Short Communication

Effects of Transforming Growth Factor-Beta/SMAD Signal Mediated by Poly-L-Lactic Acid on Biological Behavior and Collagen Expression of Dermal Fibroblasts

CHENGZHI DONG, JIAN ZHANG[1] AND LIJUAN PENG*

Department of Plastic Surgery, Tongde Hospital of Zhejiang Province, Zhejiang Province 310012, 1The Second Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou, Zhejiang Province 310053, China

To exam the effects of transforming growth factor-beta1/suppressor of mothers against decapentaplegic signal mediated by poly L-lactic acid on biological behavior and collagen expression of dermal fibroblasts. Dermal fibroblasts Hs60 were treated with different concentrations of poly L-lactic acid. Quantitative polymerase chain reaction was used to detect the collagen type I alpha 1, collagen type I alpha 2, elastin, matrix metalloproteinases-1, tissue inhibitors of metalloproteinases-1 and tissue inhibitors of metalloproteinases-2, messenger ribonucleic acid level; procollagen synthesis was analyzed using a type I prolactin inducible protein enzyme immunoassay kit, and the amount of proteins associated to the transforming growth factor-beta 1/suppressor of mothers against decapentaplegic signal pathway was measured using Western blot. The procollagen products in the high poly L-lactic acid intervention group (group C) was raised than that in the low- poly L-lactic acid intervention group (group B) and the control group (group A). The collagen type I alpha 1 and collagen type I alpha 2 protein in the group C were increased than those in the low-poly L-lactic acid intervention group and the group A. The elastin, metalloproteinases-1, tissue inhibitors of metalloproteinases-1 and tissue inhibitors of metalloproteinases-2 messenger ribonucleic acid in group C was raised than that in low-poly L-lactic acid intervention group and group A, while the metalloproteinases-1, messenger ribonucleic acid in group B was reduced than that in group C and group A. The transforming growth factor-beta1 and suppressor of mothers against decapentaplegic messenger ribonucleic acid in group C was raised than that in low-poly L-lactic acid intervention group and group A. The transforming growth factor-beta1 and suppressor of mothers against decapentaplegic protein in the group C were increased than those in the low-poly L-lactic acid intervention group and the group A. Poly L-lactic acid stimulates collagen expression and synthesis in dermal fibroblasts by activating transforming growth factor-beta1/suppressor of mothers against decapentaplegic signal pathway.

Key words: Poly L-lactic acid, transforming growth factor-beta1/suppressor of mothers against decapentaplegic, dermal fiber cells, collagen

In view of China's economic growth and the extension of life expectancy, individuals are exhorted to actively seek out bioactive chemicals that can prevent aging and enhance health in addition to pursuing beauty[1]. During skin aging, the epidermis and dermis undergo structural and functional changes. Dermal fibroblasts create collagen, the primary building block of Extracellular Matrix (ECM), to preserve the suppleness and strength of skin[2]. As a result of ultraviolet rays, inflammation, intracellular metabolites, and aging, collagen is easily inhibited, resulting in wrinkles, sagging, and relaxation of the skin. Although there is currently no way to permanently improve the anti-aging process, injectable fillers are an easy, quick, and minimally invasive cosmetic operation that has a quick recovery[3]. As the demand increases, face fillers

***Address for correspondence**
E-mail: wuhgzg19432@163.com

Accepted 07 May 2024
Revised 24 September 2023
Received 15 December 2022
Indian J Pharm Sci 2024;86(3):1161-1165

