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Original Research Article

Polysulfonic acid mucopolysaccharide exerts anti-scarring effect in rats through modulation of TGF-β 1/Smad signaling pathway

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Abstract

Purpose: *To determine the anti-scarring effect of polysulfonic acid mucopolysaccharide (MSP), and the implication of TGF-β1/Smad signal transduction route.*
Methods: *Sixty (60) male Sprague Dawley (SD) rats were assigned to control, model and polysulfonic mucopolysaccharide groups, respectively, each with 20 rats. Serum inflammatory factors, scar area and scar thickness, histopathological changes and relative concentrations of TGF-β1 Smad4, collagen types I and III, and α-SMA were determined.*
Results: *In the control group, collagen cells were closely distributed and the skin structure was intact without inflammatory infiltration. In contrast, there were numerous necrotic dermal cells on rat skin surface in model group, with obvious inflammatory infiltration and severely damaged hair follicles. In contrast, in polysulfonic mucopolysaccharide group, the thickness of skin tissue and dermis was significantly improved, with a clear layer and reduced degree of inflammatory infiltration. Types I and III collagen and α-SMA were significantly down-regulated in polysulfonic mucopolysaccharide-fed rats, relative to model rats.*
Conclusion: *Polysulfonic acid mucopolysaccharide exerts anti-scarring effect by regulating TGF-β1/Smad signal pathway, thus has the potential for use in minimizing scarring of the skin in clinical practice.*

Keywords: *Polysulfonic acid, Mucopolysaccharide, TGF-β1/ Smad signal pathway Anti scar*

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INTRODUCTION

Scar is a physiological reaction to changes in skin morphology and tissue morphology caused by healing of various wounds and trauma. The wound caused by skin trauma is repaired through healing regeneration and scar [1]. Generally, scar comprises two types: physiological scar and pathological scar. If physiological scar does not cause any marked discomfort, and does not affect physical appearance and function, it does not need treatment [2]. Excessive healing of scars leads to case scars which are divided into keloids and hypertrophic scars [3]. Under normal

circumstances, pathological scars seriously affect the function and appearance of the body, and it is usually accompanied by skin problems such as itching and pain [4].

In clinical practice, pathological scars are managed using surgery, cholesterol-like hormones, and anti-metabolic drugs. However, apart from being expensive, these methods cause severe systemic adverse reactions in patients, and they are limited in clinical practice [5]. It has been reported that transforming growth factor β1 (TGF-β1) is very closely associated with scar fibrosis [6]. The Smad protein family comprises signal proteins downstream of transforming growth factor β receptor which mediate the main pathway involved in intracellular reactions of transforming growth factor β [7].

Hiryotol, also known as MSP, is a mucosaccharide composed mainly of tissue heparin. It effectively inhibits the proliferation of collagen fiber cells, exerts anti-inflammatory and anticoagulant effects, and enhances the synthesis of hyaluronic acid [8]. However, not much is known about the anti-scarring effect of MSP, and the implication of TGF-β1/Smad signal route in this process. Therefore, this study was aimed at investigating the anti-scar formation effect of MPS, and the involvement of the TGF-β1/Smad signaling pathway in it.

EXPERIMENTAL

Animals

Sixty healthy SD male rats with mean age of 8.12 ± 2.15 weeks and mean body weight of 182.44 ± 16.74 g, were used in this study. The rats were housed 3 - 5 per cage under clean conditions, and were reared in separate cages at ambient temperature which varied from 22 to 25 ^{o}C, and humidity ranging from 55 – 60 %. The rats were exposed to equal durations of alternating light and darkness (12 h) daily in a well-ventilated environment. Feed and water were provided *ad libitum,* and the rats were fed adaptively for 7 days prior to commencement of the study. This study received approval (no. SPPH2022002) from the Animal Ethics Authority of Sichuan Provincial People's Hospital in line with NIH guidelines [9].

