old systemically healthy female individual who was used for the experiment. Based on previous studies,[15,17-19] 50 nM Tideglusib was applied to DPSCs for periods of 24 h and 1 week. Axin-2, type 1, and type 3 collagen expressions were evaluated by Western blot analysis. While the cell groups to which Tideglusib was applied were designated as the test groups, the cells without treatment served as the control groups. The study protocol was approved by the Marmara University Faculty of Dentistry Ethics Committee on November 5, 2020, with protocol number 2020-457.

Cell culture and characterization

At the commencement of the isolation procedure, the pulp tissue was washed with 1X phosphate-buffered saline (PBS) containing 1% penicillin/streptomycin and 0.1% amphotericin B. The tissue sample was then transferred into culture dishes and shredded with the help of a scalpel. A collagenase enzyme of 0.075% (weight/volume) was added to 15 tissue fragments, each of them was placed into separate vials which were incubated at 37°C for 2 h, allowing the tissue to dissociate and release the cells. After 2 h, PBS was added to the vials. After centrifugation for 10 min, the supernatant was separated. Finally, the cells were resuspended in Dulbecco's Modified Eagle's Medium (DMEM) containing 1% penicillin/streptomycin, 0.1% amphotericin B, and 10% fetal bovine serum and inoculated into 25 cm cell-culture dishes. The isolated cells were checked daily in incubators containing 5% CO_2 at 37°C for 3 days in DMEM. Then, the stem cells were removed using trypsin and collected into falcon tubes by centrifugation. Cells stained with trypan blue were counted using a Toma slide.

Flow cytometry analysis was conducted for cell characterization. 200,000 cells were placed into the tubes and stained with isotypic control IgG1 FITC/G2a PE, CD90 FITC, CD 105 FITC/CD 45 PE, and CD 14 FITC/CD 34 PE-conjugated antibodies. The tubes were incubated at room temperature for 20 minutes in the dark. Then the cells were characterized using a flow cytometer (BD FACSCalibur, BD Bioscience, CA). The cells labeled with the isotype control were utilized to locate the cells in the forward scattered light (FSC) and side scattered light; (SSC) panels. Analysis was recorded after 10,000 cells went through the gate called R1. In the analysis, it was revealed that an average of 99.6% of the cells were gathered in the R1 gate [Figure 1a].

Cells gated with R1 in flow cytometry were monitored in FL1 and FL2 channels according to the fluorescent dye used. Accordingly, histogram plot curves were drawn. M1 and M2 markers were placed on histogram plot curves of isotype controls. FITC-labeled antibodies were observed in the FL1 channel, and PE-labeled antibodies were observed in the FL2 channel. The positivity of the antibodies was evaluated according to the cell percentages in the M1 and M2 regions. Histograms shifting from M1 to M2 were considered positive, while histograms shifting from M2 to M1 were negative [Figure 1b].

Western blot

The Western Blotting technique makes it possible to identify the target protein in a complex protein structure through the specificity of antibody-antigen interaction.[20,21]

Table 1: Stem cell marker values of the analyzed samples (S1-S5)

Marker	S1	S2	S3	S4	S5
IgG_1 (%)	0.48	1.05	0.17	0.20	0.42
CD90 (%)	16.58	51.50	70.55	48.67	80.81
CD105 (%)	2.58	3.71	1.32	0.48	3.68
CD14 (%)	2.88	5.12	1.99	1.24	5.87
CD34 (%)	76.63	10.56	3.26	2.68	8.57
CD45 (%)	1.71	0.04	0.02	0.04	0.02
G2a (%)	0.04	1.76	0.25	0.12	0.94

