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ORIGINAL ARTICLE

EFFECTS OF FORMULATED TOPICAL NIFEDIPINE OINTMENT ON TGF–β AND ACCELERATION OF FACIAL SKIN WOUND HEALING IN RABBITS

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Summary

The purpose of this study was to evaluate the impact of 1% and 2% topical nifedipine ointments on TGF–β and the acceleration of facial skin wound healing in rabbits. Nifedipine ointments of 1% and 2% were prepared. Fifty healthy male rabbits were involved and distributed into two groups according to the study period: group A (7 days) and group B (14 days). Each group was subdivided into five groups (5 rabbits/group): Group I (Normal): rabbits did not undergo a surgical procedure and did not receive treatment; group II (negative control): rabbits had surgical wounds without treatment; group III (positive control): rabbits had surgical wounds with white petroleum treatment; group IV(nifedipine 1%): rabbits had surgical wounds with nifedipine 1% ointment treatment; and group V(nifedipine 2%): rabbits had surgical wounds with nifedipine 2% ointment treatment. Following euthanasia, blood samples (5 ml) were collected from all animals for TGF–β analysis using an ELISA kit. The TGF–β level in NFD 1% group was significantly higher on the 7th and14th day of the study associated with a higher rate of wound closure in comparison to other groups. Conclusions: Nifedipine 1% ointment has beneficial value for improving wound healing, while nifedipine NFD 2% has no such effect.

Key words: Nifedipine; Skin; Wound Healing; Transforming Growth Factor–β

Introduction

Nifedipine (NFD) is an antihypertensive and antianginal drug that has a negative ionotropic and chronotropic impact through the relaxation of vascular smooth muscle. This medication can block voltage-dependent calcium channels L-type in myocardial and vascular smooth muscle cells, inhibiting calcium ions from entering. Reduced intracellular calcium levels result in reduced peripheral vascular resistance and dilation of the coronary artery. These situations cause a decrease in systemic blood pressure and an increase in myocardium O_2 supply (1). Nifedipine has vasodilatory characteristics, which increase blood supply to the wound and stimulate the synthesis of growth factors (2). Gingival hyperplasia, on the other hand, is a side effect of NFD usage (3). As shown in gingival

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hyperplasia with increased proliferation and decreased apoptosis of fibroblasts, which are the primary cells that maintain and regulate connective tissue repair, nifedipine may hasten wound healing (4).

Wound healing is a complicated process, with various types of cells playing crucial, distinct roles in the four sequential phases of hemostasis, inflammation, proliferation, and remodelling (5). Inflammatory cells, cytokines, and growth factors gather at the damage site during the inflammation phase. Fibroblasts then create collagen and fibronectin as part of the creation of the new extracellular matrix. Following that, epithelial cells move over the wound bed, whereas myofibroblasts constrict the wound edges. Lastly, the remodelling phase enables granulation tissue to grow into matured connective tissue or scar. Any disturbance in the wound-healing process might lead to incomplete or delayed healing (2). TGF-ß is an important wound healing cytokine that influences many types of cells involved in responses of wound healing, including cell migration and infiltration, angiogenesis, matrix formation, and remodelling. Wound epithelization is an important part of effective healing that is organized by a complicated interaction between keratinocytes and the underlying stroma that involves many growth factors, including TGF- β (5). The sufficient synthesis of TGF-β is essential for wound healing and other biological processes, and a decreased level in the wound tissue has been linked to a slower healing process (6). This study focused on the effect of topical nifedipine ointment 1% and 2% on serum levels of TGF-β during facial skin wound healing in rabbits.

Material and Methods

Preparation of nifedipine ointment: Two different concentrations of nifedipine ointment were prepared by mixing (1 & 2 g) of nifedipine powder (Shandong Look Chemical Co., Ltd., China) in 100 gm of white petroleum to provide a final concentration (1%, 2%) W/W with continuous mixing, utilizing a spatula and glass plate, until identical ointments were made. They were kept in opaque containers and stored at 25°C ± 2°C till used (7, 8, 9).

Experimental animals: Fifty local, healthy, mature male rabbits weight between 1.25 – 1.5 kg and aged 10 – 12 months were purchased from the local market and included in the study. The animals were housed in separate cages under standard conditions (room temperature of about 25 ± 2°C with a 12:12 hr. light-dark cycle) (10) and given free access to water and a standard diet.

The rabbits were distributed randomly into two groups according to euthanizing day:

Group A included 25 rabbits euthanized on the 7th day following the surgical procedure. Group B included 25 rabbits euthanized on the 14th day following the surgical procedure. Each group was subdivided into five groups (5 rabbits/group).Group I (Normal): rabbits did not undergo a surgical procedure and did not receive treatment. Group II (negative control): rabbits having surgical wounds without any treatment.Group III (positive control): rabbits with surgical wounds were treated twice daily with white petroleum from the first day to the euthanizing day. Group IV (NFD 1%): rabbits with surgical wounds were treated twice daily with NFD 1% ointment from the first day to the euthanizing day. Group V (NFD 2%): rabbits with surgical wounds were treated twice daily with NFD 2% ointment from the first day to the euthanizing day.

