

Promotion of Random Flap Neovascularisation in Rats with Diabetes Using Botulinum Toxin Type A Through the HIF-1 α /VEGF Pathway

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Objective: This study aimed to investigate the effects of botulinum toxin type A (BoTA) on the neovascularisation of diabetic flaps through the factor-1alpha (HIF-1 α)/vascular endothelial growth factor (VEGF) pathway.

Methods: A total of 60 male Wistar rats (250–300 g) were randomly divided into 4 groups. Group A consisted of normal rats receiving saline, Group B received BoTA, Group C were diabetic rats treated with saline, and Group D were diabetic rats treated with BoTA. Random-pattern dorsal skin flaps (3 \times 9 cm) were created, and saline or BoTA was injected at proximal, mid and distal regions. Ten days later, orthotopic flap transplantation was performed. After 7 days, flap survival rate, haematoxylin–eosin (H&E) staining, and the mRNA expression of HIF-1 α and VEGF were evaluated.

Results: Flap survival area significantly increased in Group B compared to Group A ($P < 0.05$), and in Group D compared to Group C ($P < 0.05$). The highest neovascular density was observed in Group B ($P < 0.05$), while the lowest was in Group C ($P < 0.05$). No significant difference was found between Groups A and D. Reverse transcription polymerase chain reaction (RT-PCR) showed that HIF-1 α and VEGF expression levels were highest in Group B, followed by Groups A, D, and C ($P < 0.05$).

Conclusion: BoTA promotes flap survival and neovascularisation in diabetic rats by enhancing HIF-1 α and VEGF expression. These results suggest a potential therapeutic role of BoTA in improving flap outcomes in diabetic patients.

Keywords: botulinum toxin type A, flap, diabetes, factor-1alpha

Introduction

Flaps, especially random-pattern flaps, are widely used to repair local tissue defects in plastic surgery. The early blood supply of random flaps mainly relies on the pedicle, so there is a risk of ischaemic necrosis at the distal end.¹ Additionally, as the aspect ratio of the flap increases, the possibility of distal necrosis of overlength flaps also significantly increases.^{1,2} Since the abnormal structure and function of skin microcirculation caused by long-term hyperglycaemia reduce skin blood perfusion, the risk of flap necrosis in patients with diabetes increases, restricting the application of flap repair surgery. Previous studies indicate that flap necrosis in patients with diabetes is mainly attributed to impaired neovascularisation caused by insufficient ischaemia/reperfusion in hyperglycaemia.³

With these shortcomings in mind, botulinum toxin type A (BoTA) was developed to provide an alternative treatment option for promoting the flap survival rate in preclinical studies. However, our limited understanding of the therapeutic mechanisms of BoTA precludes its application to take full advantage of these mechanisms.^{4–6} A previous study indicates that the expression of vascular endothelial growth factor (VEGF) increases after the local injection of BoTA, inducing neovascularisation and increasing the number of arterioles, venules and capillaries in the treatment area.⁷ However, the

specific mechanism of the BoTA-mediated increase in VEGF expression is still unclear. It has been reported that the level of VEGF in ischaemic and hypoxic tissues is mainly regulated by its upstream factor-1alpha (HIF-1 α).⁸ Factor-1alpha is the key transcription factor regulating the expression of hundreds of genes in the hypoxic adaptive response and plays a significant role in neovascularisation.^{9,10} In addition, hyperglycaemia impairs the stability and function of HIF-1 α , thereby affecting the expression of HIF-1 α target genes that are necessary for developing new blood vessels.¹¹

Although BoTA has been widely studied in improving flap survival, most of the existing research has been conducted in healthy animal models.^{12–14} Systematic studies investigating its effects in diabetic conditions – where neovascularisation is severely compromised – are still lacking. Our study aims to fill this gap and provide new insight into the potential therapeutic role of BoTA in diabetic flap models. Therefore, we speculate that BoTA regulates the HIF-1 α /VEGF signalling pathway and promotes angiogenesis. This study aims to investigate whether BoTA can improve the survival rate of extra-long flaps in the hyperglycaemic state and its potential mechanism between BoTA and angiogenesis in flaps.