Cells seeded into petri dishes were treated with Tideglusib and incubated at 37°C for 24 h and 1 week. Following the addition of 300 µL of cell lysis buffer (150 mM NaCl, 1 mM Na_2EDTA, 1 µg/ml leupeptin, 1 mM EGTA, 1% triton X, 1 mM β-glycerophosphate, 2.5 mM sodium pyrophosphate, 1 mM Na_3VO_4, and 20 mM Tris-HCl) to petri dishes, the cells were sonicated for 1 minute and centrifuged for 10 minutes at +4°C to collect the protein-containing supernatant. The total protein concentration in the sample supernatants was determined by Bicinchoninic acid (BCA) protein assay. Water-soluble cellular proteins were separated by denatured gel electrophoresis (SDS-PAGE). Then, by using the electroblot method, 100 V current was applied for 1 h and proteins were transferred to the nitrocellulose membrane. The membrane was blocked with 5% (w/v) powdered milk (prepared in PBS with 0.05% Tween-20), incubated at +4°C for 18 h with the primary anticollagen 1, anticollagen 3, and antiaxin-2 antibodies prepared in a blocking solution at a ratio of 1:1000, and then washed. HRP-labeled secondary antibodies were applied to the membranes,

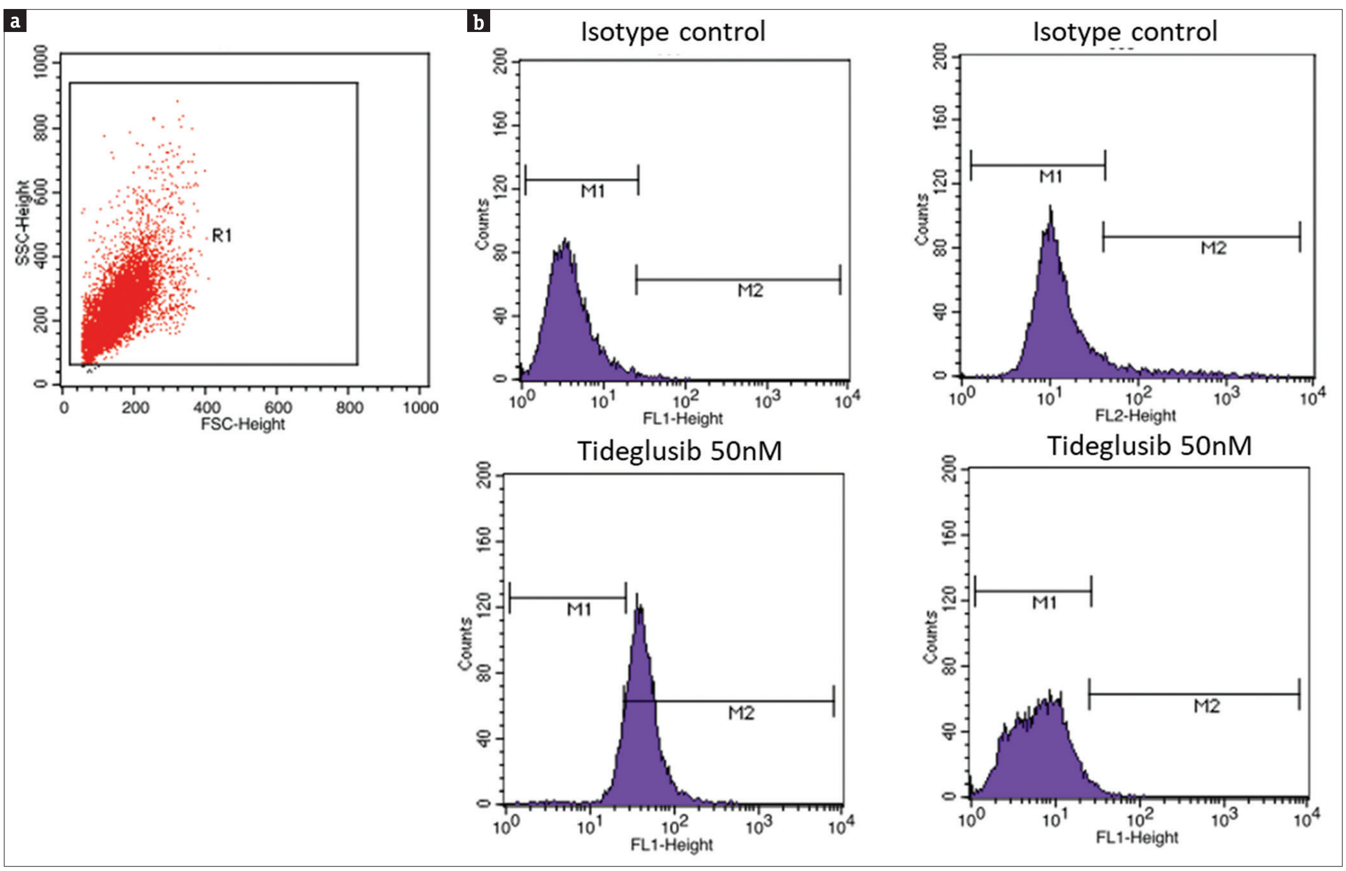

Figure 1: DPSC identification plots by flow cytometry. FSC-SSC dot plot and gate of stem cells isolated from dental pulp is indicated by R1 (a). Histogram plot of isotype control and S5 group. Histogram curve of the negative control (IgG1/G2a) used in the cell characterization experiment. Histogram curve of the CD90-FITC bound sample from the graphs obtained in the cell characterization experiment. Histogram plots shifting from M1 to M2 on histogram graphs result from positive staining (b). Representative images and quantitative results of the CD surface marker values

and the proteins were imaged using a ChemiDOC MP (Bio-Rad Laboratories, Inc., California, USA) imaging system. The experiment was repeated 4 times for each cell group. The images were transferred on films, measured by a densitometer ChemiDOC MP (Bio-Rad Laboratories, Inc., California, USA), and the optical density per square millimeter was calculated by the GraphPad Prism software (GraphPad Software, Inc., California, USA).

Statistical analysis

The data obtained in the study were analyzed using the Statistical Package for Social Sciences (SPSS) 22.0 package program. Descriptive statistics are shown as the mean, standard deviation, median, maximum, and minimum values for the variables. Intergroup difference was analyzed by Mann Whitney U Test. Statistical significance was set at $P < 0.05$ level.

