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Effects of preconditioned plasma collected during the late phase of remote ischaemic preconditioning on ventricular arrhythmias caused by myocardial ischaemia reperfusion in rats

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Abstract

Objective: The administration of preconditioned plasma collected during the late phase of preconditioning has been shown to reduce myocardial infarct size. This study aimed to investigate if preconditioned plasma could attenuate ventricular arrhythmias in a rat model *in vivo*.

Methods: Eighty rats were randomized to eight groups (10 rats/group). Two groups provided preconditioned or non-preconditioned plasma 48 h after transient limb ischaemia or the control protocol. Six groups of ischaemia-reperfusion (IR) rats received normal saline, non-preconditioned plasma, or preconditioned plasma, respectively, 1 h (groups A1, A2, A3) or 24 h (groups B1, B2, B3) before undergoing myocardial IR. Electrocardiograms were monitored using a BIOPAC system, and the incidence and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) were analysed.

Results: No significant differences existed in the incidence and duration of VT or VF among groups A1–A3 or in the incidence and duration of VT among groups B1–B3. However, there was a significantly lower incidence and shorter duration of VF in group B3 rats than in group B1 rats.

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Conclusion: Preconditioned plasma collected during the late phase of preconditioning can reduce the incidence and duration of VF compared with normal saline, suggesting its anti-arrhythmic potential.

Keywords
Remote ischaemic preconditioning, ventricular arrhythmias, ischaemia-reperfusion, plasma

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Introduction

Sudden cardiac death results primarily from lethal arrhythmias induced by acute myocardial ischaemia.[1,2] Although the restoration of blood flow to the ischaemic myocardium is beneficial when myocardial ischaemia occurs, rapid reperfusion may also induce lethal arrhythmias including ventricular fibrillation.[3] So, the arrhythmias induced by ischaemia-reperfusion together with the interventions to reduce the arrhythmias are still widely investigated.[4–8] Developing approaches to reduce the incidence and severity of ventricular arrhythmias is important for optimizing therapy in patients experiencing myocardial ischaemia.

Remote ischaemic preconditioning (RIPC) has been shown to be an effective strategy for attenuating myocardial ischaemia-reperfusion (IR) injury[9–11] and offers early- and late-phase protection.[12–15] Although many studies have demonstrated the protective effects of RIPC in reducing infarct size, the effects of RIPC on lethal arrhythmias during the myocardial IR period *in vivo* remain controversial.[7,8,16,17] In addition, the mechanisms underlying the protective effects of RIPC remain unclear. In previous studies, we found that late-phase protection of RIPC could be transferred by plasma between individuals; the transfusion of preconditioned plasma collected at the late protective phase of RIPC improved blood pressure recovery during the myocardial IR period[18] and reduced infarct size after myocardial IR.[19] These findings verified the cardioprotective role of preconditioned plasma and supported the humoral mechanisms of RIPC. However, no previous studies have investigated the anti-arrhythmia effects of late-phase preconditioned plasma of RIPC during the myocardial IR period.

This study aimed to investigate whether the transfusion of preconditioned plasma collected during the late protective phase could attenuate ventricular arrhythmias in an *in vivo* IR rat model.

Materials and methods

Animals and study groups

All animal protocols were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University, Guangzhou, Guangdong Province, China. Eighty 10–12-week-old male Lewis rats weighing 245–280 g (Vital River Company, Beijing, China) were randomized into eight groups ($n = 10$/group): a preconditioned plasma donor group, a non-preconditioned plasma donor group, and six IR groups (groups A1, A2, A3, B1, B2 and B3). One group of plasma donor rats underwent transient limb ischaemia (PDLI group), whereas the other did not (PD control group).

These two groups were the source for preconditioned and non-preconditioned plasma, respectively. The rats in groups A1, A2, and A3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 1 h before myocardial IR. The rats in groups B1, B2, and B3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 24 h before myocardial IR.

