International Journal of Cardiology 184 (2015) 140–151

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Transient carotid ischemia as a remote conditioning stimulus for myocardial protection in anesthetized rabbits: Insights into intracellular signaling

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ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form 9 December 2014
Accepted 25 January 2015
Available online 7 February 2015

Keywords:
Perconditioning
Cardioprotection
RISK
SAFE
STAT5
eNOS

ABSTRACT

Background: We investigated the effectiveness of perconditioning (Perc) applied at different time points along with the role of RISK, SAFE, STAT5 and eNOS pathways.
Methods and results: Anesthetized rabbits were subjected to 30-min ischemia/3-hour reperfusion. Perc, consisted of 4 cycles of 1-min ischemia/reperfusion, was applied in the carotid artery at different time points. Perc was started and ended during ischemia, started during ischemia and ended at the beginning of reperfusion, started at the end of ischemia and ended at reperfusion and started and ended during reperfusion. The PI3K inhibitor wortmannin, or the JAK-2 inhibitor AG490, was also applied and the infarct size was assessed. In another series assigned to the previous groups, the phosphorylation of Akt, PI3K, ERKs1/2, GSK3β, STAT3, and STAT5 was evaluated. All Perc groups had smaller infarction compared to those without Perc, independently of PI3K or JAK-2 inhibition. STAT5 was the only molecule that was phosphorylated in parallel with cardioprotection. Since Src and angiotensin II mediate the STAT5 pathway, we administered the Scr inhibitor PP1 and the angiotensin II receptor antagonist valsartan. PP1 and valsartan prevented STAT5 phosphorylation, but did not abrogate the effect of Perc. Furthermore, the NOS inhibitor L-NAME was administered and abrogated the infarct size limiting effect of Perc. In parallel, the expression of cleaved caspase-3 was elevated only in the control and Perc-A-L-NAME groups.
Conclusion: Perc reduces infarction independently of RISK, SAFE and STAT5 pathways. Src kinase and angiotensin II play a predominant role in STAT5 activation. eNOS may protect the myocardium through inhibition of apoptosis.

1. Introduction

Applying one or more short-lived episodes of ischemia and reperfusion to the heart (ischemic conditioning) or to a distal organ (remote ischemic conditioning) reduces the infarct size caused by sustained myocardial ischemia and reperfusion [1]. In this context, causing short repetitive periods of ischemia to a remote vascular territory during an evolving myocardial infarction, reduces the final infarct size and thus confers protection against reperfusion injury both in experimental models and in humans [2–4]. Clinical studies have shown promising findings that this approach termed per-conditioning (Perc) may confer protection to the heart [5] although negative outcomes with very similar interventions have also been reported [6,7].

Organ protection by Perc is still in the early stage of investigation and algorithms have hitherto been empiric [8]. No insights have been obtained so far regarding the duration of the remote ischemic stimulus of each episode or the time of its application. Moreover, despite that the mechanism of classic ischemic pre- and post-conditioning has been elucidated with the identification of several receptors, ligands and intracellular mediators, the mechanism of Perc is partially known and is still under investigation [9,10]. Experimental studies suggest a neurohormonal pathway linking the remote ischemic stimulus to the heart where established myocardial pro-survival signaling pathways are activated [11,12]. The activation of the PI3K/Akt pathway component of the Reperfusion Injury Salvage Kinase (RISK) pathway [13] at the onset of myocardial reperfusion has been implicated as a mediator of direct myocardial preconditioning [13] and postconditioning [14], although its role has been questioned by others [15]. Postconditioning has also been shown to activate the signal transducer and activator of transcription-3 (STAT3) as part of the SAFE pathway [11,16–18]. However, the translation to humans failed to prove the involvement

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http://dx.doi.org/10.1016/j.ijcard.2015.01.079
0167-5273/

of any of the above mediators and showed that only the phosphorylation of the signal transducer and activator of transcription 5 (STAT5) during reperfusion was enhanced by remote ischemic preconditioning [19]. Therefore, little is known about the biology of Perc, including the implicated triggers, mediators and effectors [8].

Bearing in mind the above gaps in evidence, we sought to determine i. whether Perc with ischemia in a remote artery applied at different time points of evolving myocardial ischemia and/or reperfusion is effective in reducing myocardial infarction and ii. to investigate the potential role of RISK, SAFE, STAT5 and eNOS pathways.

2. Materials and methods

2.1. Animals

New Zealand White male rabbits with a weight between 2.5 and 3.4 kg received proper care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health (NIH Publication No. 85-23, revised 1996). Approval from the Ethical Committee and the veterinary authorities of East Attica prefecture was obtained before the study was started.

