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Metformin improves skin flap survival through nitric oxide system

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ABSTRACT

Background: Metformin has shown cardioprotective effects in experimental models of ischemia reperfusion, which is partially mediated through nitric oxide (NO) synthesis. We investigated the effects of metformin pretreatment in a rat model of random-pattern skin flap, and the probable role of NO system.

Materials and methods: In the first experiment, the rats received increasing doses of metformin (150, 200, and 300 mg/kg), 4 h before the procedure. Dorsal skin flaps with caudal pedicles were elevated at the midline and flap survival was measured 7 d after surgery. Pathologic review of the skin flap specimen was performed in a subset of animals. In the second experiment, for evaluation of the role of NO, an NO synthase inhibitor N-nitro-L-arginine methyl ester hydrochloride (L-NAME) was administered with and without the effective dose of metformin. In the next experiment, subtherapeutic dose of NO precursor, L-Arginine, was administered with and without subeffective dose of metformin.

Results: Metformin pretreatment at doses of 200 and 300 mg/kg significantly increased skin flap survival rate. However, administration of L-NAME abolished the protective effects of metformin. On the other hand, subtherapeutic dose of L-arginine augmented the effects of low-dose metformin and significantly increased skin flap survival. Skin flaps from those rats that received 300 mg/kg metformin pretreatment and those treated with subtherapeutic doses of L-arginine and metformin showed increased vasodilation compared with control group.

Conclusions: Metformin pretreatment can improve skin flap survival through an NO dependent pathway.

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1. Introduction

Random-pattern skin flaps are widely used for closure of defects in reconstructive surgeries. However, random-pattern skin flaps are vulnerable to necrosis, dehiscence, and failure due to their restricted circulation [1]. These complications may necessitate reoperation, prolong the hospital stay, and increase the number of outpatient visits. To improve skin flap survival, different agents have been used for pharmacologic preconditioning in skin flaps, including morphine [2], adenosine [3], cyclosporine [4], pioglitazone [5], and dexamethasone [6]. However, clinical application of some of these drugs is restricted due to undesirable side effects, medication costs, or limited availability.

Metformin is among the first-line treatments for managing type 2 diabetes and preventing its vascular complications [7]. The glucose-lowering properties of metformin is mainly due to inhibition of hepatic gluconeogenesis and increased insulin-stimulated peripheral glucose uptake [8]. However, the therapeutic effects of metformin are not limited to its ability to lower blood glucose as evidence supports direct vascular effects [9,10]. The UK Prospective Diabetes Study demonstrated that metformin treatment decreased the risk of macrovascular diseases by 30% compared with other treatment modalities [11].

The therapeutic effects of metformin have been reported to be mediated through activation of AMP-activated protein kinase (AMPK) [12], a protein kinase that is activated in response to alterations in cellular energy levels [13]. When the cell is exposed to energy depletion, AMPK downregulates ATP-using pathways and promotes ATP-generating pathways to restore energy homeostasis. AMPK is also involved in regulating many cellular functions including endothelial nitric oxide synthase activation, angiogenesis, and proliferation [14–16]. Thus, it has been suggested that the cardioprotective effect of metformin on the impaired vascular reactivity in diabetes mellitus are partially due to increased activity of NO synthase [17].

Prior studies have shown the cardioprotective effects of metformin preconditioning in animal models of myocardial ischemia [17–20]. Thus, in the present study, we investigated the effect of metformin preconditioning on random skin flap tolerance to ischemia in rats. We also evaluated the involvement of NO pathway in possible effects of metformin on skin flap survival by using the NO synthase inhibitor, (N-nitro-L-arginine methyl ester hydrochloride [L-NAME]) and the NO precursor, L-arginine. We designed this study to evaluate the dose-dependent effects of metformin preconditioning on random skin flap survival, and then to examine the role of NO synthesis inhibition *versus* promotion on metformin effects.

2. Methods and materials

2.1. Drugs

Drugs were obtained from the following sources: metformin (DarouPakhsh Pharmaceutical Co, Tehran, Iran); (L-NAME; Sigma, St. Louis, MO), L-arginine (Sigma–Aldrich, London, UK); ketamine HCl (Gedoon Richter Ltd, Budapest, Hungary); xylazine HCl (BayerAG, Leverkusen, Germany). Metformin, L-arginine, and L-NAME were dissolved in 0.9% saline solution.

2.2. Animals

Eighty male Sprague–Dawley rats weighing 220–250 g were randomly divided into eight groups. Only male rats were used in our study to avoid the hormonal interaction of estrogen, which can affect skin flap survival and wound healing [21,22]. The animals were obtained from the animal house of the Faculty of Medicine, University of Tehran. They were maintained on a 12 h light–dark cycle at a room temperature of 24°C–25°C, with free access to food and water. All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, revised in 1996) and were approved by the animal care committee of the university.

2.3. Study design

In the first experiment series, the effects of increasing doses of metformin on flap survival rate were investigated. In the first four groups, animals received normal 0.9% saline solution (control group), 150 mg/kg, 200 mg/kg, and 300 mg/kg of metformin, respectively—4 h before raising the flap. In the second experiment series, we investigated the role of NO system in metformin effects on flap survival through the blockage of NO production. In the fifth and sixth group, animals received effective dose of L-NAME (10 mg/kg) with and without the most effective dose of metformin, respectively. In the third experiment, we administered subtherapeutic dose of L-arginine (100 mg/kg) with and without subtherapeutic dose of metformin to investigate possible synergistic effect. L-NAME and L-arginine were administered 30 min before flap raising and 3.5 h after metformin injection. All injections were intraperitoneal. The medication dosage and premedication timings were in accordance with the established method in our laboratory and prior literature by other groups [4,5,23,24].

2.4. Procedure

Random-pattern skin flaps were performed as previously described [4]. Briefly, the operation was performed under general anesthesia induced by intraperitoneal injections of 90 mg/kg ketamine HCl and 9 mg/kg xylazine HCl—periodic supplemental doses of ketamine were given as needed to maintain anesthesia. Bipedicled dorsal skin flaps measuring 2 × 8 cm were elevated on the midline, with the palpable hip joints as anatomic landmarks to define the caudal margin of the flap. After the elevation, the flaps were inspected and direct axial or muscular perforating cutaneous blood vessels supplying the base were cut to ensure a random skin flap. To achieve permanent ischemia, the cranial pedicle was cut in all animals and then incision lines were sutured back with continuous 4/0 silk stitches. Meanwhile, the flaps were kept wet, using saline-moist sterile gauze. One week later, rats were anesthetized and digital images were obtained from the dorsal skin flaps. Survived surface areas were demarcated and