Materials and Methods

Experimental Animals

Male Wistar rats, weighing 250–300 g, were purchased from the Laboratory Animal Centre of Guangzhou Ruige Biotechnology Co., Ltd (Guangzhou, China) and housed in a specific pathogen-free environment at approximately 23°C room temperature and 60% humidity. The experiments were conducted in strict accordance with the Ethical Review of Laboratory Animal Welfare guidelines issued by the General Administration of Quality Supervision, Inspection and Quarantine and the National Bureau of Standards. Animal experimental operations in this study were performed in accordance with the Guide for Animal Care and Use of the Guangdong Animal Experiment Association for Laboratory Animal Science (Guangdong, China). Animal experiments were approved by the Ethics Committees and Health Authorities of Hainan Medical College (No.: HYLL-2023-304).

Reagents and Instruments

Botulinum toxin type A (Botox[®] 100U, Allergan, Irvine, CA, USA) was used in this study. Haematoxylin-eosin (H&E) staining solution (100 mL) was purchased from Beijing Solebo Technology Co., Ltd, Beijing, China. A semi-automatic rotary paraffin sectioning machine (HM340E), an embedding workstation (Histosta), an automatic dyeing machine (Gemini AS), a rapid tissue processor (STP120), a refrigerated centrifuge (FRESCO 70) and a Multiskan SkyHigh microplate spectrophotometer (1510) were purchased from Thermo Fisher Inc. (Waltham, MA, USA). An electric thermostatic drying oven (Shanghai Jing Hong Laboratory Instrument Co., Ltd, Shanghai, China), an electronic analytical balance (UX2200H, Shimadzu Corporation, Tokyo, Japan) and an optical microscope (CX-23, Olympus Corporation, Tokyo, Japan) were also used in this study.

Experimental Grouping

The rats were randomly divided into four groups:

- Group A: normal rats + saline (n = 15);
- Group B: normal rats + local injection of drug A (n = 15);
- Group C: rats with diabetes + local injection of saline (n = 15); and
- Group D: rats with diabetes + local injection of drug A (n = 15).

Methods

Modelling of Rats with Diabetes

The experimental groups consisted of Wistar rats weighing 250–300 g, which were fed a high-fat and high-sugar diet, on which the type II diabetes model was induced by a single intraperitoneal injection of streptozotocin (STZ) (30 mg/kg).¹⁵ After 10 days, fasting blood glucose levels were determined using a blood glucose analyser (Johnson & Johnson, US). The modelling was deemed successful when a fasting blood glucose level of ≥ 16.7 mmol/L was maintained for 2 weeks.¹⁶

Drug Administration

Random-pattern skin flaps with caudal pedicles were designed on the rats' backs, and a rectangular flap area (3 × 9 cm) consisting of proximal, mid and distal regions was marked on the skin. Then, 2 mL saline for injection was added to the 100 U BoTA to configure it to a concentration of 50 U/mL. An intracutaneous injection of 0.1 mL BoTA was performed at the central point of each region, totalling 5 U/rat (Group B, Group D), or 0.1 mL saline was injected into each region (Group A, Group C). Orthotopic transplantation of skin flaps was performed 7 days after drug administration.

Orthotopic Transplantation of Skin Flaps

The rats were anaesthetised using an intraperitoneal injection of pentobarbital sodium (0.5 mL/100 g) and fixed on the operating table in a prone position, followed by the removal of back hair, washing with warm water and disinfection with iodophor. The skin of the flap was incised down to the subcutaneous tissue along the designed marking. The skin flap was lifted to the level of the panniculus carnosus muscle and sutured in place after complete haemostasis. The in-situ intermittent suturing method was adopted with 4-0 silk thread, and erythromycin ointment was then applied to the incision.

Evaluation of the Flap Survival Area

Flap survival conditions were examined on the 7th day after orthotopic transplantation of the flaps, with the flap necrosis criteria including tissue retraction, poor elasticity, hard texture and non-bleeding after tissue cutting.¹⁷ The anaesthetised rats were fixed to take high-definition photos using a digital camera, with a 10-cent coin placed along the flap as a reference (the diameter of the coin was 19 mm). The area of flap necrosis and the total flap area were quantified using Adobe Photoshop (Adobe, US). The pixel values of these areas were measured and then converted into actual flap lengths using the 10-cent coin as a reference. The survival area was defined as the total surface area minus the demarcated necrotic area. The flap survival rate was the percentage of the flap survival area to the total flap area.