including polycaprolactone, hyaluronic acid, non-biodegradable polymethyl methacrylate silicone, and polyacrylamide are constantly being updated[4]. Polylactic Acid (PLA) is a polymer that is immune inert, biodegradable, absorbable, flexible, and simple to produce. The compound is originated from plants and is widely used in soluble sutures, bone nails, and facial implants. It may also regulate collagen production and expression in the skin[5]. Fibroblasts are regulated in their expression of collagen genes by a range of cytokines. The primary controller of ECM synthesis is Transforming Growth Factor-Beta (TGF-β). It regulates fibroblast growth and apoptosis, and promotes collagen synthesis by inhibiting matrix degradation enzymes and p-Suppressor of Mothers Against Decapentaplegic (SMAD)[6]. In light of this, this study investigated the effects of TGF-β/SMAD signal mediated by PLA on the biological behavior and collagen expression of dermal fibroblasts, in order to provide reference for the choice of clinical treatment. Human dermal fibroblast cell line (Hs60) was from American Type Culture Collection (ATCC). The cells were kept in a culture of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1 % penicillin and 1 % streptomycin, along with 10 % Fetal Bovine Serum (FBS). The temperature of the incubator was 37° with 5 % Carbon dioxide (CO_2) present. Poly (L-lactic acid) purchased from Thermo Fisher Scientific Company of the United States. Set up control group (group A), low-PLA intervention group (group B) and high PLA intervention group (group C). Group A without any treatment, routine culture, low PLA intervention group added 0.5 mg/ml PLA, and group C added 1 mg/ml PLA. Each experiment was repeated 6 times. The cell being cultured in the incubator for 48 h, each group of cells used Ribonucleic Acid (RNA) extraction kit to extract RNA, One Step Prime Script microRNA (miRNA) complementary Deoxyribonucleic Acid (cDNA) synthesis kit was used to reverse transcription miRNA into cDNA, miRNA fluorescence Quantitative Polymerase Chain Reaction (qPCR) detection kit was used for quantitative real-time PCR, and the cycle was finished in accordance with the kit's guidelines. Following the completion of the response, the relative expression of messenger RNA (mRNA) was calculated in the software. The cells were inoculated in a 6-well plate and after 48 h of culture in the incubator, each group of cells were homogenized at 4° to make 10 % homogenate, subsequently centrifuged to ascertain the protein concentration, followed by washing, primary and secondary antibody incubation, gel preparation, electrophoretic 90 min, gel cutting, and transmembrane 90 min. Software from Bio-Rad image laboratory was used to examine the findings. Cells was inoculated and after 24 h, the supernatant of each well was collected in an incubator containing 0.5 and 1 mg/ml-oligo-L-lactic acid for 48 h, and the supernatant was determined according to the Prolyl Iminopeptidase (PIP) Enzyme-Linked Immunosorbent Assay (ELISA) commercial assay kit (TakaraBio Co., Ltd.,). The measurement data were expressed as ($\bar{x}\pm s$), the statistical program Statistical Package for the Social Sciences (SPSS) 22.0 was used for data processing and analysis, the F-variance analysis was employed for multi-group comparison, and the Least Significant Difference (LSD)-t test was utilized for pairwise comparisons between different groups. In comparison to group B, $^{b}p<0.05$ and comparison to normal group A, $^{a}p<0.05$. The level of procollagen product in group B was raised than group A, and this in high-PLA intervention group was increased than that in oligo-L-lactic acid intervention group (Table 1). The Collagen Type I Alpha (COL1A)-1 and COL1A2 protein in the group B were raised than those in the group A, and these in the group C were increased than those in the group B (Table 2). While the Matrix Metalloproteinases (MMP)-1 mRNA in the low-PLA group was lower than in the group A, the elastin, MMP-1, Tissue Inhibitor of Metalloproteinase (TIMP)-1, and TIMP-2 mRNA in the group B was higher than in the group A. The elastin, MMP-1, TIMP-1 and TIMP-2 in the group C was raised than the group B, while the MMP-1 in the group C was reduced than the group A (Table 3). The TGF-β and SMAD in the group B was raised than the group A, and these in the high-PLA intervention group was raised than the group B (Table 4). The TGF- β and SMAD protein in the group B were raised than those in the group A, and these in the group C were raised than those in the group B (Table 5). The way the skin and face look is regarded as an important index to measure happiness and health. Cosmetic surgery has the advantages of less invasion, quick recovery, less scar and can achieve the best effect[7]. PLA is a