Results

Stem cells from 5 samples were determined by flow cytometry and the sample #5 was selected for Western blot analysis. Further analysis was not carried out for the remaining four samples of stem cells which demonstrated high levels of CD34 and CD14 hematopoietic cell markers and low levels of CD90 and CD105 stem cell markers [Table 1]. The maximum CD90 level was determined in the S5 group [Table 1]. CD105 values were found to be quite low in all samples. Type 1 collagen expression in the group of DPSCs treated with 50 nM Tideglusib for 24 h was found to be significantly higher than that in the control group [Table 2 and Figure 2] (P = 0,000). There was no significant difference in the level of type 3 collagen expression in the group of cells treated with 50 nM Tideglusib for 24 h compared to the control [Table 2 and Figure 2] (P = 0,063). The amount of Axin-2 expression was found to be significantly higher in the cells treated with 50 nM Tideglusib for 24 h compared to the control group [Table 2 and Figure 2] (P = 0,001).

After applying 50 nM Tideglusib to DPSCs for 1-week, significantly lower levels of type 1 and type 3 collagen expression were detected compared to the corresponding levels in the control group (P = 0,029, P = 0,038) [Table 3 and Figure 3]. The cells treated with 50 nM Tideglusib for 1 week exhibited significantly higher levels of Axin-2 than the control group cells (P = 0,000) [Table 3 and Figure 3].

Discussion

Questions concerning the ideal state of periodontal tissue regeneration, which is intended to be achieved through the use of different techniques, remain a mystery.[22] Recently, the integration of cells from mesenchymal and epithelial origins has been intensively assessed for tooth regeneration.[23] The canonical Wnt/β-catenin signaling pathway, which stimulates the repair mechanism against

Table 2: The effect of 50 nM Tideglusib administration for 24 h

Tideglusib (Nm)		Type 1 collagen	Type 3 collagen	Axin-2
Control	*n*	4	4	4
	Mean±SD	1.00±0.00	1.00±0.00	1.00±0.00
	Median	1.00	1.00	1.00
	Minimum	1.00	1.00	1.00
	Maximum	1.00	1.00	1.00
50 Nm	*n*	4	4	4
	Mean±SD	2.20±0.59	1.11±0.19	1.55±0.23
	Median	2.38	1.06	1.51
	Minimum	1.40	0.95	1.31
	Maximum	2.65	1.39	1.86
*P**		0.000*	0.063	0.001*