Remote ischaemic preconditioning and plasma preparation and transfusion

Pentobarbital (50 mg/kg) was injected intraperitoneally for anaesthesia. Subsequently, the PDLI rats underwent four cycles of transient limb ischaemia. Transient limb ischaemia was accomplished by binding elastic rubber bands around both proximal hind limbs for 5 min, followed by 5 min of reperfusion with the release of the noninvasive ligature. Elastic bands were placed on the hind limbs of the PD control rats but were not tied. At 48 h after finishing the whole limb ischaemia or control protocol, 8–10 ml of blood was drawn from the PDLI and PD control rats. The blood was centrifuged at 1690 g for 10 min at 4 °C to obtain the preconditioned and non-preconditioned plasma (Thermo Sorvall ST 16R centrifuge; Thermo Electron LED GmbH, Osterode am Harz, Germany). According to the assigned study groups, 2 ml of normal saline, 2 ml of non-preconditioned plasma, or 2 ml of preconditioned plasma was transfused into the IR rats through the caudal vein at either 1 h or 24 h before myocardial IR.

Myocardial IR model

The myocardial IR model was achieved as described in our previous study.[18] Briefly, rats were anaesthetized with 60 mg/kg pentobarbital intraperitoneal, and then ventilated (Small Animal Ventilator Model 683; Harvard Apparatus, Holliston, MA, USA) with room air with a tidal volume of 8–10 ml/kg and a respiratory rate of 70–80 breaths/min. A left thoracotomy was performed between the third and fourth ribs, and the left anterior descending coronary artery was ligated with a 7-0 polypropylene suture slip knot tied over a section of cotton thread placed directly on the vessel to create the occlusion. After 30 min of ischaemia, the slip knot was released, and the cotton thread was removed. Reperfusion was then continued for 180 min.

Arrhythmia evaluation

Electrocardiograms were monitored continuously using a BIOPAC MP150 system (BIOPAC Systems, Goleta, CA, USA) throughout the ischaemia-reperfusion process. Arrhythmias seen in the electrocardiogram were evaluated based on the guidelines of the Lambeth Conventions.[20] Ventricular tachycardia (VT) was defined as four or more consecutive premature ventricular contractions, while ventricular fibrillation (VF) was defined as occurring when individual normal QRS waves could no longer be distinguished.[20] The incidence of VT or VF was calculated and the total duration of VT or VF during the entire IR period was measured in each group.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 22.0 (SPSS Inc., Chicago, IL, USA) for Windows®. χ^2-test was used to determine whether there was any difference among groups in the incidence of VT or VF. The total duration of VT or VF in each group was expressed as the median (interquartile range [IQR]), and the difference in the total duration of VT or VF was analysed non-parametrically using the Kruskal–Wallis test. A P-value < 0.05 was considered statistically significant.

Results

No rats died in the plasma donor groups. However, two rats died in each of the IR groups during the IR procedure, thus the sample size was eight in each IR group ($n = 8$).

Table 1. Incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) in group A rats.

	Group A rats[a]		
	A1 $n = 8$	A2 $n = 8$	A3 $n = 8$
Incidence of VT	7 (87.5%)	6 (75.0%)	6 (75.0%)
Incidence of VF	4 (50.0%)	2 (25.0%)	2 (25.0%)

Data presented as *n* of rats (%).
[a]Rats in groups A1, A2 and A3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 1 h before myocardial ischaemia.
There was no significant difference in the incidence of VT and VF among the three groups ($P \geq 0.05$); χ^2-test.

Analysis of the effect of preconditioned plasma on ventricular arrhythmias when transfused 1 h before myocardial ischaemia demonstrated that there was no significant difference in the incidence of VT among groups A1, A2, and A3. Although VF occurred in four (50%), two (25%), and two (25%) out of the eight rats in groups A1, A2, and A3, respectively, there was no significant difference in the incidence of VF among these three groups (Table 1).

The total duration of VT in groups A1, A2, and A3 was 42.40 s (IQR 6.38–98.13 s), 13.73 s (IQR 0.55–80.68 s), and 21.20 s (IQR 0.68–42.86 s), respectively. There was no significant difference in total duration of VT among these three groups (Figure 1).

The total duration of VF in groups A1, A2, and A3 was 2.75 s (IQR 0.00–29.25 s), 0.00 s (IQR 0.00–8.63 s), and 0.00 s (IQR 0.00–6.00 s), respectively. There was no significant difference in the total duration of VF among these three groups (Figure 2).