synthetic polymer made entirely of natural plants, like sugarcane and maize starch[8]. It has been found that with the degradation of PLA particles, it can gradually regain its volume by stimulating collagen synthesis. Therefore, PLA is frequently used as dermal fillers to correct flaws and enhance the look of the face and skin. Thus, it can be used in various medical fields[9,10]. In order to serve as a guide for clinical treatment choices, this study investigated PLA's putative molecular mechanism, which controls the manufacture of collagen by dermal cells in Hs68 cells. Here, we saw that PLA affected the synthesis of collagen. The procollagen products in the group B were raised than that in the group A; while this in the group C was increased than that in the group B. The PIP-EIA assay demonstrated that procollagen production in cutaneous fibroblasts rose as PLA levels increased. The COL1A1 and COL1A2 protein in the group B were raised than those in the group A, while the COL1A1 and COL1A2 protein levels in the group C were increased than the group B. As is well known, the most prevalent kind of collagen in the human body is called type I collagen, which is made up of the COL1A1 and COL1A2. The outcomes demonstrated that the relative expression of MMP-1, elastin, TIMP-1 and TIMP-2 mRNA in the group B was raised than the group A, while the MMP-1 mRNA was reduced than the group A. The MMP-1, elastin, TIMP-1 and TIMP-2 mRNA in the group C was increased than the group B, and these was reduced than that in the group B. It is suggested that the elastin also increases with the increase of the concentration of PLA. A previous study found that elastin is a cross-linked fiber that forms thicker connective tissue of the dermis, giving the skin its flexibility and mechanical structure[11]. Accordingly, collagen and elastin biomaterials should be the main components of cosmetic skin materials. While PLA gradients enhanced the levels of TIMP-1 and TIMP-2, they inhibited MMP-1. According to earlier research, overexpression of MMPs causes the skin to droop and develop rough wrinkles. It also speeds up aging by degrading the ECM[12]. Due to their tight interaction with TIMP-1, TIMP-1 and TIMP-2 have the ability to particularly block MMP-1 activity[13,14]. TGF-β/Smad pathway regulates the production of fibroblasts and collagen, as well as the synthesis of ECM, in a physiological as well as pathological manner[15]. In order to facilitate wound healing, it controls membrane fibroblast differentiation and proliferation through paracrine and autocrine processes, and secondly, it regulates MMP-1 and TIMP-1 by paracrine and autocrine action[16]. An excessive amount of TGF-β can hinder the MMP-1 expression and result in an amplified TIMP-1 response, ultimately causing an elevation in collagen expression and a decrease in ECM degradation[17]. Previous studies have found that Oyster Hydrolysate (OH) modifies the TGF-β/SMAD signal pathway to promote the synthesis of collagen[18]; by modulating the TGF-β/SMAD signal pathway, the compound extract of *Salvia miltiorrhiza* and Radix Astragali controls the production of collagen in keloid fibroblasts[19]; *Pelteobagrus fulvidraco*, a kind of seaweed, stimulates the TGF-β/SMAD signal pathway in dermal fibroblasts to increase the production of collagen[20]. Drawing from the aforementioned research context, our aim was to investigate the potential involvement of the TGF-β/SMAD signal pathway in PLA's mechanism of boosting collagen gene expression and production in dermal fibroblasts. The TGF-β and SMAD mRNA in the group B was raised than the group A, and these in the group C was increased than that in the group B. The TGF-β and SMAD protein in the group B were raised than those in the group A, while these in the group C were raised than those in the group B. It has been proposed that PLA promotes collagen gene expression and synthesis in dermal fibroblasts through the TGF-β/SMAD signal pathway. In summary, PLA stimulates collagen expression and synthesis in dermal fibroblasts by activating TGF-β/SMAD signal pathway.

TABLE 1: THE LEVEL OF PROCOLLAGEN PRODUCTS

Group	n	Procollagen products (%)
A	6	99.19±10.06
B	6	142.93±20.38[a]
C	6	171.53±32.26[ab]
F		522.412
p		0.000

Note: Comparison to group B, [a]p<0.05 and comparison to normal group C, [b]p<0.05

TABLE 2: COL1A1 AND COL1A2 EXPRESSION LEVELS IN DIFFERENT GROUPS

Group	n	COL1A1	COL1A2
A	6	0.37±0.09	0.41±0.14
B	6	0.43±0.14[a]	0.54±0.14[a]
C	6	0.86±0.16[ab]	0.78±0.12[ab]
F		80.413	47.276
p		0.000	0.000