Experimental instruments and reagents

The instruments used in this study, and their suppliers (in parenthesis) were: electronic balance (Mettler Toledo International Co. Ltd), paraffin sectioning machine (Shenyang Hengsong Technology Co. Ltd); biological tissue slicing machine (Hubei Xiaogan Kuohai Medical Technology Co. Ltd), constant temperature incubator (Jinan Chuangri New Instrument Equipment Co. Ltd), optical microscope (Northern Xinjiang Pury Seth instrument Co. Ltd), low-temperature centrifuge (Yancheng Kate Experimental Instrument Co. Ltd (Shanghai Meigu Molecular Instrument Co. Ltd) and ultra-low temperature refrigerator (Jinan Xinbexi Biotechnology Co. Ltd).

The reagents used, and their sources (in brackets) were: TGF-β1/enzyme-linked immunoassay kit (Wuhan Elite Biotechnology Co. Ltd), Smad enzyme-linked immune detection kit (Thermo Fisher Technology Co. Ltd), glycerin (Zhejiang Local Kang Hui Pharmaceutical Co. Ltd), 75 % ethanol (Beihai Guofa Marine Life Industry Co. Ltd), formaldehyde (Changsha Biotechnology Co. Ltd), chloral hydrate (Shanghai Shanpu Chemical Co. Ltd.), interleukin-10 ELISA kit (Wuhan Mercer Biotechnology Co. Ltd), MPS Cream (Mobilat Produktions GmbH, Germany), and sodium sulfide (Shandong Pulsichemical Co. Ltd).

Establishment of animal scar model and grouping

Sixty SD rats were fed adaptively for 7 days, after which they were assigned in random to control, model and MPS groups, each with 20 rats. Each rat group was fed separately in cages. Clean drinking water and feed were liberally provided, and the cages were routinely cleaned and disinfected from time to time.

In the establishment of 3rd degree scald model, rats in each group were administered chloral hydrate anesthesia at a dose calculated on the basis of 400 mL/kg, and the drug was injected into the lower left abdominal cavity of each rat. Under anesthesia, the hair of each rat was shaved off, and depilation was done by applying cotton buds moistened with 8 % sodium sulfide in a non-dripping state, to the shaven rat skins. After 5 min, the rat skin was rinsed with running water to remove residues of the drug from the depilated areas. Thereafter, the rats were rehabilitated for 24 h, followed by sterilization of the depilated skin areas with 70 % ethanol. Then, the depilated areas were scalded with steam from water heated to temperature of 95 ℃. This was done by exposing the depilated areas to steam at a distance of 10 cm above a steam pot for 10 s. During the establishment of the rat scald model, intraperitoneal injection of 0.9 % NaCl was used to mitigate high-temperature-induced shock in the rats.

Treatments

Rats in the three groups were kept in separate cages during the study to ensure easy access to feed and drinking water, and to ensure the healthy growth of the rats. Rats in MPS group were treated with 2 g of MPS ointment which was massaged on the skin twice a day for 28 days, while rats in control group received normal saline only.

Evaluation of parameters/indices

Relative levels of inflammatory factors

The levels of interleukin-10 (IL-10) and serum interferon γ (IFN-γ) were determined using ELISA.

Scar area and scar thickness

One week (7 days) after the beginning of the experiment, the rats were anesthetized, and the area of skin wound at the injury site of each rat was measured. Scar tissues were examined under a light microscope, and scar thickness in each rat was measured.

Expression levels of TGF-β1/Smad7, type I and type III collagen

The expression levels of TGF-β1/Smad7, type I and type III collagen in serum and tissue fluid samples were determined using ELISA.

Histology of scar tissues

Skin tissue samples, each of area 1.0 cm x 1.0 cm, were taken from the injured area of each rat. The sections were routinely processed for light microscopy and paraffin-embedded, followed by sectioning and staining with hematoxylin and eosin (H&E). The stained slices were processed and mounted on glass slides which were examined under the microscope.

The morphologies of the epidermis, dermis and hair follicles in the injured areas were closely examined under the microscope, and photographed.