Flap Tissue Sectioning and Staining and Evaluation of Neovascular Density and Microcirculatory Function in the Flaps

Fourteen days following the operation, the flap tissues in the proximal, mid and distal regions were collected from 5 rats, which were randomly selected from each group, and then fixed in 10% formaldehyde. After 24 hours, the specimens were dehydrated in ethanol and xylene and embedded in paraffin wax. Thick serial slices (5 µm) were obtained by microtome and stained with H&E. Neovascular density and the number of blood vessels were determined using photography with light microscopy, with 100× magnification used in the microscopy analysis (CX-23, Olympus Corporation, Tokyo, Japan). An average proportion was calculated from 10 regions in each sample and used for statistical analysis.

In addition to a histological assessment, a capillary refill test was performed on postoperative day 7 to evaluate functional microcirculation in the distal flap region. After intraperitoneal anaesthesia with sodium pentobarbital (0.5 mL/100 g), the rats were placed in the prone position. Under adequate lighting, the distal flap appearance, including colour, texture and elasticity, was observed and documented photographically. A 10-cent coin (19 mm diameter) was placed adjacent to the flap as a size reference.

To measure capillary refill, blunt forceps or a fingertip was used to gently press the distal flap area for 5 seconds, followed by immediate release and timing with a stopwatch. The time taken for the pale skin to return to its normal colour was recorded. Three sites were tested per flap and averaged. A refill time of ≤2 seconds was considered normal, whereas >3 seconds indicated impaired microcirculation.

Measurement of Factor-1alpha and Vascular Endothelial Growth Factor Protein Expressions with Fluorescent Quantitative PCR

After 7 days of the operation, 5 rats were randomly selected from each group to collect flap tissues from the proximal, mid and distal regions. The specimens were immediately frozen in liquid nitrogen and then stored at -80°C. Total RNA was extracted from the tissue using TRIzol (Cat# 10296-028, Invitrogen, CA, USA). The concentration of total RNA was determined using a UV photometer, and RNA electrophoresis was then performed to assess RNA integrity. Then, cDNA

synthesis was performed in strict accordance with the instructions of the reverse transcription kit (Cat# A3800, Promega, WI, USA). The reverse transcription conditions were set to 94°C for 25 minutes, 43°C for 5 minutes and 50°C for 5 minutes in 1 cycle and then stored at -20°C. Primers (Cat# C1181), dNTP (Cat# U1515), DNase (Cat# M6101) and RNasin ribonuclease inhibitor (Cat# N2112S) were purchased from Promega. The protein expression levels of HIF-1 α and VEGF were measured with β -actin as a reference. All reactions were repeated three times. Among the four groups, the expression of each gene was assessed as a fold change relative to the gene expression in the proximal region of the control group. The primer sequences are described in Table 1.

Immunofluorescence Staining of Vascular Endothelial Growth Factor and Factor-Ialpha in Diabetic Skin Flaps

To evaluate the protein-level expression and localisation of VEGF and HIF-1 α in diabetic skin flap tissues, immunofluorescence staining was performed on samples collected on postoperative day 14.

Skin flap tissues were harvested and washed in phosphate-buffered saline (PBS), then fixed in 4% paraformaldehyde overnight at 4°C. The tissues were trimmed, gently flattened and cryosectioned for immunostaining. The sections were permeabilised using 0.5% Triton X-100 for 30 minutes at room temperature and then washed with PBS 3 times (10 minutes each). Non-specific binding was blocked with 5% BSA for 40 minutes at room temperature, followed by another PBS wash. The sections were incubated overnight at 4°C in a humidified chamber with primary antibodies diluted in 5% BSA: anti-VEGF (1:500, Thermo Fisher, MA5-13182) and anti-HIF-1 α (1:500, Thermo Fisher, PA1-16601). After washing, the sections were incubated for 50 minutes at room temperature in the dark with FITC-conjugated goat anti-mouse IgG secondary antibody (1:400, Servicebio, GB22301). Nuclei were counterstained with DAPI, and the sections were mounted with an anti-fade mounting medium (Servicebio, S2100). Fluorescent images were captured using a Nikon Eclipse Ti-SR inverted fluorescence microscope and a DS-U3 imaging system (Nikon, Tokyo, Japan).

