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The effect of dapsone on skin flap survival depends on modulation of inflammatory response and VEGF expression

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Abstract

The random-pattern skin flap is a common method used for reconstructing skin defects. However, flap ischemia necrosis remains a significant challenge in plastic surgery. Strategies aimed at reducing persistent inflammation and promoting blood supply through angiogenesis have been identified as crucial for improving flap survival. Dapsone, a chemotherapeutic agent known for its anti-inflammatory properties through multiple pathways, is of interest in this regard. This study aims to investigate the effect of dapsone on random-pattern flap survival in rats, along with its impact on inflammation and angiogenesis. The ischemia/reperfusion (I/R) injury rat models were created using a caudal-based dorsal skin flap with delayed I/R. Twenty-four male Sprague Dawley rats were divided into control, sham, and two treatment groups receiving dapsone at doses of 12.5 mg/kg/day and 5 mg/kg/day, respectively. On the 7th post-operative day, flap survival was evaluated. Neutrophil infiltration and ulceration were measured through microscopic examination, and interleukin (IL)-8 levels through enzyme-linked immunosorbent assay. Expression levels of vascular endothelial growth factor (VEGF) and tumor necrosis factor-alpha (TNF-α) were determined using an immunohistochemistry (IHC) array. The findings revealed an increased flap survival on day 7 post-operation following systemic administration of dapsone for 5 consecutive days. Dapsone at both dosages significantly reduced the ulcer thickness, neutrophil infiltration, and IL-8 levels. The IHC results revealed that VEGF expression was significantly higher in the treatment groups compared to the control group. Moreover, TNF-α expression was significantly lower in the treatment groups compared to the control group. In conclusion, we confirmed that treatment with dapsone promotes skin flap survival, and this effect aligned with a reduction in persistent inflammation and the enhancement of VEGF. Nonetheless, more studies are required to elucidate the precise anti-inflammatory mechanism of dapsone in I/R injuries.

Keywords: Dapsone; Inflammation; Ischemia/reperfusion injury; Skin flap; VEGF

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1. Introduction

The random-pattern skin flap is commonly used to reconstruct skin defects in plastic surgery. Contrary to an axial flap, which is vascularized by a specifically identified artery, a random-pattern skin flap is designed without consideration for specific vessels and instead relies on subdermal plexuses and unnamed musculocutaneous arteries for blood supply. Following elevation from its bed, the flap attempts to restore its hemodynamic balance. Disruption in tissue blood perfusion can lead to metabolic changes that may result in distal necrosis.[1] Despite admirable advances in flap surgeries, flap necrosis remains a frequently encountered issue, posing complications for patients.[2-4] Various techniques, including surgical delay and the application of vascular endothelium growth factor (VEGF), have been clinically used to increase the flap survival rate. However, these methods are associated with high costs, time consumption, and inconvenience for patients.[5-8] In experimental contexts, the administration of diverse stem cell types or the implementation of advanced drug delivery methods, such as microneedles containing hyaluronic acid, has demonstrated promising outcomes in enhancing vasculogenesis within skin flaps.[9] Therefore, a proper pharmacologic agent represents an alternative therapy to increase the flap survival rate. The previous studies have indicated that flap necrosis primarily arises from inadequate blood supply and subsequent ischemia/reperfusion (I/R) injury, oxidative stress, inflammatory reactions, and possible surgical site infections.[10-12] Consequently, numerous potential pharmacologic agents targeting these contributing factors are being investigated clinically and experimentally to improve flap survival.

Inflammation plays a crucial role in the process of wound healing. However, during necrosis, an intense inflammatory response, characterized by excess neutrophil infiltration, exerts an inverse effect on healing. Thus, controlling inflammation is instrumental in enhancing flap survival. It is worth mentioning that while endogenously regulated neutrophil infiltration within the initial days following injury is necessary for the healing process, prolonged and persistent neutrophilia would lead to an impaired healing process.[13,14] Interleukin (IL)-8, known as a potent chemotactic agent, mediates the infiltration and activation of neutrophils. Moreover, it is well-established that secretion of IL-8 mediates the production of cytokines, including tumor necrosis factor-alpha (TNF-α).[15,16] Recent studies have suggested that, in addition to IL-8, TNF-α also mediates neutrophil recruitment to the injured tissue and induces inflammatory responses. It has been suggested that reduction in TNF-α levels and neutrophil migration alleviates the inflammatory response and increases the flap's viable area.[17,18] The previous studies have shown that the formation of new vessels in the flap is essential for increasing the survival area of random-pattern skin flaps through an improvement in tissue blood supply. Moreover, vascular endothelial growth factor (VEGF) promotes angiogenesis and significantly reduces distal flap necrosis.[19,20] Studies have shown that increased VEGF expression correlates with enhanced angiogenesis and increased flap survival area.[21] VEGF secretion is stimulated by hypoxia and inflammatory responses to an injury. Following injury, activated platelets and neutrophils release VEGF.[22,23] However, it is crucial to note that an excessive and persistent inflammatory response as a result of an I/R injury can potentially disturb angiogenesis and the healing process. It has been confirmed that inhibition of persistent inflammatory elements is accompanied by enhancement of angiogenesis and prevention of tissue necrosis.[24]