SD: Standard deviation, *Mann-Whitney *U* test, $P<0.05$

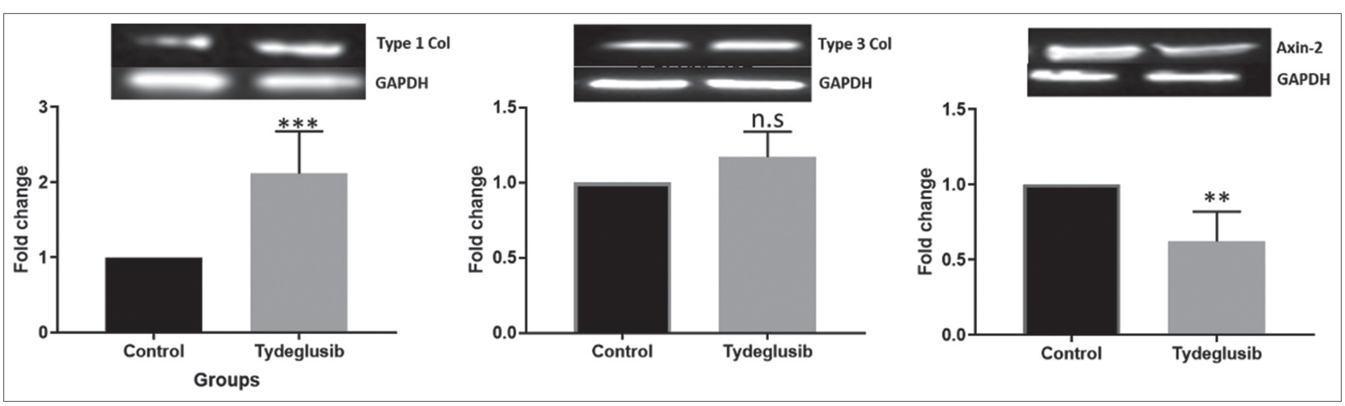

Figure 2: Western blot analysis of type 1 Collagen, type 3 Collagen and Axin 2 protein expression levels after 24 h treatment of Tideglesib 50 nM on DPSC. Band intensity was analyzed by densitometry. Fold change of protein expression levels was calculated after bands were normalized to GAPDH. Differences between means and significance of the treatments were analyzed using Mann Whitney U Test. Bar graphs represent mean ± SD, n = 3, *$P < 0.05$ versus control, **$p < 0.01$ versus control, and ***$p < 0.001$ versus control

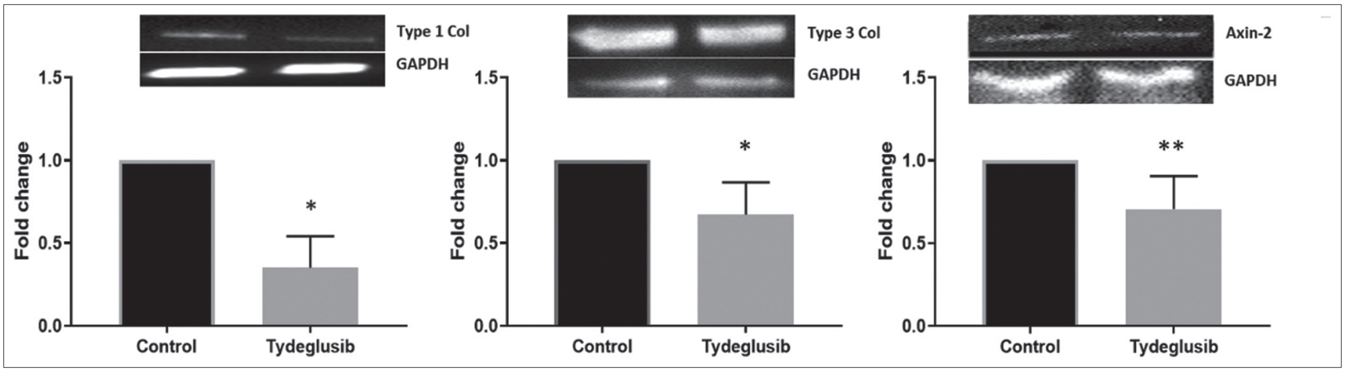

Figure 3: Western blot analysis of type 1 Collagen, type 3 Collagen and Axin 2 protein expression levels after 1 week treatment of Tideglesib 50 nM on DPSC. Band intensity was analyzed by densitometry. Fold change of protein expression levels was calculated after bands were normalized to GAPDH. Differences between means and significance of the treatments were analyzed using Mann Whitney U Test. Bar graphs represent mean ± SD, n = 3. *$P < 0.05$ versus control, **$p < 0.01$ versus control, and ***$p < 0.001$ versus control

