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Research Paper

Clinical experimental study of Arnebia Root oil in increasing FGF expression and promoting wound surface healing

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Abstract

Objective: To investigate the effection of Arnebia Root oil on the FGF expression in wound surface and the ability to promote wound surface healing. **Methods:**24 wound surfaces of patients were divided into two groups. Experimental group was treated by Arnebia Root oil and the control was treated by petrolatum gauze. Histology, histochemistry, electron microscope methods and healing rate measurement were used to show the FGF expression and wound healing process. **Results:**Endogenous FGF were expressed in both of the groups, in which of the experimental group was higher than that of the control group. the wound surface healing rate of experimental group was also higher and paralleled with FGF expression. **Conclusion:**Arnebia Root oil has effects to promote FGF expression and enhances wound surface repair. The wound healing mechanism between the action of Arnebia Root oil and function of FGF need further investigating.

Keywords: Arnebia Root; fibroblast growth factor; full-thickness skin deficiency; immunohistochemistry

INTRODUCTION

There are clinically lots of methods to heal an little area of full-thinkness skin deficiency[1-3]. A good effect was obtained in the treatment of raw surface by Arnebia Root oil[4-6]. To further investigate wound healing mechanism, randomly control studying of Arnebia Root oil repairing wound surface was performed, and expression of fibroblast growth factor (FGF) was detected for revealing initial internal relationship between FGF expression, Arnebia Root oil and wound surface healing, and to provide further theoretical support of Arnebia Root oil promoting wound surface repairing

MATERIAL AND METHODS

Arnebia root oil preparing method

Arnebia Root 100 g, Angelica Root 50 g, peanut oil 1 000 g were put into a kettle and heated for 30 min at 150℃, then cooled. When angelica root became yellow, the oil was collected and filtrated, what we got finally was the medicine we needed (made in our hospital).

Clinic data

Clinic specimen was collected from wound surface tissue of the newly enrolled patients in the orthopedic department. All patients were informed the purpose of the research and approved specimen collection, 12 patients(8 male and 4 female) with multiple wound surfaces had full-thickness skin defect. Mean wound area ranged from 4 to 8 cm^2, and 24 wound surfaces were randomly divided into two groups, one was experimental group(with Arnebia Root oil change dressings), the other was control group(with petrolatum gauze change dressings). In order to determine wound surface repairing rates of two groups, measurements were conducted by wound surface photographing incorporated with transparent square paper tracings on the 3rd, 7th, 14th and 21st day after wounded respectively. Wound sur-

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face healing rate=(original wound surface area-unhealed wound surface area)/original wound surface. When dressings were changed, tissue samples were taken including margin of the basilar part and the normal skin around the wound surface in asepsis condition, and then preserved in 10% methanal and 4% paraformaldehyde for HE dyeing. Electron microscope examination and PAP immunohistochemical stain were also performed.

Histological determination

①pathology examination: Growth of granulation and raw surface epithelization were observed. Furthermore growth of collagen and wound surface healing were also observed by HE dyeing. ② electron microscope observation: ultramicrostructure of samples including cellular and subcellular structure, and intercelluar relation were observed.

Detection method

The specimen was fixed, imbedded with paraffin, and then sliced. PAP immunohistochemistry stain was performed with rabbit anti-human FGF antidoby(SIGMA), and endogenous FGF protein contents and the change of its distribution were examined in the wound tissue in the procedure of wound surface healing. The relative amount was examined under the light microscope (400 ×) according to visible positive signal amounts, the scale of which was evaluated as follows: negative, no positive signal; weakly positive, less than 10 signals within the field of vision; positive, 11-30 positive signals within the field of vision; powerful positive, more than 30 positive signals. The scale was evaluated by the degree of coloration: negative, colorless; weakly positive, pallide-flavens; positive, buffy; powerful positive, deep buffy.

Statistical analysis

The data was presented as $\bar{x} \pm s$. Results were analyzed by one-way ANOVA. $P < 0.05$ was considered significant.

