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Role of oxidative stress, angiogenesis and chemo-attractant cytokines in the pathogenesis of ischaemic protection induced by remote ischaemic conditioning: Study of a human model of ischaemia-reperfusion induced vascular injury

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A B S T R A C T

Aims: We explored the effect of remote ischaemic conditioning (RIC) on endothelial function and on circulating mediators.

Methods and results: In 20 healthy male volunteers (mean age 31 ± 10 years), flow-mediated dilation (FMD) was measured before and after 20 min of arm ischaemia, followed by reperfusion. Remote ischaemic conditioning (RIC) was performed by applying 3 cycles of 5 min of ischaemia of the leg at the onset of index arm ischaemia. Each volunteer underwent the IR-induced vascular injury protocol with and without RIC in a crossover study design.

In the control group, IR significantly reduced FMD ($5.9 \pm 2.9\%$ before IR vs. $2.2 \pm 3.7\%$ after IR; $p < 0.001$). This effect was significantly attenuated by performing RIC (FMD of $5.5 \pm 3.1\%$ before IR vs. $4.0 \pm 3.4\%$ % after IR; p for interaction = 0.01). Serum levels of SOD and ADMA increased significantly whereas MCP-1 and VEGF levels decreased significantly.

Only changes in SOD levels were significantly related to the degree of RIC induced protection ($r^2 = 0.34$; $p = 0.018$).

Conclusion: RIC has protective effects against endothelial IR injury. Our biomarker study suggests that anti-oxidative stress mediators, such as SOD, seem to be more involved in the pathogenesis of RIC-induced protection in humans than angiogenesis factors or chemo-attractant cytokines.

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1. Introduction

The vascular endothelium regulates vasomotor, thrombotic and inflammatory mechanisms that are critical in the pathophysiology of tissue injury induced by ischaemia reperfusion (IR) injury [1]. Endothelial cells appear to be more sensitive to IR than myocytes, and during ischaemia, a state of reduced endothelial responsiveness to specific stimuli (endothelial dysfunction) temporally precedes and contributes to the appearance of IR- induced tissue necrosis [2].

In remote ischemic conditioning (RIC) brief, reversible episodes of ischemia with reperfusion in one vascular bed, tissue or organ confer a global protective phenotype and render remote tissues and organs resistant to ischemia/reperfusion injury. The peripheral stimulus can be chemical, mechanical or electrical and involves

Abbreviations: RIC, remote ischaemic conditioning; IR, ischaemia-reperfusion; FMD, flow-mediated dilation; SOD, superoxide dismutase; STEMI, ST Elevation Myocardial Infarction; ELISA, enzyme-linked immunosorbent assay; SDF-1, alpha stromal-cell derived factor-1-alpha; MCP-1, monocyte-chemoattractant protein-1; ADMA, asymmetric dimethylarginine; VEGF, vascular endothelial growth factor; SOD, superoxide dismutase; ANOVA, analysis of variance; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase.

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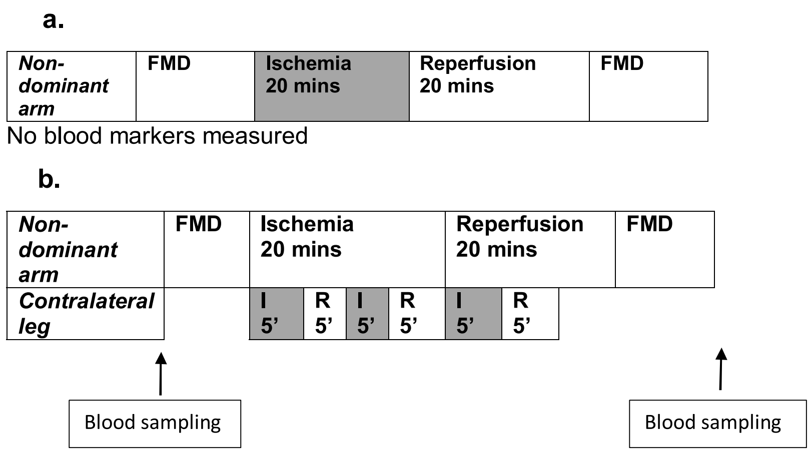

Fig. 1. Study design. a FMD of the brachial artery was assessed before 20 min of arm ischaemia (I) and after 20 min of reperfusion (R) **b.**The effect of RIC on endothelial injury was determined by applying 3 RIC cycles (3 cycles of 5 min of ischaemia (I) and 5 min of reperfusion (R) on the contralateral leg during ischaemia and early reperfusion.

activation of peripheral sensory nerves. Previous research has shown that RIC provides protection against endothelial dysfunction [3,4]. Furthermore, RIC has shown a beneficial effect on myocardial salvage in patients with acute myocardial infarction, thus demonstrating promise as a complement to angioplasty [5].

The exact mechanism of RIC remains to be elucidated, and the amount of protection seems to be dependent on various underlying factors, which are not yet fully understood. This lack of understanding gives rise to inconsistent effects when studying this procedure in the clinical setting [6,7].

RIC remains an area of scientific interest, as this simple and low-cost procedure shows promising clinical and preclinical results in various settings such as STEMI [8], stroke [9], organ transplantation [10], haemorrhagic shock [11], skin grafting [12] and renal protection [13].