Surgical procedure: Animals were anaesthetized by administering an intramuscular dose of xylazine hydrochloride (sedative and muscle relaxant) and ketamine hydrochloride (anaesthetic and analgesic) at 5, 50 mg/Kg, respectively, injected into the thigh muscle (11). The anaesthetized animal was laid on its ventral side on the surgical board. The surgical area (forehead) was shaved using scissors and a surgical scalpel, then rinsed with tap water and sterilized with povidone-iodine solutions. A full-thickness circular (1 cm in diameter) excision was carefully created using surgical blade no. 15 and forceps (12).

After that, the animals were treated topically according to their grouping twice daily at the same time with vehicle and test formulations till the day of euthanasia. The wounds were left open after the application of each treatment without receiving any antibiotics.

Blood samples (5 ml) were taken from the jugular vein of euthanized rabbits, placed into plain tubes, and stored at room temperature for thirty minutes. The serum was separated and transported by micropipette to an Eppendorf tube and stored at (-20 ˚C) until the time of analysis (13) by TGF –β ELISA kit (BT LAB, Cat. No E0133Rb).

Statistical analysis: The data were presented as mean ± SD, and the variation among the five study groups was statistically analyzed using one-way analysis of variance (ANOVA), followed by the post-hoc test. P values ≤ 0.01 were considered significant (13).

Result

Clinical observation: The animals were observed frequently following the topical application of NFD 1% or NFD 2%. No toxicity, mortality, abnormal signs and symptoms in the activity, behavioural pattern, postural irregularities, or any other clinical observations were recorded throughout the experimental period in all groups.

Clinical wound healing: The macroscopic observations show that the wound closure rates were similar in the negative control, positive control, NFD 1%, and NFD 2% groups until the 7th day. On the 14th day, the rate of wound closure in the NFD 1% group was quicker than in other groups (see Figure 1).

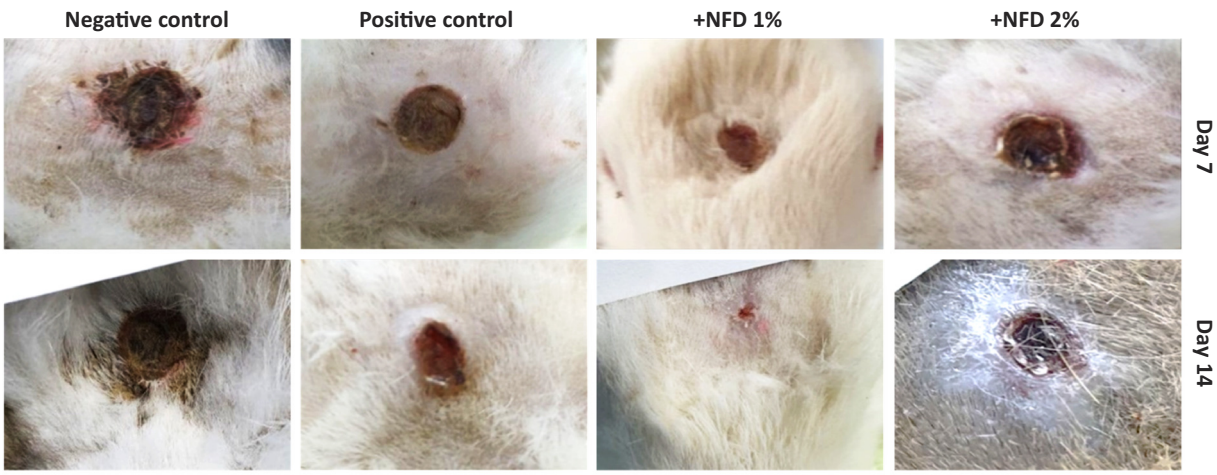

Figure 1. Cutaneous wound healing in studied groups on days 7 and 14.

The results in table (1) showed a substantial rise in TGF-β level in the NFD 1% group at the end of the 1st week compared to the other groups. There is no statistically significant difference between the positive control group and the normal, negative control, and NFD 2% groups. However, there is a substantial difference when normal and negative controls are compared to the NFD 2% group. There is no substantial difference between the normal and negative control groups.

Table 1. The level (mean ± SD) of TGF-β in the serum of study groups at the end of the 1st and 2nd weeks

Group	TGF-β (ng/L) 1st week	TGF-β (ng/L)2nd week
Normal group	405.929 ± 54.87128B	404.599 ± 35.87529AB
Negative control	408.198 ± 29.30669B	409.108 ± 15.51307AB
Positive control	352.774 ± 41.95595AB	369.748 ± 28.94025A
NFD 1%	568.684 ± 37.36063C	587.164 ± 65.15081C
NFD 2%	343.711 ± 41.96714A	431.152 ± 37.22271B
P-value	0.000**	0.000**

Each group consists of 5 animals. Data expressed as Mean ± Stander error
** Highly Significant at P ≤ 0.01
The various letters in the column indicate there are significant differences between the group at P ≤ 0.01

At the end of the 2nd week, there was no substantial difference between (normal, negative control groups) and (positive control, NFD 2% groups). There is still a substantial difference in TGF-β levels in the NFD 1% group compared to the other groups. There is a substantial difference between the positive control and the NFD 2% groups.