RESULTS

Histological manifestation

Early stage(7d after operation) :In the experimental group, granulation tissue on the raw surface grew actively, became red, soft, and filled with more fibroblast, collagen and blood capillary, compared with that in the control group which had pale and swell granulations. Middle stage(14d after operation): in the experimental group, cellular proliferation was more significant with more fibroblast, vascular endothelial cell, collagen, fibronectin and new vessels, compared with that in the control group. Later stage(21d): wound surface became epithelized, with less fibroblast, blood vessel endothelium and collagen, and cell proliferation decreased. Electron microscope detection showed that in the experimental group the raw surface had more fibroblasts, dilated endoplasmic reticulum, more cell organ such as ribosome and more blood capillary compared with the control group.

Expression of positive signal

Positive signal showed buffy in PAP immunohistochemical stain. Our experiment manifested positive signals were seen in wound tissue of two groups, the levels of FGF expression changed dynamically during the repair processes. The positive signals of FGF in experiment group were more than that of control group at various periods. At 3 day after hurt, weakly positive signals were seen at superficial wound surface tissue, and most of them were scattered within the endochylema, but no evident positive expression in the remained skin appendages epithelium , showing weakly positive or positive signals(***Fig 1***).At 7 day after hurt, positive signals were more significant , and positive cell masses could be seen, which mainly lay in superficial wound surface, neogenesis epithelium at edge of wound surface, fibroblast within derma and vascular endothelial cells. Their expressions were powerful positive(***Fig 2***). After 14 days, most positive signals reside in single cells respectively, while the levels of positive expression were a little feeble (***Fig 3***). After 21 days, although some positive cells were expressed in intracytoplasm of fibroblasts at superficial derma, levels of positive signal expression decreased significantly. Positive signals which showed weakly positive were rarely visible in the deep layer of derma, levels of positive expression was weakly positive(***Fig 4***).

Comparison of wound surface healing rate

The wound surface healing rate of the experimental group was significantly higher than that of the control group($P < 0.05$)(shown in ***Tab 1***).

DISCUSSION

In this study, in the early stage of wound surface formation(7d after operation), the raw surface inflammatory reaction was more obvious in the experimental group, with more fibroblast, collagen and blood vessel endothelium proliferation. It suggested that Arnebia Root oil had chemotaxis and stimulated the cells to immigrate to the wound.In the intermediate stage(14 d after operation), cell proliferation was more significant in the experimental group, with much more fibroblast and vascular endothelial cell. This probably derived from the effect of Arnebia Root oil, which stimulated the cells proliferation, collagen and fibronectin secretion, as well as the new vessels formation. In the later stage

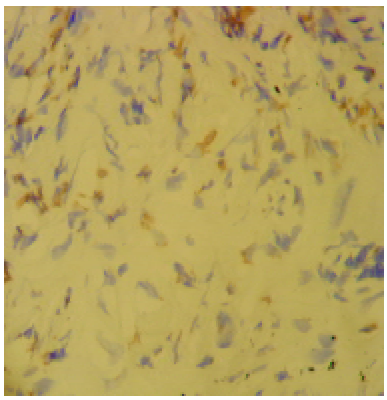

Fig 1 At 3 day, positive signals were seen at superficial wound surface tissue, and most of them were scattered within the endochylema. (PAP, × 400)

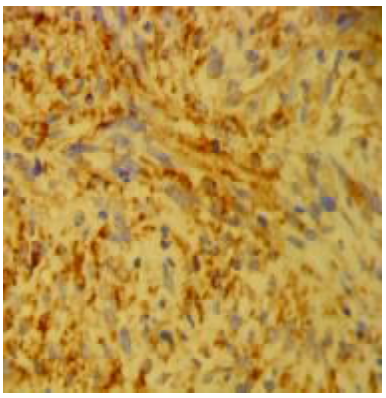

Fig 2 At 7 day, powerful positive signals could be seen in superficial wound surface, neogenesis epithelium at edge of wound surface. (PAP, × 400)

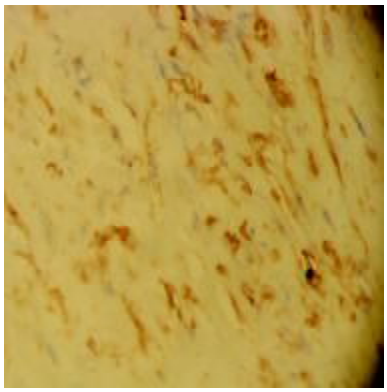

Fig 3 At 14 day, weakly positive signals were expressed at superficial wound surface tissue. (PAP, × 400)