Table 3: The effect of 50 nM Tideglusib administration for 1 week

Tideglusib (Nm)		Type 1 collagen	Type 3 collagen	Axin-2
Control	*n*	4	4	4
	Mean±SD	1.00±0.00	1.00±0.00	1.00±0.00
	Median	1.00	1.00	1.00
	Minimum	1.00	1.00	1.00
	Maximum	1.00	1.00	1.00
50 nM	*n*	4	4	4
	Mean±SD	0.43±0.11	0.75±0.11	1.64±0.39
	Median	0.39	0.76	1.60
	Minimum	0.34	0.61	1.28
	Maximum	0.57	0.87	2.06
*P**		0.029*	0.038*	0.000*

SD: Standard deviation, *Mann-Whitney *U* test, $P<0.05$

tissue damage, has an impact on cell proliferation and differentiation.[24,25] Direct use of Tideglusib promotes up-regulation of Wnt activity in pulp stem cells.[15] However, there is little information available on the Tideglusib application on DPSCs.[15,17,18] This is the first study evaluating the effect of Tideglusib on collagen expression in human DPSCs.

In a preliminary investigation by Öncü *et al*.[19] studying the effects of Tideglusib implementation on periodontal cells, it was revealed that 50 nM and 100 nM Tideglusib were not cytotoxic for periodontal cells. In a pioneering study by Neves *et al*.,[15] 50 nM was established as the common, non-cytotoxic, and most viable dosage of Tideglusib for cell types. Hanna *et al*.[17] administered 10, 50, 100, 200, and 250 nM Tideglusib to human DPSCs for 24, 48, and 72 h and reported the highest viable dosage as 100 nM at 72 h. Kornsuthisopon *et al*.[18] treated human DPSCs with 50, 100, and 200 nM Tideglusib for 1, 3, and 7 days and demonstrated no significant change in DPSC proliferation, although 50 nM and 100 nM Tideglusib boosted osteogenic marker gene expression. Based on these facts, in our research, 50 nM Tideglusib was selected as the dose and administered to DPSCs to detect the release of type 1, type 3 collagen, and Axin-2.

Axin-2 expression, which we evaluated to validate Wnt signaling pathway activation, was found to be considerably elevated in both test groups treated with Tideglusib for 24 h and 1 week compared to the control group ($P = 0.001$, $P = 0.000$). Neves *et al*.[15] utilized three small molecule GSK-3 inhibitors, CHIR99021, BIO, and Tideglusib with the help of a collagen sponge to promote tertiary dentin following experimentally induced pulp exposure. When the highest non-cytotoxic inhibitor concentration of GSK-3 inhibitors was examined by PCR, the elevation of Axin-2 expression was noticed after 30 min in all three groups and the expression of Axin-2 reached its maximum level after 1 h, which is in line with our study.[15] In another study evaluating the expression of osteogenic markers and the conversion to osteogenic origin of DPSCs, ferritin was used as a GSK-3 inhibitor for the induction of canonical Wnt activity. Although Axin was observed marginally at the outset, it was identified at higher levels than the baseline on the 24-h time display.[26] In a study with mouse epiblast stem cells, CHIR99021 was used to stabilize β-catenin through inhibition of GSK-3. The expression level of Axin-2 in mouse epiblast stem cells increased with CHIR treatment.[27] Furthermore, using BIO to epigenetically modulate Wnt β-catenin signaling in vascular smooth muscle cells, led to an increase in the expression of Axin-2.[28] Zaugg *et al*.[29] applied a collagen sponge enriched with Tideglusib or CHIR in direct contact with damaged pulp tissue in rodents to activate the Wnt signaling pathway and demonstrated an increase in the expression of Axin-2. Leone *et al*.[30] investigated the odontogenic gene expression in MSCs and reported that administration of GSK-3 inhibitor

