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Delayed therapeutic hypothermia protects against the myocardial no-reflow phenomenon independently of myocardial infarct size in a rat ischemia/reperfusion model☆

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ABSTRACT

Background: Adjunctive therapies, given in addition to reperfusion to reduce myocardial infarct size, have been disappointing based on clinical trials. New therapeutic targets independent of infarct size modification are needed. The no-reflow phenomenon occurs commonly after the infarct-related coronary artery is opened and predicts poor clinical outcome. We investigated the effects of a single application of delayed (post-reperfusion) therapeutic hypothermia (TH) in a rat model of coronary artery occlusion/reperfusion.
Methods: Rats were subjected to 60 min of coronary artery occlusion followed by 3 h of reperfusion. Rats were divided into normothermic ($n = 5$) and TH ($n = 5$) groups. In the TH, hypothermia was initiated at 1 min after coronary artery reperfusion by pumping room-temperature (22 °C) saline into and out of the thoracic cavity for 1 h. This decreased intrathoracic temperature to around 26 °C within 12 min. At 3 h after reperfusion, hearts were excised for infarct size and no-reflow zone measurement.
Results: Ischemic risk area and infarct size were similar between the 2 groups. No-reflow area (expressed as % of risk area) was significantly reduced in TH group (18.0 ± 4.4%) compared with normothermic group (39.5 ± 2.9%, $P = 0.005$). When expressed as % of necrotic area, no-reflow area was reduced by more than half in TH group (25.5 ± 6.4%) versus in normothermic group (54.4 ± 5.3%, $P = 0.01$).
Conclusions: In this preliminary study, hypothermia initiated after reperfusion following 60 min of coronary artery occlusion had no effect on infarct size yet substantially reduced the extent of no-reflow.

1. Introduction

In the setting of acute myocardial infarction, no-reflow is defined as a lack of perfusion to portions of the myocardium, despite re-opening the occluded infarct-related epicardial coronary artery [1]. In a canine model of proximal coronary artery occlusion, we showed that following 90 min of coronary artery occlusion, a fluorescent dye that stains the endothelium, failed to penetrate much of the subendocardium even after the epicardial infarct related coronary was re-opened. Perfusion defects were observed as early as 10–12 s after re-opening the proximal coronary artery and increased in size as the duration of the reperfusion phase was extended for several hours [2]. Numerous techniques have been used to confirm the presence of no-reflow including failure of carbon black, radioactive microspheres, and blue pigment to penetrate portions of the previously ischemic myocardium [1,3,4]. We have observed no-reflow in canine, rabbit, and rat models of reperfused myocardial infarction [1,5]. In the rat model, which has very limited collateral flow and a high metabolic rate, the no-reflow phenomenon is prominent after only 30 min of ischemia followed by reperfusion.

Why is treating no-reflow important? Areas of no-reflow inhibit the ability of the infarct to heal by blocking removal of necrotic debris and by preventing entry of blood-borne trophic factors and cells crucial to the healing phase of the infarct (neutrophils, macrophages, fibroblasts) from access to the zones of necrosis. In an experimental study [3] we observed that rats subjected to proximal coronary artery occlusion and reperfusion demonstrated persistent areas of no–reflow at one month and that the degree of no-reflow correlated with the severity of left ventricular remodeling, including more infarct expansion (stretching and thinning of the infarct). In humans no-reflow occurs frequently, and clinical studies have also shown that the presence of no-reflow after myocardial infarction predicts worse left ventricular remodeling and left ventricular dilatation, as well as worse prognosis that is independent of myocardial infarct size [6,7,8]. Therefore treating no-reflow might reduce adverse left ventricular remodeling. An approach to treating no-reflow

