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Remote ischemic preconditioning of the heart: Combining lower limb ischemia and electronic stimulation of the gastrocnemius muscle

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Short Title

RIPC(+) study

Abstract.

Background:

Remote ischemic preconditioning (RIPC) has been demonstrated to induce potent cardioprotection in individuals experiencing coronary ischemia. A protocol combining limb ischemia and electronic muscle stimulation of the ischemic skeletal muscle (RIPC+), performed in advance of coronary artery occlusion, was superior in terms of infarct size reduction when compared to RIPC alone.

Methods:

This study was performed to evaluate the benefit of RIPC+ in humans compared to a standard RIPC protocol and a control group. Patients with a single vessel coronary artery disease undergoing elective PCI were eligible to participate in this study. ST-segment elevations from an intracoronary ECG during 3 brief episodes of coronary artery balloon occlusions/dilatation were used as the primary endpoint.

Results:

ST-elevations significantly declined from the first to the third angioplasty in the control but remained at the same level in the RIPC and RIPC + groups. The RIPC group was characterized by the lowest ST-segment shift during coronary ischemia, which was comparable to coronary balloon occlusion number 3 in the control group, indicating successful preconditioning by the conventional RIPC method. In contrast, ST segment elevations were significantly higher in the RIPC + group. Troponin levels taken 24h after the study procedure were significantly lower in the RIPC when compared to the control and the RIPC + group.

Conclusion:

Our results again confirm the feasibility of remote ischemic preconditioning in patients undergoing coronary angioplasty. According to our results ischemia combined with electronic skeletal muscle stimulation was not superior to conventional RIPC cycles (skeletal muscle ischemia alone).

Keywords: Coronary artery disease, percutaneous coronary intervention, remote ischemic preconditioning

1. Introduction

Timely re-perfusion is the most effective intervention to prevent ischemic damage in the context of acute myocardial infarction (AMI). Paradoxically, restoration of blood flow can itself exacerbate cell death (rather than initiate salvage), a phenomenon which is termed lethal ischemia re-perfusion injury (I/R) [1]. In 1986 Murry et al. observed that the heart could be rendered resistant to lethal I/R by exposure to a brief and non-lethal, antecedent ischemic episode [2]. Subsequent studies expanded the paradigm of myocardial “conditioning” beyond the phenomenon of ischemic preconditioning to encompass post-conditioning and remote conditioning. There is a wealth of evidence that all three forms induce potent cardio-protection [3-6].

Remote ischemic preconditioning (RIPC) has been first demonstrated by application of four five-minute cycles of ischemia reperfusion to the circumflex artery which significantly reduced the infarct size caused by a sustained occlusion of the left anterior descending artery [5]. Applying ischemia-reperfusion episodes in organs remote from the heart also protected the heart from ischemia [7-9].

Despite encouraging results, RIPC has not become part of routine clinical practice yet, possibly because the efficacy of ischemic conditioning strategies in humans seem to be less profound than reported in the animal literature [10-14]. On the other hand, it could be shown that RIPC led to an improvement of the microcirculation [15, 16]. Optimizing current ischemic conditioning strategies may therefore be key to show a consistent benefit in clinical trials.

This study therefore aimed to enhance the efficacy of current remote ischemic preconditioning protocols by combining partial reduction of blood supply and rapid electrical stimulation of the gastrocnemius muscle in humans as previously demonstrated in an animal model [17].

2. Methods

The present blinded, randomized study investigated weather cardio-protective effects of standard RIPC at the lower leg can be enhanced by additional electronic stimulation of the ischemic gastrocnemius muscle. The local Ethic committee (University of Greifswald) approved the study. Details of the study protocol are published at clinical trials.gov (NCT01357499).

Thirty patients undergoing elective coronary angioplasty were randomized in a control, a standard remote ischemia (RIPC) and a RIPC + electronic muscle stimulation (RIPC +) group. After obtaining the patients' consent, a coronary angiogram was performed. In case of a suitable coronary anatomy, patients were randomly allocated in one of the three groups.

