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Original Article

Research on transforming growth factor-β in the repair process of skin burn patients

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Abstract: Objective: To investigate transforming growth factor-β (TGF-β) in the repair process of skin burn patients. Methods: A total of 164 burn patients treated in Zhujiang Hospital, Southern Medical University from September 2014 to December 2016 were enrolled as objects of study, and were divided into: control group (n=82, including 37 cases of second-degree superficial burns and 45 cases of second-degree deep burns) and an observation group (n=82, including 42 cases of second-degree superficial burns and 40 cases of second-degree deep burns), using a random number table. Patients in the control group were treated with wound dressing by using single-layer Vaseline gauze, while those in the observation group were treated with external application of chitosan wound repair membrane gel. Tetanus antitoxin was intramuscularly injected into patients in both groups to prevent tetanus, and antibiotics were used to prevent bacterial infection. Wound healing, healing time, and wound healing scar index (SI) were observed and compared between the two groups at 7, 15, and 25 days after treatment, and the expression level of TGF-β in the peripheral blood was detected via enzyme-linked immunosorbent assay before treatment and at 15 and 25 days after treatment, respectively. Results: Comparison of wound healing between the control group and the observation group revealed that the healing rate of patients with second-degree superficial burns in the observation group treated with chitosan at 7 days after treatment increased compared with that of patients with second-degree superficial burns in the control group, with a statistically significant difference ($P<0.05$). The healing rates of patients with second-degree deep burns in both groups at 15 and 25 days after treatment also increased significantly, with a statistically significant difference between the two groups ($P<0.05$). The healing time of patients with second-degree superficial burns in the observation group was obviously shorter than that of patients with second-degree superficial burns in the control group, displaying a statistically significant difference between the two groups ($P<0.05$), and the SI score of patients in the observation group treated with chitosan was obviously lower than that in the control group ($P<0.05$). Moreover, the healing time of patients with second-degree deep burns in the observation group was also shorter than that in the control group, showing a statistically significant difference between the two groups ($P<0.05$). The SI score of patients in the observation group treated with chitosan was also obviously lower than that in the control group ($P<0.05$). According to the comparison of TGF-β expression before treatment and at 15 and 25 days after treatment between the two groups of patients, expression levels of TGF-β in both groups were decreased gradually at 15 days with a difference between the two groups ($P<0.05$), but no significant difference was found in the expression level of TGF-β at 25 days between the two groups ($P>0.05$). Conclusion: External application of chitosan wound repair membrane gel significantly shortens the recovery time of patients, and it is expected that TGF-β expression level can serve as one of the evaluation indexes for patient recovery.

Keywords: Transforming growth factor-β, skin burn, C-reactive protein, chitosan wound repair

Introduction

Burns refer to the severe damage to human skin or tissue caused by high temperature, current and chemical substances [1]. According to statistics, burns rank 4th of all injuries, following traffic accidents, falls, and interpersonal violence [2]. More than 1,000,000 people get hurt in a fire in the USA every year, and there are up to 100,000 patients with moderate burns or above [3, 4]. Moreover, there have been more than 5,000,000 burn patients in China. The high incidence of burn accidents has attracted increasingly more attention, which has become a major public health problem in most developing countries [5]. Burn patients are faced with

not only psychological problems but also disfigurement, disability, and malformation, and even life risk in severe cases, greatly affecting the patients' quality of life [6].

There is no doubt that burn patients will suffer from severe pain during the treatment and healing process. However, currently there have been no better indexes for observing the prognosis of burn patients in clinic [7]. As one of the important factors involved in wound repair, transforming growth factor-β (TGF-β) is highly secreted under the stimulation of inflammatory response, and promotes angiogenesis and fibroblast proliferation, ultimately reconstructing the extracellular matrix. Therefore, a large amount of TGF-β is produced in the wound healing process, which may play a guiding role in observing the prognosis of burn patients [8, 9]. Bacterial infection occurs easily in the treatment of burns with traditional methods, resulting in a systemic inflammatory response. Therefore, in order to reduce infection in patients during the wound healing process, effective wound management is essential [10]. It has been reported in recent years that chitosan is characterized by good permeability and high absorptivity in patients, which can reduce the wound exudation effectively. Additionally, chitosan possesses broad-spectrum antibacterial activity compared with single-layer Vaseline gauze, the most commonly-used material for wound dressing in clinic [11]. Satisfactory results have been achieved in the clinical repair of burn patients with chitosan [12].

