Brief low-workload myocardial ischaemia induces protection against exercise-related increase of platelet reactivity in patients with coronary artery disease

Giancarla Scalone, Ilaria Coviello, Lucy Barone, et al.

*Heart* 2010 96: 263-268 originally published online November 5, 2009
doi: 10.1136/hrt.2009.178178

Updated information and services can be found at:
http://heart.bmj.com/content/96/4/263.full.html

These include:

**References**
This article cites 40 articles, 25 of which can be accessed free at:
http://heart.bmj.com/content/96/4/263.full.html#ref-list-1

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To order reprints of this article go to:
http://heart.bmj.com/cgi/reprintform

To subscribe to *Heart* go to:
http://heart.bmj.com/subscriptions
ABSTRACT

Objective In patients with acute myocardial infarction, pre-infarction angina is associated with smaller infarct size, probably mainly through myocardial protection induced by ischaemic preconditioning. However, in models of recurrent thrombosis myocardial ischaemia also improves arterial patency. This study investigated whether myocardial ischaemia has any effect on platelet function in patients with coronary artery disease.

Patients and design Twenty patients with low-workload myocardial ischaemia underwent, in a randomised crossover study, two treadmill exercise stress tests (EST) on two separate days: a single maximal EST (EST-1) and a maximal EST (EST-2) performed 45 minutes after a low-workload EST stopped at 1-mm ST depression (p-EST). Platelet reactivity was evaluated by measuring the closure time in response to ADP/collagen by the PFA-100 method, and monocytopleatelet aggregate (MPA) formation and CD41 platelet expression, with and without ADP stimulation, by flow cytometry.

Results Compared to resting values, closure time decreased at peak EST-1 (p<0.001) but not at peak EST-2. MPA after ADP stimulation increased more significantly at peak EST-1 compared with peak EST-2 (p<0.001). Repetition in seven patients of the p-EST/EST-2 protocol after intravenous administration of the adenosine antagonist theophylline showed prevention of the effects of p-EST on exercise-induced platelet reactivity.

Conclusions A short episode of myocardial ischaemia induces protection against an exercise-induced increase of platelet reactivity. These data also suggest a role for adenosine in this phenomenon.

Experimental studies have shown that brief episodes of myocardial ischaemia induce protection against myocardial injury caused by a subsequent prolonged ischaemic insult by determining metabolic changes in myocardial cells, which make them more resistant to ischaemia, a phenomenon called ‘ischaemic preconditioning’. 1, 2

In clinical practice, myocardial protection by ischaemic preconditioning is believed to play a role in mediating the beneficial effects of pre-infarction angina in patients presenting with acute myocardial infarction (AMI). 3, 4 Moreover, patients with prodromal angina, compared with those without, have also been shown to present a higher rate of successful coronary reperfusion following thrombolytic therapy, 5, 6 suggesting that pre-infarction angina might also be associated with a lower thrombogenic condition. Accordingly, experimental studies have shown that transient myocardial ischaemia may attenuate platelet reactivity and aggregability to damaged coronary artery vessel walls, suggesting that in the clinical setting the favourable effects of preconditioning might also be mediated by a reduction in platelet reactivity. 7, 8 In any case, no previous study has assessed the effect of ischaemic preconditioning on platelet reactivity in patients.

Exercise is known to enhance platelet aggregability in patients with obstructive coronary artery disease (CAD). 9–13 At the same time, it is known that in CAD patients an exercise stress test (EST) performed within 30 minutes of a previous positive test shows an improvement of ischaemic changes, suggesting an ischaemic preconditioning effect by the first test. 14–16

In this study we assessed whether mild transient myocardial ischaemia induced by exercise can prevent the increase of platelet reactivity in subsequent maximal efforts.

METHODS

Study groups We studied 20 patients who fulfilled the following inclusion criteria: (1) a stable pattern of angina pectoris for more than 6 months; (2) ST-segment depression at low workload (less than 1st stage of Bruce protocol) during electrocardiographic (ECG) EST; (3) significant stenosis (>75% of the lumen diameter) in at least one of the major epicardial coronary arteries. All patients were taking aspirin (100 mg). Patients were excluded if they were unable to perform EST, had ECG alterations that could interfere with ST-segment analysis (eg, bundle branch block, pacemaker rhythm, abnormal ST changes at rest), or were taking anticoagulant drugs. A careful clinical history, including cardiovascular risk factors and drug therapy, was recorded from each patient. All patients gave informed consent for participation in the study. The study complied with the Declaration of Helsinki and was approved by the institutional review board of our institute.