The transfer of the cardioprotective signal by remote ischaemic conditioning from the peripheral organ (brain, mesentery, kidney, skeletal muscle, skin) to the heart is the result of a complex neurohumoral interaction [14] with the release of an as yet unidentified humoral factor in cardiac and non-cardiac tissues.

As such, it appears that transient limb ischaemia remotely preconditions through a humoral mechanism, but neither the quantitative amount nor the exact nature of these humoral mediators in remote ischemic conditioning are currently clear [15–18]. Additionally, a change in gene expression in response to RIC has been observed in previous studies, followed by a change in different circulating proteins in plasma in humans [19,20]. The relative importance of these circulating proteins in preventing endothelial dysfunction related to ischaemia is unknown.

The purpose of this study was to explore circulating biomarkers that are involved in the process of RIC in humans. Based on the research described above, we selected a set of specific biomarkers that are thought to be related to the protection against endothelial dysfunction.

2. Methods

2.1. Clinical study protocol

The effect of remote ischaemic conditioning on IR-related endothelial dysfunction was studied using a placebo controlled randomized crossover study design in 20 healthy volunteers. All volunteers were male (mean age +/−SD, 31.5 ± 10.7 years, range 23–58 years) and free from overt cardiovascular disease or cardiovascular risk factors.

Each participant underwent endothelial function evaluation before and after prolonged arm ischaemia (20 min of arm ischemia) at the following two different times: one endothelial function evaluation with remote ischaemic conditioning (3 cycles of intermittent ischemia reperfusion of the leg) and one without remote ischaemic conditioning with a washout period of at least one week. The sequence of the two protocols (remote ischaemic conditioning or placebo) was assigned randomly. All studies repeated in the same volunteers were at least 7 days apart. (see also Fig. 1).

We used a well-studied in vivo model of ischaemic reperfusion injury leading to endothelial IR that results in transient endothelial dysfunction in conduit and resistance vessels of the arm [3] and remote ischaemic conditioning was performed at the leg.

Ischaemic reperfusion injury of the arm was induced by 20 min of upper limb ischaemia followed by reperfusion. One arm was made ischaemic using a blood pressure cuff inflated to 200 mmHg for 20 min. FMD measurements were performed just before and 20 min after arm ischaemia. The decrease in FMD was used as a measure of the extent of IR related endothelial dysfunction

Remote ischaemic conditioning was induced by inflating a blood pressure cuff placed around the upper right thigh. The cuff was inflated to 200 mm Hg for 5 min (ischaemia), followed by a 5-min deflation (reperfusion). The inflation/deflation cycle was performed three times in total.

It is important to note that blood markers were only collected when remote ischemic conditioning was applied. In view of repetitive arm ischemia for FMD measurements in the control group (and thus possibly provoking a remote ischemic conditioning stimulus) comparison of biomarkers changes between control group and RIC groups was not valuable. Hence we focused on the correlation between ischemia induced changes in biomarkers and the degree of RIC induced vascular protection expressed as the FMD difference between pre and post arm ischemia. For this reason no biomarkers were assessed in the group undergoing arm ischemia without remote ischaemic conditioning at the leg.

The study was approved by the ethics committee of the Antwerp University Hospital. Written informed consent was obtained from all participants.

2.2. Assessment of endothelial function

Endothelial function was assessed by measuring flow-mediated dilatation (FMD) of the brachial artery, as previously described [21]. A standard ultrasound system (AU5 Harmonic, Esaote Biomedica, Genova, Italy) with a 10-MHz linear array transducer was used. Radiofrequency processing was accomplished using a computer equipped with a data acquisition board and software (Wall Track System WTS, PIE Medical, Maastricht, The Netherlands). Studies were performed at the same time in the morning in a temperature-controlled room after 15 min of supine rest. Participants abstained from smoking as well as from the intake of food and caffeine-containing beverages for 24 h prior to testing. Measurements were made at baseline and during reactive hyperaemia. To induce reactive hyperaemia, a pneumatic cuff was placed around the forearm, 2 to 15 cm above the antecubital crease, approximately 10 cm distal to the site of measurement, and the cuff was inflated to a pressure of 50 mm Hg above systolic pressure for 4 min, and then deflated. The arterial end-diastolic internal diameter was measured at baseline (3 serial measurements) and during the 4 min after cuff deflation. Post-occlusion diameters were obtained continuously after deflation, up to 4 min. Brachial artery diameter was measured in millimetres, and FMD was expressed as a percentage increase from baseline diameter. The procedure was performed by a single experienced operator who was blinded to clinical and laboratory details of the participants. The coefficient of variance of the basal diameter was 4.4% (measured on 2 consecutive days at the same time), and the intraobserver variability of FMD was calculated in a subset of 10 participants and was -0.077 ± 2.2 (absolute mean difference ± SD).

2.3. Measurement of blood markers

Blood was collected by venepuncture from an arm vein contralateral to the arm undergoing reactivity testing with cuff placement. Blood was obtained just prior to the RIC session and at the end of this session. The following plasma levels were determined by ELISA: Stromal-cell Derived Factor-1-alpha (SDF-1 alpha), Monocyte-Chemoattractant Protein-1 (MCP-1), Asymmetric Dimethylarginine (ADMA), Vascular Endothelial Growth Factor (VEGF) and Superoxide Dismutase (SOD). Coefficient of variation (CV) values of the ELISA kits [22] that were used can be found in supplementary material.