Dapsone (4,4'-diamino-diphenyl sulfone) is an antibiotic agent belonging to the group of synthetic sulfones. It possesses both antimicrobial and anti-inflammatory properties. Its bacteriostatic function arises from its ability to inhibit dihydrofolate synthesis in bacteria.[25] The US Food and Drug Administration-approved indications for dapsone include leprosy, dermatitis herpetiformis, and acne vulgaris.[26] In addition, dapsone exhibits multiple anti-inflammatory, anti-oxidative, and anti-apoptotic properties. Dapsone functions by inhibiting beta-2 integrin-mediated adherence of neutrophils, thereby preventing their chemotactic migration.[27,28] In addition, it can interfere with the function of enzymes and proteins in the integrin family and demonstrates an inhibitory effect on IL-8 and TNF-α production, both crucial in neutrophil-mediated inflammation and regulation of lymphocyte and monocyte.[29,30]

Given its multiple functions in inflammation and angiogenesis, dapsone emerges as a potential candidate for preventing flap necrosis. In the present study, we aimed to investigate the random-pattern flap survival in rats treated with dapsone, along with exploring possible underlying inflammatory mechanisms. Furthermore, we aimed to assess the VEGF expression levels as an indicator of angiogenesis and flap survival,[31] and TNF-α as a key representative of the inflammatory response, respectively. An overview of the study is illustrated in Figure 1.

2. Materials and methods

2.1. Animals

Twenty-four male Sprague Dawley rats (weighing 270–300 g) were obtained from the animal house of the Department of Pharmacology, Tehran University of Medical Sciences. To attain a constant experimental

condition, the animals were individually housed in rat cages at room temperature of 24 ± 1°C and a relative humidity of 50–60%. They were subjected to a 12-h light/dark cycle and provided *ad libitum* access to food and fresh water. All procedures conducted in this study adhered to the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, revised in 1996) and were approved by the ethics committee of Tehran University of Medical Sciences (ethics approval ID: IR.TUMS.MEDICINE.REC.1399.867).

2.2. Drugs

Dapsone was procured from Sigma-Aldrich (United Kingdom) and was dissolved in 10% dimethyl sulfoxide (DMSO). The dapsone solution was freshly prepared before administration and delivered through oral gavage using 18-gauge stainless steel feeding tubes with rounded tips for rats. For anesthesia during surgical procedures, ketamine HCl and xylazine HCl were administered intraperitoneally. These anesthetic agents were purchased from Gedeon Richter Ltd. (Hungary) and Bayer AG (Germany), respectively.

2.3. Study design

To investigate the effect of dapsone on flap survival, 24 rats were randomly assigned to four equal groups: a sham group, a control group, and two treatment groups. The sham group included rats that underwent anesthesia while their whole skin remained intact. Dorsal skin, in accordance with our flap design, was acquired and utilized as the baseline for our immunohistopathological assessments. Rats in the remaining groups underwent dorsal skin flap procedures. Rats in the control group received vehicle (10% DMSO) orally, while one treatment group received dapsone at 5 mg/kg/day, and the other treatment group received dapsone at 12.5 mg/kg/day, both dissolved in 10% DMSO. Both vehicle and dapsone treatments were administered for 5 consecutive days postoperatively, with the first dose administered three hours following the operation. The dose selection of dapsone was based on our previous studies on the anti-inflammatory properties of this reagent.[32,33]

2.4. Surgical procedure

The surgical procedures were performed by an author blinded to the treatment conditions under sterile conditions. Before surgery, all rats underwent a 12 h fasting period. General anesthesia was induced by intraperitoneal injection of a solution containing ketamine HCl (90 mg/mL) and xylazine HCl (10 mg/mL). Maintenance of deep anesthesia was achieved through intermittent administration of ketamine alone. Dorsal hair was removed using an electric razor, and this surgical site was prepared using a povidone-iodine solution. To prevent hypothermia, a heat-emitting light was located next to the rats during the operation. The random-pattern skin flap model described by McFarlane *et al.*[12,34,35] was employed. The procedure involved creating a bipedicled dorsal skin flap measuring 8 cm in length and 2 cm in width, positioned on the midline of the dorsum of each rat, with the iliac crest serving as a constant anatomic landmark defining the caudal margin of the flap. Each flap was cleaved beneath the panniculus carnosus and elevated, with the cranial pedicle clamped to allow for the cauterization of perforating vessels, establishing a random ischemic pattern. Afterward, the clamp was removed, and the cranial pedicle was cut to ensure blood perfusion solely