Exercise stress test A scheme of the study protocol is shown in figure 1. Patients underwent, in a randomised crossover study...
Coronary heart disease

**Figure 1** Study protocol. Patients with stable coronary artery disease underwent two kinds of exercise stress test (EST) protocol with 4-day interval from each other. In one of the 2 days of the study (A), patients underwent maximal treadmill EST (EST-1). In the other day (B), patients first underwent a low workload EST (p-EST), which was stopped at the ischemia threshold, and then, 45 minutes later, a maximal EST (EST-2). Blood samples were obtained before EST and at peak EST-1 and EST-2 (black arrows) to evaluate closure time (CT) by the platelet function analyser (PFA-100) method, and monocyte—platelet aggregates (MPA) and CD41 mfi (CD41) by flow cytometry.

Blood sampling

In all patients blood samples were obtained immediately before each EST and within 5 minutes of peak EST-1 and EST-2 (figure 1). Through a clean, non-traumatic venipuncture, and with minimal venous occlusion, samples were drawn directly into plastic tubes containing 0.106 M trisodium citrate (blood : citrate 9 : 1), after discarding the first 2 ml to minimise the dead space. Platelets and as mean fluorescence intensity of CD41 (CD41-mfi) in the monocyte—platelet gate.

Platelet reactivity

Platelet reactivity was assessed by the platelet function analyser system (PFA-100, Dade Behring, Milan, Italy), in which the process of primary haemostasis (platelet adhesion and aggregation following vascular injury) is simulated ex vivo. Details about this method have been described elsewhere.\(^8\) Briefly, 200\,\mu l of anticoagulated whole blood is added to a standardised disposable cartridge and incubated at 37°C. The blood is then aspirated under arterial shear rates (5000 s\(^{-1}\)) through a microscopically coated with collagen (2\,\mu g equine type 1 collagen) and ADP (50\,\mu g). Platelets flowing through the ring are activated and adhere to each other to form an aggregate, thus gradually diminishing and finally stopping the blood flow. The time to reach ring occlusion (closure time), is taken as a measure of platelet reactivity (adhesion and aggregation), with shorter times indicating greater reactivity. Duplicate measures of closure time performed randomly in 10 samples showed differences of less than 5\%, in agreement with our previous studies showing good reproducibility of closure time measurements.\(^20\)\(^\text{–}\)\(^21\)

Flow cytometry

Monocyte—platelet aggregates

Blood (100\,\mu l) was labelled within 10 minutes of collection with a saturating concentration of PerCP-conjugated CD14 (lipopolysaccharide protein receptor) and FITC-conjugated glycoprotein IIb/IIIa (GP IIb CD41) for 15 minutes at room temperature. Following incubation, erythrocytes were lysed with buffered ammonium chloride and analysed by FACS. Monocyte—platelet aggregates (MPA) were identified using the logical gating facility by a combination of binding characteristics of anti-CD14 (monocyte marker) and of anti-CD41 (platelet marker) antibodies. A minimum of 5000 monocytes was counted for each test. MPA were expressed as a percentage of monocyte binding platelets and as mean fluorescence intensity of CD41 (CD41-mfi) in the monocyte—platelet gate.\(^12\)

Stimulation with ADP

The response to ADP stimulation of MPA formation and CD41 expression in the monocyte—platelet gate was also assessed. To this aim, blood samples were incubated with ADP (final concentrations 10\(^{-6}\) M) for 15 minutes at room temperature and labelled and analysed as previously described.\(^12\)

Theophylline infusion

In the last seven patients (72±5 years, six men) the pEST/EST-2 protocol was repeated with the aim of evaluating whether adenosine could be involved in the effect of pEST on platelet reactivity. This non-randomised part of the study was carried out 23.4±4 days after the second test. The exercise/sampling protocol was repeated after the administration of the non-specific adenosine receptor antagonist theophylline (240 mg in 100 ml of saline, infused intravenously over 30–45 minutes).\(^19\) At the end of infusion, according to the established protocol (figure 1), patients underwent a low-workload EST stopped at the ischaemia threshold (preconditioning EST-Th), followed 45 minutes later by a sign/symptoms limited EST (preconditioned EST-Th).