2.4. Pain scores and adverse effects

Pain scores were measured during the procedure by asking participants to rate pain on a scale from 0 to 10 every 5 min. Participants were asked to report any experience of paresthesia, paralysis or other sensations to the operator. The duration of the reported sensations was noted.

2.5. Statistical analysis

All data are expressed as mean +/− SD unless otherwise stated. Data were compared using the two-tailed Student's *t*-test or repeated measures analysis of variance (ANOVA), as appropriate. Linear regression analysis, as well as multiple forward stepwise regression analysis, was performed to correlate the change of blood markers during remote ischaemic conditioning with the degree of protection of IR endothelial dysfunction, as assessed by the difference between pre- and post-ischaemia FMD measurements. The Mann-Whitney U test was used to evaluate differences in baseline parameters before and after RIC. In all cases, $p < 0.05$ was considered statistically significant. MedCalc Statistical Software version 17.5.5 was used (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org (accessed on February, 27 2018).

3. Results

3.1. Baseline characteristics

Table 1 shows the baseline measurements for both study protocols. There were no differences in blood pressure or brachial artery diameter between the different study periods. Baseline cholesterol levels were normal (167.2 +/−32 mg/dL; normal range 150–220 mg/dL).

Table 1
Effect of RIC on blood pressure and brachial diameter Results are means +/- SD.

Variable	control n = 20	RIC n = 20	p-value
Systolic blood pressure, mmHg	135.4 (+/−10.4)	134.5 (+/−10.3)	0.24
Diastolic blood pressure, mmHg	70.9 (+/−0.5)	70.7 (+/−5.8)	0.32
Brachial artery diameter, mm	3.75 (+/−0.5)	3.95 (+/−0.56)	0.31

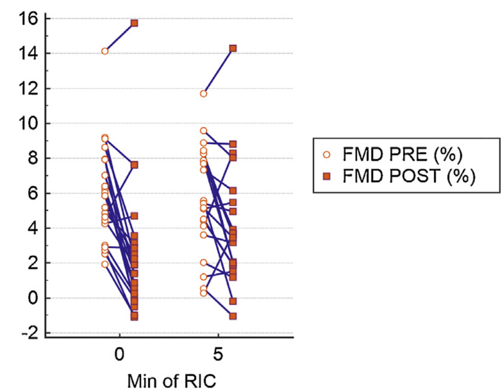

Fig. 2. Effect of RIC on vascular dilator function. FMD was 5.9 +/− 2.9% at baseline and was reduced by IR (2.2 +/− 3.7%; p < 0.001; n = 18). RIC of the leg prevented the reduction in FMD caused by ischaemia reperfusion (5.5% +/−3.1% vs. 4.0 +/−3.4% after IR; p = 0.01), though a high individual variability in response to RIC was noted as shown in this graph.

Table 2
Baseline and Post-RIC FMD data. Data are expressed as mean +/−SD.

Study	Baseline FMD	Post-IR FMD	Delta FMD	p-value
IR	5.9 +/−2.9	2.2 +/− 3.7	3.5 +/− 2.6	<0.001
IR + RIC leg	5.5 +/− 3.1	4.0 +/− 3.4%	1.5 +/− 2.9	0.01

3.2. Pain scores

All participants tolerated the procedures without any complications.

The maximum pain score on a scale from 0 to 10 was 8 (median maximum score of 5) at the leg and 7 (median maximum score of 3) at the arm. All participants experienced transient paresthesia of the arm or leg (duration 9.2 +/−3.4 min.). Five participants experienced paralysis of the arm, which was also transient with a mean duration of 2.8 +/−4.0 min. All participants tolerated the procedures without early termination and without any complications.

3.3. Effect of RIC on IR injury to the endothelium

In the control group, arm ischaemia significantly reduced FMD values from 5.9 ± 2.9% before IR to 2.2 ± 3.7% after IR ($p < 0.001$). The reduction in FMD, expressed as "delta FMD" (pre-IR FMD minus post-IR FMD), was 3.5 +/−2.6.

In the group with RIC, FMD decreased from 5.5 ± 3.1% before IR to 4.0 ± 3.4% after IR with a delta FMD of 1.5 +/− 2.9. A relative high individual variability was seen in response to RIC at the leg (see Fig. 2).

ANOVA for repeated measures revealed a significant attenuation of IR-associated endothelial dysfunction by applying RIC (p for interaction = 0.01) (see Fig. 3) (Table 2).

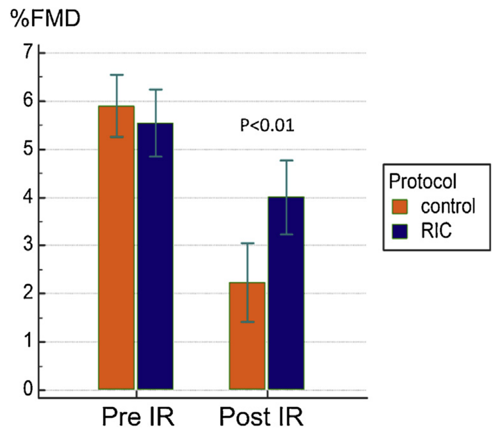

Fig. 3. Boxplot showing flow mediated dilation (FMD) before and after ischemia-reperfusion (IR) with and without remote ischemic conditioning (RIC). FMD was significantly higher after index ischemia when RIC was performed (p < 0.01).