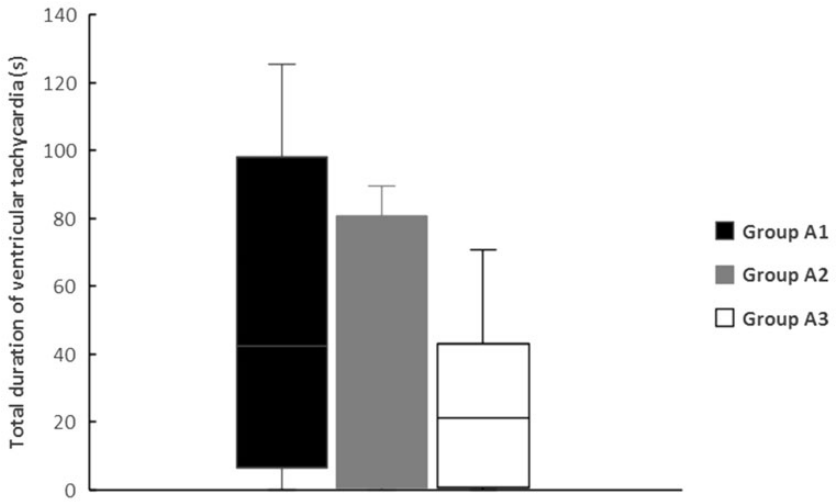

Figure 1. Total duration of ventricular tachycardia in rat groups A1, A2 and A3. Data are shown as a box plot. The length of the box is the interquartile range and the median is represented by the horizontal bar inside the box. Error bars represent minimum and maximum outliers. The rats in groups A1, A2 and A3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 1 h before myocardial ischaemia. There was no significant difference in the total duration of ventricular tachycardia among the three groups ($P \geq 0.05$); Kruskal–Wallis test.

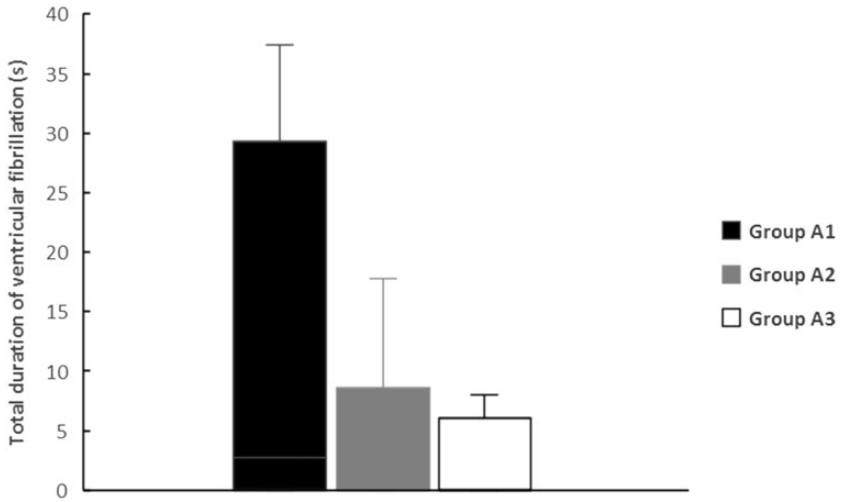

Figure 2. Total duration of ventricular fibrillation in rat groups A1, A2 and A3. Data are shown as a box plot. The length of the box is the interquartile range and the median is represented by the horizontal bar inside the box. There looks like no horizontal bar inside the box for groups A2 and A3 because that the median durations of ventricular fibrillation of the two groups are 0. Error bars represent minimum and maximum outliers. The rats in groups A1, A2 and A3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 1 h before myocardial ischaemia. There was no significant difference in the total duration of ventricular fibrillation among the three groups ($P \geq 0.05$); Kruskal–Wallis test.

Analysis of the effect of preconditioned plasma on ventricular arrhythmias when transfused 24 h before myocardial ischaemia showed that ventricular tachycardia occurred in seven (87.5%), seven (87.5%), and six (75.0%) out of eight rats in groups B1, B2, and B3, respectively. There was no significant difference in the incidence of VT among these three groups (Table 2).