Statistical Analysis

All statistical analyses were performed using SPSS Statistics software (Version 17.0, IBM, Armonk, NY, USA). Quantitative data were expressed as mean \pm standard deviation. For comparisons among multiple groups, one-way analysis of variance was used, followed by Tukey's post hoc test to assess differences between individual groups. For comparisons between two groups, an unpaired two-tailed Student's *t*-test was applied. Categorical data were compared using the chi-squared test. A P-value of less than 0.05 was considered statistically significant. All experiments were independently repeated at least three times ($n = 3$), and the sample size for each experiment is specified in the figure legends.

Experimental Results

Gross Observation and Flap Survival Rate

During the 7 days after the operation, the general behaviour and flap conditions of the rats were monitored daily. The rats remained active, with no significant change in feeding or mobility. Mild licking of the flap edge was occasionally observed, but no wound dehiscence or infection was noted, indicating stable postoperative recovery. On the 7th day, wound closure was achieved in all rats, and distal regions of the flaps showed varying degrees of colour change, including darkening and slight scab formation, suggesting ischaemic stress. Capillary refill testing revealed a delayed response in the distal portions, particularly in rats with diabetes.

The survival rate of the flaps in Group A was 67.71%, with a dark colour, poor elasticity and hard texture of the distal tissue of the flap, whereas the survival rate of the flaps in Group B was 88.31%, with good tissue activity in the back flap.

Table 1 Genes and Primer Sequences Used for q-PCR Analysis

Primer name	Forward (5'→3')	Reverse (5'→3')
VEGF	CTGCTGTACCTCCACCATGC	CTGGCTTTGGTGAGGTTTGA
HIF-1 α	GATGGCTCCCTTTTCAAGC	TTTCTGCTGCCTTGTATGGG
β -actin	AGGGAAATCGTGCGTGACAT	GAACCGCTCATTGCCGATAG

Some distal tissues from Group B showed a dull colour but with a soft texture. The flap survival rate of Group B increased in comparison with Group A ($P < 0.05$).

In the rats with diabetes, Group C showed the lowest flap survival rate at $46.64\% \pm 6.01\%$, with large areas of necrosis, pale and hardened tissue and severely compromised elasticity. Group D showed a survival rate of $72.12\% \pm 6.55\%$, significantly higher than Group C ($P < 0.05$), with macroscopically better-preserved flap tissue.

These results indicate that BoTA treatment is associated with improved flap survival in both normal and diabetic conditions. Notably, the improvement in the rats with diabetes suggests that BoTA may help mitigate the deleterious effects of hyperglycaemia on tissue viability, potentially through vascular-related mechanisms. However, further investigation is needed to confirm the underlying pathways.

Neovascular Density in Haematoxylin-Eosin-Stained Flap Tissue of Different Groups

On the 7th day after the operation, tissue sections were stained with H&E to evaluate the number of vascular ends in the proximal, middle and distal regions. Among all groups, Group B exhibited the highest average number and density of small blood vessels, distributed relatively evenly across all regions ($P < 0.05$), indicating robust and consistent neovascularisation. Group A showed moderate vascular density, predominantly in the proximal and middle segments. In Group D, neovascular structures were present but less dense than in Group B, whereas Group C displayed the lowest vascular density, with disrupted vessel morphology and the absence of identifiable microvasculature in the distal necrotic area.

Representative H&E-stained histological sections of flap tissues from Groups C and D at both $100\times$ and $400\times$ magnifications are shown in Figure 1. Group D showed increased capillary density and improved tissue integrity compared with Group C, consistent with improved angiogenesis following BoTA treatment. These histological findings suggest that BoTA promotes angiogenesis within flap tissue, particularly in areas prone to ischaemia. The contrast between the diabetic groups (C and D) and the normal groups (A and B) highlights the inhibitory impact of hyperglycaemia on vascular regeneration. Botulinum toxin type A treatment partially restored neovascular density in rats with diabetes, supporting its potential role in enhancing microcirculation under compromised metabolic conditions. Nevertheless, further quantitative and functional validation (eg perfusion imaging and collagen remodelling) will help clarify the extent and quality of vessel formation.

