Trimetazidine (TMZ) is a piperazine derivative (1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride) with anti-ischaemic properties. It is the first in a new class of metabolic agents, available for clinical use. In conditions of hypoxia or induced ischaemia, TMZ maintains homeostasis and cellular functions by selectively inhibiting 3-ketoacyl-CoA-thiolase. As a consequence, fatty acid β-oxidation is reduced and glucose oxidation is stimulated, resulting in decreased cellular acidosis and higher ATP production.

In humans, TMZ has been shown to increase the ischaemic threshold and to relieve angina pectoris in patients with coronary artery disease. These benefits have been observed without any change in heart rate, blood pressure, and rate-pressure product at rest, during submaximal and peak exercise. More recently, TMZ improved the mechanical efficiency of dysfunctional myocardium in patients with ischaemic cardiomyopathy. This effect was associated with enhanced left ventricular diastolic filling and systolic function.

There is also demonstration that TMZ has antioxidant properties. During acute and chronic ischaemia, TMZ reduces the loss of intracellular K+ induced by oxygen free radicals and also the membrane content of peroxidated lipids. In vivo, pre-treatment with TMZ (40–60 mg per day for 7 days) significantly decreases membrane malondialdehyde (MDA) content of red blood cells incubated with superoxide dismutase inhibitor diethyldithiocarbamate.

Trimetazidine improves endothelial dysfunction in chronic heart failure: an antioxidant effect

Romualdo Belardinelli*, Maridia Solenghi, Loretta Volpe, and Augusto Purcaro

Cardiac Rehabilitation and Prevention Section, Lancisi Heart Institute, Via Conca 71, 60020 Torrette di Ancona, Italy

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Aims To determine whether trimetazidine (TMZ) improves the endothelium-dependent relaxation (EDR) in chronic heart failure (CHF) and whether this effect is associated with its antioxidant properties.

Methods and results We studied 51 patients (mean age 51.4 ± 6 years) with CHF secondary to ischaemic cardiomyopathy (ejection fraction 32.5 ± 4.5%). Plasma malondialdehyde (MDA) and lipid hydroperoxides (LOOHs) were measured from an antecubital vein on study entry and after a 4 week treatment with oral TMZ (20 mg tid) (group T, n = 23) or placebo (group C, n = 22) given randomly. Endothelium-dependent vasodilatation of the radial artery (RA) was determined by intra-arterial infusion of acetylcholine (7.5, 15 and 30 μg/min). Patients receiving TMZ had an increased radial artery diameter (RAD) in response to each dose of acetylcholine infusion and a greater peak oxygen uptake (P < 0.01 vs. placebo). Plasma MDA and LOOHs levels were reduced at 4 weeks only in patients receiving TMZ (P < 0.001 for both vs. placebo). The improvement in ED-vasodilatation was correlated with changes in peak VO₂ (r = 0.68; P = 0.0001), MDA (r = −0.61; P = 0.0002) and LOOHs (r = −0.59; P = 0.005). Conclusion TMZ improves the ED-relaxation in patients with ischaemic cardiomyopathy. The antioxidant properties of TMZ may play a role.

Other in vitro studies have confirmed the antioxidant effect of TMZ. In humans, plasma levels of MDA were decreased after pre-treatment with TMZ during coronary artery bypass surgery. In conditions of high oxidative stress, such as diabetes mellitus and chronic heart failure (CHF), free radicals production is increased and contributes to endothelial dysfunction. In this setting, TMZ decreases plasma levels of both free radicals and endothelin-1. Given this effect, we hypothesize that, in CHF, the addition of TMZ to standard medications may improve the endothelium-dependent vasodilatation, which may be related to decreased plasma levels of lipid free radicals.