Ventricular fibrillation occurred in five (62.5%), two (25%), and none (0.0%) out of eight rats in groups B1, B2, and B3, respectively. There was a significant difference in the incidence of VF among these three groups ($P = 0.022$). The incidence of VF was significantly lower in group B3 than in group B1 ($P = 0.007$), and the difference between the two groups was quite large. However, there were no significant differences between groups B2 and B1 and between groups B2 and B3 (Table 2).

The total duration of VT in groups B1, B2, and B3 was 65.72 s (IQR 18.50–85.35 s), 24 s (IQR 15.44–65.35 s), and 15.79 s (IQR 2.10–43.77 s), respectively. There was no significant difference in the total duration of VT among these three groups (Figure 3).

The total duration of VF in groups B1, B2, and B3 was 15.15 s (IQR 0.00–26.60 s), 0.00 s (IQR 0.00–8.00 s), and 0.00 s (IQR 0.00–0.00 s), respectively. There was a

Table 2. Incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) in group B rats.

	Group B rats[a]		
	B1 $n = 8$	B2 $n = 8$	B3 $n = 8$
Incidence of VT	7 (87.5%)	7 (87.5%)	6 (75.0%)
Incidence of VF	5 (62.5%)	2 (25.0%)	0 (0.0%)*

Data presented as *n* of rats (%).
[a]Rats in groups B1, B2 and B3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 24 h before myocardial ischaemia.
*$P < 0.05$ compared with group B1; χ^2-test.

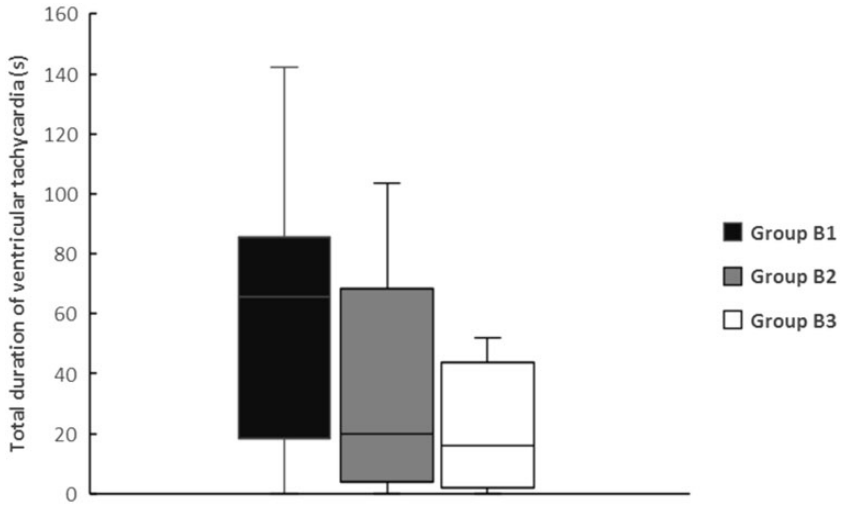

Figure 3. Total duration of ventricular tachycardia in rat groups B1, B2, and B3. Data are shown as a box plot. The length of the box is the interquartile range and the median is represented by the horizontal bar inside the box. Error bars represent minimum and maximum outliers. The rats in groups B1, B2, and B3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 24 h before myocardial ischaemia. There was no significant difference in the total duration of ventricular tachycardia among the three groups ($P \geq 0.05$); Kruskal–Wallis test.

significant difference in the total duration of VF among groups B1, B2, and B3 ($P = 0.017$). The total duration of VF was significantly shorter in group B3 than in group B1 ($P = 0.016$). However, there were no significant differences in the total duration of VF between groups B2 and B1 and between groups B2 and B3 (Figure 4).

Discussion

This current study demonstrates that the transfusion of preconditioned plasma collected during the late protective phase of RIPC into recipients 24 h before myocardial ischaemia can reduce the incidence and duration of VF during the myocardial IR period compared with that observed with the transfusion of normal saline. However, transfusion 1 h before myocardial ischaemia had no effect on the incidence or duration of ventricular arrhythmias.

