Basic Res Cardiol 95: 418 – 423 (2000)

ORIGINAL CONTRIBUTION

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Delayed attenuation of myocardial ischemia with repeated exercise in subjects with stable angina: a possible model for the second window of protection?

Received: 2 February 2000
Returned for 1. revision: 23 February 2000
1. Revision received: 3 April 2000
Returned for 2. revision: 3 May 2000
2. Revision received: 20 May 2000
Accepted: 26 May 2000

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Abstract *Aims:* A delayed myocardial protection extends between 24 and 96 h after ischemic preconditioning in animals. To test for this phenomenon in humans, subjects with stable angina were subjected to exercise test-induced myocardial ischemia and the effect of this "preconditioning" ischemic insult on the exercise-induced myocardial ischemia with the re-exercise after 24-96 hours was studied.

Methods and results: Forty-eight males with a history of infarction and positive exercise test were recruited to the study. After baseline symptom-limited exercise test, the subjects were randomized to four experimental groups (n = 12/group). The groups were allowed to recover for 24 h, 48 h, 72 h or 96 h before performing the second exercise test. Variables analyzed were heart rate-systolic blood pressure product at 1 mm ST segment depression, time to 1 mm ST segment depression, maximum ST segment depression, exercise duration, and the total ischemic time. There were no intergroup differences in baseline values for these variables. All variables were significantly improved at 24 h, the improvement peaked usually at 48 h (maximum increase in the variables by 31–46 %), and the variables returned to baseline by 96 h after the first test.

Conclusions: The exercise-induced ischemia caused transient attenuation of myocardial ischemia with re-exercise. Although the time-window and the time-course of this effect shows striking resemblance to those of the delayed preconditioning in animals, its mechanism remains speculative. The most probable mechanisms that may be involved include increased myocardial perfusion and/or some adaptive changes in the myocardium, the delayed preconditioning being one possibility.

Key words Exercise testing – preconditioning – second window of protection – angina pectoris

Introduction

Myocardial protection associated with ischemic preconditioning exhibits a biphasic pattern in most animal species studied. The early phase occurs within minutes of the preconditioning stimulus and lasts for an hour or two (17, 29). The delayed phase, known also as the second window of protection, returns about 24 h after the stimulus and wanes by 72–96 h (2, 7, 24, 25). Clinical and experimental evidence suggests that the early phase of the protection may exist also in man (12, 19, 20, 26, 30). For example, the "warm up" angina has been suggested to be one of the clinical counterparts of the phenomenon. In this context, it has been demonstrated that one exercise test or cardiac pacing improves the tolerance to a second stress performed shortly thereafter (6, 11, 16, 18, 27, 32).

The delayed protection has been studied only rarely in humans. Human fetal cardiomyocytes have been reported to exhibit both early and delayed preconditioning responses to simulated ischemia (1). However, Tomai et al. (28) have reported that, in patients with angina who underwent two consecutive exercise tests and a third test 24 h later, an improvement of ischemic threshold occurs only during the second, but not during the third exercise test. This suggested that exercise-induced myocardial ischemia triggers the early, but not the late phase of preconditioning (28). In this study, the existence of the delayed phase of preconditioning was verified further in a group of carefully selected male patients with stable angina. The subjects were subjected to the exercise test-induced myocardial ischemia and the effect of this preconditioning stimulus on the ischemia with the re-exercise after 24, 48, 72 or 96 h (i.e., a time-window of the delayed cardioprotection in animals) was examined.

Methods

Patient selection

We studied male subjects (48 ± 6 yr.) with stable angina (Canadian Cardiovascular Society Classification class I or II) and a positive exercise test for myocardial ischemia (ST-segment depression ≥ 1.0 mm). The coronary artery disease was documented by the fact that all the subjects underwent an uncomplicated transmural myocardial infarction 6–8 weeks before recruitment to the study. Subjects with hypertension, cardiac rhythm disturbances, valvular heart disease or mitral valve prolapse, left bundle branch block (at rest or during exercise), echocardiographically defined left ventricular hypertrophy, diabetes mellitus, and impaired renal or hepatic function were excluded form the study. All subjects received treatment only with aspirin, β-blocker (metoprolol or atenolol) and oral mononitrate (Table 1). The study protocol was approved by the institutional Ethics Committee and written informed consent was obtained from all subjects.

Study protocol

Forty eight subjects meeting the above inclusion criteria were recruited to the study protocol which consisted of two consecutive exercise tests (ET). At entry, all subjects performed the baseline ET and then they were randomly assigned to one of the four experimental groups (n = 12/group). The groups were allowed to recover for 24 h (group I), 48 h (group II), 72 h (group III) or 96 h (group IV) before performing the second ET.

