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Itaconic acid induces angiogenesis and suppresses apoptosis via Nrf2/autophagy to prolong the survival of multi-territory perforator flaps

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ABSTRACT

Background: Perforator flaps are widely used in hand microsurgery to reconstruct and repair soft tissue injuries. However, ischemia and subsequent ischemia-reperfusion injury may cause distal necrosis of the flap. Itaconic acid (IA) is a modulator of macrophage function, which exerts anti-inflammatory effects in macrophage activation.

Methods: The necrotic area of the flap was detected by measuring the flap temperature with an infrared thermometer. Flap cell apoptosis was detected by TUNEL staining and Western blot analysis of the apoptosis-associated proteins Bcl-2 and Bax. HE staining was used to detect angiogenesis of the skin flaps. CD31 was detected to identify positive vascular expression, and the survival of choke vessels in different areas of the skin flap was assessed by arteriography. In addition, Western blot was performed to quantify the expressions of VEGF, Nrf2, LC3II, SQSTM1, and CTSD.

Results: Itaconic acid raises VEGF protein levels in skin flaps and the number of CD31-positive vessels. The skin flaps in the IA treatment group exhibited higher neovascularization and less necrosis than those in the control group. The results of TUNEL staining and Western blot revealed that Itaconic acid attenuated apoptosis in the ischemic area of flap. The combination of itaconic acid and Nrf2 inhibitor ML385 reversed this beneficial effect. The results revealed that itaconic acid attenuated apoptosis, enhanced angiogenesis, and enhanced autophagy.

Conclusion: In summary, our findings indicate that itaconic acid might be an effective treatment to reduce flap necrosis. Additionally, this study identified a novel mechanism for the effects of itaconic acid on flap survival.

1. Introduction

In recent years, perforator flaps have widely been used to repair soft tissue defects [1–4]. However, ischemic necrosis of the distal flap portion remains a commonly encountered complication in clinical practice. Skin flap necrosis might necessitate reoperation,

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prolonging the length of hospital stay, and increasing the treatment cost. Therefore, improving the flap's survival area is a clinically relevant goal. Although delayed flap procedures have been proven to enhance the flap's viability in clinical practice, this protocol also requires a second procedure. Experimental studies on multiple pharmacological reagents have also been performed, aiming to improve skin flap survival [5,6]. However, the clinical application of some of these drugs is restricted due to undesirable side effects, medication costs, and limited availability.

Macrophages promote inflammation and are involved in the formation of blood vessels and lymphatic vessels [7–9]. Furthermore, innate immunity is mediated by macrophages. They respond quickly to pathogens, limit damage, and participate in tissue repair by promoting an anti-inflammatory phenotype [10,11]. Macrophages can promote lymphangiogenesis through paracrine and trans-differentiation into lymphatic endothelial cells [12–14]. Recent studies have found that human macrophages produce itaconic acid, which is synthesized by an enzyme encoded by the IRG1 gene [15,16].

The gene Irg1 encodes an enzyme that decarboxylates *cis*-aconitic acid, an intermediary in the tricarboxylic acid cycle, to generate itaconic acid (also known as methylenesuccinic acid) [16]. Itaconic acid is an organic substance inhibiting the activity of isocitrate lyase, a crucial enzyme in the glyoxylate shunt route, which is required for bacterial growth under certain circumstances [15,17]. Vicky Lampropoulou et al. [18] showed that itaconic acid regulates macrophage metabolism and effector function by inhibiting succinate dehydrogenase-mediated succinate oxidation. Thus, itaconate exerts anti-inflammatory effects during macrophage activation and ischemia-reperfusion injury. However, the effect and molecular mechanism of itaconic acid on perforator flaps remains unclear.

Therefore, we hypothesize that itaconic acid may enhance multi-territory perforator flap survival by promoting autophagy, attenuating apoptosis and promoting angiogenesis. The purpose of this study is to investigate whether itaconic acid enhances the survival of multiple-territory perforator flaps and to identify the underlying molecular mechanisms.

2. Methods and materials

2.1. Experimental animals and ethics statement

Efforts have been made to reduce animal suffering and discomfort and to reduce the number of animals used. The Sprague-Dawley rats were individually housed under standard conditions: temperature of 22 °C, 60% humidity, and 12 h of light and dark cycle. Food and water were freely available to all Sprague-Dawley rats. The guidelines of the Central South University Animal Care and Use Committee Guide were followed during all procedures. In addition, the study procedures have been approved by the Animal Protection Association and Central South University's Ethics Committee.

2.2. Establishment of skin flap model

Adult male Sprague-Dawley rats weighing 200–250 g were obtained from the Department of Laboratory Animals of Central South University. Eighteen male Sprague–Dawley rats were randomly divided into 3 groups (n = 6): Control, IA and IA + ML385 groups. The Sprague-Dawley rats were anesthetized with 3% sodium pentobarbital intraperitoneally and had their backs shaved with electric shavers. A 12 cm by 3 cm multi-area perforator flap was created on the rats' dorsal side, which was pedicled with iliolumbar vessels. The thoracodorsal artery and intercostal artery were ligated and bridged by a single perforator. 4/0 Mousse was used to stitch the flap. Within seven days of the procedure, the intraperitoneal injection of 6 mg/kg IA was given to the IA group. A saline solution in the same concentration was given to the control group. In addition, in the IA and ML385 cotreatment group, the rats were injected with 6 mg/kg IA and 30 mg/kg ML385.