BIO for the induction of WNT activity increased Axin-2 expression. In another study testing the role of Wnt modulators in the osteogenic differentiation of MSC, it was observed that stimulation of Wnt signaling by applying CHIR for 7 days and 21 days increased the expression of Axin-2 in MSCs.[31] Khan *et al*.[32] conducted a study in MSCs and determined that 0.5% gelatin in DMEM provided the induction of Wnt activity and accordingly, the expression of Axin-2 was increased compared to the control group. In these studies, the upregulation of Axin-2 in MSCs was considered as substantial evidence of Wnt/β-catenin pathway activation in MSCs.

Type 1 collagen is perceived as the main component of dental and periodontal connective tissues (e.g., gingiva, PDL, dentin, cementum, and bone). Type 3 collagen has been identified as collagen present in the gingiva, PDL, cementum, skin, and blood vessels, and contributes to tensile strength and the collagen production/destruction mechanism.[33] There is limited information about protein expression related to the Wnt signaling pathway.[34] In our study, after the application of 50 nM Tideglusib for 24 h in DPSCs, a significant increase was observed in the level of type 1 collagen compared to the control group (P = 0.000). DPSCs capable of reconstituting a reparative dentin-like complex are a community of progenitor cells derived from human dental pulp. In research indicated that ferritin, a GSK-3 inhibitor that epigenetically modulates canonical Wnt signaling, was demonstrated to direct DPSCs to osteogenic origin. As a result, consistent with our study, 24 h of stimulation with ferritin led to an up-regulation of type 1 collagen in DPSCs. In the same study, parallel to ferritin application and our study, it was observed that administration of another GSK-3 inhibitor, CHIR-98014, resulted in an increase in the expression of type 1 collagen by WNT signaling pathway activation.[26] In a study by Scheller *et al*.,[35] in which retrovirus-mediated infection was applied for 48 h to robustly activate Wnt signaling, type 1 collagen expression was observed to increase compared to the control group. Furthermore, it was found that canonical Wnt signaling can drive DPSC odontoblast-like development. Moreover, Wnt signaling can reprogram metabolic activity in DPSCs. It was demonstrated that after the application of the GSK-3 inhibitor BIO for 48 h, there was a significant increase in osteoblastic proliferation markers, including type-1 collagen, in DPSCs.[9] Similarly, in our study, a statistically significant increase was observed in the level of type 1 collagen in the pulp stem cell, in which the Wnt signaling pathway was activated by applying Tideglusib for 24 h.

There is limited research in the literature studying the influence of the Wnt signaling pathway on type 3 collagen release. In our study, no statistically significant difference was found in type 3 collagen expression when DPSCs were administered 50 nM Tideglusib for 24 h (P = 0.063). In our previous study, the effect of Tideglusib application on three periodontal cells was evaluated,[19] no significant difference was noticed in Type-3 collagen expression in human gingival fibroblasts and osteoblasts after 24 h. However, Ge *et al*.[36] examined the impact of elevated catenin expression on type 3 collagen secretion in hepatic fibrosis and found that the amount of type 3 collagen increased significantly when compared to the control group. Fu Xiang *et al*.[37] showed that elevated Wnt/β-catenin signaling led to increased expression of type 3 collagen in cardiac fibroblasts. However, the outcomes of these two investigations were not by our research; the variation in the applied chemicals and cell lines may be the cause for this conclusion.

In our study, a significant decrease was observed in type 1 and type 3 collagen levels compared to the control group after DPSCs execution of 50 nM Tideglusib for 1 week (P = 0.029, P = 0.038). This result is not parallel with the studies demonstrating type 1 collagen is released by stem cells, in which Wnt signaling pathway activation is achieved.[31,38] The difference in the applied molecule and cell line used in our study may be the reason for this result.