2.2. Surgical procedures

Sodium thiopentone at a dose of 30 mg/Kg was injected into a peripheral vein for anesthesia. Then the animals were intubated and connected to a respirator for small animals (MD Industries, Mobile, AL, USA) for mechanical ventilation at a rate adjusted to maintain normal arterial blood gases. More information regarding the experimental conditions has been previously described in detail [20]. At the beginning a bipolar chest lead was used for continuous electrocardiographic monitoring. The chest was surgically opened with a left thoracotomy and the beating heart was exposed. The pericardium was opened and a 3-0 silk suture was passed around a prominent coronary artery. Ischemia was induced by pulling the thread through a small piece of soft tubing, which was firmly positioned against the coronary arterial wall with the aid of a small clamp. Ischemia resulted in ST elevation on the ECG and a change in the color of the myocardium. At the end of ischemic period, the snare was opened, the artery was refilled and the myocardium was reperfused [20].

2.3. Experimental protocol

Two hundred and forty rabbits in total completed the study. Perc was accomplished by ligation of the left carotid artery with 4 cycles of

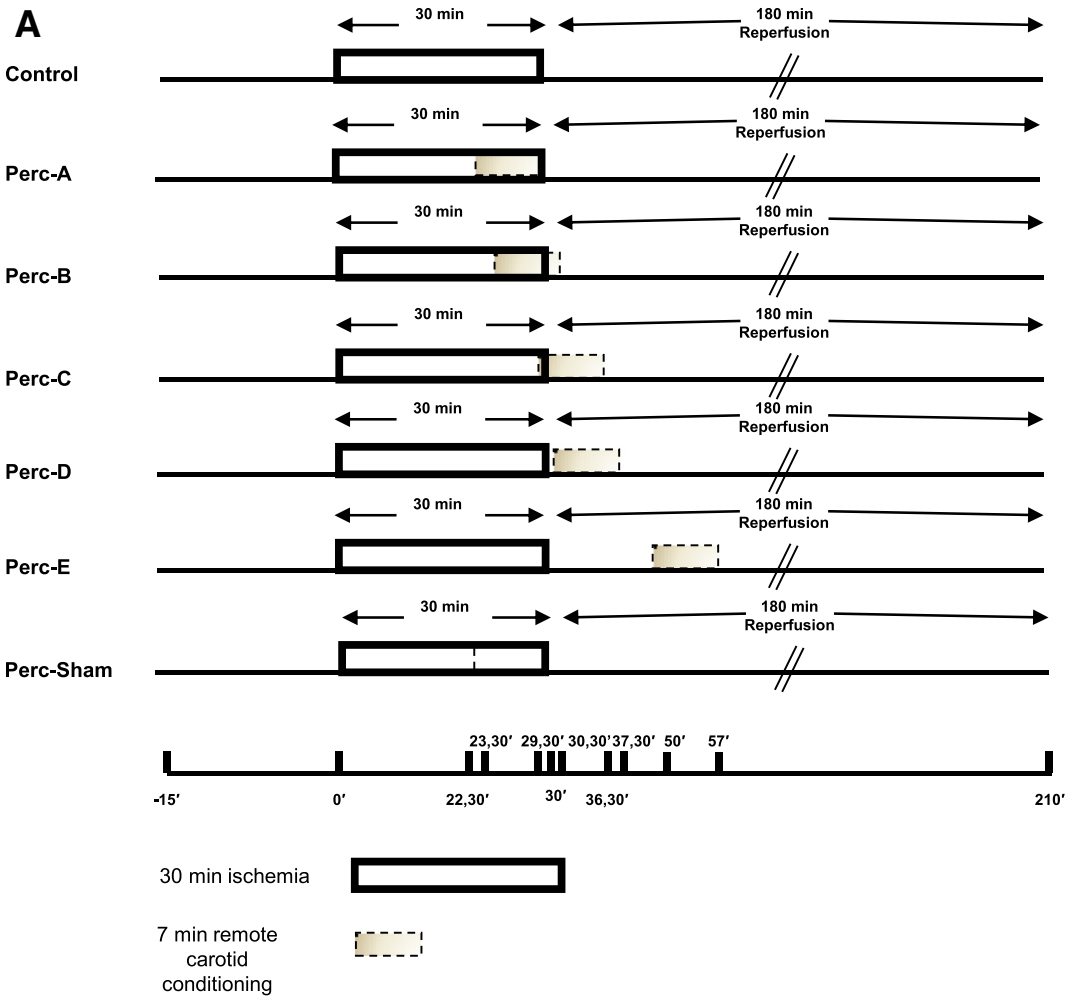

Fig. 1. (A, B): A. Schematic presentation of the study protocol at different time points. B. Schematic presentation of the experimental protocol, along with the administration of different inhibitors.