2.3. Macroscopic assessment of flap survival

On the seventh postoperative day, the appearance, color, and hair condition of the flaps were evaluated. Imaging software was used to evaluate flap survival from high-quality photographs of randomly patterned flaps. Two groups of flaps with different blood flow temperatures were analyzed using an infrared detector. Necrosis was defined as a flap area with a temperature at least 2° centigrade lower than the control group. The percentage of the viable area was calculated by the formula: viable area × 100%/total area (survival and necrotic area).

2.4. Hematoxylin-eosin staining

The flap was divided into area I, area II, and area III, arranged from proximal to distal in relation to the pedicle. Area I was the proximal area, area II was the intermediate area, and area III was the distal area. A sample of the area II was collected and stored in a 4% paraformaldehyde solution for 24 h. After paraformaldehyde fixation, the sample was dehydrated and paraffin-embedded. Subsequently, the Paraffin wax sample were sectioned into 4-μm-thick slices. The paraffin sections were then deparaffinized using xylene and rehydrated through graded alcohol solutions. In the end, the sample were stained with hematoxylin and eosin (H&E). The sections were then observed under a light microscope.

2.5. Western blot analysis

Immediately after the animals were sacrificed, the flap tissue of area II was collected and analyzed by Western blot. For Western blot analysis, each sample was treated with lysis buffer to release tissue proteins and the protein concentration was measured by a BCA method. Next, gel electrophoresis was used to separate the proteins, which were then electrotransferred to PVDF membranes and blocked with nonfat milk 5%. The samples were eluted with TBST three times and incubated at 4 °C after adding the primary antibody solution. The incubation concentration of the primary antibody is as follows: Bax (1:1000, Immunoway), Bcl-2 (1:1000, Immunoway), β-actin (1:5000, Immunoway), VEGF (1:1000, Immunoway), Nrf2 (1:1000, Immunoway), LC3B(1:2000, Abcam), SQSTM1/p62 (1:30,000, Abcam), CTSD (1:5000, Abcam), GAPDH (1:5000, Immunoway). Subsequently, secondary antibodies conjugated to HRP were incubated with the samples for 2 h at room temperature. Finally, an ECL kit was used to visualize the bands on the membrane, and the intensity of the bands was quantified using Image Lab software.

2.6. Immunohistochemistry

The paraffin-embedded tissue samples were deparaffinized in xylene and rehydrated with gradient ethanol. Following washing, the endogenous peroxidase was blocked using 3% hydrogen peroxide solution. Antigen retrieval was then performed with citrate buffer at 95 °C for 20 min. Subsequently, the specimens were blocked for 10 min with 10% bovine serum albumin PBS, incubated overnight with primary antibodies against CD31 (1:100) at 4 °C. Next, secondary antibodies conjugated to HRP were incubated with the samples. The sample slides were stained with a DAB detection kit and counterstained with hematoxylin. The stained samples were then photographed under a light microscope, and a software program was used to calculate the density of CD31-positive blood vessels.

2.7. TUNEL staining

Tissue sections were stained using the TUNEL method. The paraffin-embedded tissues were dewaxed with xylene and were soaked in gradient ethanol of decreasing concentration for rehydration. The sections were washed with PBS, proteinase K was added, and the specimens were incubated at room temperature for 15–30 min. The proteinase K reagent was then washed with PBS. TUNEL detection solution was prepared with TdT Enzyme and FITC-12-DUTP Labeling MIX. The TUNEL detection solution was added to each sample