Table 1 Clinical characteristics of the four study groups

Characteristics	Group I	Group II	Group III	Group IV
Age (years):				
Mean	49 ± 5	48 ± 7	49 ± 6	48 ± 8
Range	36 – 57	37 – 60	40 – 59	37 – 62
Coronary risk factors:				
Smokers (%)	83	83	75	92
Serum cholesterol (mg/dl)	202 ± 29	232 ± 33	226 ± 26	214 ± 34
Localization of antecedent infarction:				
Inferior (%)	83.4	66.7	75.0	66.7
Anterior (%)	8.3	8.3	8.3	16.7
Lateral (%)	8.3	25.0	16.7	16.7
History of angina before the infarction:				
No history (%)	75.0	66.7	83.4	66.7
> 6 months (%)	25.0	33.3	16.6	33.3
Medication:				
Mononitrates (%)	100	100	100	100
β-blockers – metoprolol or atenolol (%)	100	100	100	100
Aspirin (%)	100	100	100	100
ACE inhibitors (%)	0	0	0	0
Calcium antagonists (%)	0	0	0	0

Exercise testing

On the days of study, the subjects were instructed to avoid any unusual exertion; they rested for at least 30 min before ET, which was always undertaken in midafternoon, 3 h after latest dose of drug-treatment and after a light meal. ET was performed in an upright position on bicycle ergometer (Ergometric 800S, Margot Medical Ergo-Line) linked to a computerized system (Case 12, Marquette Electronics, Milwaukee, Wisconsin). The ergometric testing was started with 25 Watts and then the work-load was increased every 3 minutes by 25 Watts. The subjects were instructed to exercise to near maximum discomfort whether this was angina, fatigue or dyspnea. Other predefined indications for stopping exercise were ST-segment depression ≥ 2.5 mm, a drop in systolic pressure > 10 mmHg, a serious arrhythmia and subject's desire to stop. A standard 12-lead electrocardiogram (ECG) and blood pressure measurements were taken at baseline, at the end of each stage, at the time of 1 mm ST segment depression, at peak exercise, and at 1, 3, 5 and 10 min during recovery period. A 3-lead ECG was monitored continuously before, during, and for 10 min after the exercise. All the tests were supervised by the same cardiologist and they were interpreted in a blind fashion by two other investigators.

Table 2 Results of the baseline exercise test in the four study groups

	Time to 1 mm STD (s)	Ischemic threshold (mmHg/min)	Exercise duration (s)	Maximum STD (mm)	Total ischemic time (s)
Group I	552 ± 102	13622 ± 2499	702 ± 108	2.5 ± 0.7	242 ± 78
Group II	582 ± 108	11959 ± 2913	708 ± 132	2.2 ± 0.5	223 ± 54
Group III	534 ± 127	12713 ± 2642	672 ± 126	2.3 ± 0.7	235 ± 60
Group IV	480 ± 132	11614 ± 2321	621 ± 167	2.1 ± 0.6	245 ± 75

All values are mean ± SD; n = 12/group; STD, ST-segment depression

The ET was considered positive at first appearance of ≥ 1 mm of horizontal or downsloping depression of ST-segment (STD) 80 ms after the J point, detected in at least 2 consecutive precordial leads. The following variables were evaluated: 1) the time to onset of ET positivity; 2) the corresponding heart rate-systolic blood pressure product or ischemic threshold; 3) exercise duration; 4) maximum ST segment depression, and 5) the total ischemic time, defined as the total amount of time that there was ≥ 1 mm STD both during the exercise and recovery.

Statistical analysis

All results are expressed as mean ± SD. Mean values of quantitative variables were compared with one-way analysis of variance followed by Student t-test-either the unpaired one with Bonferroni correction, to detect differences between groups, or the paired one, to detect differences within each group over time. Since the exercise duration, the time to 1 mm STD, and the ischemic time did not show Gaussian distribution, mean values of these variables were compared with Kruskal-Wallis test followed either by the Mann-Whitney or the Wilcoxon rank test, as appropriate. Comparisons for the discrete variables between groups were performed using a Chi-square test. P value < 0.05 was considered significant.

Results

The male subjects with similar history of angina and similar clinical status were recruited to the study. Moreover, there were no differences in age, coronary risk factors, infarct localization, medication (Table 1), nor in the results of the baseline ET between the four study groups examined (Table 2).