Therefore, expression of TGF-β in the peripheral blood of burn patients treated with chitosan was detected in this study, so as to find new indexes and methods for the recovery evaluation and treatment of burn patients.

Materials and methods

Clinical data of patients

A total of 164 burn patients treated in Zhujiang Hospital, Southern Medical University from September 2014 to December 2016 were enrolled as objects of study, and divided into a control group (n=82) and an observation group (n=82), using a random number table. In the control group, there were 45 males and 37 females, aged 27-52 years, with an average of (32.4±7.5) years. In the observation group, there were 40 males and 42 females, aged 25-53 years, with an average of (33.4±7.2) years. This study was approved by the Medical Ethics Committee of Zhujiang Hospital, Southern Medical University, and patients' family members were informed and signed the informed consent.

Inclusion and exclusion criteria

Inclusion criteria: Patients aged >18 years; patients treated in Zhujiang Hospital, Southern Medical University; patients with the burn area of 5% of the total body surface area and who were admitted within 12 hours after burns; patients with flame burn and hot liquid scalding; patients without wound infection before admission; patients without familial genetic diseases.

Exclusion criteria: Patients with diabetes mellitus or cardiopulmonary insufficiency; patients with severe coma or metabolic disorders; patients with chemical burns or electrical injury; patients with allergic constitution; pregnant women; patients with hearing or cognitive impairment; patients who did not cooperate with the treatment or sign the informed consent.

Treatment methods

After admission, patients were treated with simple debridement (the clothes were cut off), washed with normal saline repeatedly and disinfected with iodophor (5%). Foreign matter and necrotic tissues were removed from the patients' wound, and the blister fluid was drained. The blister skin was retained, and the excess wound fluid was absorbed by using the sterile gauze. Patients in the control group were treated with wound dressing by using the single-layer Vaseline gauze under the above conditions, followed by gauze bandage (once a day). Patients in the observation group were treated with external application of chitosan wound repair membrane gel (Wuhan Dazheng High-Tech Biopharmaceutical Co., Ltd.), and the membrane formation was observed, after which patients were bandaged with gauze (once a day). During wound treatment, tetanus antitoxin was intramuscularly injected into all patients to prevent tetanus, and antibiotics were used to prevent bacterial infection [13].

Table 1. Clinical patient data (case)

Group	Control group (n=82)	Observation group (n=82)	t/χ^2	P
Gender			0.065	0.798
Male	45	40		
Female	37	42		
Age (years old)			0.438	0.508
≥35	25	30		
<35	57	52		
Burn degree			0.391	0.532
Second-degree superficial burns	37	42		
Second-degree deep burns	45	40		
Time from burns to admission (h)			0.101	0.751
≥6	50	47		
<6	32	35		
Burn area (%)			0.777	0.378
≥5	19	25		
<5	63	57		
Burn site			0.938	0.332
Trunk burn	34	27		
Limb burn	48	55		
Burn mode			1.227	0.260
Flame burn	55	47		
Hydrothermal burn	27	35		
Place of residence			0.890	0.346
Country	15	21		
City	67	61		
Smoking			0.610	0.435
Yes	43	37		
No	39	45		
Alcoholism			0.513	0.474
Yes	12	8		
No	70	74		
BMI (kg/m^2)	22.47±1.38	22.10±1.22	1.819	0.070

Note: BMI, body mass index.

Observation indexes

Major observation indexes: Wound healing and healing time were observed at 7, 15, and 25 days after treatment, the former of which started from the first observation time (Day 0) and was detected by using the image software Image Pro-plus. Healing rate = initial wound area before treatment - unhealed wound area during observation)/initial wound area before treatment * 100%. The scar formation (scar pigment, hardness, height and vessel) and purple ulcer in patients were scored by using the modified Vancouver scar scale [14]. Finally, the score in each group was added up to obtain the scar index (SI) of wound healing, and the higher the score, the more significant the scar proliferation. The expression level of TGF-β in the peripheral blood was detected before treatment and at 15 and 25 days after treatment via enzyme-linked immunosorbent assay (ELISA, Qingdao Jisskang Biotechnology Co., Ltd.), respectively.

