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Hydrogen sulfide modulates sub-cellular susceptibility to oxidative stress induced by myocardial ischemic reperfusion injury

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ABSTRACT

In this study, we compared the impact of H_2S pre (HIPC) and post-conditioning (HPOC) on oxidative stress, the prime reason for myocardial ischemia reperfusion injury (I/R), in different compartments of the myocardium, such as the mitochondria beside its subpopulations (interfibrillar (IFM) and sub-sarcolemmal (SSM) mitochondria) and microsomal fractions in I/R injured rat heart. The results demonstrated that compared to I/R rat heart, HIPC and HPOC treated hearts shows reduced myocardial injury, enhanced antioxidant enzyme activities and reduced the level of TBARS in different cellular compartments. The extent of recovery (measured by TBARS and GSH levels) in subcellular fractions, were in the following descending order: microsome > SSM > IFM in both HIPC and HPOC. In summary, oxidative stress mediated mitochondrial dysfunction, one of the primary causes for I/R injury, was partly recovered by HIPC and HPOC treatment, with significant improvement in SSM fraction compared to the IFM.

1. Introduction

Reperfusing the occluded coronary artery immediately is considered to be the “gold standard” for the treatment of myocardial infarction that effectively reduce the overall mortality. However, the restoration of blood flow to the ischemic myocardium resulted in cardiomyocyte dysfunction leading to cell death resulting in reperfusion injury [1]. A number of mechanisms have been proposed to mediate reperfusion injury which includes cellular calcium overload, an occurrence of a no-reflow phenomenon due to cell swelling, impaired vascular relaxation or the formation of white cell plugs, and perhaps most importantly the formation of reactive oxygen species (ROS) [2]. Low levels of ROS play an important role in cellular homeostasis, mitosis, differentiation, and signaling, while increasing radical formation following ischemia and reperfusion, helps in triggering cellular injury. Although all mammalian cells, including cardiomyocytes, express endogenous free radical scavenging enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, these antioxidant defenses are overwhelmed after ischemia and reperfusion. ROS causes lipid peroxidation, which in turn leads to cell membrane damage resulting in cell swelling [3]. A number of studies were reported to have a better cardiac function as well as reduced infarct size by either preventing or scavenging free radicals [4,5]. Classical cardioprotective regimens include ischemic pre-conditioning (IPC), first demonstrated by Murray et al. (1986) and ischemic post-conditioning (POC) as proposed by Zhao et al. (2003), could limit reperfusion injury through the activation of intrinsic pro-survival signaling cascades like PI3K/Akt (RISK pathway) and JAK2/STAT3 (SAFE pathway). Both IPC and POC protect the heart by inhibiting mitochondrial permeability transition pore opening, reducing inflammatory consequences and ameliorating oxidative stress [6].

Hydrogen sulfide (H_2S), a novel gasotransmitter, so far known for its toxicity, is synthesized by cystathionine-gamma-lyase (CSE) in the cardiovascular system and also reported to protect the myocardium against I/R injury and its cytoprotection is primarily due to its antioxidant, anti-inflammatory and antiapoptotic property [7,8]. H_2S induced pre- and post-conditioning was proven to execute by targeting mitochondria. In H_2S induced IPC, it activates antioxidant genes (Nrf2), RISK pathway, leading to the preservation of mitochondrial function and structure [9]. On the other hand, in H_2S mediated POC, mitochondria specific antioxidants were expressed significantly, along with the activation of a SAFE pathway [10].

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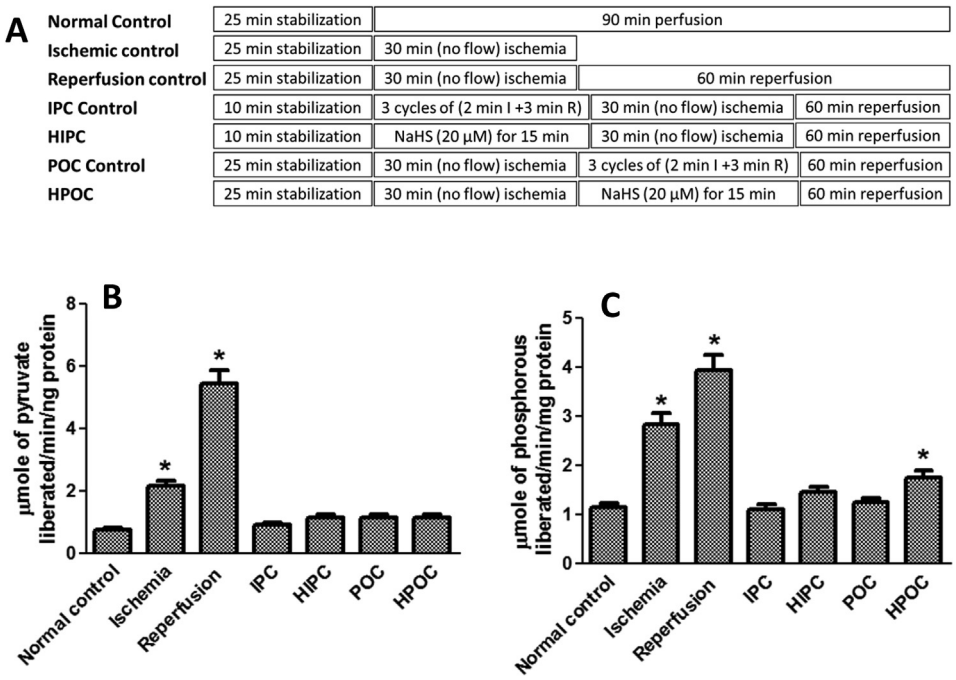

