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Experimental Study of Moist Exposed Burn Therapy/Moist Exposed Burn Ointment Combined with Zhuang Medicine Detoxification for Chronic Refractory Wound Healing

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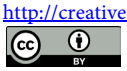

Abstract

Background: This is still a public problem that needs to be solved urgently: chronic and refractory wound healing with long course and complex pathological mechanism. At present, there is still a lack of effective clinical treatment. This study, therefore, aims at exploring moist exposed burn therapy/moist exposed burn ointment (MEBT/MEBO) combined with Zhuang medicine detoxification in the treatment of chronic refractory wound healing. **Methodology:** 100 SPF Wistar rats were randomly divided into blank control group, model control group, MEBO group, Zhuang medicine group and Combined group, with 20 rats in each group. Open wound model was established in blank control group, and chronic refractory wound model was established in other groups. Black control group and model control group were given food and water freely, MEBO group was given dressing change once a day, Zhuang medicine group was given intragastric administration once a day, and combined group was given dressing change and intragastric administration once a day. The effective rate of wound healing was observed after 12 days of continuous intervention. **Conclusion:** Skin regeneration medical technique combined with Zhuang medicine poison theory can effectively reduce the symptoms of bleeding and exudation, reduce the area of wound, shorten the healing time of wound, and achieve physiological healing of wound. It has a good effect on chronic refractory wound.

Keywords

Chronic Refractory Wounds, Wound Repair, MEBT/MEBO, Zhuang Medicine

1. Introduction

Chronic non-healing wounds on the surface of the body usually refer to the disease course of more than 1 month, unable to achieve anatomic and functional integrity through the normal orderly and timely repair process, and the condition is complex and changeable [1]. Chronic refractory wounds have a long course of disease, high cost, difficulty to cure, complex pathological mechanism, and there is still a lack of effective treatment. In recent years, with the improvement of the etiology and prevention and control techniques of chronic nonhealing wounds, the diagnosis and treatment techniques are gradually diversified, among which the moist exposed burn therapy/moist exposed burn ointment (MEBT/MEBO) is a relatively mature technology for the prevention and treatment of chronic nonhealing wounds. At present, it is widely used in clinical treatment of diabetic foot, pressure ulcers, venous ulcer, nerve ulcer and other diseases, and has achieved good efficacy in alleviating wound pain, promoting wound healing, reducing scar formation rate and amputation rate after healing [2].

The experts' study found that MEBT/MEBO can create and keep the wound wet environment, easy to combine with the wound tissue, can inhibit infection, promote healing of wound repair, the effect of hydrolysis with wound necrosis tissue, fat, chemical reactions such as esterification, promote the liquefied mixture of discharge, reduce inflammatory reaction, and protect the wound; MEBO/MEBT can also prevent the invasion of other pathogens, maintain the clean wound, inhibit the growth and reproduction of bacteria, slow down the metabolic rate of bacteria on the wound, and reduce the virulence and invasiveness [3]. MEBO/MEBT can improve the microcirculation state, stabilize blood flow, maintain oxygen consumption, similar to the traditional Chinese medicine "blood circulation and stasis" treatment method; to liquefy and nondestructively remove necrotic tissue on the wound surface and maintain the lesion in a hypoxic tension environment, which is conducive to increasing local blood flow and *in situ* regeneration of wound tissue by activating potential stem cells and increasing differentiation of *in situ* stem cells, similar to the treatment method of "removing saprogenic" in Traditional Chinese medicine. MEBO/MEBT contains a large number of essential amino acids, polysaccharides, lipids and proteins to supplement nutrient deficiency caused by wound injury and provide good nutritional support for the regeneration and repair of chronic wounds that are difficult to heal, similar to the treatment method of "simmering pus and growing flesh" in Traditional Chinese medicine [4]. The treatment principle and mode of MEBO/MEBT are in line with the syndrome differentiation and treatment of TCM external treatment, which can accelerate wound healing and affect cell proliferation, differentiation and metabolic function in the process of wound repair.

