ORIGINAL ARTICLES

The effect of pentoxifylline on IL-6 and PDGF expression in human keloid fibroblasts

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Abstract

Objective: The aim of this study was to investigate the effect of different dose of pentoxifylline (PTX) on intedeukin-6 (IL-6) and platelet-derived growth factor (PDGF) expression in human normal skin fibroblast (NF) and keloid fibroblast (KF), to explore the pathogenesis of PTX on prevention and treatment of KF.

Methods: Human NF and KF were passaged till the 5^{th} to 7^{th} generations by tissue block adhering wall method, and then were cultured in vitro with different dose of PTX. Levels of IL-6 and PDGF were detected by ELISA at various periods.

Results: (1) Compared with control group, the expressions of IL-6 in NF were significantly inhibited with 0.5 mg/ml PTX for 72 h, 1.0 mg/ml and 2.0 mg/ml PTX for 24 h, 48 h and 72 h separately ($p < .01$); The lower expressions of IL-6 were also showed in KF with 0.25 mg/ml PTX for 48 h and 72 h, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml PTX for 24 h, 48 h and 72 h separately ($p < .01$). (2) After cultured with 0.25 mg/ml PTX for 72 h, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml PTX for 24 h, 48 h and 72 h separately, the expressions of PDGF in NF were decreased significantly compared to the controls ($p < .01$); The reduced PDGF expressions were also found in KF after cultured with 0.25 mg/ml, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml PTX for 24 h, 48 h and 72 h separately ($p < .05$). Repeated measures analysis of variance indicated the difference source of cells and doses with statistical significance.

Conclusions: The expressions of IL-6 and PDGF in KF were higher than those in NF. PTX plays a notable inhibitory role in the expression of IL-6 and PDGF, which could be used as a promising medicine for Keloid treatment.

Key Words: Pentoxifylline, Keloid, Fibroblasts, IL-6, PDGF

Keloids are benign dermal tumors beyond the original margins of the scar, which caused by aggressive connective tissue proliferation and transparency variable due to cutaneous injury.[1] Scar is an important component of human self-defense system, it is regarded as a wound healing process as well as the result of the healing. The healing occurs in three phases: exudation, collagen and maturation. The pathogenesis of keloids has not been fully understood, it is widely believed that the formation of keloids is related to factors such as flbroblast (FB) and cytokines etc.[2]

Fibroblasts can secrete collagen (mainly I/III-type collagen) and a variety of cytokines, such as platelet-derived growth factor (PDGF), intedeukin-6 (IL-6), transforming growth factor-beta 1 (TGF-β1) etc.[3] Keloids are characterized by excessive collagen synthesis and deposition. The number of receptors and strength of expression of bioactive factors may also contribute to the formation of keloids. Therefore, it is vital to inhibit fibroblast growth and cytokines secretion for the prevention and treatment of keloids. Pentoxifylline (PTX) has been reported to have a wide range of anti-fibrosis capacity, it can effectively retain the fibrosis of liver, lung, renal and skin scar.[4–7] We use an in-vitro model of human NF and KF cultured with different dose of PTX, and study the effect on IL-6 and PDGF expression and further explore the potential pathogenesis of keloids.

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1 Materials and methods

1.1 Sample source

The samples were derived from surgical patients of Inner Mongolia Baogang Hospital (approved by the hospital ethics committee, and written informed consents were acquired from all patients).

Samples of NF (3 cases) and KF (3 cases) from patients between the ages of 16-50 years were obtained. The normal skin was isolated from chest, abdomen and back, while the keloid tissue was separated from neck, chest, and upper arm, respectively. Criterions for diagnosis refer to Modern Scar.[8] The three experimental subjects fit for: (1) Lesions beyond original margins of cutaneous injury; (2) Sustainable growth; (3) High skin bumps, tough, red nodular-, streak- and sheet-mass.

1.2 Reagents and instruments

DMSO, trypsin (1:250), DMEM/Ham's F12 medium (1:1), fetal calf serum (Defined FCS), double solution resistance: (100 U/ml penicillin, 100 U/ml streptomycin), dissociation solution (0.25% trypsin, 0.02% EDTA), cell freezing medium (DMEM/Ham's F12 medium: fetal calf serum: DMSO = 7:2:1), IL-6 ELISA Kit, PDGF ELISA Kit; CO_2 thermostatic incubator, inverted phase contrast microscope, electronic analytical balance.