2.1. Control group

These patients did not undergo RIPC. The angioplasty of the target lesion was started 30 min after placement of a BP cuff and the skin electrodes of the HiToP 191 (Gbo Medizintechnik AG, Rimbach, Germany) at the right lower leg (without inflating the cuff or stimulating the muscle).

Coronary intervention was performed according to standard techniques using a right femoral artery approach. The positioned coronary guide-wire was extended with a DOC-wire (0.014 inches, 145 cm; Abott vascular) and connected to the ECG unit to create an intracoronary ECG channel. Thereafter the

coronary balloon catheter was placed within the stenosis and inflated for 2 minutes. Following this inflation cycle, a 5 minutes recovery period was allowed to reestablish baseline hemodynamics and ECG conditions. This cycle was repeated 3 times [18].
To ensure complete occlusion of the coronary artery, iodinated contrast media was injected after each balloon inflation. Angioplasty was completed on the basis of the specific needs of individual patients.

2.2. Standard remote ischemic preconditioning group (RIPC group)

A blood pressure cuff was placed at the right lower leg and inflated to 200 mmHg for 5 minutes. Thereafter the cuff was deflated for 5 minutes. This cycle was repeated 3 times followed by coronary angioplasty as mentioned in the control group.

2.3. Remote ischemic preconditioning and electronic stimulation of the gastrocnemius muscle (RIPC+ group)

Electronic stimulation of the gastrocnemius muscle was performed throughout the standard RIPC cycle using the HiToP 191 device. The output of the HiToP 191 device was adjusted at the lowest level where contraction of the skeletal muscle was clearly visible. The procedure was concluded as described in the control group.

2.4. Inclusion and exclusion criteria

All Patients had to meet the following criteria to participate in the study: (1) single vessel disease, (2) vessel diameter distal of the coronary artery lesion of at least 2.5 mm), (3) 18 years of age or older, stable angina.
Candidates were excluded from the final analysis in the presence of the following characteristics: (1) presence of collateral vessels (according to rentrop criteria), (2) bundle branch blocks as determined by surface ECG, (3) multiple coronary artery lesions, (4) chronic coronary artery occlusion, (5) renal failure (eGFR (MDRD) < 50 ml/min/1.73 m²), (6) history of coronary bypass grafting, (7) history or presence of acute coronary syndrome, (8) left ventricular hypertrophy as determined by echocardiography (septal and/or posterior wall diameter ≥ 14 mm), (9) specific medications influencing ischemic preconditioning (Adenosine, Morphin und Derivates, Immunosuppressive agents, antibiotics, Theophyllin, alpha Receptor blockers, oral antidiabetics), (10) peripheral arterial disease, (11) exercise tests performed within 48 h before study inclusion.

2.5. Primary Endpoint

ST deviation of the intracoronary ECG, derived from the PCI wire was the primary endpoint in this study.

2.6. Secondary endpoints

Troponin I was taken at baseline after informed consent has been signed and 24 h after coronary intervention.
The maximum of chestpain on a visual analog scale ranging from 0 as no pain and 10 as maximum pain was recorded during each cycle of coronary balloon-inflation.

Furthermore arterial and venous blood samples were taken from the femoral artery and vein of the preconditioned leg before and after preconditioning. Venous lactate, oxygen saturations as well as arterio-venous oxygen differences were determined.

2.7. Statistics

Power calculations were based on the results of a study published by Tomai et al. who investigated the effect of phentolamin in the context of RIPC [19]. Furthermore, a dropout rate of 20 % was assumed, resulting in a study-population of 30 patients (10 in each group). After completion of the first 30 patients an interim analysis and potential further patient-enrollment was intended.