Statistics

According to the Kolmogorov–Smirnov test, variables assessed in the study showed a distribution not significantly different from normal, and were then analysed using parametric tests. According to previous studies, we hypothesised that in these patients a 20\% decrease in closure time could occur after EST-1. By assuming as significant at a \(p\) level of 0.05 a reduction of the decrease of 80\% after EST-2 (ie, a closure time decrease of only 4 s or less), we calculated that 20 patients were necessary to have a power of 60\% to detect the difference.

Comparisons of continuous variables were done by analysis of variance (ANOVA) with a repeated measure design. In case of global statistically significant differences, within-group (EST) changes were assessed by paired \(t\) tests with Bonferroni correction for multiple comparisons. SPSS version 12.02 statistical
software was used for statistical analysis. Clinical and experimental data are reported as mean±SD.

RESULTS
Clinical characteristics
Table 1 shows the main clinical characteristics of patients included in the study (16 men, four women; aged 71±9 years). Coronary angiography showed one-vessel, two-vessel and three-vessel disease in 11 (55%), two (10%) and seven (35%) patients, respectively. All but two patients had undergone one or more revascularisation procedures. All patients were taking aspirin (100 mg a day).

The main results of the EST performed during the study protocol are summarised in table 2. Significant ST-segment depression was induced during all tests. One patient only (5%) experienced angina during maximal EST (during both EST-1 and EST-2). No differences were found between EST-1 and EST-2 in heart-rate, systolic blood pressure and rate-pressure product at 1-mm ST-segment depression, as well as at peak exercise. Times to 1-mm ST depression and exercise duration were also similar.

Platelet reactivity at rest
Basal closure time, MPA and CD41-mfi did not differ before EST-1 and EST-2. A similar increase in MPA and CD41-mfi was observed after ADP stimulation before the two maximal EST (table 3).

Table 3

| Table 3 Closure time values and cytometry variables at rest and at peak of exercise |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                               |       |       |       |       |
|                               | EST-1 | EST-2 | p Value for changes |
| Closure time (s)              |       |       |       |       |
| Rest                          | 211±4 | 225±7 | <0.001 |
| Exercise                      |       |       |       |       |
| ADP                           | 390±105 | 372±117 | <0.001 |
| Theophylline infusion         | 2.0±0.5 | 2.0±0.6 | <0.01  |

Of note, although ADP stimulation induced a significant increase in MPA and CD41-mfi after both EST-1 and EST-2, the level of the increase was significantly lower after EST-2 compared with EST-1 (p<0.001 for both) (figure 3).

Platelet reactivity after theophylline infusion
In the seven patients who underwent the theophylline sub-study, EST parameters were similar for EST-1, EST-2 and EST-Th except for the time to 1 mm ST depression, which was significantly longer after theophylline administration (table 4).

The main results of platelet reactivity observed in these patients are summarised in table 5. Closure time values, MPA and CD41-mfi (with and without ADP stimulation) after theophylline administration and before EST-Th were similar to those measured before EST-1 and EST-2.

A significantly different closure time response to exercise was observed among EST-1, EST-2 and EST-Th (p<0.05) (figure 4). Indeed, as in the whole group of patients, closure time decreased significantly after EST-1 (p<0.001), but not after EST-2 (p=0.76); however, a significant reduction in closure time compared with pre-exercise was observed after EST-Th (p<0.001).

Table 2

| Table 2 Results of exercise stress testing |
|---------------------------------|-----------------|-----------------|-----------------|
|                               |       |       |       |
|                               | EST-1 | EST-2 | p EST |
| Heart rate (beats/minute)      | 67.5±13 | 68.7±12 | 66.9±10 | 0.47 |
| Systolic BP (mm Hg)            | 125.2±16 | 124.0±11 | 125.0±12 | 0.89 |
| RPP (beats/min × mm Hg)        | 843±1735 | 853±1705 | 838±1664 | 0.71 |

Results are means±SD.
BP, blood pressure; EST, exercise stress testing; RPP, rate pressure product.
The protective effects of ischemic preconditioning are well established, suggesting that they might also be significant in patients with CAD. Several studies have shown that exercise increases platelet reactivity in patients with CAD, suggesting that this effect could contribute to the small but definite increased risk of acute coronary events related to exercise. The mechanisms by which exercise increases platelet reactivity are probably multiple and may include an increase in circulating platelets, an increase of intravascular shear stress, the presence of stenoses, a reduced antiplatelet effect of dysfunctional endothelium in atherosclerotic vessels, and increased catecholamine release that can stimulate platelet activation through \( \alpha_2 \)-adrenoceptors.