Table 3
Biomarkers results.*n = 19. Results are means +/- SD.

Biomarker	Before RIC	After RIC	P-value
SOD (U/mL)	0,2 +/−0,2	0,5 +/−0,2	0,003
ADMA µmol/L	0,6 +/−0,1	0,8 +/−0,2	0,001
MCP (pg/mL)	347,2 +/−66,7	301,4 +/−65,3	<0,01
VEGF (pg/mL)	185,8 +/−90,9	173,7 +/−80,9	0,02
SDF 1 alpha*	2624,8 +/− 271,2	2561,7 +/− 303,8	0,1

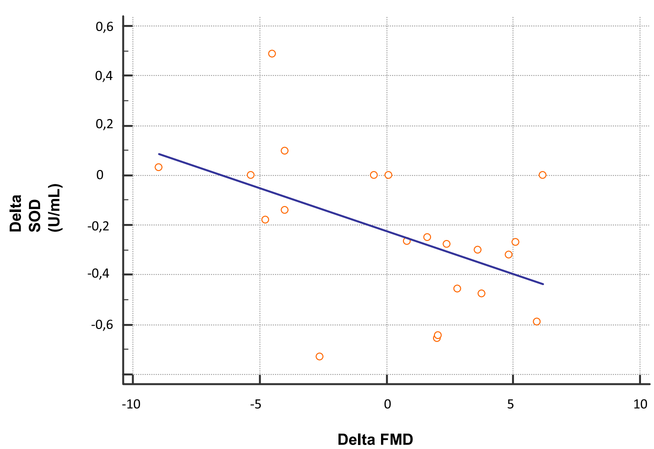

Fig. 4. Effect of RIC on circulating oxidative stress biomarkers. Scatter plot with estimated regression line showing a significant negative correlation between delta circulating superoxide dismutase plasma levels (pre- post SOD) and delta flow mediated dilation (pre – post FMD) (r^2 = 0.34; p = 0.018).

3.4. *Effect of RIC on circulating biomarkers*

There was a significant increase in SOD levels (0.24 ± 0.19 U/mL to 0.51 ± 0.24 U/mL; delta −0.26 p = 0.003) and ADMA levels (0.61 ± 0.09 µmol/L to 0.81 ± 0.19 µmol/L; p = 0.001) after leg RIC. Plasma MCP-1 decreased (348.8 ± 68.2 pg/mL to 303.4 ± 66.5 pg/mL, p < 0.01) and VEGF (181.4 ± 91.2 pg/mL to 170.9 ± 82.2 pg/mL, p = 0.02) was also significantly reduced. SDF-1 alpha levels were unchanged (2617.5 ± 277.7 pg/mL to 2548.1 ± 318.1 pg/mL, p = 0.1). (See Table 3).

Linear regression analysis showed a significant negative correlation between delta FMD and delta SOD levels (r^2 = 0.34; p = 0.018) (see Fig. 4). Participants that showed the most protection from RIC (those with the smallest decrease in FMD, this is those with the lowest delta FMD values) had the lowest increase in SOD, and some of them even showed a decrease in plasma concentration. Further analysis showed a non-significant tendency towards negative correlation between baseline SOD levels and delta FMD (r^2 = 0.11; p = 0.17) (see Fig. 4). No significant correlation could be found between the degree of protection and MCP-1, VEGF, nor SDF-1 alpha nor ADMA.

Multiple regression analysis, including changes in blood biomarkers, cholesterol level and age, confirmed that delta SOD was the only independent factor for delta FMD (p = 0.008).

4. Discussion

The level of protection against endothelial dysfunction provided by RIC is variable in humans and is dependent on various factors, such as age [23], sex [24] and tissue volume exposed to the conditioning stimulus [25]. In this study, we showed for the first time that in humans anti-oxidative stress mediators, such as SOD, seem to be more involved in the pathogenesis of RIC-induced protection in humans than angiogenesis factors or chemo-attractant cytokines.

There is an overall trend of upregulation in protein expression during the RIC procedure, and this increase in the number of upregulated peptides is cumulative with each cycle of IR of the limb [20]. Multiple animal studies have examined circulating molecules that have been proposed to play a role in RIC, such as hypoxia inducible factor 1 alpha [15], SDF-1alpha [26], interleukin 10 [16] and micro RNA-144 [27]. In humans, multiple studies have focused on circulating substances in plasma such as RNase1 [17], bradykinin [18], platelets [28] as possible circulating mediators of remote ischaemic conditioning.

The potential protective mechanism of RIC may work through inhibiting the accumulation of free radicals and reducing the inflammatory reaction caused by ischaemia reperfusion injuries. Both animal and human studies have shown that superoxide dismutase (SOD) levels increase significantly when applying remote ischaemic postconditioning, meaning that reactive oxygen species (ROS) levels decrease after RIC.