Statistical analysis was performed using SPSS version 19 for Windows (Chicago, IL, USA). Results of continuous variables are expressed as median and interquartile Range. Results of categorical data are reported as frequencies (%). Continuous variables were compared by Wilcox and Mann Whitney U-Test for inter-group comparisons whereas a Chi-square or Fisher exact test was used to compare categorical variables. For intra-group comparisons a Wilcoxon signed rank test was used. Level of significance was set at $p < 0.05$. All reported p-values are 2-sided.

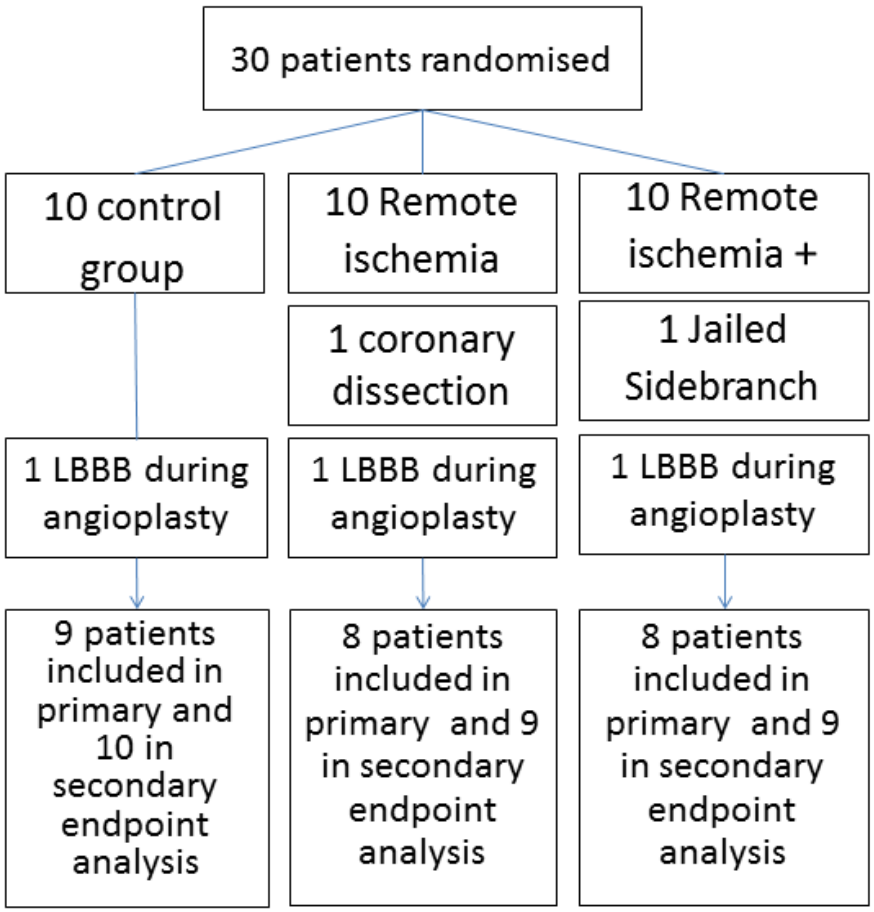

Fig. 1. Study flow chart. LBBB=left bundle branch block

3. Results

After randomization 10 patients were included in each of the 3 pre-specified groups. One patient in each of both remote preconditioning groups were excluded from the final analysis due to PCI related events (see Fig. 1). Another three patients developed a left bundle branch block during the first coronary balloon-dilatation, which precluded them from inclusion in the analysis of the primary endpoint (but not secondary endpoints) (Fig. 1). The distribution of the reported baseline-characteristics did not significantly change after exclusion of these individuals.

Baseline clinical features are depicted in Table 1. No significant differences between the investigated groups were observed with respect to baseline demographics, cardiovascular risk factors and medications. Peri-procedural data were also well balanced (Table 2).