ELISA

Coating buffer was first diluted to the required amount for hepatitis B surface antigen, added into each well (200.00 μL), and placed at 4°C overnight, followed by washing once with double-distilled water. A total of 200.00 μL blocking solution was added into each well for block-

Table 2. Wound healing of patients in both groups at each time point, n (%)

Group	Wound healing of second-degree superficial burns		P	Wound healing of second-degree deep burns		P
	Control group (n=37)	Observation group (n=42)		Control group (n=45)	Observation group (n=40)	
7 d	4 (10.81)	16 (35.56)	0.011	0	4 (10.00)	0.052
15 d	15 (40.54)	28 (66.67)	0.025	12 (26.67)	28 (70.00)	0.001
25 d	37 (100.00)	42 (100.00)	1.000	37 (82.22)	40 (100.00)	0.006

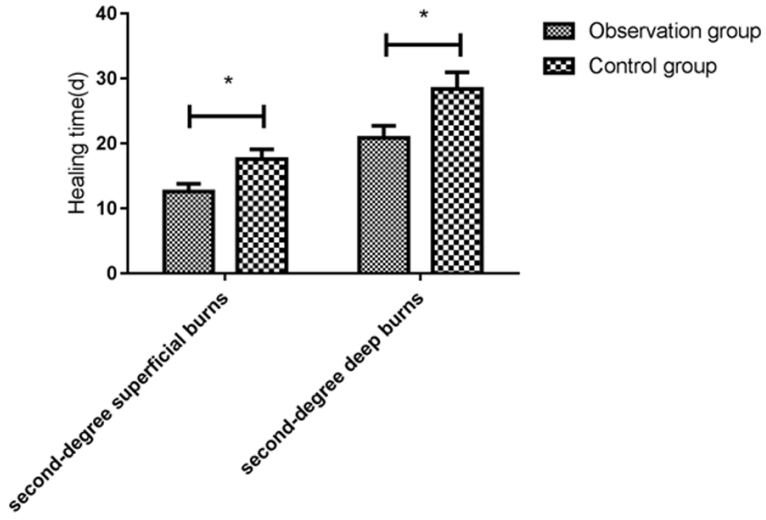

Figure 1. Healing time of patients in both groups. Comparison of healing times and between the two groups shows that, the healing time of patients with second-degree superficial burns in the observation group was obviously shorter than that in the control group, displaying a statistically significant difference (*t=15.825, P=0.001). The healing time of patients with second-degree deep burns in the observation group was also shorter than that in the control group, showing a statistically significant difference between the two groups (*t=15.112, P=0.001).

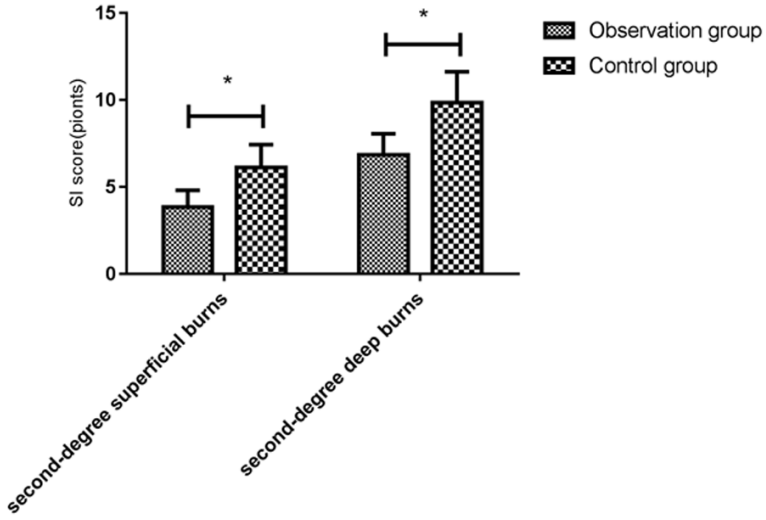

Figure 2. SI scores of patients in both groups. Comparisons of SI score between the control group and the observation group of patients manifested that, the SI score of patients with second-degree superficial burns in the observation group was obviously lower than that in the control group (*t=8.713, P=0.001). The SI score of patients in the observation group with second-degree deep burns was also obviously lower than that in the control group (*t=8.777, P=0.001). SI, scar index.