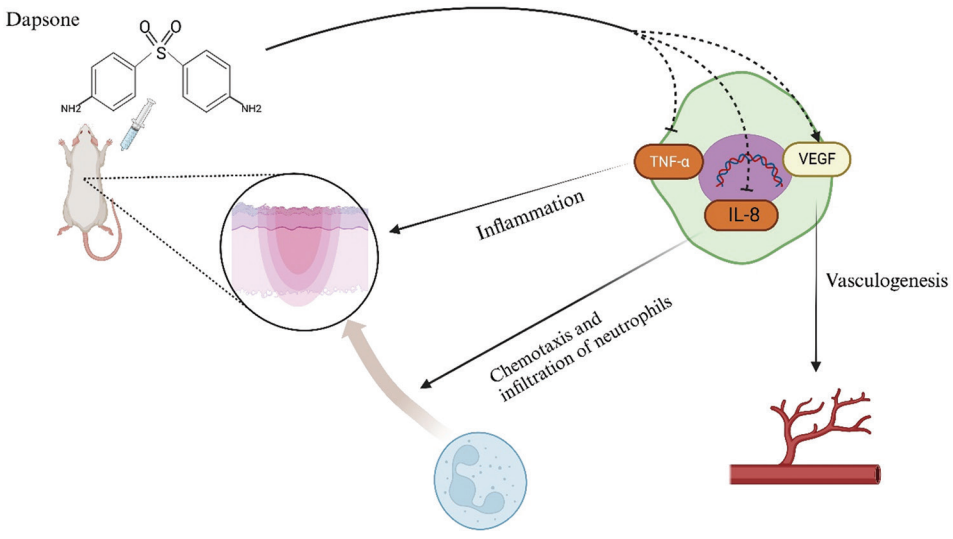


Figure 1. Overview of the study

from the caudal section. Flaps were maintained in moist, sterile gauzes during the operation. Subsequently, each flap was sutured back into its innate position using 4-0 reversed-cut nylon sutures. Corner stitches were employed to suture the cranial angles, while simple cutaneous stitches were used for all other margins. The surgical site was covered with sterile wound dressing, and each rat was housed individually in clean cages to facilitate recovery. All of the rats survived the operation and lived through 7 days of recovery.

2.5. Macroscopic evaluation

Each day, dorsal skin flaps underwent careful post-operative inspection and examination. On the 2nd day following the surgery, distal free edge necrosis was observed in a rat treated with dapsone 5 mg/kg/day. The necrotic area extended more than 1 mm from the surgical line, potentially attributed to inadvertent trauma during the procedure. Moreover, on the 5th post-operative day, infection-related necrosis was observed in another rat from the control group, likely stemming from inadequate sterilization control during the operation. No signs of circulatory failure were observed among the rats. To ensure the evaluation of skin flaps based on a comparable equal quality of procedure, these rats were excluded from the study. On the 7th post-operative day, rats were anesthetized, and the flaps were assessed by an author blinded to the treatment condition. Macroscopic changes, including appearance, skin color, texture, and hair condition of each flap, were carefully noted. Digital images were captured from the dorsal skin flaps to evaluate flap viability. Necrotic areas were characterized by dark color, edema, and eschar formation, while the total flap area was delineated by the surgical borders. ImageJ software (U.S. National Institutes of Health, USA) was used to demarcate and analyze the viable surface area. Flap survival was calculated and reported as follows:

$$\text{Flap survival (\%)} = (\text{Area of necrotic tissue}/\text{Total area of the flap}) \times 100 \quad \text{(I)}$$

2.6. Histopathological analysis and enzyme-linked immunosorbent assay

Histopathological evaluations were performed to examine the effect of dapsone on flap survival rate at a microscopic level. After the macroscopic assessments on the 7th post-operative day, the rats were anesthetized through intraperitoneal administration of ketamine HCl (90 mg/Kg) and xylazine HCl (10 mg/Kg). The necrotic tissue from the flap's cranial part and the viable tissue from the caudal part were dissected, with the obtained specimens divided into two halves: one half was fixed in 10% formalin solution for 24 h, while the other half was placed in liquid nitrogen at −70°C. Immediately after obtaining the specimens, the rats were sacrificed using a carbon monoxide (CO) chamber. The first half of the specimens were processed, embedded in paraffin blocks, sectioned into 5 μm thick slices, and prepared for hematoxylin and eosin (H&E) staining. An expert pathologist, blinded to the groups and study design, examined all H&E-stained sections for neutrophil infiltration, blood vessel distribution and angiogenesis, and ulcer thickness using an optical microscope. The second half of the specimen was homogenized using lysis buffer (2X Lysis Buffer; RayBio®, USA) and then centrifuged for 15 min (13,000 rpm, 4 °C). Subsequently, the IL-8 levels were measured using the enzyme-linked immunosorbent assay (Abcam, UK) method, adhering to the manufacturer's guidelines.

2.7. Immunohistochemistry (IHC)

The paraffin-embedded flap specimens were processed for IHC staining. These sections were then incubated with anti-TNF-α (ab6671, Abcam®, USA, 1:100) and anti-VEGF antibodies (ab46154, Abcam®, USA, 1:100). After washing with phosphate-buffered serum (PBS), specific binding to primary antibodies was visualized by enzymatic conversion of the chromogenic substrate 3,3-diaminobenzidine (DAB Substrate Kit, ab64238, Abcam®, USA) into a brown precipitate using horseradish peroxidase (ab6721, Abcam®, USA), in accordance with established protocols. Subsequently, the sections were mounted, cleared, and dehydrated. The slides were subjected to double staining using annexin-V fluorescein isothiocyanate (FITC) (Abcam, USA) and were evaluated using a fluorescence microscope. Expression levels of the desired markers were assessed and analyzed using ImageJ software (U.S. National Institutes of Health, USA).