☆ All of the 3 authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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experimentally has been therapeutic hypothermia, which when administered during the phase of ischemia, reduces both myocardial infarct size as well as no-reflow [9,10]. One problem in the clinic is that inducing hypothermia prior to reperfusion can interfere with the flow of the catheterization laboratory and increase door-to-balloon time. In one study in the rabbit model, we observed that if therapeutic hypothermia is started after reperfusion has occurred, infarct size is not reduced, yet the extent of the no-reflow zone is decreased [11]. Whether this phenomenon is unique to the rabbit model, or whether it occurs in other models such as the rat is unknown. Therefore we carried out a pilot study to determine if late (after reperfusion) therapeutic hypothermia limits no-reflow in the rat. If it did, the rat model, which is a standard model for studying long-term healing, would be useful for future studies, examining the effects of reducing no-reflow with late hypothermia on the healing phase of infarction.

2. Methods

All of the procedures outlined in this study were approved by the Institutional Animal Care and Use Committees at Huntington Medical Research Institutes. This investigation was performed in accordance with the guidelines for the care and use of laboratory animals (NIH publication No. 85-23, National Academy Press, Washington DC, revised 2011).

Sprague Dawley rats (~200 g) were anesthetized with intraperitoneal ketamine (75 mg/kg) and xylazine (5 mg/kg), intubated and mechanically ventilated. The right carotid artery was isolated and catheterized to measure heart rate and blood pressure. The left jugular vein was cannulated to inject thioflavin S solution (to assess no-reflow) and blue pigment (to assess the ischemic risk zone) at the end of the experiment. A thoracotomy was performed through the 4th left intercostal space. The proximal left coronary artery was encircled with silk suture to occlude and reperfuse the coronary artery. The rats were randomized to therapeutic hypothermia ($n = 5$) of to normothermia ($n = 5$). Rectal and thoracic cavity temperatures were monitored. In this pilot study, the rats were subjected to a 60-minute coronary artery occlusion followed by 3 h of reperfusion. At 1 min after coronary artery reperfusion, saline (22 °C) was pumped in and out of the thoracic cavity so that the surface of the heart was covered in moving fluid. This procedure decreased intrathoracic temperature to ~26 °C within 12 min (Fig. 1B). The hypothermia therapy was continued for 60 min after reperfusion, at which time the rats were allowed to rewarm. In the normothermia rats, there was no saline pumped over the heart and the rat body temperature was kept at around 37 °C using a water circulating heating pad. The size of the no-reflow zone was determined by injecting the fluorescent dye, thioflavin S, into the vasculature at 1 min prior to the end of the experiment, with the proximal coronary artery patent. Thus non-fluorescent areas represent perfusion defects or zones of no-reflow. At the end of the experiment the coronary artery was re-occluded and blue pigment (Super Imperse Blue) was injected into the vasculature to delineate areas where blood flow was present during the coronary occlusion (blue represents areas receiving blood flow and therefore non-ischemic; pink represents areas not receiving blood flow and therefore ischemic during the occlusion). Under deep anesthesia the rats were euthanized with intravenous KCl, the hearts were excised and cut into 4 transverse sections from apex to base and weighed. Heart slices were photographed under UV light to