ing. After the sample was loaded, the negative interior control group (50.00 μL), the positive interior control group (50.00 μL) and the blank control group (50.00 μL) were set, and the enzyme-labeled antibody was added and mixed evenly. The plate was then sealed, incubated, and placed in a constant temperature water box at 37°C for 45 minutes, after which the plate was washed with the prepared cleaning solution 5 times (30 s-1 min per time). Finally, 100.00 μL substrate was added into each well at 37°C for 15 minutes, and 0.05 mL sulfuric acid (2 mol/L) was added into each well to terminate the reaction, followed by color development within 15 minutes.

Statistical analysis

In this study, SPSS20.0 software package was used for the statistical analysis of all data collected, and GraphPad Prism 5 was used to draw pictures. Enumeration and measurement data in this study are presented as mean ± standard deviation, and independent-samples t test was

Table 3. TGF-β expression in patients in both groups at each time point (ng/mL, $\bar{x} \pm sd$)

Group	Control group (n=82)	Observation group (n=82)	T	P
Before treatment	64.51±14.54	62.84±13.84	0.753	0.452
15 d	42.84±8.54	32.33±5.47	9.452	0.001
25 d	18.37±3.54*	17.31±4.22*	1.743	0.083

Note: *Compared with the expression level of TGF-β before treatment, the expression level of TGF-β at 25 days after treatment was significantly different (control group: t=27.920, P=0.001; observation group: t=28.495, P=0.001). TGF-β, transforming growth factor-β.

used for measurement data in line with the normal distribution and expressed as t. Enumeration data are presented as rate (%), and chi-square test and Fisher's exact probability method was used and expressed as χ^2. ROC curve analysis was performed for TGF-β, and both specificity and sensitivity were also analyzed. P<0.05 indicates that the difference is statistically significant.

Results

Comparisons of clinical data between the two groups of patients

Comparisons of clinical data of patients between the control group and the observation group show that there are no statistically significant differences in the patient's gender, age, degree of burn, time from burns to admission, burn area, burn site, burn mode, place of residence, smoking, alcoholism and body mass index (all P>0.05). See **Table 1**.

Wound healing of patients in both groups at each time point

The comparison of wound healing between the control group and the observation group revealed that the healing rate of patients with second-degree superficial burns in the observation group treated with chitosan at 7 days after treatment increased compared with that of patients with second-degree superficial burns in the control group, and there was a statistically significant difference (P<0.05). With the passage of time, the healing rates of patients with second-degree deep burns in both groups at 15 and 25 days after treatment also increased significantly, and there was a statistically significant difference between the two groups (P<0.05). See **Table 2**.

Healing time and SI scores of patients in both groups

Comparisons of healing time and SI score between the two groups of patients showed that the healing time of patients with second-degree superficial burns in the observation group was shorter than that of patients with second-degree superficial burns in the control group, displaying a statistically significant difference (P<0.05), and the SI score of patients in the observation group treated with chitosan was lower than that in the control group (P<0.05). Moreover, the healing time of patients with second-degree deep burns in the observation group was also shorter than that in the control group, showing a statistically significant difference between the two groups (P<0.05), and the SI score of patients in the observation group treated with chitosan was also obviously lower than that in the control group (P<0.05). See **Figures 1**, **2**.

TGF-β expression in patients in both groups at each time point

According to the comparison of TGF-β expression before treatment and at 15 and 25 days after treatment between the two groups of patients, expression levels of TGF-β in both groups were decreased gradually at 15 days with a difference between the two groups (P<0.05), but no significant difference was found in the expression level of TGF-β at 25 days between the two groups (P>0.05). See **Table 3**.

Discussion

Burns are the most common trauma in daily life, which will lead to a variety of stress responses in patients. The stress responses refer to a series of metabolic changes, such as neurological, immune and endocrine disorders, caused by high-intensity stimulation and damage [15]. As the largest organ of the human body, skin consists of the epidermis, dermis, and subcutaneous tissue, whose most important function is to regulate biological invasion, secretion and excretion, body temperature, permeability and absorption [16]. The skin will repair itself when damaged, but its self-healing function will be lost under severe damage and

large damage area, leading to the body's immune dysfunction and producing a large number of inflammatory factors in patients. Moreover, a large amount of water and protein in cells will be lost, thus causing shock in patients [17]. However, patients will suffer from wound infection and multiple organ failures in most cases, so infection should be avoided in the treatment of burn patients. Infection should be treated in time if it occurs in patients, which is crucial for the treatment and prognosis of patients [18].