Table 1: Baseline characteristics

	Control group (n=10)	Remote ischemia (n=9)	Remote ischemia + (n=9)	p
Demographics				
Age (years)	71 (11)	71 (14)	75 (12)	0.716
Gender (male)	7 (70 %)	5 (56 %)	6 (67 %)	0.538
Risk factors				
BMI	30 (8)	26 (4)	29 (6)	0.570
Diabetes	2 (20 %)	0	1 (11 %)	0.589
Total cholesterol	200 (65)	188 (50)	210 (70)	0.625
Arterial HT	7 (50 %)	5 (56 %)	5 (56 %)	0.687
Clinical details				
eGFR	73 (20)	79 (24)	76 (18)	0.810
CCS grade III/IV	0	0	0	-
Medications				
Statins	10 (100 %)	7 (78 %)	8 (89 %)	0.462
ACEI/ARB	9 (90 %)	7 (78 %)	7 (78 %)	0.720
ß-Blockers	5 (50 %)	5 (56 %)	6 (67 %)	0.453

BMI=body mass index, HT= hypertension, ACEI/ARB = ACE inhibitors/angiotensin receptor blockers

Table 2: Angiographic and Peri-procedural Data

	Control group (n=10)	Remote Ischemia (n=9)	Remote Ischemia + (n=9)	p
Target vessel				
LAD	7 (70 %)	6 (67 %)	7 (78 %)	0.705
CX	1 (10 %)	2 (22 %)	1 (11 %)	
RCA	2 (20 %)	1 (11 %)	1 (11 %)	
Lesion type (ACC/AHA)				
A	8 (80 %)	7 (78 %)	7 (78 %)	0.920
B	2 (20 %)	2 (22 %)	2 (22 %)	
C	0	0	0	
Stenosis severity				
Systolic BP before PCI (mmHg)	135 (21)	130 (32)	145 (32)	
Diastolic BP before PCI (mmHg)	70 (10)	60 (19)	75 (20)	
Target vessel diameter (mm)	2.5 (0.5)	3.0 (0.5)	2.5 (0.5)	0.833
Contrast (ml)	120 (40)	110 (40)	150 (51)	0.583
Post procedural TIMI III flow	10 (100 %)	9 (100 %)	9 (100 %)	0.993

LAD = left anterior descending artery, CX = circumflex artery, RCA = right coronary artery, BP = blood pressure.

ST-segment changes during the first balloon inflation were significantly different in the investigated groups, showing the smallest excursion in patients who underwent remote preconditioning without electrical stimulation of the gastrocnemius muscle (Table 3). As depicted in Table 3 and Fig. 2, ST-elevations significantly declined from the first to the third angioplasty in the control but not in the RIPC and RIPC+ groups. ST-segments remained at a significantly higher level throughout the three coronary inflation cycles in RIPC+ patients when compared to the RIPC group.

Troponin I at 24 h following PCI was elevated in the control and the remote preconditioning+ group. In contrast, Troponin levels remained within the normal range in all patients receiving RIPC, with significant difference compared to the remaining groups.

No significant differences were observed between the investigated groups in cardiac pain severity. There was a modest trend towards decreasing chest pain from the first to the last coronary angioplasty in the control and the RIPC+ group. Although not statistically different, the RIPC cohort reported the lowest pain severity at the end of the first angioplasty.

Markers **of remote preconditioning:** As expected, venous lactate and arteriovenous difference of oxygen saturation were significantly increased in both preconditioning groups whereas none of the preconditioning methods was superior with regard to these markers. Of note, neither venous lactate nor oxygen saturations following remote preconditioning correlated with one of the pre-specified endpoints.

