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Nicorandil reduces burn wound progression by enhancing skin blood flow

Jaehoon Choi[a], Ryeolwoo Kim[a], Junhyung Kim[a], Woonhyeok Jeong[a], Sang Woo Park[b], Hyoun Wook Lee[c], Ho Yun Chung[d,*]

[a] *Department of Plastic and Reconstructive Surgery, Keimyung University School of Medicine, 56 Dalseong-ro, Jung-gu, Daegu, 41931, Republic of Korea*
[b] *Department of Plastic and Reconstructive Surgery, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, 158, Paryong-ro, Masanhoewon-gu, Changwon, 51353, Republic of Korea*
[c] *Department of Pathology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, 158, Paryong-ro, Masanhoewon-gu, Changwon, 51353, Republic of Korea*
[d] *Department of Plastic and Reconstructive Surgery, School of Medicine, Kyungpook National University School of Medicine, 130 Dongdeok-ro Jung-gu, Daegu, 41944, Republic of Korea*

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Summary We assessed whether nicorandil, an adenosine triphosphate-sensitive K+channel opener, reduces burn wound progression in a rat comb burn model. A total of 24 rats were used. Following thermal injury, one dose of nicorandil (10 or 30 mg/kg) was administered intragastrically twice daily for 3 days. At days 1 and 3 after injury, skin was harvested for histopathological examination and protein isolation. Rats treated with the 10-mg/kg and 30-mg/kg doses of nicorandil exhibited significantly increased tissue survival in the zone of stasis at days 1 and 3 after injury. The 10-mg/kg and 30-mg/kg nicorandil doses also significantly increased skin perfusion in the zone of stasis at days 1 and 3 after injury. At 30 mg/kg, nicorandil significantly reduced hypoxia-inducible factor-1α expression in the zone of stasis at day 1 after injury and reduced inflammatory responses in the zone of stasis. The latter effect included decreased polymorphonuclear neutrophil leukocyte infiltration and interleukin-1β release at day 1 after injury. At 30 mg/kg, nicorandil also significantly reduced expression of nuclear factor-κB p65, akey transcriptional factor in the regulation of inflammatory mediators, in the zone of stasis at day 1 after injury. Our study demonstrates that a 30 mg/kg dosing schedule of nicorandil increases tissue survival in the zone of stasis by attenuating ischemia-reperfusion injury. This effect is mediated by the enhancement of skin blood flow and reduction in the inflammatory

* Corresponding author. Department of Plastic and Reconstructive Surgery, Kyungpook National University School of Medicine, 130 Dongdeok-ro Jung-gu, Daegu, 41944, Republic of Korea.
E-mail address: hy-chung@knu.ac.kr (H.Y. Chung).

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response. Therefore, our findings suggest that nicorandil has potential clinical applications for patients with burns.

Introduction

Burn wound progression occurs during the first few days after a burn injury. This progression can make the initial burn wound deepen and expand. Three concentric zones of burn wound tissue have been proposed, which describe this progressive damage: the central zone of coagulation, the intermediate zone of stasis, and the outer zone of hyperemia.[1] While the zone of coagulation is characterized by direct thermal-induced irreversible necrosis, the zone of hyperemia always spontaneously recovers. However, the zone of stasis has the potential to either progress to a necrotic state or to recover normally.[2] Therefore, the extent of burn wound progression is determined by the outcome of the zone of stasis. The zone of stasis is exposed to oxidative stress resulting from ischemia–reperfusion (I/R) injury, which itself is due to the hypoperfusion, microthrombosis, and vasoconstriction/vasodilatation caused by indirect thermal injury.[3-5] If the zone of stasis can be salvaged by an appropriate intervention that modulates I/R injury, burn wound progression can be prevented. Many groups have investigated the feasibility of preventing necrotic changes in the zone of stasis with antioxidant drugs,[6-10] anti-inflammatory drugs,[11,12] or mesenchymal stem cells.[4] Although these treatments can reduce the burden of oxidation products and proinflammatory cytokines, they are insufficient to inhibit or mitigate I/R injury because this injury is derived from an upstream regulatory pathway controlling oxidative stress. Nicorandil is a hybrid drug that combines an ATP-sensitive potassium (KATP) channel opener and a nitric oxide (NO) donor and has been widely used as an anti-anginal drug. Many studies have demonstrated that nicorandil exerts a protective effect against I/R injury to several organs.[13-16] In the skin, nicorandil has been shown to enhance the survival of ischemic skin flaps by attenuating I/R injury.[17] Therefore, the aims of this study were to evaluate whether nicorandil (1) increases tissue survival in the zone of stasis in a rat comb burn model; and (2) downregulates inflammatory mediators and/or nuclear factor-κB (NF-κB), a key transcriptional factor in the regulation of inflammatory factors that are involved in tissue reperfusion injury.[18]

Materials and methods

Animals

Adult male Sprague–Dawley rats weighing 300 to 325 g were purchased from ORIENT BIO (Seongnam, Republic of Korea) and maintained in the animal facility at Keimyung University. The University Animal Care Committee for Animal Research of Keimyung University approved the study protocol (KM-2014-52). Animals were housed for at least 7 days before initiating the experiments in a well-ventilated and temperature-controlled environment. All rats had access to drinking water ad libitum. A total of 24 rats were randomly divided into a vehicle control group (n = 8, dimethyl sulfoxide alone), a 10-mg nicorandil group (n = 8, 10 mg/kg dose of nicorandil), and a 30-mg nicorandil group (n = 8, 30 mg/kg dose of nicorandil). Nicorandil was dissolved in dimethyl sulfoxide and administered intragastrically immediately after creating the thermal injury and twice daily for 3 days, with the rationale that burn wound progression often occurs within 72 hours after the burn.[1] The dose and method of nicorandil administration were chosen based on a study by Qi et al.[17]

Rat burn injury model

The rats were anesthetized with Zoletil 50® (10 mg/kg given intramuscularly; Virbac Laboratories, Carros, France). Their dorsal skin was shaved using an electric clipper, after which a commercial depilatory cream was applied to remove all remaining hair. The back of each rat was then injured according to the comb-burn model described by Regas and Ehrlich.[19] This model employs a brass comb containing four rows (1 x 2 cm) and three interspaces (0.5 x 2 cm). While the interspaces are not directly injured, they undergo progressive ischemia within hours and are necrotic at 24–48 hours.[20] The interspaces thus represent the zone of stasis. Two comb burns were created on each rat, one on each side. Postoperative analgesia was ensured by administering 5 mg/kg of ketoprofen subcutaneously. No dressings or topical treatment were applied to any of the animals. Wounds were observed daily for evidence of necrosis in the unburned interspaces. At days 1 and 3 after burn injury, the interspace skin on each side (with or without burned tissue) was harvested from four animals in each group for histological examination and protein isolation, after which the animals were humanely euthanized.

Measurement of blood perfusion

At 2 h, 1 day, and 3 days after injury, laser Doppler flowmetry (LDF) was performed using a commercial Periflux 5000 probe 404 device (Perimed, Stockholm, Sweden). LDF was performed according to the instructions in the product specification booklet provided by the manufacturer. Briefly, specialized double-faced adhesive tape was used to bond the probe and the interspace skin, after which the perfusion unit (PU) value was read by the computer. The final PU value of the interspace skin consisted of the mean value from at least three random PU values within the wound. During measurement, room temperature was maintained at 23 ± 1 °C and humidity was $45\% \pm 3\%$.