At present, Vaseline is commonly used in the clinical treatment of burn patients, but its anti-infection effect is unsatisfactory [19]. Dressing, as a kind of temporary covering, is characterized by good sealing effect on the wound and ability to maintain the wound healing environment, which also has an excellent effect in prevention and anti-infection [20]. None of the various dressings on the market can meet clinical requirements. Chitosan is characterized by good bioactivity and compatibility, which is degradable. Furthermore, chitosan can effectively realize bacteriostasis, diminish inflammation, reduce wound exudation, shorten healing time, and reduce scars when the skin is damaged [21]. There are fewer evaluation indexes for the healing of burn patients in clinic currently. TGF-β is widely distributed in animals and plants, which is also involved in regulating embryonic development, organ formation, extracellular matrix synthesis, immune response and epithelial tissue hyperplasia [22]. In case of wound in the human body, platelets will aggregate on the wound and secrete a large amount of TGF-β, and TGF-β can stimulate a variety of inflammatory cells, thereby further promoting the secretion and synthesis of TGF-β [23]. Therefore, it is speculated that TGF-β may be valuable in wound healing.

In this study, the application of chitosan in the clinical treatment of burn patients and the expression of TGF-β in burn patients during treatment were detected. First, burn patients were divided into two groups and treated with chitosan and Vaseline, respectively. Results demonstrated that the healing rate of patients with second-degree superficial burns in the observation group treated with chitosan at 7 days after treatment increased compared with that of patients with second-degree superficial burns in the control group, and the healing rates of patients with second-degree deep burns in both groups at 15 and 25 days after treatment also increased significantly, well illustrating the effectiveness of chitosan in the treatment of patients with second-degree burns. The SI score of patients in the observation group treated with chitosan wound repair membrane gel was obviously lower than that in the control group. Yoshino et al. proved that the chitosan wound repair membrane gel effectively reduced the pain score and shortened the prognosis time of scars in the clinical treatment of patients with second-degree burns by using different methods [24]. Chitosan can effectively promote the proliferation and metastasis of capillary cells around the fibroblast nuclei in patients, thus increasing the healing speed [25]. It is speculated that chitosan promotes the proliferation and migration functions of vascular endothelial cells and fibroblasts, and the gel can well maintain the humidity of the wound, thus facilitating the self-dissolution of necrotic tissues and accelerating the epithelial cell migration, so that the wounds healing speed in patients is promoted. Then the expression of TGF-β in both groups of patients at different time points was detected, and it was found that the TGF-β expression level was increased before treatment, but it was remarkably decreased in the observation group 15 days after treatment compared with that in the control group, displaying a statistically significant difference. It is speculated that the proliferation of vascular endothelial cells and fibroblasts in patients is accelerated under the influence of chitosan, and it will decline after complete healing, resulting in decreases of TGF-β secretion of inflammatory cells and TGF-β expression level in patients. The expression of TGF-β was different at 15 days between the two groups, but it was reduced at 25 days and remained similar. The possible reason is that the patient's wound was basically healed completely and the TGF-β expression level became stable. It can be proven via the above studies that the curative effect of chitosan wound repair membrane gel on patients with second-degree burns is significant, and the level of TGF-β gradually declines during treatment, so TGF-β is expected to be an observation index for the recovery of second-degree burns.

However, there are still some shortcomings in this study. For example, whether the small sample size and shorter observation cycle will produce different results remains unknown. In this clinical study, the specific mechanism of TGF-β in burn patients was not investigated. Therefore, the sample size should be expanded, and the specific mechanism of TGF-β in burn patients should be explored through a variety of detection methods in the future research, so as to further improve this experiment and prove the validity of experimental conclusions.

In conclusion, external application of chitosan wound repair membrane gel significantly shortens the recovery time of patients, and it is expected that TGF-β expression level can serve as one of the evaluation indexes for the recovery of patients.

Disclosure of conflict of interest

None.

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