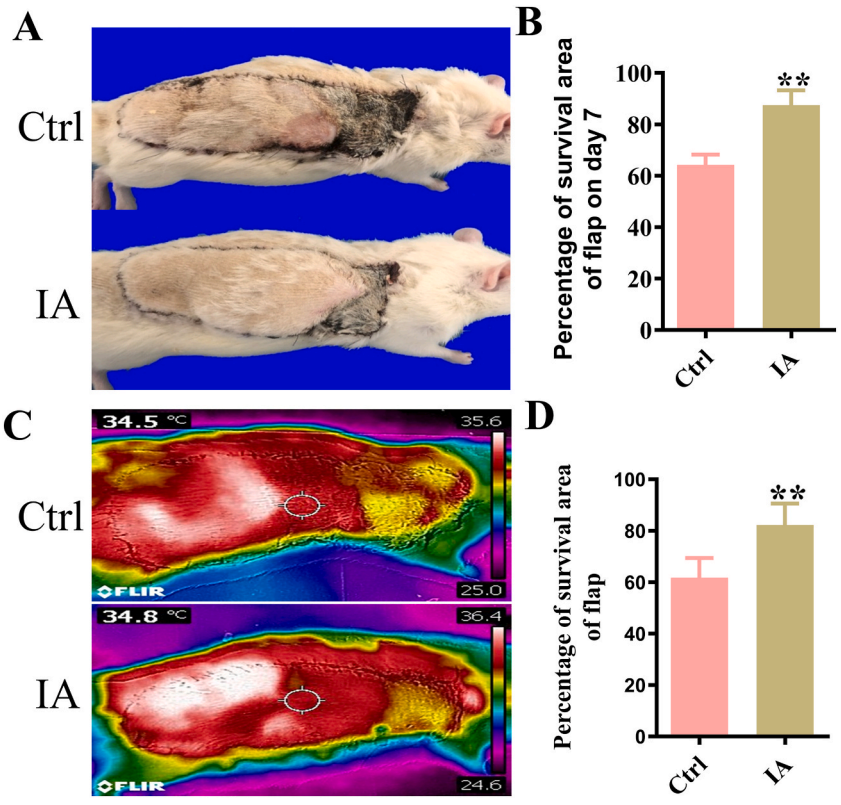

Fig. 1. IA improved multiterritory perforator flaps survival. **(A)** Image of the skin flaps taken on postoperative day 7. **(B)** Survival percentages. **(C)** Infrared detector was used to analyze the blood flow temperature of flaps. **(D)** Percentage of survival area of the flap. Values are represented as mean ± SEM, n = 6 per group. Significant differences between the groups are indicated by **P < 0.001.

and incubated at 37 °C for 60 min in the dark. After incubation, the samples were washed with PBS and DAPI staining solution was added for 5–10 min at room temperature. Then, the DAPI staining solution was aspirated and the specimens were washed 2–3 times with PBS. Finally, the slides were mounted with antibody fluorescence attenuation mounting medium. The tissue sections were then observed under a fluorescence microscope.

2.8. Angiography

Angiograms were performed seven days after surgery on the six rats of each group. First, 5 g of gelatin was dissolved in 100 mL saline solution at 40 °C, and 80 mg water-soluble lead oxide was added. The gelatin and lead oxide mixture was then injected into the rat's carotid artery until a red tinge was observed on the extremities of the rat. The flaps were then fixed at 4 °C for 24 h and photographed with an X-ray machine.

2.9. Statistical analysis

All statistical analyses were performed using GraphPad Prism 9 software. Experimental data were analyzed by using Student's t-tests or one-way ANOVA. P values < 0.05 were considered statistically significant.

3. Results

3.1. Itaconic acid improved multiterritory perforator flap survival area

All rats underwent flap surgery and recovered without developing any postoperative infection. On the seventh postoperative day, a distinct line separated the viable and necrotic areas of the flaps. Fig. 1 displays the percentages of viable flap areas in the control and IA

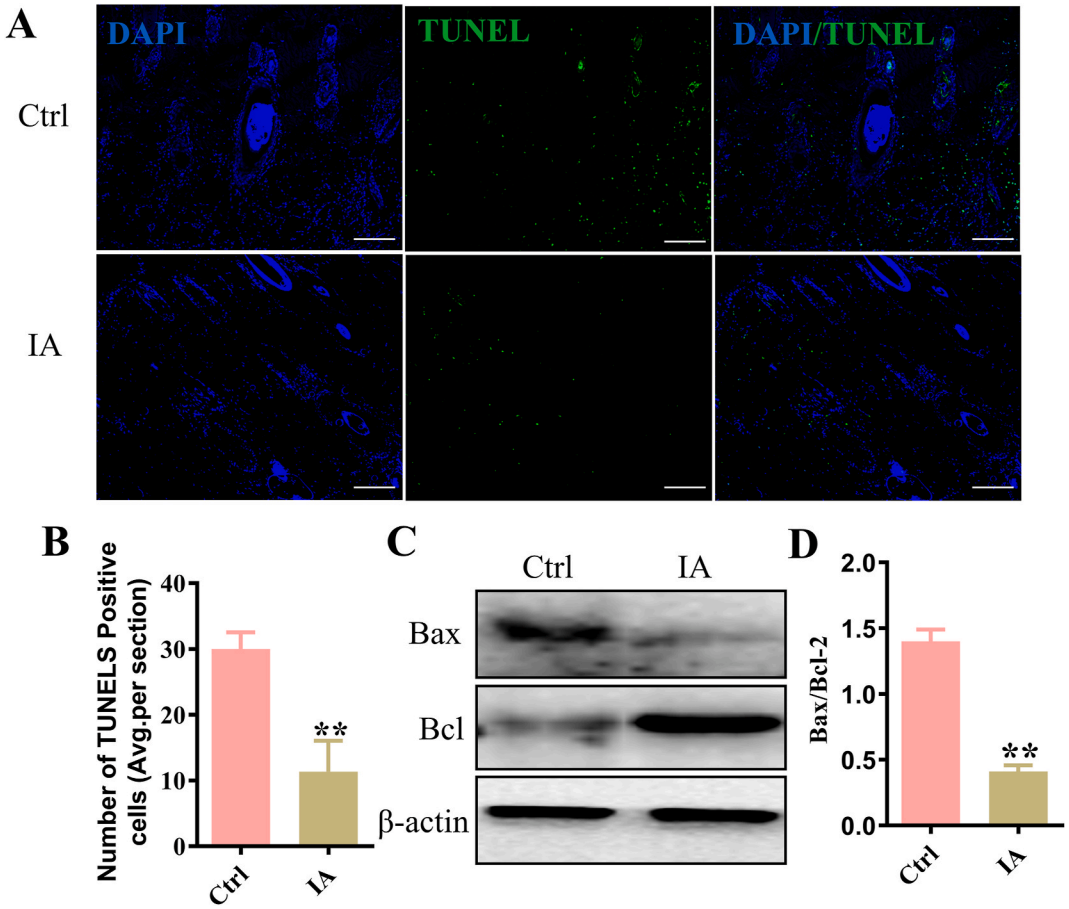

Fig. 2. IA attenuated apoptosis in the ischemic area of the flap. **(A)** TUNEL staining was used to assess apoptosis. Scale bars are 10 μm. **(B)** Quantitative estimation of the apoptotic and TUNEL-stained cells. **(C)** Representative western blots and quantification data of Bax, Bcl-2, and β-actin in each group. **(D)** The ratio of Bax/Bcl-2; the data were represented as mean ± SEM, *P < 0.05 versus the control group, n = 6.