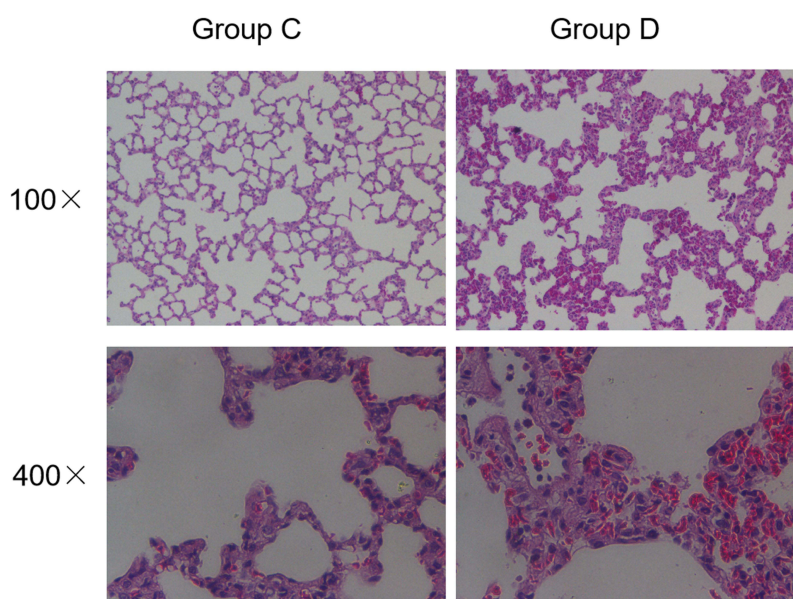


Figure 1 Representative hematoxylin and eosin (HE) staining of flap tissue sections in diabetic rats (Group C and Group D) at $100\times$ (upper panels) and $400\times$ (lower panels) magnification. Group D exhibits greater vascular density and better-preserved tissue structure compared to Group C, indicating improved angiogenesis following BoTA treatment.

Table 2 Expression Level of VEGF and HIF-1 α mRNA in Rat Flap Tissues of Different Groups

	Relative Expression of VEGF	Relative Expression of HIF-1 α
Group A	0.672 \pm 0.101	0.953 \pm 0.105
Group B	0.828 \pm 0.752	1.130 \pm 0.901
Group C	0.303 \pm 0.199	0.396 \pm 0.399
Group D	0.607 \pm 0.404	0.915 \pm 0.713

The mRNA Expression of Factor-1alpha and Vascular Endothelial Growth Factor in the Flap Tissues of Different Groups

As shown in Table 2, in normal rats receiving saline (Group A), VEGF and HIF-1 α expressions were 0.672 \pm 0.101 and 0.953 \pm 0.105, respectively. Botulinum toxin type A administration in normal rats (Group B) resulted in a slight increase, with VEGF at 0.828 \pm 0.752 and HIF-1 α at 1.130 \pm 0.901. In rats with diabetes receiving saline (Group C), the lowest expression levels were observed, with VEGF at 0.303 \pm 0.199 and HIF-1 α at 0.396 \pm 0.399, reflecting the impaired angiogenesis and tissue repair associated with diabetes ($P < 0.05$). Notably, BoTA treatment in rats with diabetes (Group D) partially restored these levels, with VEGF at 0.607 \pm 0.404 and HIF-1 α at 0.915 \pm 0.713, approaching the values seen in normal rats ($P < 0.05$).

Immunofluorescence Detection of Vascular Endothelial Growth Factor and Factor-1alpha Expression in Diabetic Flap Tissues

To validate the expression of angiogenesis-related proteins, we performed immunofluorescence staining for VEGF and HIF-1 α in diabetic flap tissues on postoperative day 14. As shown in Figure 2, both VEGF (red) and HIF-1 α (green) signals were markedly stronger in the BoTA-treated group (Group D) compared with the saline-treated diabetic control group (Group C). Blue DAPI staining confirmed consistent nuclear localisation across groups.

The merged images clearly demonstrated the co-localisation of increased VEGF and HIF-1 α expression within the tissues, suggesting enhanced angiogenic activity in response to BoTA treatment. These findings provide direct visual evidence supporting the mRNA results and reinforce the involvement of the HIF-1 α /VEGF axis in BoTA-mediated flap survival improvement under diabetic conditions.