Table 3: Endpoint parameters

	Control group (n=10)	Remote Ischemia (n=9)	Remote Ischemia + (n=9)	p
Left Bundle branch block				
After 1st Dilatation	1 (10 %)	1 (11 %)	1 (11 %)	0.911
After 2nd Dilatation	0	0	0	-
After 3rd Dilatation	0	0	0	-
ST-elevation				
Baseline (mm)	0 (2)	0 (1)	0 (2)	0.412
1st Dilatation (mm)	15.5 (21)*	6 (8)†	15.7 (22)	0.039
2nd Dilatation (mm)	13.4 (11.1)	4 (8.5)†	17.3 (12.7)	0.051
3rd Dilatation (mm)	11 (9) §	4.5 (11.6)	14.5 (10.6)	0.149
Troponin I (µg/L) at 24 h‡	0.39 (0.56)*	0.04 (1.09)	0.25 (0.45)	0.025
Cardiac pain				
1st Dilatation	5 (4)	6 (8)	7 (2)	0.794
2nd Dilatation	4 (5)	6.5 (8)	6 (9)	0.487
3rd Dilatation	3.5 (4)	8 (7)	5 (7)	0.447
Venous Lactate after preconditioning (mmol/L)	1.1 (0.3)*†	1.3 (0.8)	1.5 (0.5)	0.028
Arteriovenous oxygen difference after preconditioning (%)	21.8 (11.6)*†	36 (12.1)	36.7 (23.8)	0.003

Patients who developed a left bundle branch block during intervention were excluded from the IC-ECG analysis.

*Significantly different compared to remote ischemia; †significantly different compared to remote ischemia +;

§ significantly different compared to first Dilatation. ‡ control group (n = 10) , Remote Ischemia (n = 7), Remote Ischemia + (n = 9).