ROS play an important role in the pathophysiology of IR injury [29]. Superoxide generated by NADPH oxidase has been shown to modulate ROS production, and a recent study showed that disruption of NAPDH oxidase prevents endothelial injury in humans in vivo [25]. This finding suggests that ROS generated by NADPH oxidase are pathogenic in human IR injury. The principal dilator that accounts for FMD is endothelium-derived nitric oxide; it is therefore possible that superoxide directly inactivates nitric oxide [30], thus causing the observed reaction of IR on FMD.

In this exploratory, hypothesis-generating study an association between the level of protection by performing remote ischaemic conditioning and circulating biomarkers was looked for.

In this study, performing RIC on the leg was followed by a significant increase in SOD levels, which is consistent with previous studies. SOD levels were found to be significantly higher in patients with ST segment elevation infarction receiving RIC treatment compared to controls [31], suggesting an important association among cardiac ischaemia, RIC and SOD levels. An experimental model demonstrated that pretreatment with a SOD-mimetic protected the myocardium from damage associated with global ischaemia and reperfusion, most likely by scavenging superoxide [32].

The level of circulating SOD after RIC was lower when more protection against endothelial dysfunction was observed. Above this, we observed a tendency towards more protection when baseline circulating SOD levels where higher. This finding could suggest that a higher uptake or binding to free radicals of SOD from the circulation (as evidenced by lower increase of plasma SOD) is associated with more protection of the ischaemic (vascular) tissue. Speculatively, endothelial cells deficient in SOD might take up circulating SOD [33]. Similarly, in a prior study investigating the effect of leg

RIC on circulating nitric oxide (NO), there was an acute decrease in plasma nitrite after a single session of RIC. The hypothesis of these investigators was that NO generated by RIC of the leg is being delivered to distant arteries and stored in a form that can be released under a hypoxic stress [34].

In our study, SOD levels after ischaemia + RIC were increased, and this change in SOD levels was related to the degree of endothelial protection, which has not been investigated in previous studies to our knowledge. Further research is needed to elucidate time-dependent kinetics of SOD levels and the effect of RIC in patients suffering from acute myocardial infarction.

4.1. VEGF, SDF-1 alpha, MCP-1 and ADMA

Several lines of evidence have indicated that hypoxia enhances VEGF gene expression. The hypoxia-VEGF pathway may play an important role in angiogenesis induced by ischaemic conditioning in skeletal muscle.

Recruitment and entrapment of bone marrow-derived endothelial progenitor cells (EPCs) are important in VEGF-induced angiogenesis, and this mechanism seems to work synergistically with SDF-1alpha, which is an another important chemokine [26]. Monocyte chemoattractant protein-1 (MCP-1, also known as CC chemokine ligand 2) has a pathogenic role in animal models of IR injury [35]. MCP-1 plays a role in oxidative stress observed after ischaemia-reperfusion as NADPH oxidase activity seems to be activated through this molecule [36]. Many experimental and clinical studies have demonstrated upregulation of MCP-1 after myocardial infarction, with recruitment of monocytes/macrophages to the ischaemic myocardium [37].

Asymmetric dimethylarginine (ADMA) is a potent inhibitor of nitric oxide synthase, and increased levels of ADMA are thought to be an early hallmark of endothelial dysfunction. In this study, total levels of ADMA were significantly increased after prolonged ischemia, which could be attributed to the endothelial dysfunction induced by a period of ischaemia at the level of the arm.

In the present study we could not find a relationship between changes in plasma levels of VEGF, MCP-1, ADMA or SDF-1 alpha and the level of protection against endothelial function induced by remote ischaemic conditioning, although total levels of VEGF and MCP-1 were significantly reduced in this model of IR injury with application of RIC. This decrease in circulating levels of VEGF and MCP-1 could be attributed to a higher binding of these molecules in the ischaemic tissue, but without measurable effect on endothelial function.

4.2. Measurement of endothelial function: flow-mediated dilation

Endothelial function can be evaluated by different approaches. In this study, we used artery FMD measured by high-resolution ultrasonography, which is a common research method that is used in various settings for the examination of endothelial function [21]. Coronary endothelial function has been found to correlate with endothelial function in accessible peripheral arteries, such as the brachial artery. A close relationship has been demonstrated between FMD in the brachial artery and the coronary circulation, both in terms of morphologic lesions and functional responses to acetylcholine [38].

4.3. RIC in STEMI

Previous clinical studies have shown possible beneficial effects of RIC in STEMI patients. In a study by Botker et al., patients received remote conditioning at the arm during transport to hospital. The primary endpoint was myocardial salvage index at 30 days after primary percutaneous coronary intervention, as measured by myocardial perfusion imaging. Remote ischaemic conditioning before hospital admission increased myocardial salvage, though the effect was variable [5].

4.4. Use of leg versus arm RIC

In our study, the leg was used to avoid a subthreshold conditioning stimulus, as has been noted in previous studies investigating tourniquet-induced remote conditioning in the limbs in humans. In one study, RIC administered as 2 cycles was effective only when applied to the leg instead of the arm, and the delta FMD was lower when the leg was used versus the arm [4]. Surprisingly, maximum pain scores reached higher levels in our study, which could partly explain why this simple procedure is not yet used in patients suffering acute ischaemic events.