In this study, for the first time, we show that the significant increase in platelet reactivity induced by maximal EST in patients with obstructive CAD is significantly reduced by a short episode of myocardial ischemia induced by low-workload exercise performed 45 minutes before maximal EST. Furthermore, we provide some evidence that adenosine might play a role in this phenomenon, as this was prevented by administration of the adenosine antagonist theophylline.

**Discussion**

In this study, we provide evidence that a short episode of myocardial ischemia antagonizes the enhanced platelet reactivity induced by maximal exercise in patients with stable obstructive CAD, thus suggesting that this phenomenon might contribute to the beneficial consequences of pre-infarction angina.

It is worth noting that the effects of transient myocardial ischemia were independent of any evidence of myocardial ischemic preconditioning. Indeed, contrary to our expectations, in this study no improvement of exercise-induced ischemia was found during the symptom-limited EST (EST-2) performed after the p-EST. The reasons for the lack of ischemic preconditioning in this study are not completely clear; however, our protocol presented two major differences compared with previous studies that showed an improvement of myocardial ischemia on the second of two consecutive ESTs, which might have contributed, at least partly, to explain our failure to detect myocardial ischemic preconditioning. First, in previous studies the second EST was usually performed 10–15 minutes after the first, whereas in this study it was performed 45 minutes after the first EST, a time that was chosen, on the basis of our previous data, to allow normalisation of closure time after the EST. Second, in previous studies the first test was symptom-limited, whereas in the present study the p-EST was submaximal and stopped just as soon as ischemic ST-segment changes appeared.

The mechanisms responsible for the prevention of the exercise-induced increase of platelet reactivity by a previous effort resulting in myocardial ischemia remain to be fully elucidated. However, our observation that theophylline, a non-specific

---

**Figure 2** Individual and mean (±SD) closure time values before and at peak of control exercise stress test (EST-1) and preconditioned exercise stress test (EST-2) in 20 coronary artery disease patients. PFA-100, platelet function analyser.

**Figure 3** Individual and mean (±SD) monocyte–platelet aggregates (left) and CD41 expression (right) following ADP stimulation before and at peak of control exercise stress test (EST-1) and preconditioned exercise stress test (EST-2) in 20 patients with coronary artery disease. PFA-100, platelet function analyser.
Adenosine antagonist, was able to blunt this favourable effect suggests that adenosine may indeed play a role.

Adenosine is the main mediator of metabolic coronary blood flow regulation, and therefore is released in large amounts during myocardial ischaemia.31–32 Adenosine, however, also has antiplatelet effects through A2 adenosine receptor stimulation33–35 and, importantly, it is also believed to play a significant role in myocardial protection induced by ischaemic preconditioning.36–38

We might thus speculate that adenosine released during a short myocardial ischaemic episode (as in our p-EST) might induce changes in the number/affinity of membrane receptors and/or in the intracellular signalling pathways of platelets that result in a reduction in the increased platelet reactivity after subsequent exercise-induced myocardial ischaemia.

Limitations of the study
Some questions following our study remain open. First, we show that ischaemic low-workload EST induces protection against a subsequent effort-related increase in platelet reactivity and monocyte–platelet interaction. However, we cannot exclude that exercise per se, rather than EST-induced myocardial ischaemia, is responsible for this effect. Second, the time course of the protective effect of transient myocardial ischaemia on platelet reactivity remains to be established.

All our patients were on aspirin therapy, therefore we cannot exclude that different results might have been obtained without the antiplatelet effect of aspirin. However, due to ethical concerns, aspirin could not be withdrawn in our patients. Furthermore, the influence of aspirin on exercise-related changes in platelet reactivity may be limited.13 39 40 It should be noted that aspirin is an obligatory treatment in CAD patients and, therefore, our data may have more relevant clinical implications than those that would have been obtained in the absence of aspirin treatment.

CONCLUSIONS
In this study we show that in patients with stable CAD, a short episode of myocardial ischaemia prevents the increase in platelet reactivity during subsequent symptom-limited effort, suggesting that this phenomenon might be involved in the favourable clinical effects of pre-infarction angina. Our data also suggest that adenosine might be involved in the mechanisms responsible for this beneficial phenomenon.

Acknowledgements The authors are very grateful to Mrs Helen Raiswell for kindly reviewing the manuscript.

Competing interests None.

Ethics approval This study was conducted with the approval of the review board of the Istituto di Cardiologia, Università Cattolica del Sacro Cuore, Rome, Italy.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES
Coronary heart disease