Methods

As shown in Figure 1, patients assessed for eligibility were initially 60 (45M/15F). Of them, nine were excluded and 51 were randomized. Inclusion criteria were clinical stability during the last 3 months, and ability to perform exercise. Exclusion criteria were a recent acute coronary syndrome and/or coronary interventions, renal insufficiency (serum creatinine > 2.5 mg/dL), liver abnormalities, uncontrolled hypertension, and orthopedic and/or neurological limitations. None of our patients regularly assumed multivitamin/mineral tablets or relevant amount of foods particularly rich in antioxidants.

Study design

The protocol was approved by the local Ethical Committee. Patients were randomized into two homogeneous groups after a run-in period.
of 1 week, during which they signed an informed written consent, and were visited by a cardiologist. The protocol was designed according to CONSORT statement suggestions. In detail, sequence generation was computerized, sequence was concealed until interventions were assigned, and implementation was performed by physicians and nurses not involved in the study design. Patients were assigned on an individual basis to either TMZ or placebo treatments. They remained on the same allocation throughout the study period. A computer-generated randomization list was drawn up by an independent statistician and given to the central hospital pharmacy. Each patient received one or the other treatment directly from the pharmacy. The code was revealed to the researchers, once recruitment, data collection, and all analyses were completed. Both physicians involved in the study and patients were blinded and unaware of group assignment. A group (T, n = 27) received TMZ 20 mg tid for 4 weeks, and a group (C, n = 22) received placebo. (Table 1) Standard medications were allowed and remained unchanged during the study. On study entry and at 4 weeks, all patients underwent a symptom-limited cardiopulmonary exercise testing, a study of the vasomotor reactivity of the radial artery (RA), and blood chemistry analysis. Primary endpoint of the study was to determine whether TMZ might improve the endothelium-dependent relaxation (EDR). Secondary endpoints were the effect of TMZ on functional capacity and indices of cardiovascular efficiency assessed by cardiopulmonary exercise testing.

Cardiopulmonary exercise testing

After a familiarization test, a symptom-limited cardiopulmonary exercise test was performed on an electronically braked cycle-ergometer using a ramp-pattern increase in work rate. Expired gases and volumes were analysed, breath-by-breath, with a metabolic cart (QUARK PFT, COSMED, Rome). Heart rate and blood pressure were measured every minute during increasing work rate exercise and recovery. A 12-lead ECG was recorded every minute. The exercise test was stopped when one or more of the following criteria were present: predicted heart rate, fatigue, dyspnoea, excessive systemic blood pressure increase (>230/130 mmHg), >2 mm ST-depression in at least two adjacent leads and/or angina. The anaerobic threshold was measured by the V-slope method. Peak oxygen uptake was the average oxygen uptake during the last 15 s of exercise.

Radial artery vasomotor function

All studies were performed in a room with constant temperature (23 °C), barometric pressure (760 mb), and humidity (50%). Patients were evaluated in the morning in fasting condition after withdrawal of medications for four times the half-lives. Under local anaesthesia with lidocaine, a 20-gauge cannula was inserted into the brachial artery to determine the effects of infusion of an endothelium-dependent vasodilator, acetylcholine (Ach), and an endothelium-independent vasodilator, nitroglycerine, on radial artery diameter (RAD) and blood flow. The Ach infusion was administered at doses of 7.5, 15, and 30 µg/min for 90 s each, followed by 0.9% saline for 5 min and by nitroglycerine at doses of 0.2 µg/min for 5 min. The sequence of infusions was randomly selected before each study. After 15 min of relax in supine position, a 7.5 MHz ultrasound probe was positioned over the dominant arm to detect good quality RA images (ESAOTE Challenge, Florence, Italy). Acquisition started after fixation of the probe in a stereotaxic arm in order to avoid artefacts due to operator movements. Images were taken at baseline for 30 s, at the end of each 5 min infusion phase. Endothelium-dependent and independent responses have been determined on the basis of percent change in diameter from baseline. Images were entered online into a dedicated personal computer and were processed for analysis after digital conversion. We used a software designed by us allowing beat-to-beat analysis of diameter changes during the test. Images were evaluated by two independent experienced operators unaware of the clinical picture and blinded to each other’s interpretation. Intra-observer and inter-observer variability were assessed in 25 consecutive subjects with a variety of conditions, and results were acceptable and in agreement with those of other laboratories (1.2 ± 0.8% and 1.9 ± 0.9%, respectively).