4.5. Limitations

In view of repetitive arm ischemia for FMD measurements in the control group, comparison of biomarkers changes between control group and RIC groups was not valuable. Hence we focused on the correlation between ischemia induced changes in biomarkers and the degree of RIC induced vascular protection expressed as the FMD difference between pre and post arm ischemia. For this reason, no biomarkers were assessed in the group undergoing arm ischemia without remote ischemic conditioning at the leg.

Only male participants were considered as it is known that hormonal changes can affect the effect seen by applying remote ischemic conditioning. It would be very interesting to repeat this study in females assessing the difference between males and females and consequent effects attributable to hormonal differences. Above this, most studies investigating effects of remote ischemic conditioning in healthy humans are done in males, so this remains an area of scientific interest.

5. Conclusion

Remote ischaemic conditioning in humans can be induced by applying short cycles of ischaemia of one leg, and it has protective effects against endothelial IR injury. Our biomarker study suggests that antioxidative stress mediators, such as SOD, seem to be involved in the pathogenesis of RIC-induced protection in humans more than angiogenesis factors or chemo-attractant cytokines. However, more research is needed to draw definitive conclusions regarding the pathophysiological involvement in patients suffering cardiovascular disease.

Conflict of interest

None.

Informed consent statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration of 1975, as revised in 2000 and the study was approved by the local ethics committee of the Antwerp University Hospital. Informed consent was obtained from all volunteers for being included in the study.

Animal studies statement

No animal studies were carried out by the authors for this article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pathophys.2018.11.001.