Discussion

Topical NFD for the treatment of cutaneous wounds is indicated in medical practice in isolated case reports, with no standard concentrations, i.e., it is prescribed off-label (14). The potential effects of topical NFD on wound healing might be attributed to its ability to modulate microcirculation, accelerate epithelization and microvascular neogenesis, influence extracellular matrix and collagen synthesis, and stimulate growth factors (4).

The findings of this study showed that NFD at 1% concentration promoted wound healing, but NFD at 2% concentration had no such impact. The wound closure rate and TGF-β value were different between the NFD 1% group and the other groups.

The expression level of TGF-β in the NFD 1% group was significantly increased in the 1st and 2nd week in comparison to other groups. This finding supports prior studies that established a relationship between NFD use and gingival hyperplasia. Two major mechanisms have been proposed for nifedipine-induced gingival overgrowth. Non-inflammatory mechanisms include reduced folic acid absorption and impaired collagenase activity. In the inflammatory pathway, inflammation may result in the overexpression of many cytokine factors, including TGF-β (15).

The research demonstrated that TGF-β causes an increase in gingival fibroblast periostin, a matricellular protein that regulates connective tissue's function and structure. It also promotes the proliferation of fibroblasts and the deposition of fibronectin and glycosaminoglycan extracellular matrix. The aetiology of NFD-induced gingival hyperplasia is characterized by an excess of collagen deposition caused by the blockage of collagen intracellular and extracellular degrading pathways (1).

TGF-β plays a role in all phases of physiological skin wound healing (16), which is a response to damaged tissue that leads to restoration of tissue integrity. Thus, wound contracting and closing are important indicators of healing in open wounds (17).

Inflammation is a critical stage in wound healing. Nevertheless, a prolonged and severe inflammatory reaction frequently results in a delayed wound. TGF-ß is a powerful inflammatory system regulator that appears to have a role in both the onset and resolution of general inflammatory reactions. It increases monocyte motility and growth factor production, but once an inflammatory reaction is initiated, it also displays potent anti-inflammatory effects such as inhibiting neutrophil and T-lymphocyte adherence to endothelium, downregulating macrophages, and antagonizing TNF- α function (6).

TGF-ß also stimulates the differentiation of macrophages into alternatively activated macrophages (M2) capable of active efferocytosis, and this activity may play a role in the resolution of inflammation (18).

Re-epithelialization is a vital stage in the healing process that permits keratinocytes to move over the wound bed and restore epidermal layers (19). TGF-β can facilitate re-epithelialization, promote fibroblast cell migration and proliferation, improve myofibroblast function, and involve in wound healing improvement (6).

Angiogenesis is recognized as an essential contributor during the proliferative stage of healing, which is considered to be associated with vascular endothelial growth factor (VEGF) stimulation. TGF- β appears to play an important role in angiogenesis (20) because it induces high amounts of angiogenetic growth factors such as VEGF (21). Increased secretion of TGF-ß by fibroblasts could be responsible for the improvement of angiogenesis in diabetic wounds (19).

Collagen, the most prevalent protein in the extracellular matrix, is essential for wound healing (22) and aids in the strengthening and support of connective tissues, as well as the processes of hemostasis and epithelialization. As far as we know, TGF-β stimulates collagen formation and accumulation (23).

The studies revealed that the absence of TGF-ß up-regulation in venous ulcers and diabetic foot ulcers may explain why these chronic wounds heal slowly. Indeed, the effect of the therapy on TGF-ß levels has been employed as an effective criterion in various therapeutic studies on diabetic foot ulcers (16).

The wound healing effect of NFD 1% might be attributed to the activation of growth factors, which are necessary for the wound healing process (15). whereas NFD at 2% concentration has no significant effect on TGF-ß levels. We believe that NFD at 2% concentration may cause chronic inflammation due to upregulation of inflammatory cells in the inflammatory phase (14) and that staying wound healing in the inflammatory phase rather than advancing to the proliferative phase prevents, rather than promotes, wound healing (24). However, more research is needed to determine the mechanisms involved in the NFD 1% healing capability and why NFD 2% has no such effect, this might be explained in term of over-vasodilation resulting in reduced blood supply and thereby reduced growth factor supplied in the lesion vicinity (25).

Conclusions

Topical application of NFD1% ointment showed more positive healing activity in a full-thickness wound model through an increase in TGF-ß level and accelerated wound healing, whereas NFD2% ointment had no such effect.

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Conflict of Interest

The authors declare no conflict of interest.

Adherence to Ethical Standards

The experimental work for this research was done in an animal house and scientific laboratories and approved by the Research Ethics Committee and Scientific Committee/Department of Dental Basic Science/College of Dentistry/University of Mosul (approval number: UoM.Dent/A.L.6/22 on 08.02.2022).

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