α-Smooth muscle actin (α-SMA)

Immunohistochemistry was used to measure α-SMA. Paraffin sections of injured tissue were prepared, dehydrated and rinsed with distilled water. Then, the sections were incubated with 3 % hydrogen peroxide for 15 min, followed by addition of pepsinogen for antigen repair. After 10 min, the sections were incubated with primary antibody for 12 h at 4 ^{o}C. On the second day, the sections were rinsed thrice in phosphate solution, each time for 3 min, followed sequentially with DAB staining for 3-5 min, counter-staining with hematoxylin, dehydration, clearing and sealing. Four fields were selected at high magnification under a microscope. Brownish-yellow color indicated positive stain for α-SMA.

Statistical analysis

The SPSS 20.0 software was employed for data processing. Measured data are presented as mean ± SD). Paired comparison was done with *t*-test and LSD tests. Values of $p < 0.05$ indicated statistically significant differences.

RESULTS

Serum levels of inflammatory factors

The expression level of IL-10 level in model group was significantly higher than that in control group, while IFN-γ level was significantly lower. However, IL-10 level was significantly lower in MPS group than in model group, while IFN-γ level was markedly higher in MPS group than in the model group ($p < 0.05$). These data are presented in Table 1.

Table 1: Comparison of changes of serum inflammatory factors in three groups of rats (mean ± SD, n = 20)

Group	IL-10 (ng/L)	IFN-γ (ng/L)
Control	182.45±31.46	724.13±91.45
Model	254.94±28.33*	516.78±91.22*
Polysulfonic mucopolysaccharide	204.75±24.78$^{*\#}$	618.33±75.61$^{*\#}$
F	34.37	28.79
P-value	<0.001	<0.001

$^{*\#}P < 0.05$, $^{\#}$vs control; $^{\#}$vs model rats

Post-therapy scar area and thickness

As shown in Table 2, scar area and scar thickness were markedly reduced in MPS group, relative to model rats.

TGF-β1 and Smad4 levels in rat skin

There were relatively higher amounts of TGF-β1 and Smad4 in model rats than in control rats. However, TGF-β1 and Smad4 levels were markedly down-regulated in MPS-exposed rats, relative to model rats. These data are shown in Table 3.

Table 2: Comparison of scar area and scar thickness amongst the three groups post-treatment mean ± SD, n = 20)

Group	Scar area (mm^2)	Scar thickness (mm)
Control	—	—
Model	8.23±0.61	1.35±0.51
Polysulfonic mucopolysaccharide	6.12±0.58#	0.75±0.12#
F	11.210	5.121
P-values	<0.001	<0.001

#Compared with model group

Table 3: Expression levels of TGF-β1 and Smad4 in skin tissues of rats in the three groups (mean ± SD, n = 20)

Group	TGF-β1	Smad4
Control	78.44±10.56	1.35±0.22
Model	258.46±20.33*	3.35±1.09*
Polysulfonic mucopolysaccharide	123.45±11.85*#	1.83±0.11*#
F	791.60	52.39
P-value	<0.001	<0.001

*#$P < 0.05$, *vs control; #vs model rats.

Pathological changes in rat skin

Collagen was closely distributed in control rats, and skin structure was intact and without inflammatory infiltration. In the model group, several dermal cells were necrotic on the skin surface, with obvious inflammatory infiltration, and the hair follicle structure was severely damaged. However, relative to the model group, the skin tissue and dermis thickness in the MPS group were significantly improved, with clear layers and reduced inflammatory infiltration. These results are shown in Figure 1.

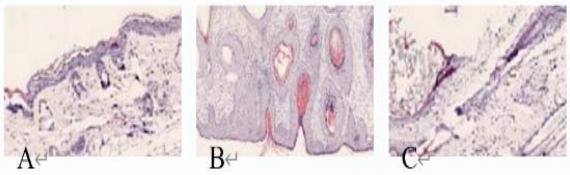

Figure 1: Pathological changes in rat skin (x100). A: control, B: model, C: MPS

Expressions of types I and III collagen, and α-SMA

As shown in Table 4, types I and III collagen and α-SMA levels were markedly up-regulated in model rats, relative to control rats. However, relative to the model group, levels of type I and type III collagen and α-SMA in MPS group were significantly lower ($p < 0.05$).

