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ORIGINAL RESEARCH

TOPICAL TRETINOIN 0.05% ENHANCES ANGIOGENESIS IN FULL-THICKNESS WOUNDS IN RATS: A COMPARATIVE STUDY OF SINGLE AND REPEATED APPLICATION IN RATS

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ABSTRACT

Background: Angiogenesis plays a crucial role in the proliferative phase of wound healing by supplying oxygen and nutrients to regenerating tissue. Vascular Endothelial Growth Factor (VEGF) is the key mediator in this process. Tretinoin, a vitamin A derivative commonly used in dermatology, may influence angiogenesis in a dose- and context-dependent manner, but its role in acute wound models remains underexplored.
Methods: This experimental study involved 27 male Rattus norvegicus (Wistar strain), randomized into three groups (n=9): control (no tretinoin), single-dose topical tretinoin 0.05%, and repeated-dose for four consecutive days. Full-thickness excisional wounds (20 mm diameter) were created on the dorsal skin. On day five, tissue samples were collected. Neovascular density was assessed using hematoxylin-eosin staining with a semi-quantitative 0–4 scale, while VEGF expression was measured via immunohistochemistry using digital quantification.
Results: Repeated tretinoin application significantly increased neovascular density (mean score 2.67) and VEGF expression (72.54 ± 15.72) compared to single-dose (1.67; 30.42 ± 15.52) and control groups (1.1; 11.07 ± 2.89). Statistical analysis confirmed significant differences among all groups ($p < 0.05$) using Kruskal-Wallis and ANOVA, with post hoc tests showing consistent intergroup significance.
Conclusion: Topical tretinoin 0.05% enhances angiogenesis in full-thickness wounds, with repeated application producing greater effects. These findings support its potential use as a pro-angiogenic agent in acute wound management.

Keywords: Wound healing; tretinoin; angiogenesis; neovascularization; vascular endothelial growth factor

INTRODUCTION

Acute wounds remain a major clinical concern, particularly in trauma-related cases, where postoperative complications are more frequent compared to non-trauma patients. These complications often lead to prolonged hospital stays and increased healthcare costs.[1] Under normal conditions, acute wounds typically heal within three weeks, provided no complications occur. However, persistent inflammation, infection, or vascular impairment may prolong healing and increase the risk of transition into chronic wounds.[2] Modern wound care strategies such as negativepressure wound therapy (NPWT) have been widely adopted in acute settings. Nevertheless, treatment failure still occurs, particularly in patients with comorbidities such as atherosclerosis, diabetes, peripheral vascular disease, and pressure-related injuries.[3] These limitations highlight the need for adjunctive therapies that can promote faster and more effective healing.

Tretinoin, a derivative of vitamin A, has been shown to support various aspects of wound repair, including angiogenesis, epithelialization, fibroplasia, and

collagen synthesis. Animal studies demonstrate that vitamin A deficiency impairs these processes, resulting in delayed healing.[4]

Tretinoin exerts its biological effects by binding to nuclear retinoic acid receptors, activating gene transcription, and stimulating the release of vascular endothelial growth factor (VEGF), a major regulator of angiogenesis. VEGF enhances vascular permeability and supports endothelial proliferation and migration, ultimately facilitating new blood vessel formation.[5]

This study aims to evaluate the effect of single versus repeated topical application of 0.05% tretinoin on angiogenesis in full-thickness acute wounds using a rat model. Angiogenesis is assessed by both neovascular density and VEGF expression.

METHODS

Study design

This study was an experimental, post-test only design using male Wistar rats (Rattus norvegicus). The primary aim was to evaluate the effect of single versus repeated topical application of 0.05% tretinoin on angiogenesis in acute full-thickness wounds.

Experimental animals

Healthy male Wistar rats, aged 3–4 weeks and weighing 200–250 g, were selected as experimental subjects. Inclusion criteria included normal physical appearance and activity level. Rats with anatomical deformities or skin infections were excluded. Additional dropout criteria included infection or complications at the wound site, or animal death during the study.