Myocardial IR injury can induce lethal ventricular arrhythmias, leading to circulatory collapse and ultimately sudden death. At present, it is well established that ischaemic preconditioning (IPC) can attenuate ventricular arrhythmias induced by myocardial IR.[21–23] RIPC induced by a tourniquet or a blood-pressure cuff is a safe and noninvasive technique to exert a myocardial protective effect, and the effect of RIPC in limiting infarct size against IR injury has been shown in previous studies.[9–11] However, the influence of RIPC on IR-induced arrhythmias remains controversial. A previous study found that RIPC at the early protective phase induced by lower limb ischaemia reduced the incidence and duration of IR-induced ventricular arrhythmias.[8] However, another study found that RIPC induced by transient limb ischaemia in rats had no significant effect on arrhythmia score.[16] RIPC induced by 15-min mesenteric

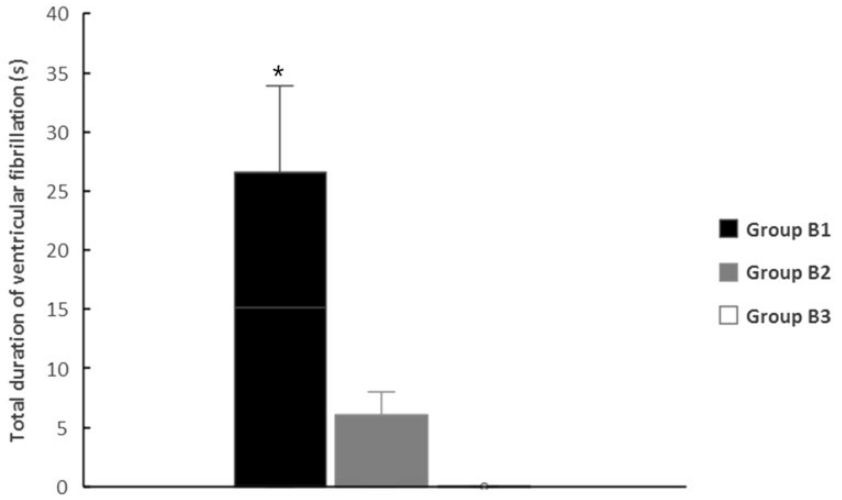

Figure 4. The total duration of ventricular fibrillation in rat groups B1, B2 and B3. Data are shown as a box plot. The length of the box is the interquartile range and the median is represented by the horizontal bar inside the box. Error bars represent minimum and maximum outliers. The rats in groups B1, B2, and B3 received normal saline, non-preconditioned plasma, and preconditioned plasma, respectively, 24 h before myocardial ischaemia. The total duration of ventricular fibrillation was shorter in group B3 compared with that seen in group B1. However, there were no significant differences in the total duration of ventricular fibrillation between groups B2 and B1 and between groups B2 and B3 ($P \geq 0.05$). *$P < 0.05$ compared with group B3; Kruskal–Wallis test.

artery occlusion and 15-min reperfusion only delayed the onset of the first episode of arrhythmia, but had no influence in reducing the incidence and severity of ischaemia-induced arrhythmias.[17]

Our previous study found that the transfusion of preconditioned plasma collected during the late protective phase of preconditioning reduced infarct size after myocardial IR compared with the transfusion of either non-preconditioned plasma or normal saline.[19] Arrhythmia is an important index that reflects the electrical activity of the heart, and is the main complication after myocardial IR, and is also the main concern of both physicians in clinics and researchers in the laboratory faced with myocardial IR. The present study investigated the transfusion of preconditioned plasma on arrhythmias induced by IR, and the current results show that the transfusion of preconditioned plasma can reduce the incidence and duration of VF compared with that observed with the transfusion of normal saline. These results are similar to previous findings,[8] and partly verify our previous hypothesis that preconditioned plasma may be a reservoir of cardioprotective factors released by RIPC, and that the transfusion of preconditioned plasma may have cardioprotective effects.[18] It remains unclear how the protective signal is transferred from the remote organ to the heart during RIPC. Three hypotheses have been suggested;[24] the neural hypothesis,[25] humoral hypothesis,[26] and systemic protective response hypothesis.[27] The humoral hypothesis proposes that humoral mediators of RIPC are released into the circulatory system from the remote organ and are then carried to the heart where they ultimately activate cardioprotective pathways

in cardiac myocytes.[26] The results of the present study support the humoral hypothesis.