2.8. Statistical analysis

Data are expressed as mean ± standard deviation (SD) and were analyzed using one-way and two-way analyses of variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparisons between groups. All statistical analyses were performed using GraphPad Prism software version 6.07. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Assessment of skin flap survival

Figure 2 illustrates the percentage of necrosis as the survival rate of the skin flap among the rats. A one-way ANOVA confirmed a significant difference between study groups ($F[3, 13] = 28.01$, $P < 0.001$). Tukey's test revealed that treatment with dapsone at 12.5 mg/kg significantly reduced the necrosis percentage compared with the control group

(*MD* = 35.21, *P* < 0.001). However, no significant difference was observed between the control and dapsone 5 mg/kg groups (*P* = 0.620). These results indicate that dapsone at 12.5 mg/kg exhibited statistically higher effectiveness than dapsone at 5 mg/kg (*MD* = 26.48, *P* < 0.01) (Figure 2).

3.2. Microscopic ulceration and neutrophil infiltration

Figure 3 illustrates the microscopic evaluation of flap injury and ulceration using H&E staining in our histopathologic assessment. Results revealed variations in ulcer thickness among the study groups (*F*[3,8] = 113.9, *P* < 0.001). *Post hoc* analysis revealed significant effects of dapsone administration at both doses on ulcer thickness (*MD* = 88.67, *P* < 0.001; *MD* = 178.3, *P* < 0.001, respectively) (Figure 3B). Moreover, significant differences were observed among the four groups regarding neutrophil infiltration (*F*[3,8] = 29.20, *P* < 0.00) (Figure 3A). Tukey's analysis confirmed that the I/R injury in the random-pattern flap model promoted neutrophil infiltration at the injury site (*MD* = −146.00, *P* < 0.001). Notably, systemic administration of dapsone in both the 5 and 12.5 mg/kg groups resulted in significantly reduced neutrophil infiltration (*MD* = 98.33, *P* = 0.002; *MD* = 128.330, *P* < 0.001, respectively). This effect did not exhibit significant dose dependence (*P* = 0.357) (Figure 3A).

3.3. Assessment of IL-8 level

As depicted in Figure 4, IL-8 levels were measured and compared among groups. A one-way ANOVA confirmed a statistically significant difference between study groups (*F*[3,20] = 20.54, *P* < 0.001). The induction of I/R injury in our skin flap model led to an increase in IL-8 levels, as expected (*MD* = 80.5, *P* < 0.001). Administration of dapsone significantly reduced IL-8 levels, regardless of the dose administered (*MD* = 30.17, *P* = 0.039; *MD* = 38.67, *P* = 0.006; for doses of 5 and 12.5 mg/kg, respectively). However, the effect of dapsone on the IL-8 levels did not appear to be dose-dependent (*P* = 0.84) (Figure 4).

3.4. Skin flap expression of VEGF and TNF-α

Figure 5 presents the expression of VEGF and TNF-α in the skin flap using IHC staining. We observed significant differences in VEGF expression among the four groups (*F*[3,8] = 83.04, *P* < 0.001) (Figure 5A), with the highest mean VEGF expression observed in the sham group. Moreover, the results revealed that the induction of I/R injury in our skin flap model led to a decrease in the expression of VEGF (*MD* = 35.45, *P* < 0.001). In addition, the IHC assessment revealed higher VEGF expression in rats receiving dapsone at either 5 or 12.5 mg/kg compared with the control group (*MD* = 13.72, *P* = 0.002; *MD* = 22.08, *P* < 0.001; respectively)