Other concentrations and different compounds that could not be included in the research might be proposed as a limitation of the present investigation. However, this work is the first to identify the phenomenon of protein expression, which indicates as a possible regenerative response of DPSCs through activation of the Wnt signaling pathway.

Conclusions

Within the limits, it can be considered that the Tideglusib molecule may have a positive regenerative effect because of an increase in type 1 collagen in DPSCs when applied for 24 h. The fact that Tideglusib had a varied impact on type 1 and type 3 collagen increases when administered to DPSCs for 24 h and 1 week suggests that the short and long-term outcomes of this treatment may be disputed. However, our study is the first in which the impact of Tideglusib on human DPSCs has been investigated with different observation periods. Further studies are needed to reveal the effect of Wnt signaling pathway activation on the regenerative response of DPSCs.

Acknowledgements

We thank our colleagues from Marmara University Genetic and Metabolic Diseases Research and Implementation Center who provided insight and expertise that greatly assisted the research. No funding was received to assist with the preparation of this manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proceed Natl Acad Sci 2000;97:13625-30.
2. Gervois P, Struys T, Hilkens P, Bronckaers A, Ratajczak J, Politis C, *et al*. Neurogenic maturation of human dental pulp stem cells following neurosphere generation induces morphological and electrophysiological characteristics of functional neurons. Stem Cells Dev 2015;24:296-311.
3. Bronckaers A, Hilkens P, Martens W, Gervois P, Ratajczak J, Struys T, *et al*. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. Pharmacol Ther 2014;143:181-96.
4. Karbanová J, Soukup T, Suchánek J, Pytlík R, Corbeil D, Mokrý J. Characterization of dental pulp stem cells from impacted third molars cultured in low serum-containing medium. Cells Tissues Organs 2011;193:344-65.
5. Guo H, Wei J, Yuan Y, Liu C. Development of calcium silicate/calcium phosphate cement for bone regeneration. Biomedical Materials 2007;2:S153-9.
6. Luisi SB, Barbachan JJD, Chies JAB, Sant'Ana Filho M. Behavior of human dental pulp cells exposed to transforming growth factor-beta1 and acidic fibroblast growth factor in culture. J Endod 2007;33:833-5.
7. Laino G, Carinci F, Graziano A. Dâ aquino R, Lanza V, De Rosa A, *et al*. *In vitro* bone production using stem cells derived from human dental pulp. J Craniofac Surg 2006;17:511-5.
8. Cossu G, Borello U. Wnt signaling and the activation of myogenesis in mammals. EMBO J 1999;18:6867-72.
9. Uribe-Etxebarria V, Agliano A, Unda F, Ibarretxe G. Wnt signaling reprograms metabolism in dental pulp stem cells. J Cell Physiol 2019;234:13068-82.
10. Whyte JL, Smith AA, Helms JA. Wnt signaling and injury repair. Cold Spring Harb Perspect Biol 2012;4:a008078.
11. Morvan F, Boulukos K, Clément-Lacroix P, Roman SR, Suc-Royer I, Vayssière B, *et al*. Deletion of a single allele of the Dkk1 gene leads to an increase in bone formation and bone mass. J Bone Miner Res 2006;21:934-45.
12. Han P, Wu C, Chang J, Xiao Y. The cementogenic differentiation of periodontal ligament cells via the activation of Wnt/β-catenin signalling pathway by Li+ions released from bioactive scaffolds. Biomaterials 2012;33:6370-9.
13. Gao C, Holscher C, Liu Y, Li L. GSK3: A key target for the development of novel treatments for type 2 diabetes mellitus and Alzheimer disease. Rev Neurosci 2011;23:1-11.
14. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med 2004;10:55-63.
15. Neves VC, Babb R, Chandrasekaran D, Sharpe PT. Promotion of natural tooth repair by small molecule GSK3 antagonists. Sci Rep 2017;7:39654.