Fig. 1. A) Schematic diagram explaining the experimental groups viz., Normal perfusion, Ischemic control, Ischemia-reperfusion control (I/R), Ischemic preconditioning (IPC) control, Ischemic post-conditioning (POC) control, H_2S preconditioning (HIPC) and H_2S post-conditioning (HPOC), B) Lactate dehydrogenase activity and C) Creatine kinase activity. (*) represents statistically different ($P < 0.05$) from the normal control.

Despite the known great potential of hydrogen sulfide as a pre- or post-conditioning agent, the exact mechanism behind its cardioprotective effect remains unclear, primarily due to the lack of complete understanding of the pathophysiological feature of I/R. Recently, our lab had shown that cardiac mitochondrial subpopulation, namely interfibrillar and subsarcolemmal mitochondria had experienced the different magnitude of damage during I/R. Mitochondrial dysfunction, being the pivotal pathological reason for I/R, specific mitochondrial subset damage can be detrimental to the measurable outcome on the efficacy of H_2S as a cytoprotective agent in myocardial ischemia-reperfusion injury. Detailed understanding of the effect of oxidative stress specifically on IFM and SSM is essential, as ROS plays a key role in I/R injury. Thus, the aim of this manuscript is to provide definite experimental evidence for the impact of I/R injury and H_2S induced pre or post-conditioning on different compartments of the myocardium, in particular, IFM and SSM individually.

2. Materials and methods

2.1. Animals

The study protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. In polycarbonate cages, animals were kept at a controlled temperature of 25 ± 3 °C and 60± 10% relative humidity with a 12 h dark/light cycle. Before the start of the experiment, rats were acclimatized for a week with standard laboratory diet and drinking water given *adlibitum*.

2.2. Isolated perfused rat heart preparation

Male Wistar rats (230–280 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (80 mg/kg). Heparin (1000 IU) was administered i.p. 30 min prior to anesthesia to prevent coagulation during excision of the heart. The heart was excised, mounted on a Lagendorff apparatus (AD instruments, Australia) and perfused retrogradely through the aorta with Krebs Hensleit (KH) buffer (pH 7.4, 37 °C) at a constant flow rate of 8 ml/min as previously described [11]. Each heart was allowed to stabilize (in terms of LVEDP) before the commencement of any experiment.

The experiment was carried as per the protocol shown in Fig. 1A. Briefly, in the normal perfusion group (NP), heart was perfused for 90 min with KH buffer. In case of ischemic control group, after stabilization, hearts were subjected to no-flow ischemia for 30 min.

Table 1
Myocardial hemodynamic measurement during the time of experiment.

Groups	LVEDP[1]	LVDP[1]	RPP[2]
Normal Perfusion (n = 6)	6 ± 2	98 ± 4	95 ± 2
I/R (n = 6)	45 ± 3*	42 ± 3*	32 ± 2*
IPC (n = 6)	22 ± 4#	90 ± 4#	82 ± 3#
HIPC (n = 6)	23 ± 3#	93 ± 3#	83 ± 2#
POC (n = 6)	18 ± 4#	95 ± 4#	87 ± 3#
HPOC (n = 6)	17 ± 3#	96 ± 3#	88 ± 2#

Data represented as mean ± SE; *p<0.05 vs normal control. #P<0.05 vs I/R control. LVEDP– Left ventricular end diastolic pressure; LVDP– Left ventricular developed pressure; RPP– Rate pressure product. 1–mmHg; 2–mmHg x bpm x $10^{-}3$.

Table 2
Infarct size measurement using TTC staining.

Groups	Infarct size (% of total heart)
Normal control (n = 6)	4 ± 0.8
Ischemia (n = 6)	8 ± 0.2
I/R (n = 6)	24 ± 0.9*
IPC (n = 6)	7 ± 0.7#
HIPC (n = 6)	9 ± 0.5#
POC (n = 6)	7 ± 0.7#
HPOC (n = 6)	6 ± 0.5#

Data are represented as mean ±SE; *p<0.05 Vs normal control; #p<0.05 vs I/R control.