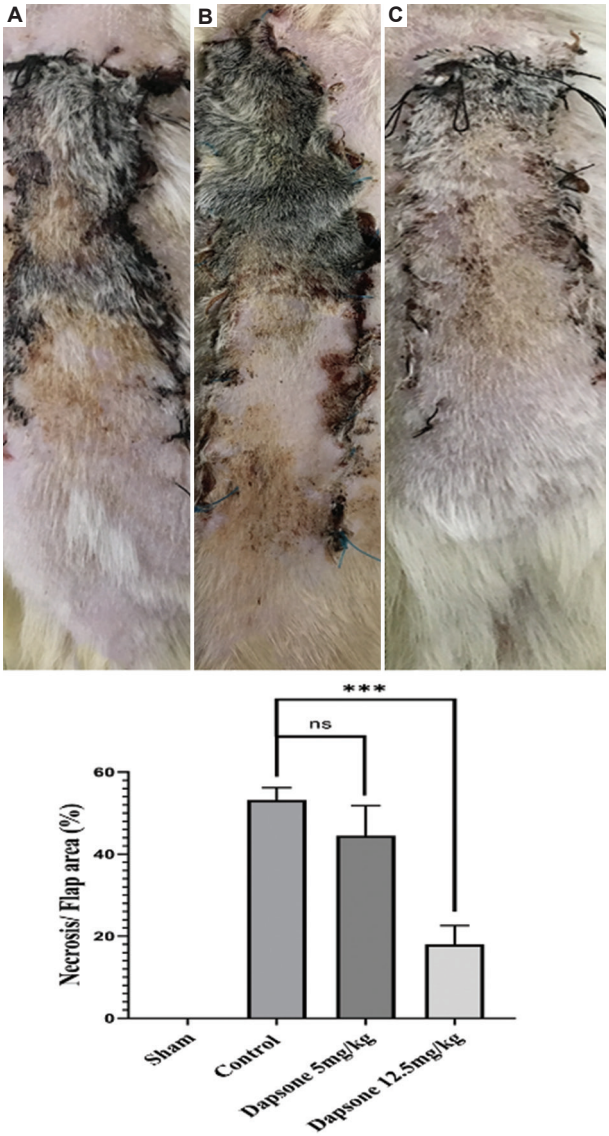


Figure 2. Flap survival investigations following an ischemia/reperfusion (I/R) injury. Upper panel: Dorsal skin flaps on day 7 following surgery: (A) Control group; (B) dapsone 5 mg/kg group; and (C) dapsone 12.5 mg/kg group. The necrotic area was defined by a dark color, edema, and eschar formation, and the total flap area was delineated by the surgical borders. Lower panel: The survival rate of the skin flap is quantified as the percentage of necrosis over the area of the surgical flap. Administration of dapsone 12.5 mg/kg had a significant effect on the flap necrosis (*P* < 0.001). Notes: ns: Non-significant; ***P < 0.001.

(Figure 5A). ANOVA analysis also confirmed statistically significant differences in TNF-α expression between the study groups (*F*[3,8] = 16.06, *P* = 0.001) (Figure 5B). As expected, induction of I/R injury increased the expression of TNF-α in the skin flap (*MD* = 26.48, *P* < 0.01). Notably, both doses of dapsone significantly reduced the expression level of TNF-α (*MD* = 19.35, *P* = 0.008; *MD* = 24.38, *P* = 0.002; respectively) (Figure 5B). Interestingly, the effect

