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Research report

Remote ischemic preconditioning improves post resuscitation cerebral function via overexpressing neuroglobin after cardiac arrest in rats

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ABSTRACT

In this study, we investigated the effects of remote ischemic preconditioning on post resuscitation cerebral function in a rat model of cardiac arrest and resuscitation. The animals were randomized into six groups: 1) sham operation, 2) lateral ventricle injection and sham operation, 3) cardiac arrest induced by ventricular fibrillation, 4) lateral ventricle injection and cardiac arrest, 5) remote ischemic preconditioning initiated 90 min before induction of ventricular fibrillation, and 6) lateral ventricle injection and remote ischemic preconditioning before cardiac arrest. Reagent of Lateral ventricle injection is neuroglobin antisense oligodeoxynucleotides which initiated 24 h before sham operation, cardiac arrest or remote ischemic preconditioning. Remote ischemic preconditioning was induced by four cycles of 5 min of limb ischemia, followed by 5 min of reperfusion. Ventricular fibrillation was induced by current and lasted for 6 min. Defibrillation was attempted after 6 min of cardiopulmonary resuscitation. The animals were then monitored for 2 h and observed for an additionally maximum 70 h. Post resuscitation cerebral function was evaluated by neurologic deficit score at 72 h after return of spontaneous circulation. Results showed that remote ischemic preconditioning increased neurologic deficit scores. To investigate the neuroprotective effects of remote ischemic preconditioning, we observed neuronal injury at 48 and 72 h after return of spontaneous circulation and found that remote ischemic preconditioning significantly decreased the occurrence of neuronal apoptosis and necrosis. To further comprehend mechanism of neuroprotection induced by remote ischemic preconditioning, we found expression of neuroglobin at 24 h after return of spontaneous circulation was enhanced. Furthermore, administration of neuroglobin antisense oligodeoxynucleotides before induction of remote ischemic preconditioning showed that the level of neuroglobin was decreased then partly abrogated neuroprotection of remote ischemic preconditioning. These date suggested that neuroglobin involved in neuroprotective effect of remote ischemic preconditioning. In conclusion, remote ischemic preconditioning attenuated post resuscitation cerebral dysfunction and the neuroprotection was mediated partly by high level of neuroglobin in a rat model of cardiac arrest and resuscitation.

1. Introduction

Cardiac Arrest (CA) remains to be a leading cause of disability and death worldwide (Neumar et al., 2015). Despite the skills and methods of cardiopulmonary resuscitation develop fast recent years, such as the rapid utilization of defibrillation and a focus on high-quality chest compression, the mortality or morbidity of CA remains high (Neumar et al., 2008). Even if a small number of victims achieve a return of spontaneous circulation successfully, most of them suffer from post-cardiac arrest syndrome which causes cardiovascular instability and neurological injury (Neumar et al., 2008). More survivors may undergo permanent brain damage with an unacceptable quality of life (Herlitz et al., 2000). Global ischemia-reperfusion injury of brain during cardiopulmonary resuscitation is a significant contributor to post–cardiac arrest neurological dysfunction and a definite contributor to morbidity and mortality of CA patients (Pußwald et al., 2000). Mild hypothermia partly improves human survival and neurological outcomes after cardiac arrest, but it is limited by expensive costs as well as potential side effects (Nolan et al., 2003). Therefore, novel interventions are needed to study for purpose of improving

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neurological outcomes after CA.

Remote ischemic preconditioning (RIPC) is applied directly to a remote organ or tissue by applying one or more cycles of brief, nonlethal ischemia and reperfusion, that has been proven to protect brain or other organs to against subsequent lethal ischemia and reperfusion injury in many studies (Ren et al., 2008; Zhou et al., 2010).

The underlying mechanism of RIPC has not been elucidated adequately, the fashionable concepts suggest that humoral factors, neural factors or immune factors which is produced in the nonlethal ischemic organ or tissue, subsequently transmitted protective effect to the target organ (N et al., 2015; Dirnagl et al., 2009). The animal focal brain or global brain ischemic models are often adopted in plenty of previous RIPC studies (N et al., 2015; Stagliano et al., 1999; Pérez-Pinzón et al., 1997), however, quite a few studies are involved in the brain injury model of CA. Amazingly, a recent study demonstrates that RIPC via transient limbs ischemia markedly improves postresuscitation myocardial and cerebral function in a rat CA model and resuscitation (Xu et al., 2015). But, in this study, the mechanism of organ protection provided by RIPC is not been investigated. Obviously, more and deeper details about endogenous mechanisms of neuroprotection induced by RIPC should be studied in cerebral ischemia insults of CA.