groups. The mean surviving areas were 87.50 ± 2.344% and 64.2 ± 1.64% in the IA and control groups, respectively. The flap survival area in the IA group was considerably larger than in the control group (Fig. 1A and B; $p < 0.001$). Additionally, the blood flow temperature analysis revealed that necrosis was present in the areas of flaps with temperatures less than 2° centigrade. The percentage of flap survival regions in the IA group (82.2 ± 3.39%) was significantly greater than in the control group (61.7 ± 3.13%; Fig. 1C and D; $p < 0.05$), indicating that IA treatment might increase flap survival area.

3.2. Itaconic acid attenuated apoptosis in the flap ischemic area

The Western blot analysis of Bax and Bcl-2 and the TUNEL test were performed to evaluate the effect of IA on cellular apoptosis in the ischemia area of the flap. The TUNEL stain results are displayed in Fig. 2A and B, showing significantly more apoptotic cells in the control group than in the IA group. Additionally, the Western blot analysis revealed significant downregulation of the proapoptotic protein Bax, whereas the antiapoptotic protein Bcl-2 was significantly upregulated. The data also showed that IA therapy resulted in a decrease in the Bax/Bcl-2 ratio (Fig. 2C and D). The above findings suggested that IA injection successfully reduced apoptosis in the ischemia region of the flap.

3.3. Itaconic acid promoted angiogenesis in the ischemic area of the flap

On histological analysis, the mean vessel densities of area II and area III was noticeably higher in the IA group than in the control group (Fig. 3A). The skin flaps in the IA treatment group exhibited higher neovascularization and less necrosis than those in the control group (Fig. 3A). According to the H&E staining results, mean vessel densities (MVDs) were 18.50 ± 1.75 and 8.3 ± 1.14, respectively (Fig. 3B; $p < 0.001$). Endothelial cells frequently express the marker CD31. Therefore, the quantity of CD31-positive vessels directly reflects the MVDs of the two groups. In the multiterrory skin flap model, a higher number of CD31-positive vessels was found in the IA group compared to the control group (16.33 ± 2.24 vs. 9.17 ± 1.14, $p < 0.05$), as shown in Fig. 3D.

Previous research showed that choke vessels at the boundaries of the vascular territory provide blood and nutrients to the distal part of the skin flaps. On the seventh postoperative day, a whole-body angiography was carried out to visualize the vascular networks in the skin flap. The flaps in the two groups had clear, well-perfused microvessels. The microvascular imaging ranges of area II and area