Note: Comparison to group B, [a]p<0.05 and comparison to normal group C, [b]p<0.05

TABLE 3: THE ELASTIN, MMP-1, TIMP-1 AND TIMP-2 mRNA LEVEL

Group	n	Elastin	MMP-1	TIMP-1	TIMP-2
A	6	0.77±0.09	0.33±0.14	0.31±0.03	0.38±0.20
B	6	1.32±0.35[a]	0.44±0.14[a]	0.49±0.04[a]	0.66±0.12[a]
C	6	2.05±0.46[ab]	0.78±0.12[ab]	0.54±0.03[ab]	0.79±0.15[ab]
F		321.687	218.481	258.235	157.823
p		0.000	0.000	0.000	0.000

Note: Comparison to group B, [a]p<0.05 and comparison to normal group C, [b]p<0.05

TABLE 4: THE TGF-β AND SMAD mRNA LEVEL

Group	n	TGF-ß mRNA	SMAD mRNA
A	6	1.24±0.32	0.83±0.35
B	6	1.65±0.40[a]	1.76±0.43[a]
C	6	2.47±0.54[ab]	2.59±0.67[ab]
F		212.744	318.451
p		0.000	0.000

Note: Comparison to group B, [a]p<0.05 and comparison to normal group C, [b]p<0.05

TABLE 5: EFFECT OF TGF-β AND SMAD IN EACH GROUP

Group	n	TGF-ß	SMAD
A	6	0.28±0.04	0.30±0.03
B	6	0.57±0.08[a]	0.52±0.09[a]
C	6	0.86±0.28[ab]	0.76±0.11[ab]
F		115.521	245.156
p		0.000	0.000

Note: Comparison to group B, [a]p<0.05 and comparison to normal group C, [b]p<0.05

Acknowledgement:

This research received grant from Zhejiang Medical and Health Science and Technology Program Project (No: 2024KY870).

Conflict of interests:

The authors declared no conflict of interests.

REFERENCES

1. Michel P, Zbikowska HM, Rudnicka K, Gonciarz W, Krupa A, Gajewski A, *et al.* Anti-inflammatory, antioxidant and photoprotective activity of standardised *Gaultheria procumbens* L. leaf, stem, and fruit extracts in UVA-irradiated human dermal fibroblasts. J Ethnopharmacol 2024;319(2):117219.
2. Hong YR, Kim TH, Park KH, Kang J, Lee K, Park EK, *et al.* RhBMP-2-conjugated three-dimensional-printed poly (L-lactide) scaffold is an effective bone substitute. Tissue Eng Regen Med 2023;20(1):69-81.