In Zhuang medicine, chronic non-healing wounds are categorized as "ecthyma", "skirt sore", "heat toxic disease" and so on. According to the principle of

"deficiency poison causes all kinds of diseases" in Zhuang medicine, "poison" can be divided into tangible poison and invisible poison. When the visible poison is infiltrated or the external invisible poison is interwoven with the endogenous invisible poison, which is accumulated in the fire and dragon road of the skin, the qi and blood are frozen and blocked, the skin vein is blocked and the drainage is not good, and the "three channels and two paths" are damaged, resulting in the three qi can't be synchronized and lead to the generation of chronic wounds. Therefore, the treatment of chronic refractory wounds in Zhuang Medicine is based on the internal treatment principle of "seeking the cause from poison, treating the disease with poison, identifying poison and treating with detoxification", and the treatment of chronic refractory wounds by detoxification and eliminating evil [5]. Zhuang medicine detoxification method can effectively promote the physiological healing of wound by removing the "toxin" in the wound.

The conduction process of signal pathway involves a variety of biological effects, and activating stem cells to differentiate into epidermal cells, fibroblasts and vascular endothelial cells is a key step in the process of wound healing. Our previous study showed that ERK1/2 (Extracellular regulatory protein kinases), p38 MAPK (p38 mitogen-activated protein kinas) and other mediated signaling pathways can reconstruct the vascular network by activating stem cells, and the activation of stem cells is not only affected by ERK1/2, p38 MAPK and other signaling pathways, but also affected by multiple wound repairs related signaling pathways. Therefore, combined with our previous studies, we speculated that WNT (Extracellular factor)/β-catenin (Important regulatory proteins of the WNT signaling pathway) and Hippo/YAP (Yes-associated protein) signaling pathways also play a role in promoting the differentiation of stem cells into epidermal cells, fibroblasts, and vascular endothelial cells for the treatment of chronic refractory wounds. The interaction between WNT/β-catenin and Hippo/YAP signaling pathway often plays a key role in maintaining cell stability. WNT/β-catenin signaling pathway plays a regulatory role in the construction of neovascularization network, remodeling of epithelial tissue, and promoting wound repair. Hippo/YAP signaling pathway mainly regulates cell proliferation and differentiation, influences cell apoptosis cycle, and maintains cell stability in injured tissues [6]. The role of WNT signaling pathway in wound repair is to guide stem cells to carry out orderly proliferation and differentiation, guide stem cells to carry out functional differentiation and prognosis that promote the regeneration and repair of blood vessels and nerves in wound injury [7]. β-catenin, a multifunctional protein encoded by CTNNB1 gene, is a core regulatory factor in the classical WNT signaling pathway, which can control the generation cycle of potential stem cells and epidermal stem cells, accelerate the proliferation, division and directed differentiation of stem cells, and promote tissue regeneration and repair of chronic refractory wounds [8]. Hippo signaling pathway is highly conservative and induces transcription and expression of target genes related to

cell growth, proliferation and apoptosis by regulating the cell generation cycle. The downstream core factor of this pathway is transcription coactivator (YAP). [9]. When YAP1 is phosphorylated in Hippo signaling pathway, the content of free β-catenin is decreased, and then the conduction of WNT/β-catenin signaling pathway is blocked. Therefore, when YAP phosphorylation is inhibited, it can promote the expression of WNT/β-catenin signaling pathway and play a negative regulatory role [10]. WNT/β-catenin and Hippo/YAP signaling pathways downstream of the common factors of Cyclin D1 has the function of regulating the cell cycle change at the stage of cell division can promote fibroblasts proliferation, vascular endothelial cells from the early stage of the DNA synthesis to DNA synthesis phase shift, activate the cell vitality, restrain premature cell apoptosis, activate the differentiation of epidermal stem cell proliferation of fibroblasts, vascular endothelial cells, give play to the role of its metabolism, build new blood vessels and nerves system, promote injury regenerative repair of the wound [11].

On the basis of previous studies, this study for combining with the detoxification method of Zhuang medicine to explore the repair mechanism of chronic refractory wounds. Therefore, a rat model of chronic nonunion wound was established to explore the mechanism of MEBT/MEBO combined with Zhuang medicine detoxification in the treatment of chronic nonunion wound.