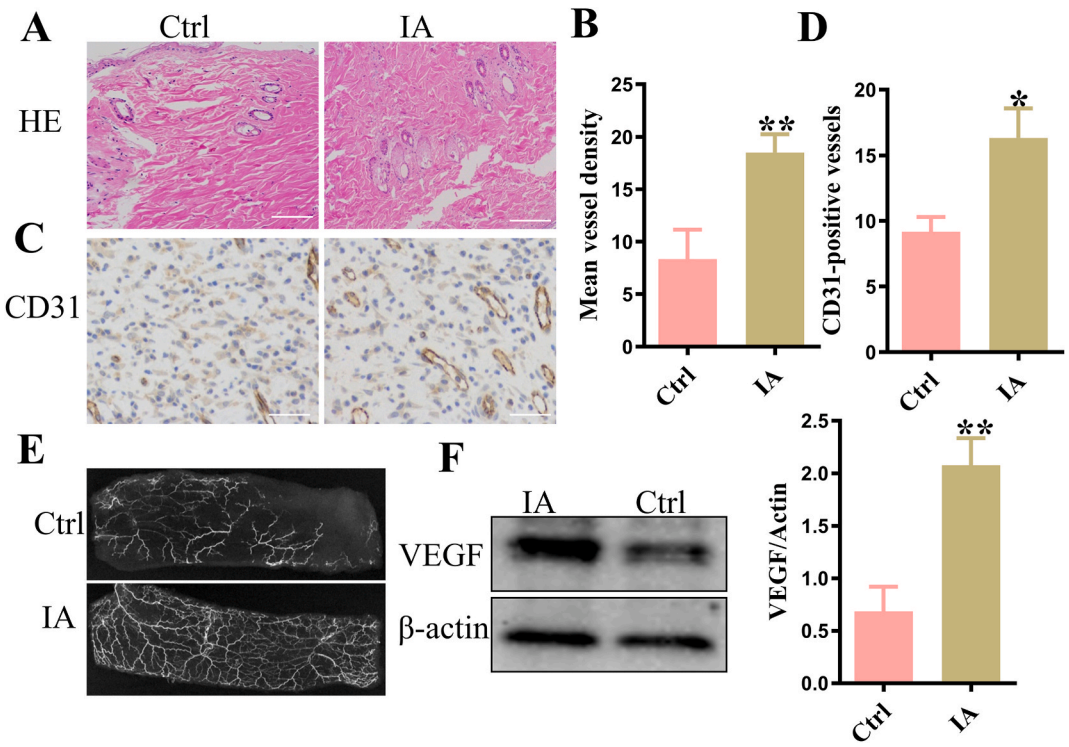

Fig. 3. IA promoted angiogenesis in the ischemic area of the flap. **(A)** On the 7th postoperative day, angiogenesis in the ischemic flap areas was evident by H&E staining (original magnification × 100). Scale bar: 100 μm. **(B)** MVDs assessed by H&E staining. **(C)** Immunochemical detection of CD31, which is a marker of angiogenesis (original magnification × 400). Scale bar: 25 μm. **(D)** The numbers of CD31-positive vessels. Values are represented as mean ± SEM, n = 6 per group. Significant differences between the groups are indicated by *P < 0.001. Postoperative angiography showed the lateral thoracodorsal (TD), posterior intercostal (IC) and iliolumbar (IL) vascular territories. Significant enhancement in dilation of choke vessels was observed in the second choke zone. The vasculature was invisible in the necrosis area. **(F)** Representative western blots and quantification data of VEGF and β-actin in each group; Columns represent mean ± SEM, **P < 0.01 versus the control group.

III were substantially wider in the IA group than in the control group. The findings demonstrated that the iliolumbar artery had expanded its territory to serve surrounding vascular territories in the IA group as compared to the control group, resulting in greater dilatation of the choke vessel zone I. According to angiography, the choke vessels in zone II of the IA group were significantly dilated, which was not observed in the control group. However, there was a noticeable improvement in the dilation of choke arteries in the second choke zone (Fig. 3E).

IA raises VEGF protein levels in skin flaps. The second choking zone was collected and a Western blot analysis was performed to investigate the impact of IA on VEGF protein expression. In comparison to the control group, both IA groups had significantly higher levels of VEGF protein (Fig. 3F; $P < 0.001$).

3.4. Itaconic acid improved the survival of multiterritory perforator flap via the Nrf2/autophagy signaling pathway

Rats were treated with Nrf2 inhibitor ML385 to test the pro-angiogenic ability of Itaconic acid through the Nrf2/Autophagy signaling pathway. Western blot analysis was used to evaluate the Nrf2 levels. The findings showed significantly higher levels of Nrf2 protein in the IA group compared to the control group and IA + ML385 group (Fig. 4A; P 0.05). Furthermore, the expression of proteins was related to autophagy in tissues. In this investigation, the autophagosomal (LC3II), lysosomal (CTSD), and autophagic substrate (SQSTM1/p62) proteins were primarily examined. The LC3II protein levels were significantly higher in the IA group than in the control group. Additionally, inhibiting the Nrf2 protein might lower the expression level of the LC3II protein (Fig. 4B; $P < 0.05$). The integral absorbance of the proteins SQSTM1/p62 and CTSD was then examined. Western blotting revealed that the level of p62 was lower in the IA group than in the control group, while the CTSD levels were greater in the IA group (Fig. 4C).

The VEGF, Bax, and Bcl-2 levels were detected by Western blot analysis, further supporting the hypothesis that IA increased angiogenesis and suppressed apoptosis by activating the Nrf2/Autophagy signaling pathway. The MVDs were also assessed by IHC, and the TUNEL test was performed. The experiment results demonstrated that Nrf2 protein inhibition resulted in decreased angiogenesis and VEGF protein downregulation (Fig. 5A and B). Similarly, following treatment with the Nrf2 inhibitor ML385, the proapoptotic protein Bax was elevated, the antiapoptotic protein Bcl-2 was downregulated, resulting in an increased Bax/Bcl-2 ratio (Fig. 5E). According to the TUNEL labeling, the IA + ML385 group had a considerably larger number of apoptotic cells (Fig. 5C). Inhibiting Nrf2 protein reversed the improvement in choke vessel dilation (Fig. 5D). These findings indicated that IA might activate the Nrf2/Autophagy signaling pathway to promote angiogenesis and reduce apoptosis in the ischemic region. Additionally, blocking Nrf2 protein reversed the effects of IA on flap viability (Fig. 6).

Collectively, these findings showed that the Nrf2/Autophagy signaling pathway was extensively activated in the ischemia flap area in response to IA, which was reversed upon administration of AKT inhibitor ML385.

