Acute Effects of Heparin Administration on the Ischemic Threshold of Patients With Coronary Artery Disease

Evaluation of the Protective Role of the Metabolic Modulator Trimetazidine

Gabriele Fragasso, MD,* Pier Marco Piatti, MD,† Lucilla Monti, MD,† Altin Palloshi, MD,* Chunzeng Lu, MD,* Gianpietro Valsecchi, PhD,† Emanuela Setola, MD,† Giliola Calori, MD,‡ Guido Pozza, MD,† Alberto Margonato, MD, FESC,∗ Sergio Chierchia, MD, FESC, FACC∗

Milano, Italy

OBJECTIVES We sought to assess the effects of heparin and the potential protective effects of trimetazidine (TMZ) on exercise performance, plasma nitric oxide (NO), endothelin-1 (ET-1) and free fatty acid (FFA) release in patients with stable coronary artery disease (CAD).

BACKGROUND Heparin has been shown to reduce the ischemic threshold in patients with CAD. Trimetazidine may affect myocardial substrate utilization by shifting energy production from FFA to glucose oxidation.

METHODS In four consecutive days, nine patients with CAD each received one of the following four regimens: 1) one tablet of placebo the evening before and at 8 AM and 4 PM on the day of the study, 10 ml of saline in a bolus 10 min before exercise, followed by an infusion of the same preparation; 2) placebo at the same times as in the first regimen, 5,000 IU of heparin 10 min before exercise, followed by 1,000 IU/h; 3) 20 mg TMZ at the same times as in the first regimen, 5,000 IU of heparin 10 min before exercise, followed by 1,000 IU/h; or 4) TMZ at the same times as in the first regimen, 10 ml of saline 10 min before exercise, followed by an infusion of the same preparation.

RESULTS During placebo (test 2), heparin reduced the time to 1-mm ST-segment depression and prolonged the recovery time, as compared with the results of test 1. When heparin was administered after TMZ (test 3), the time to 1-mm ST-segment depression and the recovery time were similar to those recorded during saline (test 1). Finally, compared with all study phases, TMZ during saline (test 4) prolonged the time to 1 mm. No changes in NO release were found, whereas ET-1 was decreased at peak exercise and during recovery, when the patients were receiving TMZ (tests 3 and 4). Free fatty acids increased after heparin, both with placebo and TMZ.

CONCLUSIONS In patients with CAD, heparin reduces the ischemic threshold. Trimetazidine reduces the effects of heparin, probably by inhibiting FFA oxidation and enhancing glucose metabolism. The concomitant novel observation of reduced ET-1 release is likely to be also dependent on TMZ-induced improvement of endothelial metabolism or reduction of myocardial ischemia. (J Am Coll Cardiol 2002;39:413–9) © 2002 by the American College of Cardiology

Heparin is universally employed as an antithrombotic agent in patients with acute coronary syndromes. However, its administration is known to increase circulating free fatty acids (FFAs) (1), which may adversely affect myocardial energetics, especially during ischemia (2). In addition, although low-dose heparin infusion can increase nitric oxide (NO) levels and forearm blood flow in normal subjects (3), recent studies have shown that high-dose heparin, at concentrations often achieved in acute cardiovascular conditions, increases platelet aggregation (4,5) and impairs NO production and vasomotion in rats (6). These observations suggest the possibility that heparin exerts a prothrombotic effect and may imply, theoretically at least, that high-dose heparin could also negatively affect myocardial perfusion by interfering with the production of constitutive NO. Recently, our group has shown that standard unfractionated heparin may reduce the ischemic threshold in patients with coronary artery disease (CAD), probably by increasing FFA release (7). Although low-molecular-weight heparin has been shown to yield a weaker plasma lipolytic potential (8,9), unfractionated heparin, by mobilizing lipoprotein lipase, which hydrolyzes serum triglycerides and increases nonesterified fatty acid availability, may further worsen the metabolic milieu of the ischemic myocardium. In fact, exogenous fatty acids, the main metabolic fuel of the myocardium under aerobic conditions, are detrimental under oxygen deprivation, because their presence further augments the accumulation of long-chain acyl esters in the myocytes. The accumulation of lipids and their degradation...
may contribute to the progression of injury. However, if glucose oxidation could be stimulated during ischemia-reperfusion, this would result in a significant increase in cardiac efficiency, as determined by a reduction of lactate production and acidosis, and an increase in adenosine triphosphate (ATP) content, with a parallel improvement in cardiac function and lesser injury.