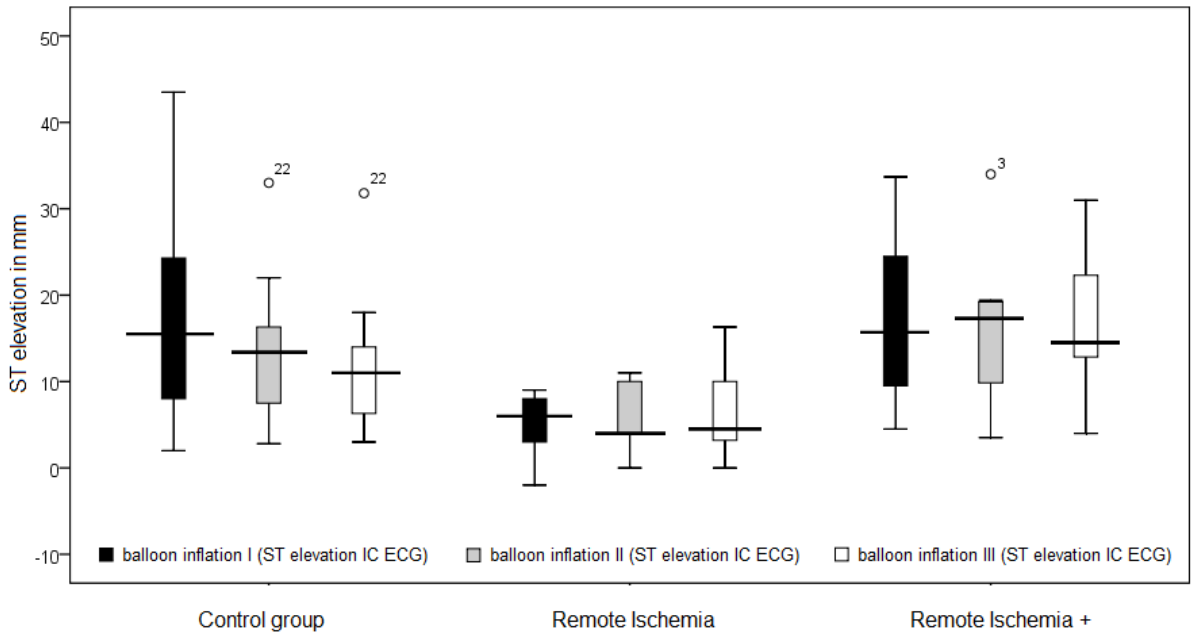

Fig. 2. ST segment changes of IC-ECG. IC-ECG = intracoronary ECG

4. Discussion

This study intended to maximize the effect of remote ischemic preconditioning of the heart by combining lower limb ischemia and electrical muscle stimulation of the ischemic skeletal muscle. Most important, our results again confirm the efficacy of remote ischemic preconditioning in reducing the ischemic burden of the heart in patients with acute coronary artery occlusion, caused by intracoronary balloon inflation in our study. However, the combination of lower leg ischemia and electrical stimulation of the ischemic muscle did not enhance the preconditioning efficacy when compared to ischemia alone which is in contrast to a previous investigation in animals [18].

In the present study, two RIPC strategies were evaluated and compared to a control group. Intracoronary ECG, which has been demonstrated to predict microvascular obstruction and myocardial infarct size in primary PCI, was used to demonstrate the ischemic burden of the heart during 3 brief episodes of coronary ischemia, induced by inflation of a coronary angioplasty balloon [19, 20].

All study participants had stable coronary artery disease and comparable baseline characteristics. As previously demonstrated, the ST-segment shift in our control group significantly declined from the first to the third balloon inflation, indicating a significant preconditioning effect caused by the first and the second ischemic episodes. Of note, in our RIPC group ST-elevations during balloon-inflation one to three were significantly lower compared to the first inflation in the control group, but similar to inflation number 3 in the control group. These results demonstrate preexistent ischemic conditioning caused by

the preliminary RIPC cycles. It also implies similar underlying mechanisms in ischemic preconditioning at the same vascular drainage and those remote from the heart.

As observed in our RIPC patients, no significant changes in ST segment shifts were found from balloon-inflation cycle 1 to 3 in the RIPC+ group. However, ST segment elevations in these patients were comparable to those measured during the (not preconditioned) first balloon-inflation in the control group and therefore significantly higher when compared to RIPC.
These findings suggest that additional electronic muscle stimulation may inhibit certain preconditioning pathways and may therefore abolish the preconditioning effect [21]. On the other hand, despite the randomized design of the present study, sample size and inhomogeneities between the analyzed groups like microvascular dysfunction due to atherosclerotic vessel wall changes on a subclinical level cannot be excluded and could also account for our findings [22, 23]. According to this hypothesis, preconditioning may still be present at a different level.
However, Troponin, a marker for myocardial damage, which was taken 24h after the procedure, was also higher in the RIPC+ group when compared to the RIPC patients who showed the lowest levels. In terms of Troponin, no difference was observed between the RIPC+ and the control group.

Cardiac pain during coronary artery occlusion or caused by significant coronary stenosis, is another marker of coronary ischemia and RIPC has been demonstrated to effect pain severity in a positive way [13, 19]. In our study, only a non-significant trend towards decreasing pain severity from the first to the third coronary balloon inflation was observed in the control-group. In contrast to the above-mentioned parameters, patients in the RIPC and the RIPC+ group reported increased pain severity.
As pain is a subjective parameter, the applied RIPC/RIPC+, which may at least have been inconvenient for some patients, could have influenced the reported cardiac pain severity.

4.1. Limitations of the study

First, PCI was performed in different vascular drainages and was not restricted to one coronary artery. This may be important as the supplied muscular tissue and therefore the derived ST segment shifts may differ between different coronary arteries. Furthermore, the included number of patients may not have been sufficient to detect a difference between our RIPC and RIPC+ groups. Our findings regarding the RIPC+ group may therefore not be conclusive.
On the other hand, the present study is a randomized study showing well-balanced baseline characteristics. The number of included patients corresponds to the initially performed power-calculations. Despite the mentioned limitations confounders seem to be restricted as far as possible.

4.2. Conclusion

Our results again confirm the feasibility of remote ischemic preconditioning in patients undergoing coronary angioplasty. According to our results ischemia combined with electronic skeletal muscle stimulation was not superior to conventional RIPC cycles (skeletal muscle ischemia alone).

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