DISCUSSION

The skin, being the first line of defense against external injury, has the requisite capacity for wound repair. When the skin is damaged, the wound formed on the skin surface destroys its structure and function, and leads to changes in secretion, proliferation and differentiation in the environment of tissue cells [10]. Scar is a pathological wound healing reaction of the skin caused by a variety of traumas. Wound repair has become a serious and difficult issue in the field of skin plastic surgery, and the demand for wound repair is significantly higher in females than in males [11].

With modernity and improvements in standard of living, the quest for beauty is gradually increasing. The presence of scar tissue has a significant impact on the psychology and self-esteem of an individual. It is known that MPS effectively inhibits various enzymes involved in metabolism, and it exerts anti-inflammatory effects through the prostaglandin and complement systems. Moreover, MPS is involved in the synthesis of mesenchymal cells, and it has the capacity to retain intercellular water, thereby enhancing connective tissue regeneration [12]. In this study, a rat scald model was established, and the anti-scar effect of MPS, and the mechanism involved, were investigated in this model.

Interleukin is activated by the immune system, and it mediates collective immune response and inflammatory response in the form of intercellular signals.

Table 4: Expression levels of type I and III collagen and α-SMA in rats in the 3 groups (mean ± SD, n = 20)

Group	Type Ⅰ collagen	Type III collagen	α-SMA
Control	0.59±0.05	0.46±0.03	0.76±0.08
Model	1.33±0.19*	1.46±0.31*	1.25±0.31*
Polysulfonic mucopolysaccharide	1.13±0.12*#	1.15±0.11*#	1.05±0.12*#
F	165.89	144.11	31.15
P-value	<0.001	<0.001	<0.001

*#$P < 0.05$, *vs control; #vs model group

It is an anti-inflammatory factor which is closely associated with the formation of scar tissue, and it suppresses the secretion of chemokines and inflammatory factors by inhibiting eosinophils, neutrophils and MHCII monocytes, thereby inhibiting antigen presentation [13]. A study has shown that IFN-γ negatively regulates scar formation and inhibits fibroblasts in scar formation [14]. In this study, the level of IL-10 in MPS-exposed rats was markedly reduced, relative to model rats, but IFN-γ was markedly higher, relative to that in model rats. Moreover, scar area and scar thickness were markedly lower in MPS-fed rats than in model rats. Thus, MPS effectively inhibited the expression of serum IL-10, up-regulated the expression of IFN-γ, and reduced scar area and scar thickness, thereby effectively inhibiting scar formation. It has been reported that IFN-γ inhibited the expression of TGF-β, reduced scar volume and inhibited the development of fibrosis [15]. The major components of fibroblast are α-SMA cells, and they participate in scar formation. After stimulation, fibroblasts are activated, proliferated and transformed into fibroblasts characterized by α-SMA. Tissue fibrosis is closely associated with TGF-β which plays a role through the signaling pathway composed of TGF-β receptor and Smad protein [16]. In the Smad family, Smad3 is a positive regulator of the TGF-β-Smad channel pathway, and it directly interacts with TGF-β, which, on phosphorylation, combines with Smad4 to form a dimer which is translocated into the nucleus [17]. It is known that Smad7 is an inhibitor of TGF-β-Smad signal transduction pathway, and it directly binds to R-Smad to prevent its phosphorylation, thereby ultimately blocking TGF-β [18]. In this study, there were markedly reduced TGF-β1 and Smad4 levels in MPS-exposed rats, relative to model rats, while collagen types I and III and α-SMA were lower in MPS-exposed rats than in model group. These results indicate that MSPS regulated TGF-β1/Smad signal route, thereby reducing the expressions of types I and III collagen and α-SMA.

CONCLUSION

The results obtained suggest that MPS exerts anti-scar effect in a rat scar model by regulating TGF-β1/Smad signaling pathway. Therefore, MPS may be used for the management of scarring in clinics.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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