All subjects achieved ET positivity during two ET performed. As summarized in Table 3, severe chest pain was the main reason to interrupt the baseline ET. This was true also for the ET performed after 96 h. However, all ETs performed after 24 h, 48 h, and 72 h were interrupted because of a physical exhaustion.

Table 3 The reasons to interrupt the exercise tests in the study groups

	Baseline test (no of subjects)	Second test (no of subjects)
Group I	11 – angina 1 – pressure drop	12 – fatigue
Group II	10 – angina 2 – dyspnea	12 – fatigue
Group III	11 – angina 1 – arrhythmia	12 – fatigue
Group IV	12 – angina	11 – angina 1 – fatigue

Double product at 1 mm STD as well as other results of ET showed a similar time-course of changes. All results were significantly improved at 24 h, this improvement peaked usually at 48 h and the results returned to values not significantly different from the baseline by 96 h after the first ET (Figs. 1 and

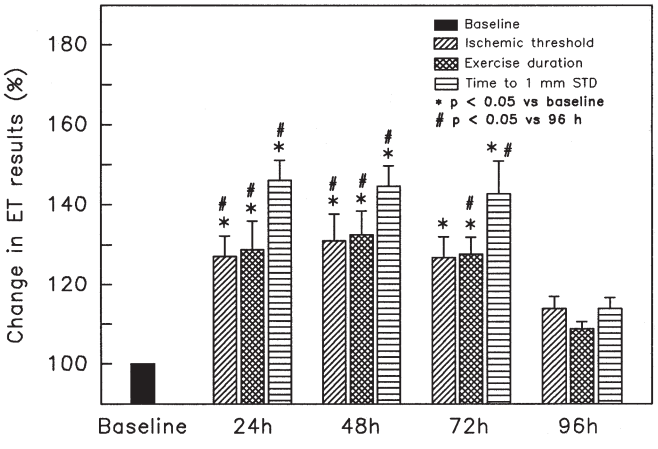

Fig. 1 Time-course of changes in the ischemic threshold (heart rate x systolic blood pressure at 1-mm STD), the exercise duration, and the time to 1-mm ST depression, in the subjects performing consecutive exercise tests (ET). All 48 subjects performed the baseline ET first. Then they were assigned to one of the four experimental groups (n = 12/group). The groups performed the second ET after either 24 h, 48 h, 72 h or 96 h. Percent changes from the results of the baseline ET are plotted.
All values are mean ± SD; *p < 0.05 vs. baseline; # p < 0.05 vs. 96 h.

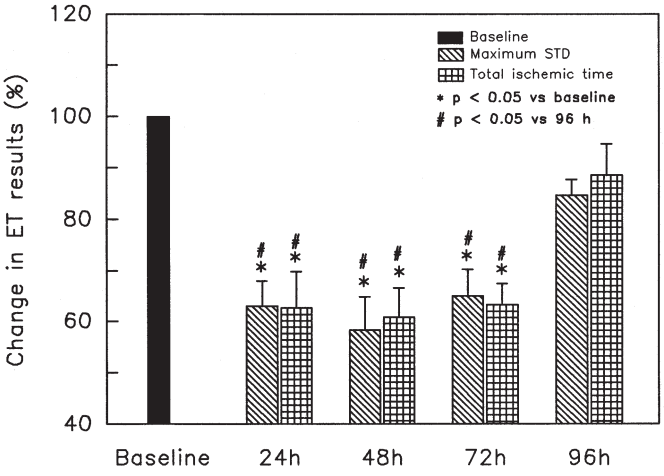

Fig. 2 Time-course of changes in the maximum ST segment depression (STD) and the total ischemic time. See Fig. 1 for more details. Percent changes from the results of the baseline ET are plotted.
All values are mean ± SD; *p < 0.05 vs. baseline; # p < 0.05 vs. 96 h.

2). The peak increase in the double product amounted to 31 %, that in the exercise duration to 33 %, and that in the time to 1 mm STD (which as an only exception was observed at 24 h) amounted to 46 % (Fig. 1). The peak reduction in the maximum STD amounted to 41 % and that in the total ischemic time to 39 % (Fig. 2).

Discussion

The main finding of this study is that in the rigorously selected and therefore relatively homogenous group of male subjects with stable angina: 1) ET-induced episode of demand myocardial ischemia resulted in the marked improvement of the ischemic threshold as well as of the exercise tolerance with the re-exercise after 24 h, 48 h and 72 h, but not after 96 h and 2) that the peak improvement in these variables was usually evident only after 48 h. Thus, both the time-window and the time-course of the improvement showed striking resemblance to those of the infarct- and stunning-limiting effect of the delayed preconditioning in animals (2, 7, 24, 25). This raises an intriguing question hether the phenomenon described here is a clinical counterpart of the delayed phase of the ischemic preconditioning or is related to some other mechanism?