Blood chemistry

Venous blood samples were placed in tubes containing sodium EDTA (1 mg/mL) and in polystyrene tubes. The tubes were promptly chilled in ice bath and then immediately centrifugated at 3100 rpm at 4°C for 10 min; serum was separated at 1000 rpm at
room temperature for 10 min. MDA levels in plasma samples were measured using HPLC with fluorescence detection.\textsuperscript{17} A TBA reaction was initiated by mixing 200 $\mu$L of dipotassium EDTA plasma with 750 $\mu$L of phosphoric acid (0.15 mol/L), 300 $\mu$L of water, and 250 $\mu$L of TBA (42 mmol/L). The reaction mixture was incubated in a boiling water bath for 60 min, cooled on ice, and then neutralized and precipitated by mixing 500 $\mu$L of the sample with 500 $\mu$L of a NaOH-methanol solution. The MDA/TBA complex was then separated by injecting 20 $\mu$L of the sample on a HPLC column. Fluorescence was measured with an excitation wavelength of 532 nm and an emission wavelength of 553 nm. Total plasma hydroperoxide absorbance of the blank and test samples. In our laboratory, oxidative stress variability was assessed in 25 healthy subjects. Intra-assay variability was found to be 7.4% for lipid hydroperoxides (LOOHs) and 9.5% for MDA. Inter-assay variability was 18% for LOOHs and 19% for MDA.

**Statistical analysis**

Data were analysed by SPSS 12.0 (for Windows) statistical package. Intergroup comparisons were performed using the two-way repeated measures analysis of variance followed by Tuckey post hoc test. Data were tested for normal distribution using the Kolmogorov–Smirnov test and for homogeneity of variances with Levine’s test. From previous studies from our laboratory, we obtained a standard deviation (SD) of 80 mm for measurements of radial diameter changes at 30 $\mu$m/min Ach in patients with similar clinical characteristics to those enrolled in the present study ($n = 5$, age 59 $\pm$ 9 years, ejection fraction 32 $\pm$ 6%). We therefore estimated a SD of 80 $\mu$m for the assessment of the EDR. A difference in RA diameter exceeding two SDs (i.e. 160 $\mu$m) was considered to be statistically relevant. We then calculated a number of patients of 20 per group for a power of 80% and $\alpha = 0.05$. In order to adjust the probability of type I error for multiple testing, we applied the Bonferroni correction, obtaining a value of $P = 0.016$. Linear regression analysis was used to assess relationships between the endothelium-dependent vasorelaxation and changes in oxidative stress. Data are presented as mean and SD. Statistical significance was considered for a value of $P < 0.05$.

**Results**

Of 51 patients, 45 (23 T/22 C) completed the study. (Figure 1) Four patients in group T and two patients in group C were withdrawn from the study, two for gastrointestinal problems (1T, 1C), two for personal reasons (1T, 1C), and two for work-related problems (2T). Only one of the two patients with gastrointestinal problems was treated with TMZ. The proportion of patients with 1/2/3 coronary risk factors was similar between treated and control patients (Table 1).

**Response to acetylcholine**

Patients treated with TMZ had a significant improvement in arterial diameter during Ach infusion (Figure 1). No changes were observed in control patients. In more detail, an increased diameter was observed in 19 out of 23 patients, and it was evident at each dose of Ach infusion. The arterial diameter at baseline was similar on initial evaluation and at 4 weeks in both groups (group T: 3.22 $\pm$ 0.05 mm and 3.25 $\pm$ 0.05 mm; group C: 3.28 $\pm$ 0.06 mm and 3.32 $\pm$ 0.06 mm, $P = 0.55$) (Figure 2).