The animals were housed in polypropylene cages (30×40×15 cm) lined with husk bedding and covered with a wire mesh. They were maintained at approximately 32°C, fed 20 g/day of pellet feed (PAR-G), and allowed ad libitum access to drinking water. Animal handling complied with ethical guidelines including 3R and 5F principles. All experimental protocols utilized in this study were approved by the Animal Care and Use Committee (ACUC) of Airlangga University, Surabaya, Indonesia (No. 2.KEH.21.02.2025).

Sample size and grouping

Sample size was determined using Federer's formula: $(t-1)(r-1)>15$. With three treatment groups ($t = 3$), a minimum of nine animals per group was calculated ($r = 9$), yielding a total of 27 rats. Animals were randomized into three groups: a control group with no topical treatment, a single-application group that received 0.05% topical tretinoin only on the first day after wounding, and a repeated-application group that received 0.05% topical tretinoin once daily for five consecutive days.

Wound creation and intervention

Following randomization, the rats were anesthetized via intramuscular injection of ketamine at a dose of 10 mg/kg. The dorsal area was shaved and disinfected using 10% povidone-iodine followed by diluted Savlon solution (1:30). A full-thickness excisional wound measuring 10×10 mm was created on the dorsal skin using a sterile surgical blade. To minimize wound contraction, a silicone stent of the same size was sutured to the wound margins with nylon 5-0. In the treatment groups, 0.05% topical tretinoin was applied directly to the wound surface and left for five minutes before being rinsed with 0.9% sodium chloride. Each wound was then covered with petroleum-impregnated gauze and sealed using transparent dressing to maintain hygiene and prevent contamination. The control group received no tretinoin application and was treated with dressing only, while the single-application group received tretinoin on the first day post-injury, and the repeated-application group received daily applications of tretinoin for five consecutive days. All animals were housed individually in controlled environments with standard lighting, temperature, food, and water, and were observed daily for signs of complications. On day five, corresponding to the proliferative phase of wound healing, all animals were euthanized by intraperitoneal injection of phenobarbital at a dose of 60–200 mg/kg. Full-thickness skin samples, including subdermal fat, were excised from the wound area and fixed in 10% buffered formalin for further histological and immunohistochemical analysis. Study design and experimental workflow as shown in Figure 1.

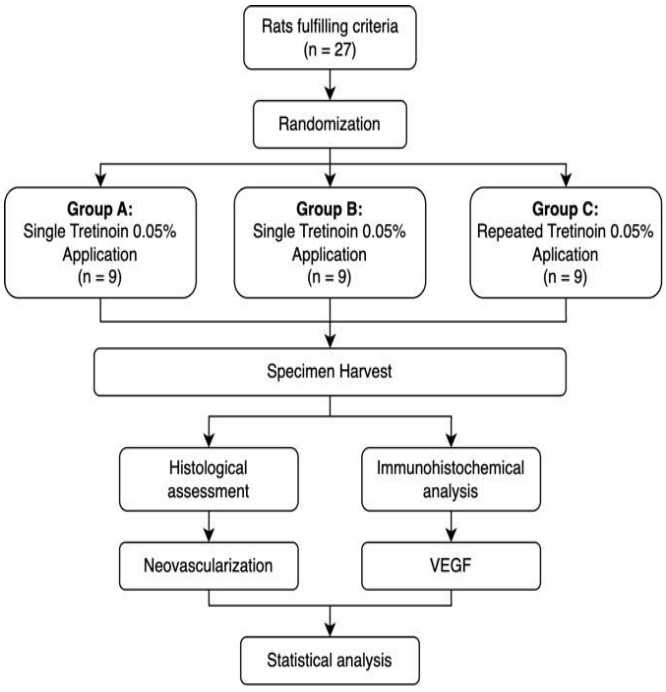

Figure 1. Study design and experimental workflow

Histological evaluation of neovascularization

The collected tissue samples were processed using standard paraffin embedding techniques. Sections with a thickness of 4–6 µm were stained with hematoxylin-eosin (HE) and observed under 400×

magnification using a Triscope microscope (AccuScope). Neovascularization was assessed semi-quantitatively using a five-point scale, where 0 indicated no visible capillaries, 1 indicated sparsely distributed capillaries, 2 represented moderate capillary density, 3 indicated dense capillary networks, and 4 reflected very dense neovascular formations. All evaluations were conducted by an anatomical pathology specialist.

