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Opening of calcium-activated potassium channels improves long-term left-ventricular function after coronary artery occlusion in mice

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ABSTRACT

Background: Opening of mitochondrial calcium-activated potassium channels (BK_{Ca}) reduces infarct size after myocardial ischemia/reperfusion injury (I/R). It is unknown if targeting BK_{Ca}-channels improves cardiac performance in the long-term after I/R.
Methods: Experiments were conducted in compliance with institutional and national guidelines in C57BL/6 mice ($n = 7–8$/group). Animals were randomized into two groups. Preconditioning was induced by intraperitoneal application of NS1619 (NS, 1 μg/g bw) 10 min before ischemia, control animals (Con) received the vehicle. All animals underwent 45 min of myocardial ischemia and four weeks of reperfusion. Transthoracal Echocardiography (TTE) was conducted one and four weeks after ischemia (TTEW1/TTEW4) and additionally a cardiac MRI was done in week four. At the end of experiments the infarction scar was determined by AZAN staining.
Results: TTE revealed that NS1619 improved ejection fraction one week (Con: 36 ± 4%, NS: 45 ± 4%; $P < 0.05$) and four weeks after I/R (Con: 33 ± 11%, NS: 46 ± 8%; $P < 0.05$). Preconditioning with NS1619 reduced end-diastolic volume at both time points (TTEW1: Con: 60 ± 12 μl, NS: 45 ± 8 μl; TTEW4: Con: 82 ± 31 μl, NS: 44 ± 8 μl; each $P < 0.05$) and increased fractional shortening after four weeks (TTEW4: Con: 12 ± 6%, NS: 24 ± 8%; $P < 0.05$). MRI-analysis after four weeks confirmed the echocardiographic results. NS1619 increased ejection fraction by 45% (MRI: Con: 29 ± 6%, NS: 42 ± 9%; $P < 0.05$ vs. Con) and reduced end-diastolic and -systolic volume (EDV, ESV) compared to control (MRI: EDV: Con: 110 ± 19 μl, NS: 88 ± 16 μl; ESV: Con: 79 ± 19 μl, NS: 53 ± 18 μl; each $P < 0.05$). Preconditioning reduced infarction scar after four weeks by 25% (Con: 12 ± 3%, NS: 9 ± 2%; $P < 0.05$).
Conclusions: Preconditioning by opening of BK_{Ca}-channels with NS1619 improves cardiac performance after four weeks of reperfusion and reduces myocardial infarction scar.

1. Introduction

Cardiovascular disease is the major cause of premature death in Western countries, of which ischemic heart disease is the main contributor [1]. In 1986 Murry discovered the phenomenon called ischemic preconditioning (IPC), whereby short sublethal periods of ischemia and reperfusion before a sustained myocardial ischemia reduce infarct size up to 75% [2]. Although there is strong evidence for the cardioprotective effect of IPC in various experimental settings [3–5], its translation to clinical efficacy has not been successful [6]. Preconditioning interventions are affected by numerous factors including comorbidities [6], medication [7] and age [8–10] all of which might contribute to the failure of translating these protective effects to the clinical practice.

We and others [8,11–15] have reported that calcium-activated potassium channels (BK_{Ca}) are critical factors for various cardioprotective interventions, e.g. ischemic and anesthetic pre- and postconditioning. These channels are located in the inner mitochondrial membrane in cardiomyocytes and are members of the voltage-gated K^+ channel superfamily. In addition, we previously demonstrated that direct pharmacological activation of BK_{Ca}-channels by NS1619 initiated a strong infarct size reduction not only in young but also in aged rat hearts in vivo [11]. This finding provides evidence that BK_{Ca}-channel opening

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could be a useful therapeutic strategy to reduce cardiac damage when administered in vivo clinically.

However, in addition to limiting initial myocardial necrosis, long-term preservation of cardiac performance after myocardial infarction is essential for reducing the course of disease. Adverse myocardial remodeling with the consequence of reduced cardiac function, left ventricular dilatation and the development of heart failure are the major clinical problems caused by myocardial ischemia and reperfusion injury. Therefore, it is even more important that a potential therapeutic strategy improves cardiac function and prevents the development of heart failure in the long-term after myocardial infarction than it reduces infarct size in the subacute phase after ischemia and reperfusion. Here, we aimed to investigate whether BK_{Ca}-channel opening by NS1619 before ischemia reduces adverse cardiac remodeling and preserves left ventricular function long term after myocardial infarction.

2. Methods

The current investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication number 85–23, revised 1996) and was performed after obtaining approval from the Animal Ethics Committee of the University of Düsseldorf, Germany. C57BL/6 mice (2–3 months, Janvier Laboratories), weighing 27 ± 1 g were housed on a 12:12 light/dark schedule with free access to standard chow and water. All chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany).