**Response to nitroglycerine**

At 4 weeks, in both TMZ patients and controls there were no changes, as compared with baseline, in the endothelium-independent vasorelaxation. On study entry, percent change in diameter was 18.4 $\pm$ 1.4% in the TMZ group and 17.8% $\pm$ 1.6% in the placebo group ($P = 0.18$), while at 4 weeks the change was 19.1 $\pm$ 1.3% and 18.6 $\pm$ 1.6%, respectively ($P = 0.26$).

**Cardiopulmonary exercise testing**

As shown in Table 2, at 4 weeks, treated patients had significant improvements in peak VO$_2$ (12.6 $\pm$ 2.3%), anaerobic threshold (26.6 $\pm$ 2.9%), ventilation (31.6 $\pm$ 4.2%), and O$_2$ pulse (22.2 $\pm$ 2.3%) ($P < 0.01$ vs. placebo for all comparisons). Heart rate was unchanged at rest and at peak exercise in both groups. Systolic blood pressure was significantly increased at peak exercise only in patients receiving TMZ ($+16.6$ $\pm$ 1.9%, $P < 0.01$ vs. placebo). No changes were observed in the control group.
Table 2  Cardiopulmonary exercise testing results

<table>
<thead>
<tr>
<th></th>
<th>TMZ</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study entry 4 weeks</td>
<td>Study entry 4 weeks</td>
</tr>
<tr>
<td>Peak VO2 (mL/kg/min)</td>
<td>15.8 ± 1.9</td>
<td>17.8 ± 1.4*</td>
</tr>
<tr>
<td>AT VO2 (mL/kg/min)</td>
<td>10.9 ± 1.6</td>
<td>13.8 ± 1.7*</td>
</tr>
<tr>
<td>Ventilation (L/min)</td>
<td>52.5 ± 18</td>
<td>69.1 ± 19*</td>
</tr>
<tr>
<td>Peak O2 pulse (mL/beat)</td>
<td>9.9 ± 1.5</td>
<td>12.1 ± 1.5*</td>
</tr>
<tr>
<td>ΔVO2/ΔW (mL/min/W)</td>
<td>7.8 ± 0.9</td>
<td>8.7 ± 0.9*</td>
</tr>
<tr>
<td>Peak work rate (W)</td>
<td>108 ± 22</td>
<td>128 ± 20*</td>
</tr>
<tr>
<td>Resting heart rate (beats/min)</td>
<td>73.2 ± 11</td>
<td>70.4 ± 9</td>
</tr>
<tr>
<td>Resting systolic blood pressure (mmHg)</td>
<td>131 ± 10</td>
<td>131 ± 10</td>
</tr>
<tr>
<td>Peak heart rate (beats/min)</td>
<td>134 ± 21</td>
<td>140 ± 19</td>
</tr>
<tr>
<td>Peak systolic blood pressure (mmHg)</td>
<td>144 ± 18</td>
<td>168 ± 19*</td>
</tr>
</tbody>
</table>

Forty-five patients completed the protocol. TMZ, trimetazidine. *P < 0.01 vs. placebo (absolute changes at 4 weeks).

Systemic oxidative markers

In patients treated with TMZ, there was a significant decrease in both plasma MDA levels (from 3.98 ± 0.69 to 2.15 ± 0.59 μmol/L) and LOOHs levels (from 3.72 ± 0.9 to 2.06 ± 0.6 μmol/L) as compared with placebo (P < 0.001 for both vs. placebo). No changes were observed in the control group.

Correlations

The improvement in Ach-induced vasodilation was correlated with changes in peak VO2 (r = 0.79; P = 0.0001) (Figure 3), MDA (r = −0.61; P = 0.0002) and LOOHs (r = −0.59; P = 0.005) (Figure 4).