16. Tolosa E, Litvan I, Höglinger GU, Burn D, Lees A, Andrés MV, *et al*. A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. Mov Disord 2014;29:470-8.
17. Hanna S, Aly R, Eldeen GN, Adanero Velasco A, Perez Alfayate R. Small molecule GSK-3 inhibitors safely promote the proliferation and viability of human dental pulp stem cells-in vitro. Biomedicines 2023;11:542.
18. Kornsuthisopon C, Tompkins KA, Osathanon T. Tideglusib enhances odontogenic differentiation in human dental pulp stem cells *in vitro*. Int Endod J 2023;56:369-84.
19. Öncü B, Yilmaz AM, Yilmaz BK, Altunok EÇ, Kuru L, Ağrali ÖB. Cytotoxicity and collagen expression effects of tideglusib administration on human periodontal cells: An in-vitro study. Clin Exp Health Sci 2020;10:153-62.
20. Burnette WN. "Western blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 1981;112:195-203.
21. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proceed Natl Acad Sci 1979;76:4350-4.
22. Trofin EA, Monsarrat P, Kémoun P. Cell therapy of periodontium: From animal to human? Front Physiol 2013;4:325.
23. Baranova J, Büchner D, Götz W, Schulze M, Tobiasch E. Tooth formation: Are the hardest tissues of human body hard to regenerate? Int J Mol Sci 2020;21:4031.
24. Cadigan KM, Nusse R. Wnt signaling: A common theme in animal development. Genes Dev 1997;11:3286-305.
25. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell 2012;149:1192-205.
26. Rolph DN, Deb M, Kanji S, Greene CJ, Das M, Joseph M, *et al*. Ferutinin directs dental pulp-derived stem cells towards the osteogenic lineage by epigenetically regulating canonical Wnt signaling. Biochim Biophy Acta Mol Basis Dis 2020;1866:165314.
27. Kim H, Wu J, Ye S, Tai C-I, Zhou X, Yan H, *et al*. Modulation of β-catenin function maintains mouse epiblast stem cell and human embryonic stem cell self-renewal. Nat Commun 2013;4:2403.
28. Saito Y, Nakamura K, Miura D, Yunoki K, Miyoshi T, Yoshida M, *et al*. Suppression of Wnt signaling and osteogenic changes in vascular smooth muscle cells by eicosapentaenoic acid. Nutrients 2017;9:858.
29. Zaugg L, Banu A, Walther A, Chandrasekaran D, Babb RC, Salzlechner C, *et al*. Translation approach for dentine regeneration using GSK-3 antagonists. J Dent Res 2020;99:544-51.
30. Leone A, Volponi AA, Renton T, Sharpe PT. In-vitro regulation of odontogenic gene expression in human embryonic tooth cells and SHED cells. Cell Tissue Res 2012;348:465-73.
31. Frith JE, Titmarsh DM, Padmanabhan H, Cooper-White JJ. Microbioreactor array screening of Wnt modulators and microenvironmental factors in osteogenic differentiation of mesenchymal progenitor cells. PLoS One 2013;8:e82931.
32. Khan IU, Yoon Y, Kim WH, Kweon O-K. Gelatin positively regulates the immunosuppressive capabilitiesof adipose-derived mesenchymal stem cells. Turkish J Biol 2017;41:969-78.

33. Narayanan AS. Connective tissues of the periodontium: A summary of current work. Coll Relat Res 1983;3:25-31.
34. Minear S, Leucht P, Jiang J, Liu B, Zeng A, Fuerer C, *et al*. Wnt proteins promote bone regeneration. Sci Transl Med 2010;2:29ra30.
35. Scheller E, Chang J, Wang C. Wnt/β-catenin inhibits dental pulp stem cell differentiation. J Dent Res 2008;87:126-30.
36. Ge W-S, Wang Y-J, Wu J-X, Fan J-G, Chen Y-W, Zhu L. β-catenin is overexpressed in hepatic fibrosis and blockage of Wnt/β-catenin signaling inhibits hepatic stellate cell activation. Mol Med Rep 2014;9:2145-51.
37. Xiang F-L, Fang M, Yutzey KE. Loss of β-catenin in resident cardiac fibroblasts attenuates fibrosis induced by pressure overload in mice. Nat Commun 2017;8:712.
38. Huang X, Zhong L, Hendriks J, Post JN, Karperien M. The effects of the WNT-signaling modulators BIO and PKF118-310 on the chondrogenic differentiation of human mesenchymal stem cells. Int J Mol Sci 2018;19:561.