References

[1] K. Laude, P. Beauchamp, C. Thuillez, V. Richard, Endothelial protective effects of preconditioning, Cardiovasc. Res. 55 (2002) 466–473.
[2] P.S. Mankad, M. Amrani, S. Rothery, N.J. Severs, M.H. Yacoub, Relative susceptibility of endothelium and myocardial cells to ischaemia-reperfusion injury, Acta Physiol. Scand. 161 (1997) 103–112, http://dx.doi.org/10.1046/j.1365-201X.1997.00195.x.
[3] S.P. Loukogeorgakis, R. Williams, A.T. Panagiotidou, S.K. Kolvekar, A. Donald, T.J. Cole, D.M. Yellon, J.E. Deanfield, R.J. MacAllister, Transient limb ischemia induces remote preconditioning and remote postconditioning in humans by a K(ATP)-channel dependent mechanism, Circulation 116 (2007) 1386–1395, http://dx.doi.org/10.1161/CIRCULATIONAHA.106.653782.
[4] S.P. Loukogeorgakis, A.T. Panagiotidou, M.W. Broadhead, A. Donald, J.E. Deanfield, R.J. MacAllister, 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. 46 (2005) 450–456, http://dx.doi.org/10.1016/j.jacc.2005.04.044.
[5] H.E. Bøtker, R. Kharbanda, M.R. Schmidt, M. Bøttcher, A.K. Kaltoft, C.J. Terkelsen, K. Munk, N.H. Andersen, T.M. Hansen, S. Trautner, J.F. Lassen, E.H. Christiansen, L.R. Krusell, S.D. Kristensen, L. Thuesen, S.S. Nielsen, M. Rehling, H.T. Sørensen, A.N. Redington, T.T. Nielsen, Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial, Lancet Lond. Engl. 375 (2010) 727–734, http://dx.doi.org/10.1016/S0140-6736(09)62001-8.
[6] D.J. Hausenloy, L. Candilio, R. Evans, C. Ariti, D.P. Jenkins, S. Kolvekar, R. Knight, G. Kunst, C. Laing, J. Nicholas, J. Pepper, S. Robertson, M. Xenou, T. Clayton, D.M. Yellon, ERICCA trial investigators, remote ischemic preconditioning and outcomes of cardiac surgery, N. Engl. J. Med. 373 (2015) 1408–1417, http://dx.doi.org/10.1056/NEJMoa1413534.
[7] S.L. McLeod, A. Iansavichene, S. Cheskes, Remote ischemic perconditioning to reduce reperfusion injury during acute ST-segment-elevation myocardial infarction: a systematic review and meta-analysis, J. Am. Heart Assoc. 6 (2017), http://dx.doi.org/10.1161/JAHA.117.005522.
[8] D.J. Hausenloy, R. Kharbanda, M. Rahbek Schmidt, U.K. Møller, J. Ravkilde, L. Okkels Jensen, T. Engstrøm, J.M. Garcia Ruiz, N. Radovanovic, E.F. Christensen, H.T. Sørensen, M. Ramlall, H. Bulluck, R. Evans, J. Nicholas, R. Knight, T. Clayton, D.M. Yellon, H.E. Bøtker, Effect of remote ischaemic conditioning on clinical outcomes in patients presenting with an ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention, Eur. Heart J. 36 (2015) 1846–1848.
[9] D.C. Hess, R.A. Blauenfeldt, G. Andersen, K.D. Hougaard, M.N. Hoda, Y. Ding, X. Ji, Remote ischaemic conditioning-a new paradigm of self-protection in the brain, Nat. Rev. Neurol. 11 (2015) 698–710, http://dx.doi.org/10.1038/nrneurol.2015.223.
[10] J. Jia, J. Li, L. Jiang, J. Zhang, S. Chen, L. Wang, Y. Zhou, H. Xie, L. Zhou, S. Zheng, Protective effect of remote limb ischemic perconditioning on the liver grafts of rats with a novel model, PLoS One 10 (2015), e0121972, http://dx.doi.org/10.1371/journal.pone.0121972.
[11] J. Huang, D. Xu, Q. Guo, B. Ou, Q. Ling, J. Li, Z. Yang, W. Tang, Remote ischemic post-conditioning improves myocardial dysfunction via the risk and safe pathways in a rat model of severe hemorrhagic shock, Shock Augusta Ga (2017), http://dx.doi.org/10.1097/SHK.0000000000000940.
[12] R. Kraemer, J. Lorenzen, M. Kabbani, C. Herold, M. Busche, P.M. Vogt, K. Knobloch, Acute effects of remote ischemic preconditioning on cutaneous microcirculation–a controlled prospective cohort study, BMC Surg. 11 (2011) 32, http://dx.doi.org/10.1186/1471-2482-11-32.
[13] C. Zhou, Y. Jeon, P. Meybohm, A. Zarbock, P.J. Young, L. Li, D.J. Hausenloy, Renoprotection by remote ischemic conditioning during elective coronary revascularization: a systematic review and meta-analysis of randomized controlled trials, Int. J. Cardiol. 222 (2016) 295–302, http://dx.doi.org/10.1016/j.ijcard.2016.07.176.
[14] S.M. Davidson, P. Selvaraj, D. He, C. Boi-Doku, R.L. Yellon, J.M. Vicencio, D.M. Yellon, Remote ischaemic preconditioning involves signalling through the SDF-1α/CXCR4 signalling axis, Basic Res. Cardiol. 108 (2013) 377, http://dx.doi.org/10.1007/s00395-013-0377-6.
[15] H. Kalakech, S. Tamareille, S. Pons, D. Godin-Ribuot, P. Carmeliet, A. Furber, V. Martin, A. Berdeaux, B. Ghaleh, F. Prunier, Role of hypoxia inducible factor-1α in remote limb ischemic preconditioning, J. Mol. Cell. Cardiol. 65 (2013) 98–104, http://dx.doi.org/10.1016/j.yjmcc.2013.10.001.
[16] Z.P. Cai, N. Parajuli, X. Zheng, L. Becker, Remote ischemic preconditioning confers late protection against myocardial ischemia-reperfusion injury in mice by upregulating interleukin-10, Basic Res. Cardiol. 107 (2012) 277, http://dx.doi.org/10.1007/s00395-012-0277-1.
[17] H.A. Cabrera-Fuentes, B. Niemann, P. Grieshaber, M. Wollbrueck, J. Gehron, K.T. Preissner, A. Böning, RNase1 as a potential mediator of remote ischaemic preconditioning for cardioprotection, Eur. J. Cardiothorac. Surg. 48 (2015) 732–737, http://dx.doi.org/10.1093/ejcts/ezu519, discussion 737.
[18] R. Sharma, P.K. Randhawa, N. Singh, A.S. Jaggi, Bradykinin in ischemic conditioning-induced tissue protection: evidences and possible mechanisms, Eur. J. Pharmacol. 768 (2015) 58–70, http://dx.doi.org/10.1016/j.ejphar.2015.10.029.