Discussion

The results of the present study demonstrate that TMZ improves the endothelium-dependent vasodilation in patients with ischaemic cardiomyopathy and CHF, and this effect was correlated both with decreased plasma levels of MDA and LOOHs and with enhanced functional capacity. No changes in the endothelium-independent vasorelaxation were detected. Moreover, TMZ improved functional capacity and cardiovascular efficiency, as shown by cardiopulmonary exercise testing results (Table 2).

To our knowledge, this is the first demonstration that TMZ, a metabolic agent with well-documented anti-ischaemic properties, is able to improve the endothelial vasomotor function in CHF. This potentially beneficial effect may depend on its antioxidant properties.

As a matter of fact, at 4 weeks, the increase in RAD in response to Ach was more marked in patients treated with TMZ with more than two coronary risk factors (Figure 5), who had higher plasma levels of MDA and LOOHs than patients with one or no risk factors (Figure 6). According to previous observations, this subgroup of patients is more responsive to antioxidant treatment. Heitzer and co-authors demonstrated that co-infusion of vitamin C improved Ach-induced vasodilation in patients with coronary artery disease and high oxidative stress, and this response was associated with an adverse outcome.19

A blunted vasodilator response to Ach mirrors the oxidative stress imposed on the vascular wall, which in turn determines endothelial dysfunction.20 The addition of an antioxidant has beneficial effects on the endothelium, because it improves nitric oxide biological activity. Gokce et al.21 have demonstrated that increased plasma ascorbic acid concentrations after single-dose (2 g per os) and long-term treatment with ascorbic acid (500 mg/day) were both associated with improvements in flow-mediated dilation of the brachial artery.

Previous studies have shown that TMZ has antioxidant properties. In one study, long-term administration of TMZ significantly reduced superoxide anions generation and MDA after ischaemia-reperfusion.22 In vivo, pre-treatment with TMZ (40–60 mg per day for 7 days) significantly decreased membrane MDA content of red blood cells incubated with superoxide dismutase inhibitor diethyldithiocarbamate.9 However, this antiradical effect is not observed when TMZ is added in vitro to isolated erythrocytes from untreated volunteers. The fact that TMZ antiradical activity occurs after oral administration and that it requires a longer duration than acute addition in the perfusate of isolated cells suggests that it is mediated by one or more of its metabolites. Two metabolites of TMZ have been identified in plasma after oral administration of [14C]-TMZ,23
and 10 metabolites have been detected in urine after 80 mg orally.24

There is evidence that free radicals are increased in CHF in both experimental and clinical studies.25 Prasad et al.26 have found that leucocyte-mediated production of oxygen-derived free radicals is increased four-fold in heart failure patients as compared with controls. Free radicals generated under conditions of oxidant stress induce significant tissue damage and modifications of lipids and proteins in the vasculature. Lipid peroxidation generates hydroperoxides from polyunsaturated fatty acids (PUFA), when hydroxyl radicals (OH·) come into contact with PUFA, a PUFA radical, which in turn rearranges to form a conjugated diene reacting with molecular oxygen to form PUFA-peroxyl radical. Lipid peroxides can yield lipid peroxyl radicals (LOO·), which can react with nitric oxide to form lipid peroxinitrites (LOONO·). In this study, an antioxidant effect of TMZ is suggested by a reduction in systemic markers of oxidant stress, such as MDA and LOOHs. Moreover, we found a relationship between changes in plasma concentrations of MDA and LOOHs and the response to Ach (Figures 3 and 4).