It has been difficult to prove the existence of preconditioning in man, and recent experimental studies have even questioned the validity of using ST segment elevation during balloon inflation as a marker of the early phase of preconditioning (3). Likewise the validity of most of the exercise ECG variables as markers of ischemia remains uncertain. Thus, the total ischemic time probably reflects the intensity of exercise-induced ischemia (4, 5). The exercise duration, maximum ST segment depression, and the time to 1-mm STD are considered as global indices of exercise tolerance. Some of them are, however, subjective, and their improvement might have been the consequence of a training or learning (e.g., exercise duration, although the subjects were already familiar with exercise-testing) or improved hemodynamic conditions on re-exercise (e.g., time to 1-mm STD) rather than changes in the ischemic threshold (10, 27, 31). Consequently, the heart rate-systolic blood pressure product at the onset of 1-mm STD, which is considered a valid and relatively constant index of the ischemic threshold (10, 22, 23), was the only quantifiable exercise ECG indicator of myocardial ischemia analyzed in this study. Altogether, our results strongly suggest that in the subjects examined in this study, ET-induced myocardial ischemia triggered the protection against ischemia and improved exercise tolerance with the re-exercise performed 24–72 h thereafter. This seems to contrast with the observation of Tomai et al. (28) that in patients who underwent two consecutive ETs and a third one 24 h later, only the exercise tolerance (as indicated by the increased time to 1.5-mm STD), but not the ischemic threshold, showed the improvement during the third ET. It remains to be established whether it is some difference between the study groups (e.g., patients with chronic stable angina and no previous infarction in Tomai's study vs. patients with recent infarction in this study) or rather between ET protocols (three ETs in Tomai's study vs. two ETs in this study) which explains these discrepant results.

The physiologic basis for the delayed protection against ischemia described here as well as for that occurring on re-exercise after very short rest periods, as described before (6, 11, 16, 18, 32), is uncertain. If it is taken for granted that the double product is the objective index of the ischemic threshold, its marked improvement on re-exercise observed here (by about 30 % at maximum) indicates that for the same cardiac work-load the subjects developed less myocardial ischemia. Theoretically, this kind of the effect might result either from an increase in myocardial perfusion or from some adaptive changes in the cardiac muscle that make myocardial contraction more energetically effective or from a combination of the two above.

Available evidence suggests that an increase in myocardial perfusion does not explain the improvement on re-exercise performed within minutes (18, 32). It is, however, uncertain if the same is true for our model where the consecutive ETs were separated by days of rest. For instance, longer time-gaps between the ETs, as in this study, may allow for the development of new collaterals. Another mechanism may involve an upregulation of the coronary endothelial function and related improvement in myocardial perfusion. Indeed, it has been demonstrated that 10 min coronary artery occlusion in dog induces enhanced coronary endothelial function, which is delayed in onset (6 h), reaches peak after 1 to 2 days, and is prolonged in duration (5 days) (14). Moreover, ischemic pre-

conditioning has been demonstrated to afford the early (9, 15, 21) as well as the delayed phase (13) of the coronary endothelial protection in various experimental models.

Evidence suggests that an adaptive down regulation of regional myocardial contractile function and related reduction in oxygen demand does not explain the improvement on re-exercise performed within minutes (6). It remains to be established whether this is also pertinent to our model. Another attractive possibility would be that it is some kind of myocardial adaptation mediated by ischemic preconditioning which accounts for the delayed attenuation of myocardial ischemia described in this study.

Unfortunately, resolving these possibilities may prove to be difficult. Perhaps the most important reason for this is that the studies in man are greatly hindered by the present inability to adequately assess myocardial perfusion (8).

In conclusion, this study demonstrates an intriguing phenomenon that ET-induced ischemia exerts the delayed anti-ischemic effect in human heart. Although the time-window and the time-course of this effect show a striking resemblance to those of the delayed preconditioning in animals (2, 7, 24, 25), its mechanism remains speculative. The most probable mechanisms that may be involved include increased myocardial perfusion and/or some adaptive changes in the myocardium, with delayed preconditioning being one possibility. Whatever the mechanism of the phenomenon might be, its strong anti-ischemic potential may prove to be clinically exploitable.

Acknowledgments This study was supported by the KBN 4 PO5A 015 15 grant.

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