Immunohistochemical analysis of VEGF expression

To assess VEGF expression, formalin-fixed paraffin-embedded tissue sections were processed through heat-induced epitope retrieval (HIER), followed by incubation with monoclonal mouse anti-VEGF antibody (clone C-1). Detection was performed using diaminobenzidine (DAB) chromogen, and hematoxylin was used as a counterstain. Slides were analyzed under 400× magnification and digitally captured for quantitative evaluation using the AccuView software. VEGF expression was quantified based on the intensity and proportion of brown staining in relation to the blue counterstained background.

Data analysis

All data were processed and analyzed using SPSS version 27 (IBM Corp.). The distribution of each dataset was first tested for normality to determine the appropriate statistical approach. For normally distributed data, parametric analysis using one-way analysis of variance (ANOVA) was applied, whereas non-parametric methods including Kruskal-Wallis and Mann-Whitney tests were used for non-normally distributed data. Statistical significance was defined as $P < 0.05$.

RESULTS

Neovascular density

Histological examination of full-thickness wound tissues stained with HE demonstrated a progressive increase in neovascular density across treatment groups. Representative microscopic images (Figure 2) showed that the control group exhibited sparse capillary formation with scattered endothelial nuclei, while the single-dose tretinoin group demonstrated a moderate increase in capillary density. The most prominent angiogenic response was observed in the repeated-application group, which displayed dense and well-organized networks of newly formed vessels within the granulation tissue.

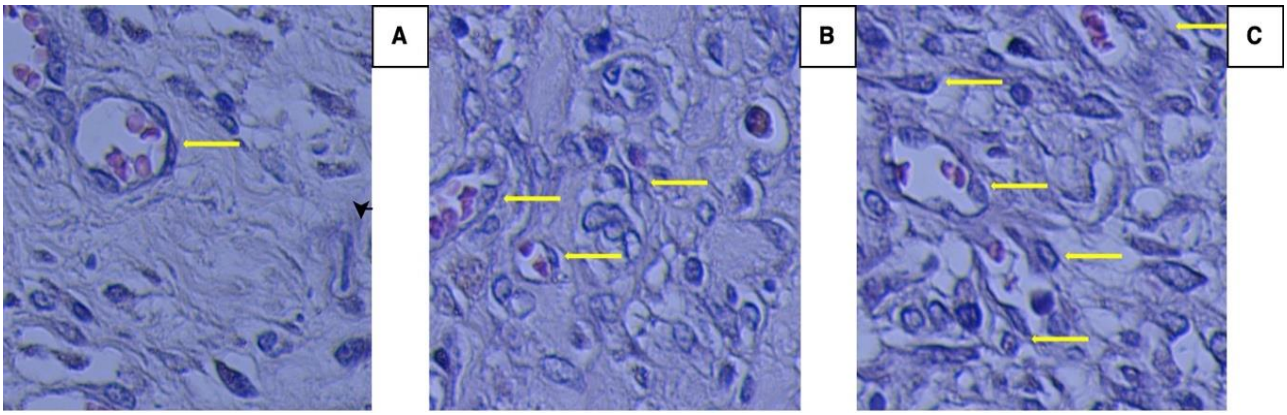

Figure 2. Histological appearance of neovascularization (H&E, ×400). Representative images of wound tissue stained with HE on day 5 post-injury. Endothelial cells with bluish-purple nuclei identified by yellow arrows. (A) Control group showing sparse capillary structures; (B) Single-dose tretinoin group with moderate capillary presence; (C) Repeated-dose tretinoin group showing dense neovascularization throughout granulation tissue.

Quantitative analysis using a semi-quantitative scoring system revealed that the median neovascular density score increased from 1.0 in the control group to 2.0 in the single-application group and remained at 2.0 in the repeated-application group, with a wider distribution toward higher scores. Notably, two samples in the repeated-application group reached the maximum score of 4, indicating very dense vascular proliferation, which was not observed in the other groups (Table 1).

Table 1. Neovascular density scores among groups. Values represent the number of animals per score category (+1 to +4), as well as the minimum, median, and maximum scores observed in each group.