Neuroglobin (Ngb) is a recently discovered heme-binding globin. This globin is abundantly expressed in high-energy demanded organs or tissues, like brain and retina (Burmester and Hankeln, 2009). It is been proven that Ngb is an endogenous critical neuroprotective molecule against ischemia reperfusion injury (Garry and Mammen, 2003). Some studies demonstrate that Ngb overexpression can confer neuroprotection against oxidative stress and enhance cell survival under ischemic condition (Sun et al., 2001; Sun et al., 2003). In other respect, some studies demonstrate that inhibition of Ngb expression could exacerbate neurons injury to stress (Ye et al., 2009). Strategies which could be used to up-regulate the level of Ngb are expected to be neuroprotective. Some findings demonstrate that Ngb levels can be enhanced by pharmacological patterns, such as hemin and cobalt (Jin et al., 2011). In addition, one study demonstrates that RIPC could induce Ngb high expression in global ischemic brain (Li et al., 2013). However, there is no report about the Ngb expression and inner relationship between Ngb with RIPC in CA model.

In this study, we aim to determine whether RIPC could be indeed induced in a rat 6 min ventricular fibrillation (VF) induced CA model. We examine neuron apoptosis and necrosis in CA1 region of the hippocampus caused by cerebral ischemic injury of CA to determine the mechanism underlying the effect of RIPC on cerebral function following ROSC. Furthermore, the Ngb expression in the CA1 hippocampus is also been investigated and Ngb antisense oligodeoxynucleotides (AS-ODNs) is used to strengthen comprehension of the role of Ngb which indicated as a potential endogenous protein in RIPC.

2. Results

2.1. RIPC improved resuscitation outcomes in CA Rats

A total of 215 rats were prepared for the study (Fig. 1). Among them, 188 underwent VF induced CA/CPR, whereas the rest 15 rats received sham operation and other 12 rats received Ngb AS-ODNs with lateral cerebral ventricle injection. Finally, 130 experimental rats were successfully resuscitated. In addition, 33 rats that resuscitated from CA but failed to survive for time point of investigation. The rest 97 living experimental rats which including rats from sham group and Ngb AS-ODNs group were investigated at 24 h, 48 h and 72 h for time points.

There was no significant difference in physiologic variables among six groups at baseline (Table 1), whereas acidosis was observed in both groups and RIPC could reduce acidosis ($^{c}p < 0.01$) (Table 2). Furthermore, the needed shock times during CPR and the ratio of return of spontaneous circulation were comparative among all groups. The results showed that the requirement of shock time in RIPC group was less compared to CA group ($^{a}p < 0.01$) and the ratio of return of spontaneous circulation was higher in RIPC group (Table 2).

2.2. RIPC preserved brain function after CA/CPR

The rats in all groups were evaluated through a scale of modified neurologic deficit score by an observer blinded to the distribution of rats of groups at 72 h after ROSC (Fig. 3). The CA group had significantly depressed neurological scores compared to sham

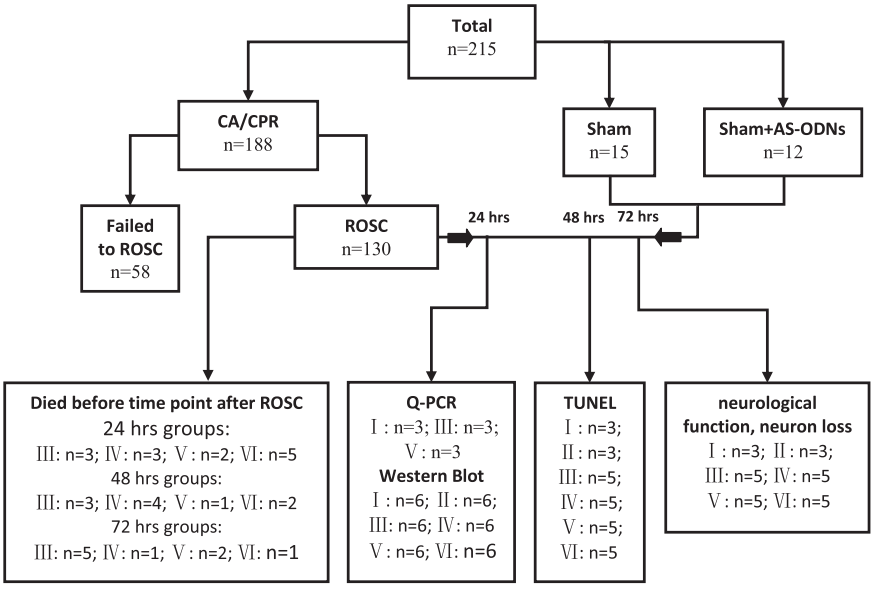

Fig. 1. Flow diagram of the experimental groups. Q-PCR = quantitative polymerase chain reaction, TUNEL = terminal deoxynucleotide transferase–mediated dUTP-biotin nick-end labeling. I: sham group, N=15; II: Ngb AS ODNs + CA group, N=12; III: CA group, N=19; IV: Ngb AS-ODNs + CA group, N=16; V: RIPC + CA group, N=19; VI: Ngb AS-ODNs + RIPC + CA group, N=16.