In the present study, when compared with the transfusion of non-preconditioned plasma, the transfusion of preconditioned plasma had no significant effect on ventricular arrhythmias. This may be related to the relatively small sample size in this current study. Furthermore, some studies have indicated that the mechanisms that limit infarct size and those responsible for the anti-arrhythmic effect of IPC may be different;[28–30] and one could postulate that the mechanisms for the ability of preconditioned plasma to limit infarct size and those that create an anti-arrhythmic effect are also different. Further studies are needed to confirm this hypothesis. Our previous study found that transfusion of non-preconditioned plasma could also reduce myocardial infarction size compared with transfusion of normal saline, and we speculated that the plasma advantage of maintaining blood volume may be involved in the mechanism.[19] However, in this current study, non-preconditioned plasma did not significantly reduce the duration and incidence of arrhythmias compared with normal saline, suggesting that the plasma advantage of maintaining blood volume may not affect ventricular arrhythmias.

This current study found that preconditioned plasma transfused 1 h before myocardial ischaemia had no effect on ventricular arrhythmias. This may have occurred because the protective factors in preconditioned plasma may need time to activate and amplify the protective pathways, a possibility we have previously suggested,[19] which requires further investigation. These current findings are also consistent with the findings of our previous study that found that the preconditioned plasma transfused 1 h before myocardial ischaemia had no effect on the infarct size induced by IR.[19]

In conclusion, this study suggests the anti-arrhythmic potential of preconditioned plasma collected during the late protective phase of RIPC.

Author contributions

San-Qing Jin designed and supervised the experiments and revised the manuscript. Yang Zhao, Zhi-Nan Zheng, and Gang Dai performed the experiments. Yang Zhao wrote the first draft of the manuscript. Xiang Liu took part in analysing the data and revising the manuscript. All authors have seen and approved the final version of this paper.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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References

1. Napolitano C. Genetic testing of inherited arrhythmias. *Pediatr Cardiol* 2012; 33: 980–987.
2. Zipes DP and Wellens HJ. Sudden cardiac death. *Circulation* 1998; 98: 2334–2351.
3. Yellon DM and Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007; 357: 1121–1135.
4. Hu Z, Chen M, Zhang P, et al. Remote ischemic preconditioning differentially attenuates post-ischemic cardiac arrhythmia in streptozotocin-induced diabetic versus nondiabetic rats. *Cardiovasc Diabetol* 2017; 16: 57–59.
5. Alburquerque-Bejar JJ, Barba I, Ruiz-Meana M, et al. Additive effects of exenatide, glucose-insulin potassium, and remote ischemic conditioning against reperfusion ventricular arrhythmias in pigs. *Rev Esp Cardiol (Engl Ed)* 2016; 69: 620–622.