Trimetazidine, 1(2,3,4-trimethoxybenzyl-piperazine dihydrochloride; TMZ), has been reported to exert anti-ischemic properties without affecting myocardial oxygen consumption and blood supply (10). The beneficial effect of this agent has been attributed to preservation of phosphocreatine and ATP intracellular levels (11) and a reduction of cell acidosis (12,13), calcium overload (13) and free radical–induced injury caused by ischemia (14). More importantly, TMZ affects myocardial substrate utilization by inhibiting oxidative phosphorylation and by shifting energy production from FFA to glucose oxidation (15,16). Recent evidence indicates that this effect is predominantly caused by selective blockade of long-chain 3-ketoacyl coenzyme A thiolase activity, the last enzyme involved in beta-oxidation (16).

Several clinical studies have shown that TMZ alone or in combination with other anti-anginal drugs can substantially improve exercise tolerance and increase the ischemic threshold in patients with effort angina (17–22).

The aims of the present study were to investigate, in patients with chronic stable angina, the effects of short-term heparin administration on the ischemic threshold, plasma NO, endothelin-1 (ET-1) and FFA release and, in this context, to evaluate the potential protective effect of TMZ.

**METHODS**

**Patients.** Nine consecutive patients (all males, age 63 ± 6 years) with angiographically proven CAD awaiting percutaneous revascularization were selected for the study. All had a reproducibly positive exercise test. Patients with a previous myocardial infarction or any cardiac condition potentially interfering with the unequivocal interpretation of the 12-lead electrocardiogram (ECG) were excluded from the study. Anti-ischemic medications and statins were withdrawn at least 72 h before the beginning of the study. All patients were taking salicylates, which were not withdrawn for study purposes. None of the patients had overt diabetes mellitus. Three had mild glucose intolerance, controlled by diet only. All patients gave written, informed consent to participate in the study.

**Protocol.** The study consisted of four randomized, double-blinded phases conducted on consecutive days. In separate sessions, patients underwent exercise testing under the following medications, given in random order: test 1: one tablet of matching placebo given the evening before and at 8 AM and 4 PM on the day of the study and a 10-ml bolus of 0.9% NaCl given 10 min before exercise testing (according to the Bruce protocol, at 5 PM), followed by an infusion of the same solution (12 ml/h) until the end of recovery; test 2: one tablet of placebo the evening before, at 8 AM and 4 PM and a 5,000-IU bolus of heparin given 10 min before exercise, followed by an infusion of 1,000 IU/h; test 3: 20 mg TMZ given the evening before and at 8 AM and 4 PM and a 5,000-IU bolus of heparin given 10 min before exercise, followed by an infusion of 1,000 IU/h; and test 4: 20 mg TMZ given the evening before and at 8 AM and 4 PM and a 10-ml bolus of 0.9% NaCl given 10 min before exercise testing, followed by infusion of the same solution. Treadmill exercise testing (CASE 12-Marquette Electronics, Milwaukee, Wisconsin) was performed at 5 PM, in the fasting state, according to the Bruce protocol. Blood pressure (measured by a cuff sphygmomanometer) and the 12-lead ECG were recorded at baseline, after the heparin and NaCl boluses, during the third minute of each exercise step and throughout recovery. Exercise was terminated when there appeared ≥2-mm rectilinear or downsloping ST-segment depression or severe angina, fatigue, ventricular tachycardia or a blood pressure decrease >10 mm Hg. Heart rate, systolic and diastolic blood pressures and rate–pressure product (RPP) were measured at rest, at the appearance of 1-mm ST-segment depression and at peak exercise. The time to 1-mm ST-segment depression and to peak exercise and the recovery time (time to ST-segment return to baseline during recovery after exercise) were recorded. Achieved percent age-adjusted maximal predicted heart rate (220 – patient age), maximal ST-segment depression, cumulative maximal ST-segment depression and the number of ECG leads showing diagnostic changes were also measured.