2.1. Surgical preparation

Mice were anesthetized by intraperitoneal injection of ketamine (50 mg/kg bw), medetomidine (0.2 mg/kg bw) and atropine (0.06 mg/kg bw). Mechanical ventilation was performed after endotracheal intubation and animals were placed on a heating plate to maintain body temperature at 38 °C throughout surgery. Heart rate was measured continuously by electrocardiography (ECG) and digitized using an analogue to digital converter (PowerLab/8SP, ADInstruments Pty Ltd., Castle Hill, Australia) at a sampling rate of 500 Hz. Data were continuously recorded on a personal computer using Chart for Windows v5.0 (ADInstruments Pty Ltd., Castle Hill, Australia). Hearts were exposed via left-sided mini-thoracotomy and pericardium was gently removed. A suture was placed around a major branch of the left coronary artery (LAD) and the ends of the suture were threaded through a propylene tube to form a snare. All animals were allowed to recover for 15 min before starting the experimental protocol. Myocardial ischemia was achieved by tightening the snare and successful coronary occlusion was verified by epicardial cyanosis, hypokinesia and ST-elevations in ECG-analysis. Subsequently, reperfusion started by opening the snare.

Preconditioning was induced by intraperitoneal administration of NS1619 (NS, 1 μg/g bw) 10 min before ischemia and control animals (Con) received the vehicle at the same time.

2.2. Short-term experiments

In the first set of experiments, acute infarct size reduction of preconditioning with NS1619 was confirmed in mice hearts in vivo. Animals were randomly assigned to 2 groups: Con (n = 7) and NS (n = 6), respectively. All mice underwent 45 min of regional myocardial ischemia followed by 120 min of reperfusion as illustrated in Fig. 1A. At the end of experiments, hearts were excised and infarct size was stained by Evan's Blue and TTC as described previously [16]. The area of risk and the infarcted area were determined by planimetry using SigmaScan Pro 5® computer software (SPSS Science Software, Chicago, IL).

2.3. Long-term experiments

In the second set of experiments, the long-term effect of preconditioning with NS1619 was evaluated. Animals were randomly assigned to one of the treatment groups, Con (n = 7) and NS (n = 8), respectively. As described above, mice underwent 45 min of myocardial ischemia by tightening the snare, but the observation period of reperfusion was extended to four weeks as shown in Fig. 1B. Accordingly, at the onset of reperfusion the different layers of the thorax (i.e. ribs, pectoral muscles and skin) were sewed up individually to regain anatomical location and physiological function. After onset of spontaneous breathing, mice were extubated and post-operative analgesia was achieved by intraperitoneal application of buprenorphine (0.06 mg/kg bw) every 8 h for two days. Four weeks after ischemia hearts were excised, fixed in formalin and myocardial infarction scar was stained with azorcarmine and anillin blue (AZAN), which stains myocardium red and collagen blue. Subsequently, infarction scar [% of the left ventricle] was determined by planimetry using SigmaScan Pro 5® computer software (SPSS Science Software, Chicago, IL).

2.4. Transthoracic echocardiography

A detailed description of this method is available in the Online Data Supplement.

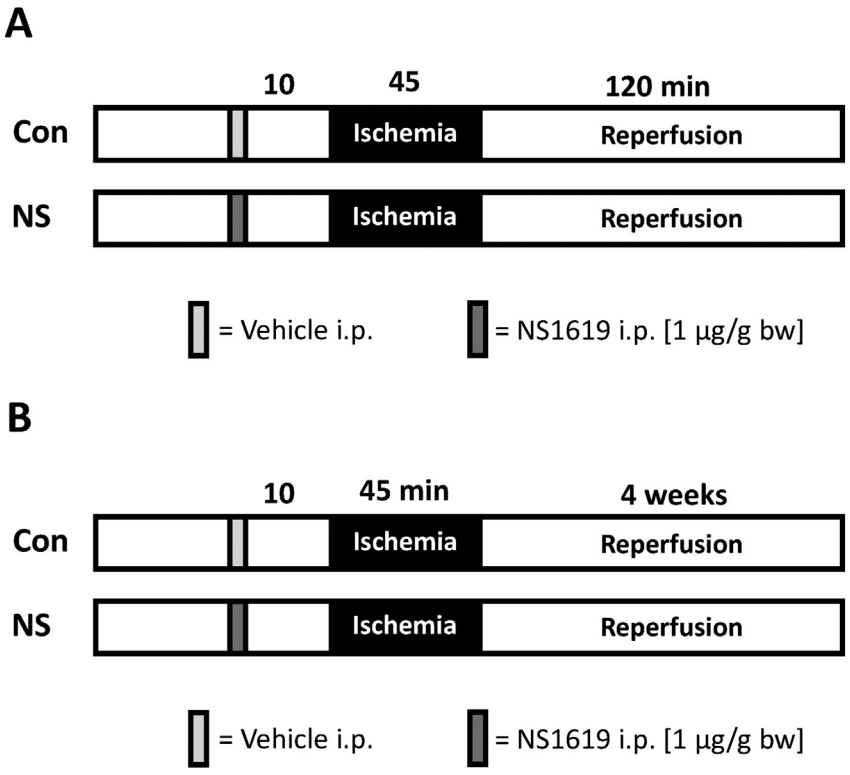

Fig. 1. A) Experimental design of short-term experiments: Con = Control, NS = NS1619. After 15 min of recovery period vehicle or NS1619 (1 μg/g bw) was administered 10 min before 45 min of regional myocardial ischemia followed by 120 min of reperfusion. B) Experimental design of long-term experiments: Con = Control, NS = NS1619. After 15 min of recovery period vehicle or NS1619 (1 μg/g bw) was administered 10 min before 45 min of regional myocardial ischemia followed by 4 weeks of observation period.

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