[19] H.K. Kim, S.W. Kang, S.H. Jeong, N. Kim, J.H. Ko, H. Bang, W.S. Park, T.-H. Choi, Y.-R. Ha, Y.S. Lee, J.B. Youm, K.S. Ko, B.D. Rhee, J. Han, Identification of potential target genes of cardioprotection against ischemia-reperfusion injury by express sequence tags analysis in rat hearts, J. Cardiol. 60 (2012) 98–110, http://dx.doi.org/10.1016/j.jjcc.2012.02.004.
[20] M. Hepponstall, V. Ignjatovic, S. Binos, P. Monagle, B. Jones, M.H.H. Cheung, Y. d'Udekem, I.E. Konstantinov, Remote ischemic preconditioning (RIPC) modifies plasma proteome in humans, PLoS One 7 (2012), e48284, http://dx.doi.org/10.1371/journal.pone.0048284.
[21] A.L. Moens, I. Goovaerts, M.J. Claeys, C.J. Vrints, Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? Chest 127 (2005) 2254–2263, http://dx.doi.org/10.1378/chest.127.6.2254.
[22] G.F. Reed, F. Lynn, B.D. Meade, Use of coefficient of variation in assessing variability of quantitative assays, Clin. Diagn. Lab. Immunol. 9 (2002) 1235–1239, http://dx.doi.org/10.1128/CDLI.9.6.1235-1239.2002.
[23] L. Moro, C. Pedone, A. Mondì, E. Nunziata, R. Antonelli Incalzi, Effect of local and remote ischemic preconditioning on endothelial function in young people and healthy or hypertensive elderly people, Atherosclerosis 219 (2011) 750–752, http://dx.doi.org/10.1016/j.atherosclerosis.2011.08.046.
[24] P. Paradis-Deschênes, D.R. Joanisse, F. Billaut, Sex-specific impact of ischemic preconditioning on tissue oxygenation and maximal concentric force, Front. Physiol. 7 (2016) 674, http://dx.doi.org/10.3389/fphys.2016.00674.
[25] S.P. Loukogeorgakis, M.J. van den Berg, R. Sofat, D. Nitsch, M. Charakida, B. Haiyee, E. de Groot, R.J. MacAllister, T.W. Kuijpers, J.E. Deanfield, Role of NADPH oxidase in endothelial ischemia/reperfusion injury in humans, Circulation 121 (2010) 2310–2316, http://dx.doi.org/10.1161/CIRCULATIONAHA.108.814731.
[26] J.-X. Yu, X.-F. Huang, W.-M. Lv, C.-S. Ye, X.-Z. Peng, H. Zhang, L.-B. Xiao, S.-M. Wang, Combination of stromal-derived factor-1alpha and vascular endothelial growth factor gene-modified endothelial progenitor cells is more effective for ischemic neovascularization, J. Vasc. Surg. 50 (2009) 608–616, http://dx.doi.org/10.1016/j.jvs.2009.05.049.
[27] K. Przyklenk, microRNA-144: the 'what' and 'how' of remote ischemic conditioning? Basic Res. Cardiol. 109 (2014) 429, http://dx.doi.org/10.1007/s00395-014-0429-6.
[28] A. Stazi, G. Scalone, M. Laurito, M. Milo, G. Pelargonio, M.L. Narducci, R. Parrinello, S. Figliozzi, G. Bencardino, F. Perna, G.A. Lanza, F. Crea, Effect of remote ischemic preconditioning on platelet activation and reactivity induced by ablation for atrial fibrillation, Circulation 129 (2014) 11–17, http://dx.doi.org/10.1161/CIRCULATIONAHA.113.005336.
[29] F. Bagheri, V. Khori, A.M. Alizadeh, S. Khalighfard, S. Khodayari, H. Khodayari, Reactive oxygen species-mediated cardiac-reperfusion injury: mechanisms and therapies, Life Sci. 165 (2016) 43–55, http://dx.doi.org/10.1016/j.lfs.2016.09.013.
[30] S. Lavi, E.H. Yang, A. Prasad, V. Mathew, G.W. Barsness, C.S. Rihal, L.O. Lerman, A. Lerman, The interaction between coronary endothelial dysfunction, local oxidative stress, and endogenous nitric oxide in humans, Hypertension 51 (2008) 127–133, http://dx.doi.org/10.1161/HYPERTENSIONAHA.107.099986.
[31] H. Lotfollahi, M. Mohammadi, S. Ghaffari, R. Badalzadeh, B. Sohrabi, N. Aslanabadi, A. Separham, A. Golmohammadi, A. Abbasnejad, M. Roshani, Effect of remote ischemic post-conditioning on oxidative stress in blood of STEMI patients treated with primary angioplasty, J. Cardiovasc. Thorac. Res. 8 (2016) 113–118, http://dx.doi.org/10.15171/jcvtr.2016.24.
[32] K. Kilgore, G.S. Friedrichs, C.R. Johnson, C.S. Schasteen, D.P. Riley, R.H. Weiss, U. Ryan, B. Robert Lucchesi, Protective effects of the SOD-mimetic SC-52608 against Ischemia/Reperfusion damage in the rabbit isolated heart, J. Mol. Cell. Cardiol. 26 (1994) 995–1006, http://dx.doi.org/10.1006/jmcc.1994.1120.
[33] H. Ohta, T. Adachi, K. Hirano, Internalization of human extracellular-superoxide dismutase by bovine aortic endothelial cells, Free Radic. Biol. Med. 16 (1994) 501–507.
[34] J.M. Rifkind, E. Nagababu, D.A. Dobrosielski, M.T. Salgado, M. Lima, P. Ouyang, H.A. Silber, The effect of intermittent pneumatic compression of legs on the levels of nitric oxide related species in blood and on arterial function in the arm, Nitric Oxide Biol. Chem. 40 (2014) 117–122, http://dx.doi.org/10.1016/j.niox.2014.06.007.

[35] T. Kakio, A. Matsumori, K. Ono, H. Ito, K. Matsushima, S. Sasayama, Roles and relationship of macrophages and monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 in the ischemic and reperfused rat heart, Lab. Investig. J. Technol. Methods Pathol. 80 (2000) 1127–1136.
[36] T. Hayasaki, K. Kaikita, T. Okuma, E. Yamamoto, W.A. Kuziel, H. Ogawa, M. Takeya, CC chemokine receptor-2 deficiency attenuates oxidative stress and infarct size caused by myocardial ischemia-reperfusion in mice, Circ. J. 70 (2006) 342–351.
[37] I. Martinovic, N. Abegunewardene, M. Seul, M. Vosseler, G. Horstick, M. Buerke, H. Darius, S. Lindemann, Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease, Circ. J. 69 (2005) 1484–1489.
[38] H. Teragawa, K. Ueda, K. Matsuda, M. Kimura, Y. Higashi, T. Oshima, M. Yoshizumi, K. Chayama, Relationship between endothelial function in the coronary and brachial arteries, Clin. Cardiol. 28 (2005) 460–466.