**Biochemical testing.** Blood sampling for NO, ET-1, lactate and FFA determination was performed at −15 min, at 0 time, at peak exercise and after 10, 15 and 20 min from the end of exercise. Levels of NO were evaluated by measuring the end products of their metabolism (i.e., nitrite and nitrate levels), using enzymatic catalysis coupled with the Griess reaction. Specifically, NO3 was reduced to NO2 by 0.1 U nitrate reductase, 5 × 10−6 mol/l flavin adenine dinucleotide and 250 × 10−6 NADPH. The samples were incubated at 37°C for 3 h, then 8.8 U lactic dehydrogenase and 10−2 mol/l pyruvate was added, and the samples were incubated for an additional 90 min at 37°C. Finally, the Griess reaction was added to each well, and the samples were read at 540 nm (23).
Table 1. Ergometric Parameters

<table>
<thead>
<tr>
<th></th>
<th>MPH (%)</th>
<th>RPP at 1-mm ST-Segment Depression</th>
<th>RPP at Peak Exercise</th>
<th>Time (s) to 1-mm ST-Segment Depression</th>
<th>Time (s) to Peak Exercise</th>
<th>Recovery Time (s)</th>
<th>Maximal ST-Segment Depression (mm)</th>
<th>No. of ECG Leads*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo + NaCl</td>
<td>96 ± 8</td>
<td>28,873 ± 5,119</td>
<td>30,658 ± 3,601</td>
<td>321 ± 179</td>
<td>408 ± 161</td>
<td>518 ± 263</td>
<td>2.02 ± 1.18</td>
<td>4.33 ± 1.73</td>
</tr>
<tr>
<td>Placebo + Heparin</td>
<td>95 ± 10</td>
<td>26,337 ± 7,021</td>
<td>30,613 ± 5,947</td>
<td>259 ± 185†</td>
<td>409 ± 174</td>
<td>704 ± 164†</td>
<td>2.31 ± 1.13</td>
<td>5.22 ± 1.79</td>
</tr>
<tr>
<td>TMZ + Heparin</td>
<td>95 ± 7</td>
<td>26,816 ± 8,009</td>
<td>30,720 ± 5,790</td>
<td>318 ± 174†</td>
<td>420 ± 174</td>
<td>472 ± 186</td>
<td>1.74 ± 0.90</td>
<td>4.67 ± 2.55</td>
</tr>
<tr>
<td>TMZ + NaCl</td>
<td>99 ± 4</td>
<td>29,367 ± 5,428</td>
<td>31,327 ± 2,035</td>
<td>468 ± 198‡</td>
<td>558 ± 99</td>
<td>493 ± 80‡</td>
<td>1.70 ± 1.04</td>
<td>4.33 ± 1.52</td>
</tr>
</tbody>
</table>

*Number of electrocardiographic leads showing diagnostic ST-segment depression. Dunn’s multiple comparisons: ‡p < 0.05 versus placebo + NaCl and TMZ + heparin; †p < 0.05 versus placebo + NaCl and placebo + heparin and TMZ + heparin; and ‡p < 0.05 versus placebo + heparin. Data are presented as the mean value ± SD of ergonomic parameters in the four phases of the study.

ECG = electrocardiographic; NaCl = sodium chloride; MPH = maximal predicted heart rate; RPP = rate-pressure product; TMZ = trimetazidine.

For measurement of ET-1, the samples were extracted on a SepPack C18 mini-column (Amprep, Amershams International, Buckinghamshire, U.K.), and the eluate was evaporated in a Speed VAC SC 110 (Savant Instruments, Inc., Farmingdale, New York). The samples were reconstituted with 250 µl of radioimmunoassay (RIA) buffer and assayed with an RIA kit (Du Pont de Nemours, Boston, Massachusetts). Typical intra- and inter-assay coefficients of variation were 3.0% and 11.9%, respectively (24).

Levels of FFA and lactate were determined by the fluorometric enzymatic method, adapted to a Cobas Fara Centrifugal Analyzer (Roche, Basel, Switzerland) (25).