2. Material

2.1. Experimental Animals

A total of 100 SPF Wistar rats (male, 300 - 350 g/rat) were reared in a single cage in the Animal Experiment Center of Guangxi University of Traditional Chinese Medicine between January 2019 and March 2020. The experiment was carried out after 7 days of adaptive feeding at 23˚C.

2.2. Main Drugs and Reagents

Moist exposed burn ointment (MEBO), 40 g/piece, National medicine approval Z20000004, provided by Shantou MEBO Pharmaceutical Co., LTD. Zhuang Potion decoctions (50 g fresh tooth leaf nihua Hua, 25 g Diao Zhu, 15 g Red peony root, diao Ao Sticks 20 g, jispatholobi 15 g, tudangshen 15 g, Polygonum multiflorum 15 g, Huanghua water lotus 15 g) are provided by the central pharmacy of Guangxi University of Traditional Chinese Medicine Affiliated International Zhuang Medical Hospital.

2.3. Experimental Methods

2.3.1. Model Building

Refer to Huang Xu-Sen *et al.* [12]. The full-thickness skin defect injection method was improved, and a circular area with a diameter of about 25 mm was cut off on the back of the rat as an open wound, and then glucocorticoid was injected around the wound edge for 7 consecutive days to establish the rat model

of chronic refractory wound.

2.3.2. Grouping and Medication

According to the random number table method, 20 of 100 rats were randomly selected as blank control group to establish open wounds without glucocorticoid injection. The other rats were first established chronic refractory wound model, and then randomly divided into model control group, MEBO group, Zhuang medicine group and combined group, with 20 rats in each group. Blank control group and model control group and normal breeding, MEBO group according to the handbook of burn skin regeneration medical technology clinical methods described daily switching time, Zhuang medicine group according to the experimental animals and human equivalent dose conversion method described the dose of 3200 mg/kg/d Zhuang medicine decoction lavage daily once, joint group used at the same time treatment and drug lavage once a day.

2.4. Effectiveness Evaluation

After 12 days of intervention, the effective rate was analyzed according to wound healing area and bleeding and exudation. 1) Complete remission: the wound disappeared completely; 2) Significant effect: the wound healing area was more than 50%, and there was no bleeding and exudation on the wound; 3) Effective: wound healing area in 10% - 50%, bleeding and exudation can still be seen on the wound; 4) Invalid: the wound healing area is less than 10%, bleeding and exudation can still be seen on the wound. The effective rate is calculated as follows:

$$\text{Response rate} = \frac{(\text{complete remission} + \text{significant effect} + \text{effective})}{(\text{complete remission} + \text{significant effect} + \text{effective} + \text{ineffective}) \times 100\%}.$$

2.5. Statistical Treatment

SPSS 24.0 statistical software was used for analysis. Rank-sum test of group design grade data was used for comparison between multiple groups. $P < 0.05$ was considered as statistically significant difference.

3. The Results

There were differences in wound healing efficiency among all groups (χ^2 = 50.953, $P < 0.001$), there was no difference between the combined group and the blank control group (Z = 1.310, $P = 0.190$), but there were differences between the combined group and the MEBO group (Z = −3.072, $P = 0.002$), and between the combined group and the Zhuang medicine group (Z = −2.934, $P = 0.003$). Therefore, Combination is oral plus topical, the effect is better than oral or topical alone.

With the development of science and technology, modern physicians have combined modern medical technology with the idea of syndrome differentiation

Table 1. Effective rate of each group ($\bar{x} \pm s$).