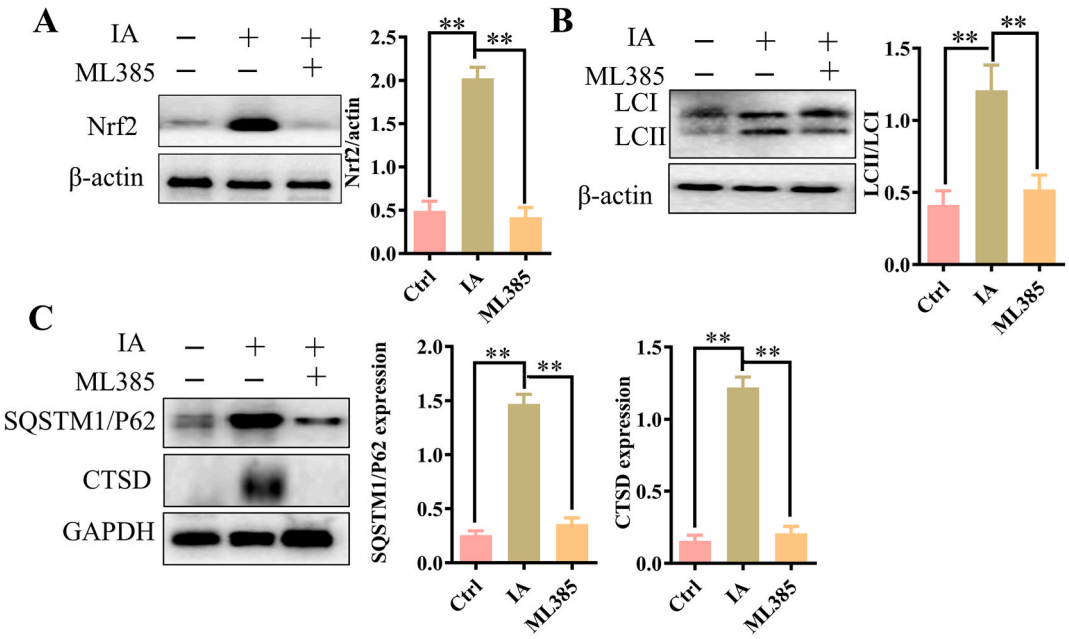

Fig. 4. IA improved the survival of skin flap via the Akt/Nrf2 signaling pathway. **(A)** The Nrf2 expression was assessed by Western blot analysis; Columns represent mean ± SEM, **P < 0.01 versus the control group or IA + ML385 group; **(B)** The LCII expression was assessed by Western blot analysis; Columns represent mean ± SEM, **P < 0.01 versus the control group or IA + ML385 group; **(C)** Representative western blots and quantification data of SQSTM1/P62, CTSD, and GAPDH in each group; Columns represent mean ± SEM, **P < 0.01 versus the control group or IA + ML385 group.

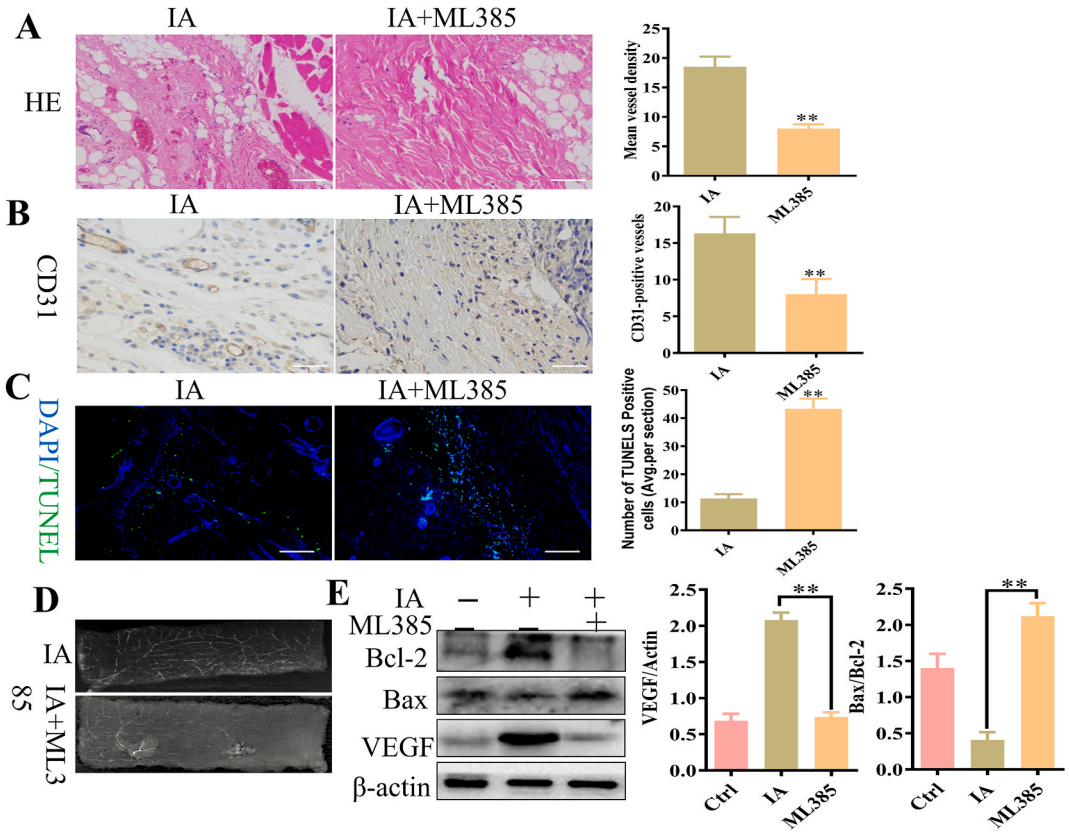

Fig. 5. Inhibition of Nrf2 protein reversed the effects of IA on apoptosis and angiogenesis in the ischemic area of the flap. **(A)** Neovascularization in the IA and IA + ML385 groups by H&E staining (magnifcation × 100). Scale bar: 100 μm. Columns represent mean ± SEM, **P < 0.01 versus the IA + ML385 group. **(B)** CD31-positive vessels in the IA and IA + ML385 groups as assessed by immunohistochemistry (magnifcation × 400). Scale bar: 25 μm. Columns represent mean ± SEM, *P < 0.05 versus the IA + ML385 group. **(C)** TUNEL staining to assess apoptosis at 7 days. Columns represent mean ± SEM, *P < 0.05 versus the IA group. Scale bars are 10 μm. **(D)** Postoperative angiography showed vascular territory of lateral thoracodorsal (TD), posterior intercostal (IC) and iliolumbar (IL). **(E)** The expressions of angiogenesis-related protein VEGF and apoptosis-related protein Bax, Bcl-2 in each group by Western blot analysis. Columns represent mean ± SEM, **P < 0.01 versus the IA group.