6. Noorbakhsh MF, Arab HA and Kazerani HR. Liver ischemia preconditions the heart against ischemia-reperfusion arrhythmias. *Iran J Basic Med Sci* 2015; 18: 80–88.
7. Hu Z, Hu S, Yang S, et al. Remote liver ischemic preconditioning protects against sudden cardiac death via an ERK/GSK-3β-dependent mechanism. *PLoS One* 2016; 11: e165123.
8. Dow J, Bhandari A, Simkhovich BZ, et al. The effect of acute versus delayed remote ischemic preconditioning on reperfusion induced ventricular arrhythmias. *J Cardiovasc Electrophysiol* 2012; 23: 1374–1383.
9. Wu Q, Wang T, Chen S, et al. Cardiac protective effects of remote ischaemic preconditioning in children undergoing tetralogy of fallot repair surgery: a randomized controlled trial. *Eur Heart J* 2017. doi: 10.1093/eurheartj/ehx030. [Epub ahead of print].
10. Elbadawi A, Ha LD, Abuzaid AS, et al. Meta-analysis of randomized trials on remote ischemic conditioning during primary percutaneous coronary intervention in patients with ST-segment elevation myocardial infarction. *Am J Cardiol* 2017; 119: 832–838.
11. Bromage DI, Pickard JM, Rossello X, et al. Remote ischaemic conditioning reduces infarct size in animal in vivo models of ischaemia-reperfusion injury: A systematic review and meta-analysis. *Cardiovasc Res* 2017; 113: 288–297.
12. Yoon YE, Lee KS, Choi KH, et al. Preconditioning strategies for kidney ischemia reperfusion injury: implications of the "time-window" in remote ischemic preconditioning. *PLoS One* 2015; 10: e124130.
13. Liu Z, Wang YL, Hua Q, et al. Late remote ischemic preconditioning provides benefit to patients undergoing elective percutaneous coronary intervention. *Cell Biochem Biophys* 2014; 70: 437–442.
14. Cai ZP, Parajuli N, Zheng X, et al. Remote ischemic preconditioning confers late protection against myocardial ischemia-reperfusion injury in mice by upregulating interleukin-10. *Basic Res Cardiol* 2012; 107: 277.
15. Loukogeorgakis SP, Panagiotidou AT, Broadhead MW, et al. Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: Role of the autonomic nervous system. *J Am Coll Cardiol* 2005; 46: 450–456.
16. Ahmed LA, Salem HA, Attia AS, et al. Comparative study of the cardioprotective effects of local and remote preconditioning in ischemia/reperfusion injury. *Life Sci* 2012; 90: 249–256.
17. Galagudza MM, Sonin DL, Vlasov TD, et al. Remote vs. local ischaemic preconditioning in the rat heart: infarct limitation, suppression of ischaemic arrhythmia and the role of reactive oxygen species. *Int J Exp Pathol* 2016; 97: 66–74.
18. Zhao Y, Zheng ZN, Jin SQ, et al. Effects of plasma collected 48 h after transient limb ischemia on blood pressure recovery in homogenic rats after myocardial ischemia reperfusion in vivo. *Chin Med J (Engl)* 2013; 126: 2894–2899.
19. Zhao Y, Zheng ZN, Cheung CW, et al. Transfusion of plasma collected at late phase after preconditioning reduces myocardial infarct size induced by ischemia-reperfusion in rats in vivo. *Chin Med J (Engl)* 2017; 130: 303–308.
20. Walker MJ, Curtis MJ, Hearse DJ, et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. *Cardiovasc Res* 1988; 22: 447–455.
21. Vegh A, Papp JG, Szekeres L, et al. Are ATP sensitive potassium channels involved in the pronounced antiarrhythmic effects of preconditioning? *Cardiovasc Res* 1993; 27: 638–643.
22. Hagar JM, Hale SL and Kloner RA. Effect of preconditioning ischemia on reperfusion arrhythmias after coronary artery occlusion and reperfusion in the rat. *Circ Res* 1991; 68: 61–68.
23. Liu Y and Downey JM. Ischemic preconditioning protects against infarction in rat heart. *Am J Physiol* 1992; 263: H1107–H1112.
24. Hausenloy DJ and Yellon DM. Remote ischaemic preconditioning: underlying mechanisms and clinical application. *Cardiovasc Res* 2008; 79: 377–386.

25. Tang ZL, Dai W, Li YJ, et al. Involvement of capsaicin-sensitive sensory nerves in early and delayed cardioprotection induced by a brief ischaemia of the small intestine. *Naunyn Schmiedebergs Arch Pharmacol* 1999; 359: 243–247.
26. Shimizu M, Tropak M, Diaz RJ, et al. Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: evidence suggesting cross-species protection. *Clin Sci (Lond)* 2009; 117: 191–200.
27. Konstantinov IE, Arab S, Kharbanda RK, et al. The remote ischemic preconditioning stimulus modifies inflammatory gene expression in humans. *Physiol Genomics* 2004; 19: 143–150.
28. Sun W and Wainwright CL. The potential antiarrhythmic effects of exogenous and endogenous bradykinin in the ischaemic rat heart in vivo. *Coron Artery Dis* 1994; 5: 541–550.
29. Lu H, Remeysen P and De Clerck F. The protection by ischemic preconditioning against myocardial ischemia- and reperfusion-induced arrhythmias is not mediated by ATP-sensitive potassium channels in rats. *Coron Artery Dis* 1993; 4: 649–657.
30. Lu HR, Remeysen P and De Clerck F. Does the antiarrhythmic effect of ischemic preconditioning in rats involve the L-arginine nitric oxide pathway? *J Cardiovasc Pharmacol* 1995; 25: 524–530.