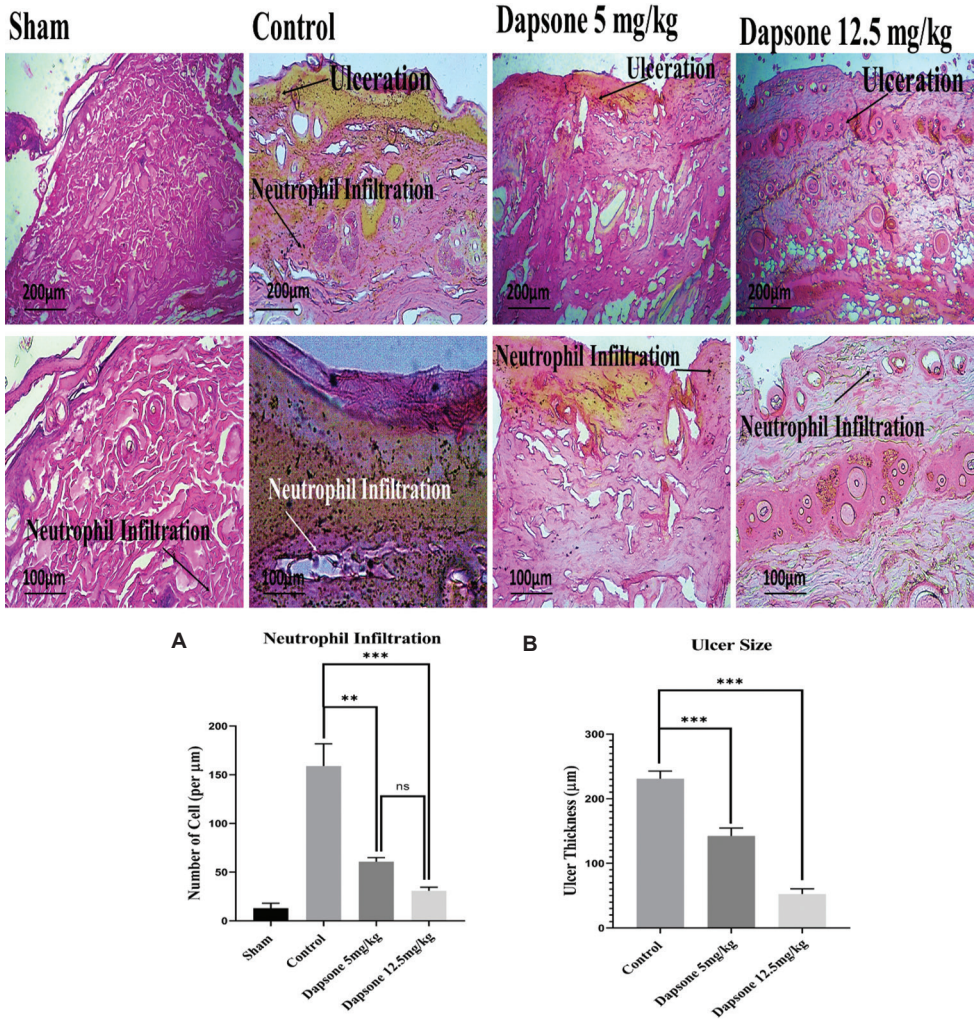


Figure 3. Neutrophil infiltration and ulceration following an ischemia/reperfusion (I/R) injury. Upper panel: Histopathological evaluation of skin flaps using hematoxylin and eosin staining on day 7 following surgery. The ulcerated area and infiltration of neutrophils are indicated by arrows. Scale bars: 200 μm, magnification ×40; 100 μm, magnification ×100. Lower panel: (A) Comparison of neutrophil infiltration among the groups, quantified as the number of cells per μm. As expected, I/R injury increased neutrophil infiltration. Both doses of dapsone inhibited neutrophil infiltration. (B) Comparison of ulceration among the groups. The mean of ulceration thickness (μm) in each group is compared between groups. Administration of dapsone in both doses ameliorated ulceration thickness significantly ($P < 0.001$).
Notes: ns: Non-significant; $^{**}P < 0.01$, $^{***}P < 0.001$.

of dapsone on the inhibition of TNF-α did not exhibit dose dependence ($P = 0.65$).

4. Discussion

This study, for the first time, evaluates dapsone as a possible therapeutic approach to flap survival in a well-established experimental model of a random-pattern skin flap. Interestingly, we observed that dapsone promoted flap survival following induction of I/R injury. Although the decrease in flap necrosis was not statistically significant at the dose of 5 mg/kg regarding macroscopic evaluation, a histopathologic study of dermal ulceration suggested favorable outcomes at both doses. Moreover, we employed inflammatory and angiogenetic examination on flap survival, following the trend among experimental skin flap models,[36] in an effort to explore the therapeutic mechanisms of dapsone. In summary, our results suggested inhibition of neutrophil infiltration, TNF-α production, and an increase in VEGF expression following 5 days of treatment with dapsone after the initial I/R injury in our skin flap model.

The employment of pharmacological strategies serves as a pivotal methodological avenue for researchers seeking to augment their comprehension of the fundamental mechanisms governing the viability of skin flaps subsequent to I/R injury. Various pharmacological agents

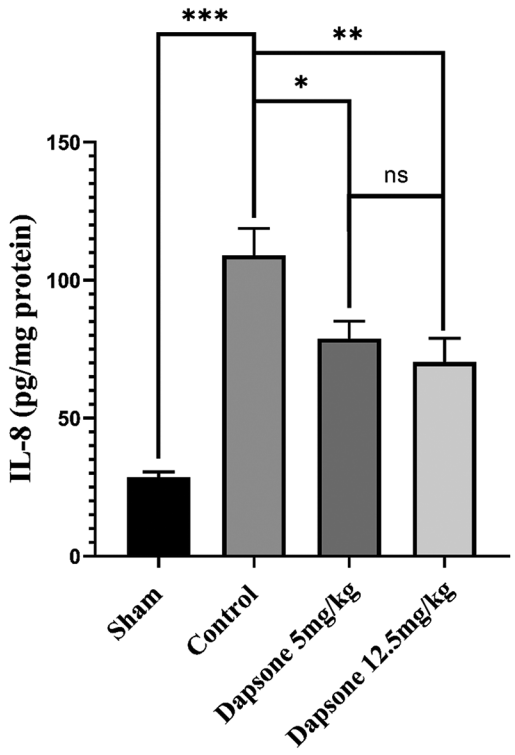


Figure 4. Assessment of interleukin (IL)-8 levels in the skin flaps using enzyme-linked immunosorbent assay. The mean level of IL-8 (pg/mg protein) is compared among the study groups. Performing a random-pattern skin flap increased the levels of IL-8. Administration of dapsone in both doses decreased the levels of IL-8 significantly. The effect of dapsone on IL-8 was not dose-dependent.
Notes: ns: Non-significant; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

have been suggested as exhibiting promising outcomes in promoting skin flap survival. Notably, modafinil has been demonstrated to mitigate necrotic manifestations within this context by activating ATP-sensitive potassium channels, thereby enhancing the anti-inflammatory properties inherent to the nitric oxide (NO) pathway.[37] In a parallel vein, an alternative investigation has propounded the favorable efficacy of ivermectin through modulation of gamma-aminobutyric acid receptors, subsequently leading to the downregulation of the TNF-α/IL-1β inflammatory cascade.[38] It has been demonstrated that the survival of skin flaps following I/R injury depends on factors including oxidative stress, neutrophil infiltration, angiogenesis, and a proinflammatory cytokine response. It is worth mentioning that sometimes it is hard to differentiate the causational outcomes of these factors from one another as one may also contribute to other pathways as well.[9,39] Neutrophil infiltration or influx is widely known as a critical feature of I/R injuries in general and skin flaps in particular. The initial release of reactive oxygen species in the skin flap I/R injury leads to chemotaxis and activation of neutrophils and, therefore, the production of free radicals and additional apoptosis.[36,40] Moreover, experimental studies of I/R injury have revealed that TNF-α is a key component of inflammation-mediated neutrophil migration, activation, and, consequently, the progression of the inflammatory response.[41-43] Expression of TNF-α not only leads to more cumulative apoptosis following I/R injury but also potentially enhances the expression of adhesion markers, chemokines, and cytokines, which lead to more infiltration of neutrophils into the injured site.[44]