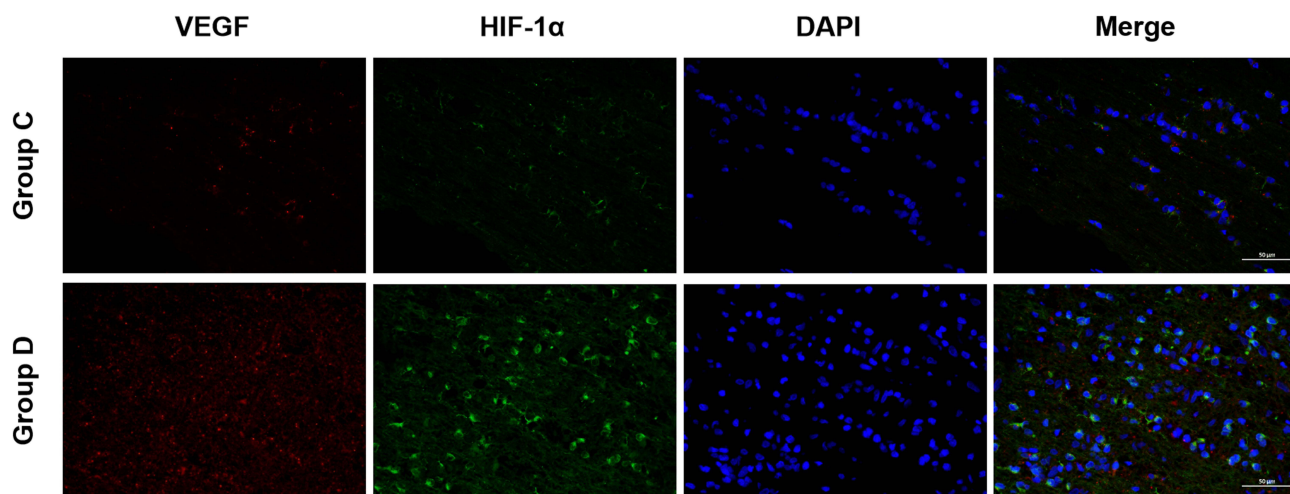


Figure 2 Immunofluorescence staining of flap tissues from diabetic rats. VEGF (red), HIF-1 α (green), and DAPI-stained nuclei (blue) in Group C and Group D. Merged images show colocalization of VEGF and HIF-1 α , which is notably stronger in Group D, suggesting BoTA-induced upregulation of angiogenic markers.

Discussion

Patients with diabetes are confronted with slow-healing wounds, smaller flap survival areas and high necrosis rates, making it one of the most common challenges in clinical wound repair and reconstructive surgeries.^{18,19} In this study, we found that the local injection of BoTA increased random flap viability in rats with diabetes induced by STZ and enhanced neovascular density in flap tissues. Furthermore, we found that BoTA could promote the mRNA expression of VEGF and HIF-1 α . Although previous studies have explored the role of BoTA in improving flap survival, most of them were conducted in non-diabetic models.^{12–14} To our knowledge, there has been limited investigation into the effects of BoTA in diabetic settings, where impaired neovascularisation and wound healing present unique challenges. This study provides new data in this context.

This study found that BoTA significantly increased the survival rate of skin flaps, which was not affected by diabetic conditions, indicating that BoTA can be used as an effective auxiliary treatment option for skin flap transplantation. Additionally, we found that BoTA could promote an increase in the flap survival rate by enhancing angiogenesis. Although the hyperglycaemic state inhibits new blood vessel regeneration in flaps, BoTA can significantly mitigate this effect. Previous studies reported that BoTA had a chemical delay effect on random, axial and myocutaneous flaps, thus promoting skin blood flow and the flap survival rate,^{20–22} which was consistent with our results.

More importantly, this study provides novel insight into the underlying mechanisms, suggesting that BoTA promotes angiogenesis in diabetic skin flaps through the HIF-1 α /VEGF signalling pathway. Factor-1 α is a transcription factor that mediates the expression of multiple genes, including those related to blood vessel growth and repair.²³ Under hyperglycaemic conditions, the expression of HIF-1 α decreases, thereby constraining vascular regeneration.¹¹ However, it is still unclear whether the improved neovascularisation induced by BoTA in diabetic flaps results from HIF-1 α accumulation. In this study, the expression levels of HIF-1 α and VEGF in rats with diabetes (Groups C and D) were significantly higher than those in the control group, especially in the distal end of the flap with severe ischaemia and hypoxia. However, there was no statistical difference between Groups A and B, which may be attributed to the fact that HIF-1 α is more easily activated in a hypoxic state. Hence, we speculate that BoTA may exert its therapeutic effects partly by restoring HIF-1 α expression under diabetic conditions, thereby enhancing VEGF-mediated neovascularisation.