Statistical analysis. Data are reported as the mean value ± SD. Comparisons among groups were performed by one-way analysis of variance (ANOVA). When variables did not show a Gaussian distribution, nonparametric analysis was undertaken to investigate the differences among groups. Friedman’s test for paired samples was performed, and Dunn’s multiple comparisons method was used for post hoc comparisons. The FFA, ET-1, NO2/NO3 and lactate results were analyzed by using two-way ANOVA with repeated measures of one factor. When the time–group interaction was significant, tests for simple effects were performed (paired t test: time 0 vs. peak exercise in different groups and one-way ANOVA among groups at different times), and the Bonferroni adjustment for post hoc comparisons was performed. Pearson and Spearman correlation coefficients were also calculated, where appropriate. Analysis was performed with SAS 6.12 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Exercise testing. Table 1 shows detailed results pertaining to the ergometric parameters. Compared with test 1, test 2 administration of heparin reduced the time to 1-mm ST-segment depression (259 ± 185 vs. 321 ± 179 s, p < 0.05) and prolonged recovery time (704 ± 164 vs. 518 ± 263 s, p < 0.05) (Fig. 1), although it did not significantly increase maximal ST-segment depression (Fig. 2).

When heparin was administered after TMZ (test 3), the time to 1-mm ST-segment depression (318 ± 174 s) and the recovery time (472 ± 186 s) were similar to those recorded during saline administration (test 1) (Fig. 1). No significant differences in basal and peak heart rate or systolic and diastolic blood pressure were found. Accordingly, the RPPs at 1-mm ST-segment depression and at peak exercise were similar in all study phases (Fig. 3). Finally, compared with all study phases, TMZ during saline (test 4) induced a significant prolongation of the time to 1-mm ST-segment depression.

Biochemical results. Figure 4 shows the profile of biochemical results. No significant differences were found in the plasma levels of FFA, ET-1 and NO2/NO3 and lactate before the beginning of the tests. Plasma FFA levels were different among tests (F = 28.9, p < 0.001); the main effect of time was significant (F = 75.7, p < 0.001); and the rate of increase was different among different tests (time–test interaction: F = 26.3, p < 0.001). Plasma FFA increased by 10-fold during tests 2 and 3 (heparin, with and without TMZ, p < 0.001), but only a slight increase was observed during tests 1 and 4 (p = 0.01).

The time effect was significant for ET-1 levels (F = 7.5, p < 0.001). The main effect of the tests was not significant (F = 2.22, p = 0.1). Moreover, there was a significant interaction between the effect of changes in ET-1 levels and different treatments (F = 4.86, p < 0.007). During the first 10 min of saline or heparin infusion, the ET-1 levels remained unchanged in all patients. The paired t test, used to investigate the difference between time 0 and peak time, showed that at the end of the exercise period, ET-1 levels increased in test 1 (from 7.6 ± 0.6 to 9.9 ± 0.9 pg/ml, p <
0.005) and increased further during test 2 (from 7.6 ± 0.7 to 11.2 ± 1.9 pg/ml, p < 0.01). Conversely, during test 3, ET-1 levels remained unchanged (from 7.7 ± 0.7 to 8.1 ± 0.9 pg/ml, p = NS) and decreased during test 4 (from 7.0 ± 0.6 to 5.8 ± 0.3, p < 0.01). Fifteen minutes after the end of exercise, ET-1 levels were still significantly less in test 4 than in test 2 (5.1 ± 0.3 vs. 9.5 ± 1.6 pg/ml, p < 0.01).

Finally, the increment in ET-1 levels at peak exercise was positively correlated with the duration of ST-segment depression during the recovery period (r = 0.31, p < 0.02).

Plasma NO$_2$/$\text{NO}_3$ remained unchanged during all exercise (F = 1.56, p = 0.21), and the test effect was not significant (F = 0.08, p = 0.97).

Two-way ANOVA with repeated measures showed highly significant differences in lactate levels during the exercise period for the four tests (F = 49.4, p < 0.0001). No test effect was found (F = 0.21, p = 0.9), and no significant interaction between the effect of changes in lactate levels and different treatments was found (F = 0.9, p = 0.7).

During the first 10 min of infusion, blood lactate levels remained unchanged in all tests, although they increased during exercise, with a peak at peak exercise, and thereafter declining in all tests.

**DISCUSSION**

The results of this study confirm our previous observation (7) that, in patients with stable CAD, unfractionated heparin may significantly decrease the ischemic threshold. The administration of TMZ blunts the deleterious effects of heparin, probably by inhibiting oxidative phosphorylation...
and FFA oxidation and by enhancing glucose metabolism (16).