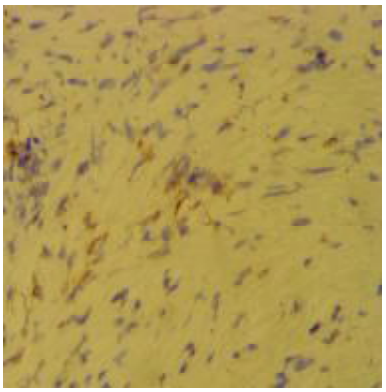

Fig 4 At 21 day, weakly positive signals were expressed in intracytoplasm of fibroblasts at superficial derma. (PAP, × 400)

Tab 1 **Wound surface healing rate of the Arnebia Root oil treated group and control group** (%, $\bar{x} \pm s$)

groups	wound surface healing rate in different time			
	1~3d	3~7d	7~14d	14~21d
experimental group	$0.16 \pm 0.15^{*}$	$0.34 \pm 0.12^{*}$	$0.29 \pm 0.22^{*}$	$0.26 \pm 0.14^{*}$
control group	0.13 ± 0.09	0.30 ± 0.13	0.28 ± 0.06	0.20 ± 0.15

Compare the experimental group with the control group, $^{*}P < 0.05$

(21d), wound surface was already close to epithelization, and cell proliferation became so slow and almost stopped, which made the difference of two groups less than before.

In this experiment, endogenous FGF expression and distribution were examined in the wound tissue in the procedure of wound surface healing. The results manifested endogenous FGF were express positively in wound tissue of both groups, which suggested FGF played an important role in traumatic agglutination [7-9]. Since wound surface healing rates paralleled FGF expression in experiment group. The high FGF expression at epithelium of wound edge, superficies of wound surface, fibroblast in the superficial layer of derma and capillary endothelium cells manifested endogenous FGF protein participated cell metabolism and proliferation in these positions, and promoted wound repairing. FGF expression evidently enhanced at the 3rd day after wound, and kept increasing to the peak at 7th day, which illustrated migration and proliferation of repaired cells were most active in early term so that wound surface healing rate increased obviously.After 14 day, expression of FGF decreased and kept decreasing, which was probably due to the degradation of stimulating response in wound surface tissue, which caused less FGF synthesis. At 21st day, FGF expression decreased continuously, which was probably due to self-restriction of the organism growth. When wound surfaces were gradually repaired, the capability of self-adjustment to repair trauma in decreased histiocytes. However, FGF expressions in experimental group were higher than that of control group at various stages, which suggested Arnebia Root oil could induce secretion of FGF and promote its expression. The high FGF expression par-

alleling wound surface healing rate indicated FGF participated wound surface repairing as a positive regulatory factor.

The main components of Arnebia Root oil are Arnebia Root, Angelica root [10], and so on. Traditional Chinese medicine presumes that it has the effect of removing corrosion, promoting tissue regeneration, promoting blood flow, detoxication and disinfections, alleviating pain, contracting vascular and astricting tissue. The oil component of the traditional Chinese drug could moisten and nourish the wound surface. So Arnebia Root oil was mainly applied to treat fire burn in the past [11]. But in view of the modern medical science, Arnebia Root oil promotes wound surface healing and tissue repairing with an extremely complicated biology process, which is associated with provocation of various growth factors[12,13].The results suggested Arnebia Root oil accelerated wound surface healing might by means of stimulating cells to self-secrete and synthesis.

Many factors impacted the agglutination of tissue[14,15]. As the multifunctional regulatory factor for cell growth, FGF promoted wound healing with others factors[16], and played important roles in many aspects. Some study showed FGF could stimulate fibroblast proliferation, collagen synthesis, blood capillary proliferation, apoptosis and granulation tissue formation. FGF played a facilitative role in epithelial regeneration on the wound surface. Both endogenous and exogenous FGF could enhance tissue healing rate, and FGF expressions were different in various periods in repairing procedure[17-20].

On the whole, Arnebia Root oil had evident effect to promote FGF that could regulate and enhance raw surface repair process.

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