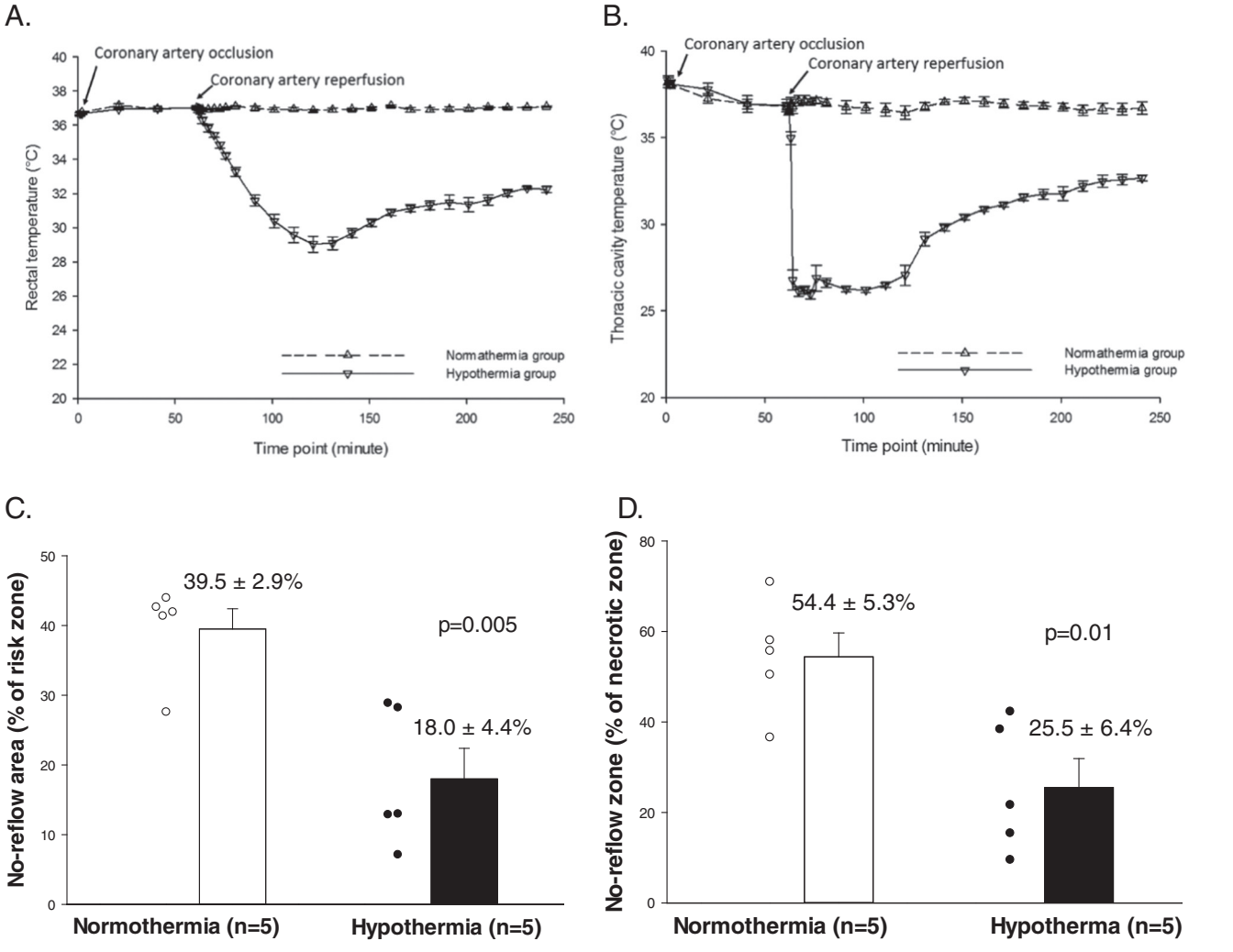

Fig. 1. Panels A and B: The rectal and thoracic cavity temperature versus time. In the hypothermic group, room temperature (22 °C) saline was circulated to rat thoracic cavity at 1 min after coronary artery reperfusion. A: In the hypothermic group, the rectal temperature slowly dropped to 30 °C within 50 min, while in the control group the rectal temperature maintained at 37 °C. B: The temperature in the thoracic cavity rapidly dropped to 26 °C within 12 min and kept at 26 °C to 1 h after reperfusion in the hypothermic group, while the thoracic cavity temperature maintained at 37 °C in the control group. In the hypothermic group, the cooling was stopped at 1 h after reperfusion, and both rectal and thoracic cavity temperature slowly increased to 32 °C at 3 h after reperfusion. Panels C and D: Rats were randomized into normothermic and hypothermic group. Delayed hypothermic treatment significantly reduced no-reflow zone compared with control group. C: The no-reflow zone was expressed as percentage of left ventricle ischemic risk zone. D: The no-reflow zone was expressed as % of necrotic zone in the two groups.

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