4. Discussion

Itaconic acid is induced by LPS, at least in part through type I IFN receptors, and through Nrf2 activation. Itaconic acid supports anti-inflammatory programs [19,20] and may reduce inflammation by blocking succinate dehydrogenase in lipopolysaccharide-activated macrophages [18]. This study explored the possible molecular mechanism of itaconic acid on wound healing of multi-territory perforator flaps.

Perforator flaps are one of most popular procedure for reconstructive surgeons to repair soft-tissue defects caused by trauma or tumor ablation. However, Ischemic necrosis of the flaps is a common complication and often results in significant cosmetic and functional defects. This study detected the effects of itaconic acid-treatment on the viability of skin flap, our study provides new evidences that itaconic acid improves flap survival by promoting angiogenesis and inhibiting apoptosis. This process might be involved in the Nrf2/autophagy signaling pathway. Our results not only suggest that itaconic acid may be an effective treatment for promoting flap survival, but also provide new insights into the pathway involved in activating autophagy. Our results indicated that itaconic acid might provide potential therapeutic intervention for improving the multi-territory perforator flap survival.

Apoptosis is a form of programmed cell death responsible for the removal of damaged cells in an efficient and orderly manner, such as active cell death brought on by DNA damage or during growth and development. Apoptosis affects variables that encourage cell division, tissue regeneration, and wound healing, as well as tissue homeostasis and human health [21]. It is mainly triggered by the intrinsic and extrinsic pathways [22]. The intrinsic apoptosis process, also referred to as the mitochondrial pathway, is mediated by intracellular signals that react to various stress situations, including severe oxidative stress, hypoxia, extremely high cytosolic Ca concentrations, and irreversible genetic damage [23].

Subsequent activation of the pro-apoptotic proteins Bax and Bak in the Bcl-2 family neutralizes the anti-apoptotic proteins bcl-2, bcl-xl and Mcl-1, leading to changes in mitochondrial membrane permeability. This allows proteins normally present within the intermembrane space to diffuse into the cytosol [22,24]. TUNEL staining was used to explore the effect of itaconic acid on cell

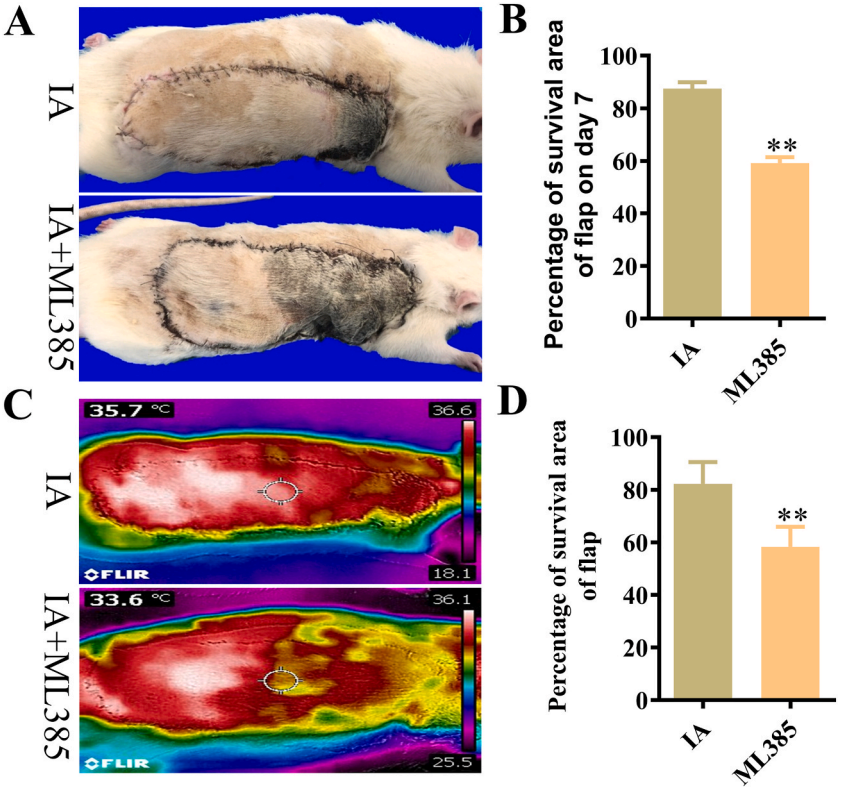

Fig. 6. Inhibition of Nrf2 protein reversed the effects of IA on flap survival. **(A)** The postoperative flaps of the IA and IA + ML385 groups on postoperative day 7. **(B)** The percentages of survival area in the IA group (88.17 ± 2.25%) and IA + ML385 group (59.21 ± 2.21%). Columns represent mean ± SEM, *P < 0.001 versus the IA + ML385 group. **(C)** Infrared detector was used to analyze the blood flow temperature of flaps. **(D)** Percentage of survival area of the flap. Columns represent mean ± SEM, *P < 0.001 versus the IA + ML385 group.

apoptosis, revealing fewer apoptotic cells in the itaconic acid-treated group. Then, Western blotting analysis showed that the Bax/Bcl-2 ratio in the itaconic acid treatment group was significantly lower than that in the control group, indicating that itaconic acid attenuated apoptosis. These results suggest that itaconic acid inhibits apoptosis and exerts a protective effect on multidomain perforator flaps.