1.3 Cell culture

Tissue block adhering wall method was used. Tissues were placed in the sterile culture chamber, and were minced into 1-mm^3 small pieces after washing with phosphate buffered saline solution (PBS) then put the pieces into the culture flasks. Turned over the bottles to slip the liquid and stabled the pieces in the bottom. After incubated for 2-3 h with 5% CO_2 at 37°C, slipped, aspirated the liquid and removed poorly adherent cells. After adding 1-2 ml culture media, turned off the flasks again and stored in the incubator for 18-24 h. Explant tissue culture media was changed every 2-3 days. The cells were restored and passaged after adequate growth of fibroblasts was detected (filled out 75% space of the flask). The 5^{th} to 7^{th} generations were used for future work.[9]

1.4 Models and treatment

The experimental NF and KF were performed as two groups, with no test drug as the control group seperately. PTX with concentrations of 0.25 mg/ml, 0.5 mg/ml, 1.0 mg/ml, and 2.0 mg/ml were added to the experimental group. Levels of IL-6 and PDGF were detected by ELISA. Cells were seeded in the 24-well plates with the concentration of 5×10^4/ml. Three replicates were made for each concentration sample.

The plates were placed in a incubator for 24 h, and then were changed to the culture media with the final concentration of 0.25 mg/ml, 0.5 mg/ml, 1.0 mg/ml, 2.0 mg/ml PTX for 24 h, 48 h, and 72 h. The cell-free supernatants were collected and stored in microcentrifuge tubes at –20°C for later analysis. IL-6 and PDGF levels of supernatant of each well from each time point were evaluated with solid phase ELISA. Optical density (OD) was measured at 450 nm. Standard regression curve was made by means of the Curve Expert 1.3 software, and cytokine concentrations were determined by comparison with known standards.

Statistical analysis data were performed to determine the statistical significance using SPSS 13.0. The parameters were expressed as $\bar{x} \pm$ s. Comparison of mean in multiple groups was conducted with oneway analysis of variance (ANOVA) between groups. Further statistical method of multiple comparison of mean in each group was used by repeated measures ANOVA if there was significant difference. A value of $p < .05$ was considered significant.

2 Results

2.1 Effects of PTX treatment on IL-6 expression in human NF and KF

KF secreted more IL-6 than NF, and PTX with certain concentrations could inhibit the production. Compared with the control group, NF treated with 0.5 mg/ml PTX for 72 h, 1.0 mg/ml and 2.0 mg/ml PTX for all time points separately had statistical signicance in reducing the production of IL-6 ($p < .01$); The significant lower levels of IL-6 were also expressed in KF with 0.25 mg/ml PTX for 48 h and 72 h, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml PTX for all time points separately ($p < .01$) (see Table 1).

2.2 Effects of PTX treatment on PDGF expression in human NF and KF

KF secreted more PDGF than NF, and PTX with certain concentrations could inhibit the production. After cultured with 0.25 mg/ml PTX for 72 h, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml PTX for all time points separately, the expressions of PDGF in NF were decreased significantly compared to the controls ($p < .01$) (see Table 2). The reduced PDGF expressions were also found in KF after cultured with 0.25 mg/ml, 0.5 mg/ml, 1.0 mg/ml and 2.0 mg/ml PTX for all time points separately ($p < .05$) (see Table 2).

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Table 1: Effects of PTX treatment on IL-6 expression (pg/ml) in human NF and KF

Time (h)	Concentration (mg/ml)	IL-6 (pg/ml)	*p*
	0	19.20 ±2.09/25.57 ±1.64	
	0.25	17.09 ±1.73/23.02 ±1.63	/.012
24	0.5	15.70 ±1.27/17.99 ±2.66*	/.004
	1.0	13.94 ±1.48*/15.67 ±1.73*	.000/.005
	2.0	11.79 ±1.43*/10.48 ±1.76*	.000/.000
	0	22.13 ±1.95/30.71 ±1.26	
	0.25	19.65 ±0.52/26.99 ±0.42*	.030/.000
48	0.5	16.64 ±1.25/23.01 ±0.57*	.011/.001
	1.0	15.66 ±1.70*/20.39 ±1.35*	.002/.000
	2.0	13.44 ±1.45*/14.52 ±2.18*	.000/.000
	0	23.97 ±0.35/33.25 ±1.39	
	0.25	20.18 ±1.50/29.20 ±3.77*	.014/.000
72	0.5	18.08 ±0.73*/25.69 ±2.48*	.001/.000
	1.0	16.88 ±0.61*/23.56 ±0.88*	.000/.000
	2.0	12.98 ±1.30*/13.92 ±1.83*	.000/.000