On the other hand, the application of dapsone has exhibited anti-inflammatory properties over recent decades. Conventionally, dapsone is well-known for its anti-microbial and anti-protozoa effects. However, its clinical application in autoimmune settings has spurred the exploration of anti-inflammatory properties targeting both molecular and cellular elements of inflammation.[45] Indeed, the potential of dapsone to affect specific elements of inflammation led us to formulate our hypothesis. Modschiedler *et al.* have the first to conclude that dapsone could suppress the adhesion of neutrophils to epidermal binding sites.[46] Subsequent research revealed that dapsone not only inhibits the chemotaxis of neutrophils but also reduces neutrophil activity and functionality through an intracellular calcium-dependent signaling pathway.[47,48] As mentioned earlier, IL-8 is widely recognized as a potent chemotactic agent that facilitates neutrophil infiltration and activation. IL-8 also mediates the production and release of other cytokines, including TNF-α, by activated neutrophils. Previous experimental and clinical studies have revealed that dapsone inhibits the secretion of IL-8, resulting in the suppression of neutrophil infiltration.[44,49] In this study, dapsone exhibited a similar effect on the IL-8 levels, consistent with predictions from previous studies. Our results indicated that IL-8 levels were in alignment with neutrophil infiltration and TNF-α expression among study groups.

Moreover, the administration of dapsone for treating cutaneous lupus erythematosus patients has been observed to significantly inhibit the production of TNF-α.[29,50,51] Subsequently, it was also revealed that dapsone exhibits anti-TNF-α properties in other inflammatory dermal conditions.[52,53] For instance, although the etiology of necrobiosis lipoidica remains unclear, clinical research suggests that the administration of dapsone exhibits effectiveness and is accompanied by a decrease in dermal ulceration.[54,55] In addition, an *in vitro* assessment of lipopolysaccharide-induced inflammation has confirmed that dapsone inhibits the production of TNF-α in isolated bone marrow cells.[56] Interestingly, in alignment with the aforementioned underlying inflammatory mechanism of