Group	Sample size, n	Neovascular density score, n				Min-Max	Median
		+1	+2	+3	+4		
A (control)	9	8	1	0	0	1-2	1
B (Single dose tretinoin)	9	4	4	1	0	1-3	2
C (Repeated dose tretinoin)	9	0	5	2	2	2-4	2

Boxplot analysis (Figure 3) visualized the trend, with a notable upward shift in median and interquartile range among tretinoin-treated groups. This observation was further supported by categorical distribution (Figure 3), which showed that the control group was dominated by low-density scores (+1), whereas the repeated-application group showed a broader spread toward scores +3 and+4.

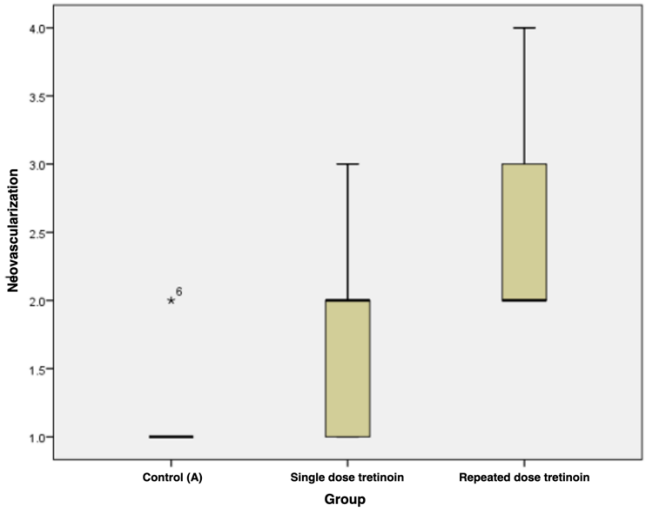

Figure 3. Boxplot of neovascularization scores. Semi-quantitative scores of capillary density in each group. The repeated-dose tretinoin group demonstrates the highest median and interquartile range compared to the control and single-dose groups.

Statistical testing using the Kruskal-Wallis test confirmed a significant difference in neovascular scores across the three groups (P = 0.001). Post hoc Mann-Whitney comparisons demonstrated that each pairwise comparison (control vs. single-dose, control vs. repeated-dose, and single-dose vs. repeated-dose) yielded statistically significant differences (P < 0.05), indicating a consistent increase in neovascular density associated with tretinoin application, especially with repeated exposure (Table 2).

Table 2. Mann–Whitney U test comparing neovascular density scores between treatment groups.

Comparison	Sample size per group, n	Median (Min-Max)	P-value
Group A vs Group B	9	1 (1-2)	0.048*
Group A vs Group C	9	2 (1-3)	<0.001*
Group B vs Group C	9	2 (2-4)	0.02*

*P-value significant at < 0.05

VEGF expression

Immunohistochemical analysis of VEGF expression revealed marked differences between groups. Figure 4 shows representative tissue sections, in which VEGF-positive staining appeared as brown coloration in cytoplasmic regions. The control group displayed minimal VEGF expression, confined to isolated cells with weak staining intensity. In contrast, the single-application group showed moderate brown staining distributed more extensively. The repeated-application group exhibited robust and diffuse VEGF expression throughout the wound bed, with high-intensity staining.

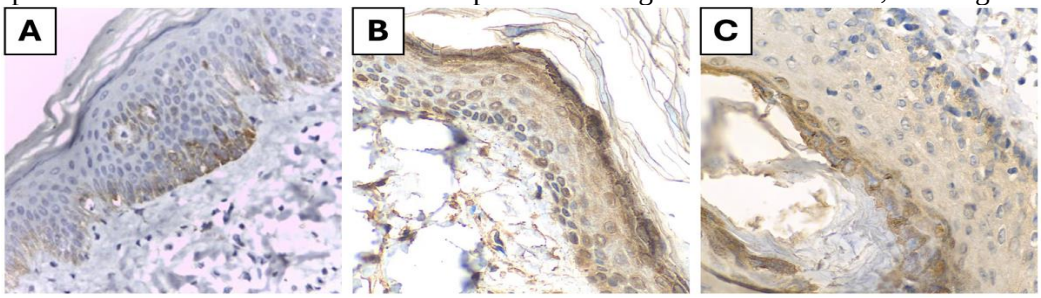

Figure 4. VEGF expression by immunohistochemistry (×400). Tissue samples stained for VEGF expression showing cytoplasmic brown signals. (A) Control group with minimal VEGF-positive cells; (B) Single-dose tretinoin group with moderate expression; (C) Repeated-dose tretinoin group showing intense and widespread VEGF staining.