3. Heo H, Lee H, Yang J, Sung J, Kim Y, Jeong HS, *et al.* Protective activity and underlying mechanism of ginseng seeds against UVB-induced damage in human fibroblasts. Antioxidants 2021;10(3):403.
4. Cho CS, Jo I. Bone morphogenic protein-2-conjugated three-dimensional-printed Poly (L-Lactic Acid) (PLLA) scaffold is likely promising as an effective bone substitute. Tissue Eng Regen Med 2023;20(2):155-6.
5. Xie J, Wu X, Zheng S, Lin K, Su J. Aligned electrospun poly (L-lactide) nanofibers facilitate wound healing by inhibiting macrophage M1 polarization *via* the JAK-STAT and NF-κB pathways. J Nanobiotechnol 2022;20(1):342.
6. LL E, Cheng T, Li CJ, Zhang R, Zhang S, Liu HC, *et al.* Combined use of recombinant human bmp-7 and osteogenic media may have no ideal synergistic effect on leporine bone regeneration of human umbilical cord mesenchymal stem cells seeded on nanohydroxyapatite/collagen/poly (l-lactide). Stem Cells Dev 2020;29(18):1215-28.
7. Muniyandi P, Palaninathan V, Veeranarayanan S, Ukai T, Maekawa T, Hanajiri T, *et al.* ECM mimetic electrospun porous Poly (L-Lactic Acid) (PLLA) scaffolds as potential substrates for cardiac tissue engineering. Polymers 2020;12(2):451.
8. Janik-Karpinska E, Ceremuga M, Niemcewicz M, Synowiec E, Sliwiński T, Bijak M. Mitochondrial damage induced by T-2 mycotoxin on human skin-fibroblast Hs68 cell line. Molecules 2023;28(5):2408.
9. Marine-Casado R, Teichenne J, Tobajas Y, Caimari A, Villar A, Zangara A, *et al.* Pomegranate natural extract Pomanox® positively modulates skin health-related parameters in normal and UV-induced photoaging conditions in Hs68 human fibroblast cells. Int J Food Sci Nutr 2023;74(1):51-63.
10. Gogele C, Vogt J, Hahn J, Breier A, Bernhardt R, Meyer M, *et al.* Co-culture of mesenchymal stem cells and ligamentocytes on triphasic embroidered poly (l-lactide-co-ε-caprolactone) and polylactic acid scaffolds for anterior cruciate ligament enthesis tissue engineering. Int J Mol Sci 2023;24(7):6714.
11. Kim HW, Jung YA, Yun JM, Kim Y, Kim SA, Suh SI, *et al.* Effects of poly-l-lactic acid on adipogenesis and collagen gene expression in cultured adipocytes irradiated with ultraviolet B rays. Ann Dermatol 2023;35(6):424.
12. Chuang FJ, Wang YW, Chang LR, Chang CY, Cheng HY, Kuo SM. Enhanced skin neocollagenesis through the transdermal delivery of poly-L-lactic acid microparticles by using a needle-free supersonic atomizer. Biomater Adv 2023;154:213619.
13. Nowag B, Schafer D, Hengl T, Corduff N, Goldie K. Biostimulating fillers and induction of inflammatory pathways: A preclinical investigation of macrophage response to calcium hydroxylapatite and poly-L lactic acid. J Cosmetic Dermatol 2024;23(1):99-106.
14. Liu C, Jiang S, Xu W, Chen X, He R, Deng K, *et al.* Poly-l-lactide-co-ε-caprolactone (PLCL) and poly-l-lactic acid (PLLA)/gelatin electrospun subacromial spacer improves extracellular matrix (ECM) deposition for the potential treatment of irreparable rotator cuff tears. In J Biol Macromol 2023;245:125522.
15. Kalvand E, Bakhshandeh H, Nadri S, Habibizadeh M, Rostamizadeh K. Poly-ε-Caprolactone (PCL)/Poly-L-Lactic Acid (PLLA) nanofibers loaded by nanoparticles-containing TGF-β1 with linearly arranged transforming structure as a scaffold in cartilage tissue engineering. J Biomed Mater Res Part A 2023;111(12):1838-49.
16. Hur GH, Ryu AR, Kim YW, Lee MY. The potential anti-photoaging effect of photodynamic therapy using chlorin e6-curcumin conjugate in UVB-irradiated fibroblasts and hairless mice. Pharmaceutics 2022;14(5):968.
17. Lee JJ, Ng SC, Hsu JY, Liu H, Chen CJ, Huang CY, *et al.* Galangin reverses H_2O_2-induced dermal fibroblast senescence *via* SIRT1-PGC-1α/NRF2 signaling. Int J Mol Sci 2022;23(3):1387.
18. Lee JJ, Ng SC, Ni YT, Liu JS, Chen CJ, Padma VV, *et al.* Protective effects of galangin against H_2O_2/UVB-induced dermal fibroblast collagen degradation *via* hsa-microRNA-4535-mediated TGFβ/SMAD signaling. Aging (Albany NY) 2021;13(23):25342.
19. Fang CL, Paul CR, Day CH, Chang RL, Kuo CH, Ho TJ, *et al. Poria cocos* (Fuling) targets TGFβ/SMAD7 associated collagen accumulation and enhances Nrf2-antioxidant mechanism to exert anti-skin aging effects in human dermal fibroblasts. Env Toxicol 2021;36(5):729-36.
20. Kim JM, Chung KS, Yoon YS, Jang SY, Heo SW, Park G, *et al.* Dieckol isolated from *Eisenia bicyclis* ameliorates wrinkling and improves skin hydration *via* MAPK/AP-1 and TGF-β/SMAD signaling pathways in UVB-irradiated hairless mice. Marine Drugs 2022;20(12):779.