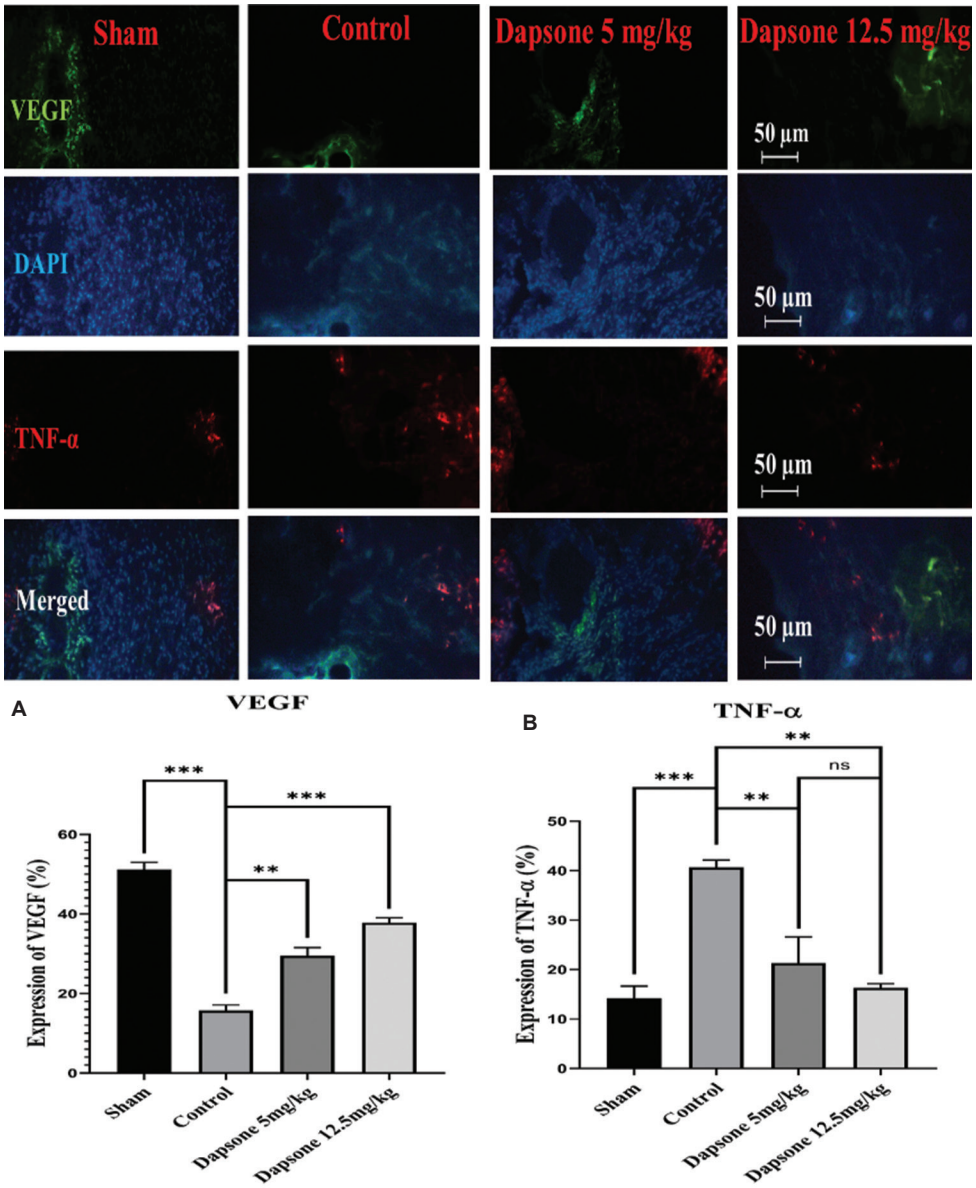


Figure 5. Expressions of vascular endothelial growth factor (VEGF) and tumor necrosis factor-alpha (TNF-α) in skin flaps following an ischemia/reperfusion (I/R) injury. Upper panel: Immunohistochemical assessment of VEGF and TNF-α on day 7 post-operation. Scale bars: 50 μm, magnification ×400. Lower panel: Mean VEGF and TNF-α expression comparisons for each group. As expected, induction of I/R injury and the ensuing inflammation reduced VEGF levels (A) while promoting TNF-α levels (B) in the skin flap. Oral administration of dapsone at either 5 or 12.5 mg/kg significantly ameliorated these trends in both markers. However, the effect of dapsone on TNF-α did not exhibit dose dependency.
Notes: ns: Non-significant; $^{**}P < 0.01$, $^{***}P < 0.001$.

I/R injury in skin flaps, it has been suggested that dapsone may provide protective effects against inflammation in kidney[57] and cardiac[58] I/R injury settings through the downregulation of TNF-α, oxidative stress, and neutrophil activity. In our study, we observed that the effects of dapsone on TNF-α levels, neutrophil infiltration, and, correspondingly, dermal necrosis and flap survival are consistent with previous findings. However, the precise mechanism of dapsone's anti-inflammatory properties remains unclear. Other studies have concluded that the anti-inflammatory effects of dapsone correspond to the inhibition of NF-κB and substantial downregulation of downstream cytokine production.[32]

In addition, our histopathological and IHC assessments indicated an increase in angiogenesis and VEGF expression

levels in our treatment groups. Although the precise impact of dapsone on angiogenesis remains poorly understood, our results suggest a dual effect: inhibition of excess inflammation and neutrophil infiltration on the one side, and enhancement of angiogenesis on the other. There is solid evidence supporting the role of dapsone in the inhibition of progressive inflammation and cytokine production.[59-61] Numerous studies have sought to explore the underlying mechanisms of wound healing and the formation of scar tissue and fibrosis as undesirable outcomes of impaired healing. Various factors contribute to regulating the process of appropriate healing and minimizing scar formation. The previous research has indicated that a balanced interplay between inflammation and angiogenesis plays a critical role in preventing impaired wound healing. For instance, persistent neutrophil infiltration and overexpression of TNF-α not only impede angiogenesis and downregulate VEGF expression but also decrease the viability and survival of keratinocytes.[24,62,63] Similarly, our results also suggest a correlation between prolonged inflammation and the status of angiogenesis.

Before drawing a conclusion, several factors should be taken into consideration. First, dapsone is associated with adverse side effects, including hematologic (methemoglobinemia and hemolysis), dermatologic (exfoliative dermatitis and erythema multiform), neurologic (peripheral neuropathy), and gastrointestinal (anorexia and nausea/vomiting) effects.[45] Second, patients with peripheral vascular disease (e.g., Buerger's disease) are known to possess a higher risk of flap surgery failure. It has been suggested that diabetic or atherosclerotic patients may benefit from preconditioning to mitigate their risk of flap failure. Pharmacologic or ischemic preconditioning, alone or in combination, could serve as a therapeutic approach for patients with compromised perfusion and, consequently, an increased risk of flap failure.[64-66] However, to translate pharmacologic agent studies into clinical practice, large-scale animal studies involving various flap types and sizes are required.

5. Conclusion

In this study, we explored the effects of dapsone on skin flap survival, focusing on its anti-inflammatory properties. Our results revealed that systemic administration of dapsone effectively prevented skin flap necrosis and ulceration. Subsequent assessments demonstrated that dapsone decreased neutrophil infiltration, IL-8 levels, and TNF-α expression, as well as enhanced the VEGF expression. However, future investigations are warranted to fully elucidate the promising anti-inflammatory effects of dapsone.

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Conflict of interest

The authors declare no conflicts of interest.

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Ethics approval and consent to participate

The study protocol was executed in agreement with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, Eighth Ed.) and institutional and governmental concerns for animal care and use (Approval ID: IR.TUMS.MEDICINE.REC.1398.922).

Consent for publication

Not applicable.

Availability of data

The datasets used during this study are available on reasonable request from the corresponding author.

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