It should be noted, however, that gene expression alone may not fully elucidate the mechanistic pathway of BoTA's action. Although our data support a potential role for the HIF-1 α /VEGF axis, further studies involving protein-level validation, functional assays (eg perfusion imaging) and pathway inhibition experiments are needed to confirm this mechanism. Therefore, our findings should be interpreted as preliminary mechanistic insights rather than conclusive evidence.

In terms of BoTA administration, Kim TK et al²⁴ adopted 2 \times 8 cm random flaps and injected 1.5 U BoTA into the middle third of the flap margins. Kim YS et al randomly injected 20 U BoTA into the proximal, mid and distal regions along the 3 \times 10 cm random flaps 7 days before the surgery, resulting in 8.3% and 31% increases in random flap survival areas of the rats.²⁵ We found that these two protocols had no significant differences in the rat strain (Sprague–Dawley), weight (300–400 g) and age (10 weeks), and both adopted the Botox produced by the US-based Allergan. Thus, it can be inferred that the 20 U dosage of BoTA has more pronounced effects on the flap survival rate. Using Wistar rats weighing 250–300 g, slightly lighter than those used in the above literature, we set the dose of BoTA to 5 U and administered it at 3 points to ensure uniform diffusion of the drug across all areas of the skin flap. Due to the diffusion range of Botox being 1.44 cm², injecting it into the pedicle of the skin flap may not allow it to diffuse to the distal end. Therefore, it is speculated that the higher rate of skin flap necrosis in the former may be attributed to this.

The optimisation of BoTA dosing and injection sites in our study may enhance the uniformity of its distribution and overall therapeutic efficacy. These practical adjustments, though subtle, improve the translational relevance of this approach for diabetic wound repair. Although our current study focused on angiogenesis-related parameters, future studies incorporating hydroxyproline quantification may further elucidate the role of BoTA in enhancing collagen deposition and overall tissue regeneration under diabetic conditions.

In future investigations, several aspects warrant attention. A quantitative assessment of collagen content, such as hydroxyproline, could help clarify BoTA's role in extracellular matrix remodelling. Moreover, functional evaluations of neovascularisation, including perfusion imaging or endothelial marker expression, may provide deeper mechanistic

insights. To further validate the activation of angiogenic pathways, future studies will incorporate protein-level assays such as the Western blot and immunohistochemistry to confirm the expression and localisation of key factors such as HIF-1 α and VEGF. In addition, extending the observation period beyond 7 days to include a second time point at 14 days may help capture later-stage tissue remodelling and long-term flap viability. Studies with extended observation periods and larger animal models may better reflect clinical outcomes. It would also be valuable to examine BoTA's influence on inflammatory and immune responses under chronic hyperglycaemia. Clinical studies are needed to validate these findings and determine optimal dosing strategies in patients with diabetes undergoing reconstructive procedures. Additionally, in this study, the assessment of flap texture was based on gross visual inspection and tactile evaluation, which may introduce subjectivity. Although this approach is commonly used in preclinical and clinical practice, we acknowledge that the absence of objective instrumentation, such as a texture analyzer, limits the precision of texture quantification. Future experiments will aim to incorporate texture analysis equipment to provide more standardized and quantitative data on flap biomechanical properties.

Conclusion

To sum up, this study reveals that BoTA promotes flap neovascularisation and improves the survival rate of random flaps through the HIF-1 α /VEGF pathway. This research provides a theoretical foundation for the use of BoTA in treating wounds in patients with diabetes and increasing the survival rate of flap transplantation.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethics Approval

The experiments were conducted in strict accordance with the Ethical Review of Laboratory Animal Welfare guidelines issued by the General Administration of Quality Supervision, Inspection and Quarantine, and the National Bureau of Standards. Animal experimental operations in this study were performed in accordance with Guide for Animal Care and Use of Guangdong Animal Experiment Association. Animal experiments were approved by Ethics Committees and Health Authorities of Hainan Medical College (No.HYLL-2023-304).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Title: Exploring the effect of botulinum toxin type A on the survival rate of skin flap transplantation in rats with diabetes; Project: Hainan Medical and Health Research Project; No.: 2001320275A2001.

Disclosure

All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately.

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