Postoperative flap angiogenesis is a major factor in flap survival, and induction of angiogenesis is essential in improving flap survival. Vascular endothelial growth factor (VEGF) is necessary for embryonic development of the blood and lymphatic vasculature and is a master regulator of vascular growth in disease [25]. When expressed as a single factor, VEGF initiates a cascade of events from endothelial activation to the creation of new functional and stable vascular networks [26]. The effect of VEGF-A depends on the concentration of its local microenvironment, with low concentrations of VEGF reducing thrombosis and dilating blood vessels, while high concentrations of VEGF can promote angiogenesis [27]. H&E staining and immunochemical detection of CD31 were used to confirm angiogenesis, showing higher angiogenesis in the itaconic acid group than in the control group. Western blotting analysis showed that the VEGF levels in the itaconic acid treatment group was significantly higher than in control group.

Autophagy is a dynamic process of subcellular degradation that is critical for cell survival under nutrient-deficient conditions. During autophagy, parts of the cellular components are sequestered in double-membrane-bound vesicles called autophagosomes, which are subsequently degraded upon fusion with lysosomes to provide essential elements for maintaining cellular metabolism [28]. Phosphatidylethanolamine combines with LC3I in the cytoplasm to form LC3II, which can be used as an indicator of the level of autophagy [29]. The autophagy-related proteins LC3II and CTSD were detected by Western blot, showing higher levels in the itaconic acid treatment group compared to the control group. These results indicated that itaconic acid promotes flap survival by increasing autophagy. Autophagy can help cells overcome nutrient deficiencies, metabolic stress, and maintain energy balance [30]. Past studies have shown that the activation of autophagy enhances angiogenesis in endothelial cells through ROS generation and activation of AKT [29]. Increased autophagy has protective effects under hypoxic and chronic ischemic conditions [29]. Induction of autophagy promotes angiogenesis, while inhibition of autophagy inhibits angiogenesis, including vascular endothelial growth factor-induced angiogenesis [29]. Therefore, autophagy is beneficial for flap survival under hypoxic and chronic ischemic conditions. Autophagy promotes angiogenesis by inducing pro-vascular endothelial growth factor. Autophagy induction may be a potential treatment for oxidative stress-mediated injuries, including ischemic flap necrosis.

This study demonstrated that itaconic acid promotes flap survival in mice via the Nrf2 pathway. The Nrf2 inhibitor ML385 was used in combination with itaconic acid to intervene in rats, which reversed the effect of itaconic acid on flap survival. Our findings suggest

that itaconic acid is a potent pro-angiogenic agent that acts through NRF2 to reduce apoptosis and promote the expression of the pro-angiogenic factor VEGF. Itaconic acid is produced by immune responsive gene 1 (IRG1)-coded enzyme in activated macrophages and known to play an important role in metabolism and immunity via regulating the NRF2. Nuclear factor E2-related factor 2(NRF2) is the most important transcriptional regulator in its regulation of stress response and maintains homeostasis of vascular endothelial cells. It has been demonstrated that NRF2 played an important role in angiogenesis and could up-regulate the expression of VEGF which was the most important factor in vascular remodeling. Our previous study also has presented that the protective effects on survival of multi-territory perforator flap via Nrf2-induced autophagy, inhibition of apoptosis and promotion of angiogenesis, thereby promoting the survival of multi-territory perforator flaps.

However, the limitations of this study should also be acknowledged. Only a single concentration of itaconic acid was investigated, and the optimal concentration for postoperative administration should be determined in further studies. In addition, research in larger animal models (as proof of concept for preclinical studies) would be beneficial for further translational research. Therefore, rabbits or pigs can be used in the future to verify the beneficial effects of itaconic acid on perforator flap survival.

In conclusion, our study provides new evidence that itaconic acid improves flap survival by promoting angiogenesis and inhibiting apoptosis. This effect is mediated by the activation of autophagy, which may be induced by triggering the Nrf2 signaling axis. Our results not only suggest that itaconic acid may be an effective treatment for promoting flap survival, but also provide new insights into the pathway involved in activating autophagy.

Author contribution statement

Liming Qing: Conceived and designed the experiments, Contributed reagents, materials, analysis tools or data. Panfeng Wu, Juyu Tang: Conceived and designed the experiments. Gaojie Luo: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Zekun Zhou, Chengxiong Huang, Peiyao Zhang, Nianzhe Sun, Wei Chen, Chao Deng,: Performed the experiments. Xiaoxiao Li: Conceived and designed the experiments.

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Ethics statements

The guidelines of the Central South University Animal Care and Use Committee Guide were followed during all procedures. In addition, the study procedures have been approved by the Animal Protection Association and Central South University's Ethics Committee.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e17909.

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