Note. * means $p < .01$

Table 2: Effects of PTX treatment on PDGF expression (ng/ml) in human NF and KF

Time (h)	Concentration (mg/ml)	PDGF (ng/ml)	*p*
	0	71.69 ±2.29/77.85 ±2.66	
	0.25	66.98 ±1.05/73.35 ±1.21*	.033/.000
24	0.5	64.39 ±1.28*/72.03 ±0.93*	.000/.000
	1.0	59.95 ±0.58*/63.35 ±4.24*	.007/.009
	2.0	48.42 ±1.61*/57.64 ±2.55*	.000/.007
	0	68.72 ±1.43*/79.90 ±3.31	
	0.25	68.72 ±1.43*/73.66 ±0.95*	.004/.000
48	0.5	66.89 ±0.90*/70.16 ±5.75*	.000/.000
	1.0	62.35 ±1.74*/65.84 ±7.37*	.000/.000
	2.0	53.20 ±4.83*/59.82 ±2.28*	.000/.006
	0	77.01 ±2.59/85.93 ±1.58	
	0.25	71.48 ±1.74*/76.29 ±2.49*	.003/.000
72	0.5	69.38 ±2.62*/71.56 ±4.38*	.000/.000
	1.0	64.44 ±1.88*/64.88 ±1.25*	.000/.001
	2.0	54.45 ±5.00*/64.24 ±0.60*	.000/.000

Note. * means $p < .01$

3 Discussion

Currently, keloid is regarded as a common clinical disease, however, the pathogenesis of keloid disease has not been fully elucidated, and the therapeutic approaches are difficult to resolve the problem of recurrence. keloid generally can affect the patients' appearance, normal work and rest, some can even induce degeneration and canceration, which aggravate the patients' psychological burden and psychosomatic health. To date, it is still a research hotspot to improve and reduce the recurrence of keloid.

PTX is an unselective phosphodiesterase inhibitor, which has a strong anti-inflammatory, anti-immune and anti-fibrosis effect. PTX has been attributed to its clinical influence on the sequelae of cerebrovascular disorder, stroke or trauma. PTX has the function of expanding the brain and peripheral blood vessels, reducing blood viscosity and improving blood circulation in the brain and limbs. Recently, PTX has been widely used to treat cutaneous vascular, infectious diseases, skin tumors, connective tissue disease, allergic and metabolic skin diseases, with a good curative effect, less side effect and safety clinical medication.[6] In addition, studies show that PTX appears to be beneficial in anti fibrosisarterial diseases, it can effectively inhibit the keloid of liver, lung, kidney fibrosis and skin scar.[4–7] Feng HY et al.[10] reported that levels of I/III procollagen in keloids were higher than those in normal skin, while PTX could reduce the proliferation of fibroblasts and gene transcription of I/III procollagen, as well as TGF-βl levels obviously. In clinical research, PTX has been used in the treatment of keloid, but relevant basic research has been less reported.

The proliferation of fibroblasts is influenced by multiple cytokines, numbers and strength of expression of these biological active factors are vital to the formation of keloid. In recent years, numerous studies have demonstrated that immune factors such as cytokines (e.g. IL-6, PGDF), pancreatic cells, mast cells, macrophages, lymphocytes and immunoglobulin are closely related to scar. Xue et al.[11] implied the over expression of IL-6 was attributed to its influence on keloid.

Fibroblasts are the major cells to produce PDGF. PDGF plays an important role in the process of wound healing and scar formation. PDGF also has a strong chemotaxis and mitogenic effect on fibroblasts, which can promote wound healing, formulate muscle fiber fibroblasts and increase the expression of TGF-β R1 and TGF-β R2.[12] Zhou et al.[13] used real-time quantitative PCR method and found that the protein and mRNA expression of PDGF-α increased significantly in KF, but those were not apparent in the expression of PDGF-β. PDGF is a chemotactic factor of mononuclear cell and fibroblast, Lee[14] indicated that the significant increasing level of PDGF-α receptor mRNA in scar was related to the formation of keloid.

Our studies showed that: (1) PTX could reduce the expression of IL-6 and PDGF in KF, which were higher at all time spot in NF. Compared with control group, 0.5 mg/ml PTX could significantly depress the IL-6 secretion, 0.25 mg/ml PTX could apparently inhibit the expression of PDGF with dose manner, performed progressive inhibitory effect accompanied with the increased concentrations of PTX with significant difference ($p < .01$). Likely, the expressions of IL-6 and PDGF were diminished in NF but with less inhibition than KF.

(2) IL-6 and PDGF have a close relationship with the formation of keloid. PTX can inhibit the keloid by significantly reducing the expression of IL-6 and PDGF in KF. These results may provide experimental evidence and theoretical

basis in the treatment of keloid.

Conflicts of Interest Disclosure

The authors have no conflict of interest related to this article.

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