The improvement in conduit artery endothelium-dependent vasodilation after TMZ may be the consequence of both a direct and an indirect effect of the drug on the endothelium. The indirect effect is related to the improvement in cardiovascular dysfunction due to TMZ-induced metabolic changes. In ischaemic cardiomyopathy, TMZ is able to improve myocardial contractility of dysfunctional myocardium through inhibition of 3-ketoacyl transferase, which optimizes cellular energy metabolism. Both a reduction in intracellular acidosis and preservation of phosphocreatine and ATP intracellular levels after TMZ improve cardiovascular function and are associated with decreased endothelin-1 plasma levels in patients with ischaemic cardiomyopathy and diabetes.7 In the present study, both functional capacity (peak VO₂ + 12.6 ± 2.3%), anaerobic threshold +26.6 ± 2.9%) and cardiovascular efficiency (O₂ pulse + 22.2 ± 2.3%, ΔVO₂/ΔWR + 12 ± 1.6%, peak exercise systolic blood pressure +17 ± 1.9%) were improved in the timetazidine group. Both effects may contribute to a more active lifestyle and a better quality of life.

In CHF, endothelin-1 plasma levels are increased in parallel with the severity of cardiovascular dysfunction, and the endothelium-dependent vasorelaxation is impaired due to an altered vascular homeostasis. Nitric oxide seems to blunt signalling by endothelin, the principal peptide responsible for vascular smooth muscle cell contraction, by slowing its release. In conditions of chronic shear stress, nitric oxide appears to be a critical mediator of the decrease of endothelin release.27 In fact, endothelin decrease is

![Figure 4](image-url) Pre–post changes in the endothelium-dependent response vs. pre–post changes in MDA and LOOHs in TMZ-treated patients (closed circles) and controls (open circles).

![Figure 5](image-url) Effects of TMZ on the arterial diameter response to crescent doses of Ach on study entry (circles) and at 4 weeks (squares) in patients with one or no (< RF) coronary risk factors and more than two coronary risk factors (> 2RF).

![Figure 6](image-url) Pre–post changes in plasma MDA and LOOHs in TMZ-treated patients on study entry (filled bars) and at 4 weeks (crossed bars) in patients with one or no cardiovascular risk factors and with more than two risk factors. P < 0.01 vs. placebo.
abrogated by nitric oxide inhibitors, and potentiated by phosphodiesterase inhibitors. A possible mechanism may be that TMZ inhibits endothelin-1 release by decreasing the deleterious effects of chronic ischaemia. This effect may improve nitric oxide-mediated vasorelaxation.

On the other side, TMZ may exert a direct effect on endothelial cells, acting as a lipid barrier permeable transition metal chelator, protecting the endothelium from free radicals. This action may be of particular importance in diabetic and in conditions of high oxidative stress, in which LDL oxidation and DNA oxidative damage may directly injure endothelial cells and cause abnormal gene expression and altered signal transduction. Lipid peroxyl radicals combine readily with nitric oxide with a rate constant of $\sim 1-3 \times 10^9 \text{M}^{-1}\text{s}^{-1}$, leading to formation of lipid peroxynitrite derivatives. Observations that nitric oxide-mediated inhibition of platelet aggregation is potentiated by glutathione peroxidase suggest that reduction of LOOHs enhances nitric oxide bioactivity.

Limitations
We did not measure neither reactive oxygen species nor enzymes implicated in their regulation, such as superoxide dismutase. This could help in the interpretation of the effects induced by TMZ. The fact that TMZ reduces lipid free radicals in the plasma is not sufficient for a causative role in improving Ach-mediated vasorelaxation. Heart failure per se causes endothelial dysfunction and oxidative stress. However, given the potential for lipid peroxidation to impair nitric oxide bioactivity in the setting of vascular disease, it is reasonable to speculate that reducing vascular lipid peroxidation should be associated with enhanced nitric oxide bioactivity.

In summary, the results of the present study demonstrate that TMZ improves the EDR of a conduit artery in patients with CHF and ischaemic cardiomyopathy. This beneficial effect is correlated with decreased plasma levels of both MDA and LOOHs, suggesting a possible link between TMZ’s antioxidant properties and improved nitric oxide bioactivity. The improvement in cardiovascular efficiency induced by TMZ may have potential therapeutic and prognostic implications. An important effect can be an increase in stroke volume during daily submaximal physical activities that can allow a more active life style and improve functional capacity and quality of life.

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Conflict of interest: none declared.

References