**Effects of heparin on ischemic myocardium: role of FFAs.** A variety of mechanisms have been suggested to explain the deleterious effects of fatty acids and their derivatives on myocardial function during ischemia and reperfusion (26). Indeed, in the ischemic myocardium, long-chain fatty acids accumulate quickly. The rate of fatty acid uptake and oxidation by the heart is controlled by the availability of fatty acids (27). Exogenous fatty acids, the main metabolic fuel of the myocardium under aerobic conditions, are detrimental under oxygen deprivation, because their presence further augments the accumulation of long-chain acyl esters in the myocytes. The accumulation of lipids and their degradation may contribute to the progression of injury. Furthermore, during reperfusion, fatty acid oxidation can quickly recover and become the dominant source of ATP production. A high rate of fatty acid oxidation contributes to a marked decrease in cardiac efficiency during the ischemia-reperfusion period. Therefore, an increase in fatty acid release induced by heparin and, to a lesser extent, by its low-molecular-weight fragments (8,9) may further worsen the local metabolic milieu, and although the net effect of unfractionated heparin administration remains beneficial, mainly because of its antithrombotic effect, it is not known yet whether the adverse effects outlined in this study and previously (7) have any clinical relevance in the setting of acute coronary syndromes. The fact that the RPP at 1-mm ST-segment depression was similar during all study phases indicates that the observed decrease in the ischemic threshold after heparin was likely caused by poor management of energetic resources, rather than the result of alterations in the supply/demand ratio. However, if glucose oxidation is stimulated during ischemia-reperfusion, a significant increase in metabolic efficiency ensues, which causes a parallel increase in cardiac function and lesser injury. When translating these findings into clinical practice, it appears that drugs acting as FFA inhibitors may play a significant role in the treatment of ischemic heart disease (28–31).

**Manipulation of cardiac metabolism.** A number of different approaches have been used to manipulate energy metabolism in the heart (32). These involve both indirect measures and the use of agents that act directly on the heart to shift energy substrates utilization away from fatty acid metabolism and toward glucose metabolism. One way to increase glucose oxidation and to decrease fatty acid metabolism in the heart is to decrease circulating fatty acid levels. This can be achieved by the administration of glucose/insulin solutions (33), nicotinic acid (34) and beta-adrenergic blocking agents (35,36). Another approach consists of directly modifying substrate utilization by the heart. Experimental studies with metabolic modulators suggest that inhibition of oxidative phosphorylation and fatty acid substrates can shift substrate utilization from fatty acid oxidation to glucose (37–39). In our study, the beneficial effects of TMZ were operative, regardless of the levels of FFA which were not affected by TMZ. Rather than reducing FFA blood levels, TMZ probably inhibited the utilization of fatty acid substrates.

**Effects of TMZ on cardiac metabolism.** In isolated rat hearts undergoing ischemia/reperfusion, TMZ delays the occurrence of ischemic contracture and improves recovery of post–ischemic left ventricular dysfunction (40), as well as accelerates the recovery of mitochondrial oxidative phosphorylation and phosphocreatine resynthesis (41). Fantini et al. (15) observed that the utilization of palmitoyl-carnitine by isolated cardiac mitochondria is inhibited by high doses of TMZ, in the absence of significant changes in pyruvate and citrate oxidation. Additional studies also suggest that TMZ acts by affecting myocardial substrate utilization, because the drug inhibits oxidative phosphorylation and utilization of fatty acid substrates and shifts metabolism from fatty acid to glucose oxidation (42). Specifically, the agent inhibits beta-oxidation by selectively blocking the activity of 3-ketoacyl coenzyme A thiolase, the last enzyme of the oxidative chain (16). The preferential oxidation of glucose observed during TMZ infusion affords even greater protection during reperfusion than during acute ischemia (39). The reported reduction, by TMZ, of cellular acidosis induced by ischemia (12) is probably secondary to the improvement of mitochondrial function. All of these effects may contribute to reduce the deleterious effects of the ischemic insult, and, because they occur in the absence of detectable changes in systemic and coronary hemodynamic variables, the in vivo effects of TMZ on the ischemic myocardium are likely to depend on direct cytoprotection.