Descriptive analysis showed a clear gradient of VEGF levels, with mean ± SD values of 11.07 ± 2.89 in the control group, 30.42 ± 15.52 in the single-dose group, and 72.54 ± 15.72 in the repeated-dose group (Table 3).

Boxplot analysis (Figure 5) illustrated these findings with minimal overlap in the confidence intervals between groups, suggesting consistent and reproducible expression patterns.

Table 3. Descriptive statistics and one-way ANOVA results comparing VEGF expression levels among groups. Values are presented as mean ± standard deviation.

Group	Sample size, n	Mean ± SD	F statistic	P-value
A	9	11.07 ± 2.89		
B	9	30.42 ± 15.52	53.7	<0.001*
C	9	72.54 ± 15.72		
Total	27	38.01 ± 28.92	-	-

*P-value significant at < 0.05

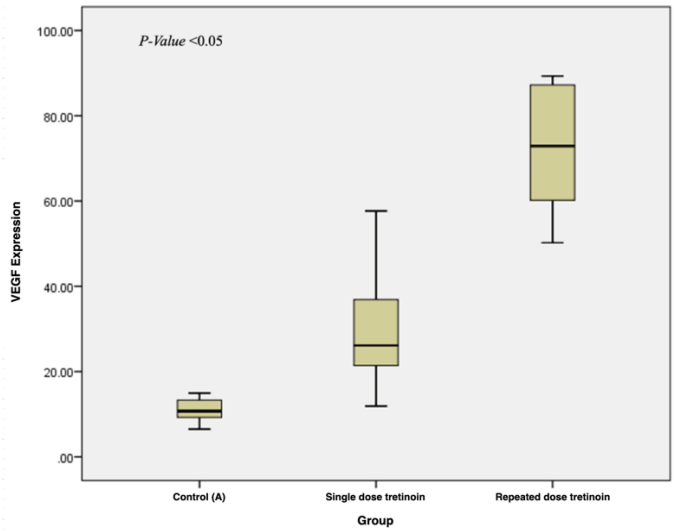

Figure 5. Boxplot of VEGF expression levels. Quantitative analysis of VEGF expression measured via digital image software. The repeated-dose group shows the highest expression level, with distinct separation from the other two groups.

Prior to inferential analysis, normality of VEGF data distribution was confirmed using the Shapiro-Wilk test, which returned P-values > 0.05 for all groups, allowing for parametric analysis. One-way ANOVA revealed a highly significant difference among groups ($F = 53.70$, $P < 0.001$), indicating that tretinoin application had a significant impact on VEGF expression (Table 3). Further post hoc analysis using Tukey's HSD test showed that each group differed significantly from the others, with VEGF expression in the repeated-dose group being significantly higher than in the single-dose group, which in turn was significantly higher than in the control group (Table 4).

Table 4. Tukey HSD subset results. Groups not sharing a common subset are significantly different at the 0.05 level.

Group	Sample size, n	Subset for alpha = 0.05		
		1	2	3
A	9	11.07	-	-
B	9	-	30.43	-
C	9	-	-	72.55

These results collectively suggest that topical tretinoin 0.05%, particularly when applied repeatedly, promotes angiogenesis in full-thickness wounds by enhancing both neovascular density and VEGF expression in a statistically significant and biologically meaningful manner.

DISCUSSION

This study investigated the effect of topical tretinoin 0.05% on neovascular density and VEGF expression in full-thickness acute wounds. The results demonstrated that repeated application of tretinoin significantly enhanced both neovascularization and VEGF expression compared to single-dose or no

treatment groups, suggesting a pro-angiogenic role for topical tretinoin under these conditions.