Group	n	Complete remission	significant effect	effective	ineffective	effectively	χ^2	*P*
Blank control group	20	18	2	0	0	100%		
Model control group	20	0	0	3	17	15%		
MEBO group	20	6	4	2	8	60%	50.953	0.000
Zhuang medicine group	20	7	2	2	9	55%		
Combined group	20	15	3	1	1	95%		

and treatment that applied a variety of technical means to the prevention and treatment of diseases. It is an important method for the prevention and treatment of chronic refractory wounds by external treatment of traditional Chinese medicine in contemporary times, which can better promote wound repair. According to the results in Table 1, after 12 days of intervention, there was a significant difference in the effective rate of each group ($\chi^2 = 50.953$, $P < 0.001$), indicating that there were differences in wound repair efficiency assessment among the 5 groups. Further pairwise comparison between groups using Bonferroni correction showed that there were differences in each group compared with the model control group, indicating that both MEBT/MEBO and Zhuang detoxification could improve the wound surface. There was no difference between the combined group and the blank control group (Z = 1.310, $P = 0.190$), but there was difference between the combined group and the MEBO group (Z = −3.072, $P = 0.002$), and between the combined group and the Zhuang medicine group (Z = −2.934, $P = 0.003$), indicating that the combined group achieved the same recovery effect as the blank control group. Combined application of MEBT/MEBO and Detoxification method of Zhuang medicine in the treatment of chronic refractory wounds can better improve local symptoms, reduce the symptoms of bleeding and exudation, reduce the area of the wound, shorten the healing time of the wound faster, make the wound achieve physiological healing as soon as possible, and have a good effect on the chronic refractory wounds.

4. Discussion

Our previous studies have shown that MEBT/MEBO can create and maintain a moist environment on the wound surface, which is easy to combine with the wound tissue. It has the function of inhibiting infection aggravation, promoting wound healing and reducing inflammatory reaction. It not only protected the wound surface but also promoted the regeneration of stem cells *in situ*. It can induce stem cells to differentiate into epidermal cells, fibroblasts, vascular endothelial cells and so on. MEBT/MEBO can reconstruct blood vessels and nerves to achieve *in situ* regeneration of injured wounds [3]. The detoxification method of Zhuang medicine has a good advantage in the treatment of chronic refractory wound and has a good effect on local neurodegenerative diseases. The detoxifi-

cation method of Zhuang medicine can activate vascular endothelial factor (VEGF), promote the formation of vascular endothelial cells, form new granulation to repair the wound, inhibit the aggregation of inflammatory cells and improve the symptoms of bleeding and exudation in the treatment of chronic refractory wounds.

The results of this study also confirmed that MEBT/MEBO combined with Zhuang detoxification method has certain effects and advantages in the treatment of chronic refractory wounds, but the mechanism of MEBT/MEBO combined with Zhuang detoxification method to promote the repair of chronic refractory wounds has not been very clear. Our previous studies have shown that chronic difficult wound repair is associated with the conductive effect of signaling pathways, wound repair involving stem cells, fibroblasts, endothelial cells, epithelial keratinocytes and other processes of cell metabolism, and this series of physiological processes is dependent on the intracellular signal transduction pathways to various stimulus from extracellular to intracellular [13]. Therefore, the repair of chronic non-healing wounds can promote the proliferation and differentiation of fibroblasts and vascular endothelial cells, improve the ultra-pathological structure of cells, and accelerate the process of wound healing under the influence of ERK1/2, p38 and other mediated signaling pathways [14]. This is currently a hot topic in wound repair.

5. Conclusion

It is an important method to treat chronic non-healing wounds by combining modern medical technology with traditional Chinese medical thought. MEBT/MEBO and Zhuang detoxification not only have a profound historical basis for wound repair, but also have a good clinical effect. In spite of all aspects of mechanism and many research results, many areas are still worthy of in-depth discussion and research, Therefore, exploring the mechanism of MEBT/MEBO and Zhuang detoxification to regulate WNT/β catenin and Hippo/YAP signaling pathways in the treatment of chronic wounds is also our next research direction. The results of this study will provide a theoretical basis for the improvement of chronic wound repair difficulties, and we hope to achieve new results.

Limitations of the Study

This study is still in the preliminary stage and basic research, and there are few experimental animals, which is also the limitation of this paper.

Ethical Approval of the Project

Ethical approval will be obtained from Guangxi University of Chinese Medicine, Ethics Committee.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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