**Effects of heparin and TMZ on ET-1 release.** In patients with stable CAD, heparin may significantly decrease the ischemic threshold, probably by increasing plasma concentrations of FFAs, which can adversely affect the metabolism of the ischemic myocardium (7). The administration of TMZ appears to completely abolish the deleterious effects of the heparin-induced increase of FFAs, probably by delaying the mitochondrial utilization of glucose and nonfatty substrates, allowing improvement of its ability to sustain ischemia. Besides altering the metabolic balance of the ischemic myocardium, increased FFA release may adversely influence endothelial function (43). In our study, all patients exhibited a marked increase in ET-1 during exercise. However, ET-1 release significantly decreased with exercise when patients were taking TMZ. This decrement was greater during saline than during heparin. It has been shown (44) that, in physiologic conditions, coronary endothelial cells predominantly utilize exogenous glucose for energy production. In these cells, glucose effectively suppresses the oxidation of lactate and palmitate, which are the preferred substrates for the whole heart. Similar to the cardiac muscle, both ischemia and an increased availability of FFA may negatively affect the endothelial cells’ metabolism and cause the release of endothelial factors. One hypothesis is that improved myocardial and endothelial...
metabolism, together with the recent confirmation that TMZ reduces intracellular acidosis during ischemia (45), which could not only influence myocardial but also endothelial membranes, may help to explain the reduction in exercise-induced ET-1 release (and ischemia) observed with TMZ.

A second hypothesis is that, by just decreasing the severity of myocardial ischemia, TMZ can inhibit ET-1 release. Experimental data support the hypothesis that myocardial ischemia, by itself, contributes to the release of ET-1 in plasma (46,47). In fact, apart from endothelial cells (48) and vascular smooth muscle cells (47), cardiomyocytes (49) have also been shown to produce ET-1 in response to myocardial ischemia. Furthermore, it has been shown very recently that endothelin release is a marker of ischemic severity rather than ischemia itself (50), and that TMZ, in the presence of high triglycerides levels, may improve both myocardial contractile recovery and ET-1 release after low-flow ischemia (51). Therefore, the decrease in ET-1 release observed with TMZ could likely be linked to a TMZ-induced reduction of myocardial ischemia, although, conversely, the increase in ET-1 release after heparin could be related to a greater magnitude of ischemia during this arm of the study.

The hypotensive effects of endothelin-converting enzyme inhibitors and endothelin receptor antagonists could be useful in the treatment of different cardiovascular diseases. Development of such agents will increase our knowledge of the physiologic and pathologic roles of the endothelins and should generate drugs with novel benefits. Whether TMZ could also play a role in this setting has yet to be determined.

Effects of TMZ in ischemic heart disease. Trimetazidine is an anti-anginal agent that increases cell tolerance to ischemia. The beneficial effects of oral TMZ on the frequency and severity of anginal attacks, as well as exercise capacity, have been shown in several clinical studies (17–22). The results of the present study confirm previous findings, by showing that, compared with placebo, TMZ increased the ischemic threshold when patients were receiving saline (test 1 vs. test 4). Furthermore, this study shows that in the experimental setting of increased FFA availability, as generated by unfractionated heparin, the addition of TMZ partly reverses the negative metabolic effect of these metabolites. These findings are consistent with the notion that TMZ, in addition to providing symptom relief and functional improvement in patients with angina pectoris, also has a cytoprotective action during ischemia.

Conclusions. In patients with stable CAD, heparin reduces the ischemic threshold. Trimetazidine appears to reduce the effects of heparin, probably by inhibiting oxidative phosphorylation and FFA oxidation and by enhancing glucose metabolism. The concomitant novel observation of reduced ET-1 release is likely to be also dependent on TMZ-induced improvement of endothelial metabolism. Both mechanisms are likely to contribute significantly to the beneficial effect of TMZ. We believe that our observations are important and potentially relevant to the management of patients with a variety of cardiovascular conditions.

Reprint requests and correspondence: Dr. Gabriele Fragasso, Dipartimento di Cardiologia e Scienze Cardiovascolari, Istituto Scientifico/Università San Raffaele, Via Olgettina 60, 20132 Milan, Italy. E-mail: gabriele.fragasso@hsr.it.

REFERENCES