The observed increase in neovascular density in the repeated-dose group indicates enhanced angiogenic activity during the proliferative phase of wound healing. Neovascularization is essential for supplying oxygen and nutrients to regenerating tissue, and its acceleration is a critical marker of effective wound repair.[6]

These findings are consistent with a previous study that reported increased retinal vascular development after retinoic acid administration through VEGF upregulation.[7] The results also support the context-dependent effects of retinoids, where specific concentrations and application regimens may stimulate rather than suppress angiogenesis. Conversely, earlier studies observed VEGF suppression in keratinocytes exposed to tretinoin, highlighting that its effects are influenced by the biological environment, dose, and exposure duration.[8,9] In the present study, the repeated short-duration topical application may have provided adequate angiogenic stimulation without inducing VEGF inhibition.

Immunohistochemical analysis confirmed significantly higher VEGF expression in the repeated-treatment group, suggesting that topical tretinoin may enhance angiogenesis at the molecular level. Retinoic acid, the active metabolite of tretinoin, acts by binding to nuclear retinoic acid receptors (RARs), which heterodimerize with retinoid X receptors (RXRs) to regulate gene transcription.[10] One key target gene is *VEGFA*, the primary mediator of angiogenesis in wound healing.[11] Activation of RAR/RXR signaling enhances VEGF transcription, leading to increased endothelial proliferation, vascular permeability, and neovessel formation.[12-14]

Moreover, tretinoin may indirectly influence angiogenesis by modulating inflammatory and extracellular matrix remodeling pathways. It has been shown to upregulate MMP-2 and MMP-9, which facilitate endothelial migration by degrading basement membrane components, thereby promoting capillary sprouting.[15,16] In parallel, tretinoin may enhance fibroblast-derived VEGF secretion through TGF-β signaling activation in dermal stromal cells.[17] This dual action, direct gene upregulation and indirect paracrine stimulation, creates a local environment conducive to neovascularization.

Interestingly, tretinoin also suppresses AP-1, a transcription factor that normally inhibits keratinocyte differentiation and wound matrix remodeling.[18] Inhibiting AP-1 may reduce negative regulation of VEGF in keratinocytes under inflammatory stress, allowing VEGF expression to proceed unopposed in the early healing phase.[19] These mechanisms might explain why short-term, repeated application resulted in increased VEGF and neovascular density, whereas prolonged or systemic use of retinoids in other models produced conflicting results due to AP-1 over-suppression or VEGF receptor desensitization.

Similarly, the findings align with prior studies showing increased dermal vascularity and epidermal VEGF levels following short-term topical tretinoin use, despite its suppressive role in other settings, such as UV-induced angiogenesis.[20] A previous study also demonstrated tretinoin's ability to modulate early angiogenic signaling pathways and vascular responsiveness to VEGF.[21] The dual behavior of tretinoin underscores its complex pharmacological profile, warranting cautious modulation of dose and application schedule.

While a previous study demonstrated that repeated topical tretinoin 0.05% enhanced fibroplasia but did not promote epithelialization by day 5 in full-thickness wounds, our study provides further evidence that repeated application can support angiogenesis during wound healing, positioning it as a potentially useful therapeutic option in managing acute wounds.[22] However, given its bidirectional effects, further studies are necessary to define the optimal concentration and duration of treatment. Future research should extend to chronic wound models, clinical human trials, and comparative evaluations with other pro-angiogenic agents. Investigations into potential adverse effects, including irritation or long-term VEGF suppression, are also essential.

CONCLUSION

In conclusion, repeated topical application of tretinoin 0.05% significantly increases neovascular density and VEGF expression in full-thickness acute wounds, suggesting a favorable role in promoting angiogenesis and accelerating tissue repair. These results support the therapeutic potential of tretinoin in wound healing, while also emphasizing the importance of precise dosing strategies.

DECLARATION

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Conflict of interest

The authors declare no potential conflict of interest relevant to this article.

Ethical approval

Ethical approval was granted by the Animal Care and Use Committee (ACUC) of Airlangga University, Surabaya, Indonesia (No. 2.KEH.21.02.2025).

Informed consent

Not applicable.

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