

Trimetazidine, a Metabolic Modulator, with Cardioprotective Effects Against Myocardial Ischemia

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Metabolic therapy constitutes a relatively new therapeutic option for ischemic heart disease (IHD). Metabolic agents including Trimetazidine [1-(2,3,4-trimethoxybenzyl) piperazine] (TMZ) are currently used as anti-ischemic cardiac drugs to alleviate this internal metabolic disturbance. The present study focused on the assessment of the beneficial effects of TMZ as additional medication to the conventional medical therapy in patients with myocardial infarction. The obtained results showed an increase of superoxide dismutase (SOD) activities and a faster normalization of total sialic acid (TSA) levels in patients with TMZ add-on therapy, in comparison to the control group, indicating a stabilization of the ischemic process under TMZ therapy, with decreased free radical production and restoration of the antioxidant capacity and of the membrane lesions. The present findings emphasize the importance of TMZ administration to prevent reperfusion-mediated cardiac injury and dysfunction and demonstrate that this promising therapeutic approach is worthy of further research.

Keywords: Trimetazidine (TMZ), myocardial infarction, sialic acid, superoxide dismutase, metabolic modulators

Modulation of myocardial energetic metabolism represents a new and promising approach for the treatment and management of ischemic heart disease (IHD) [1-3].

Under normal conditions, the healthy heart obtains about 95% of its energy through the production of ATP from mitochondrial oxidative metabolism, while 5% originates from glycolytic ATP production [4]. Fatty acid oxidation (FAO) provides approximately two-thirds of cardiac ATP in the fasting state, with the other sources of energy being derived from glucose oxidation and lactate. However, FAO is a less efficient energy source compared with glucose oxidation, theoretically requiring 11-12% more oxygen for a given amount of ATP produced [1, 5].

Myocardial ischemia radically alters the balance of cardiac fuel metabolism by switching in substrate metabolism away from fatty acids toward glucose to maximize efficiency. Because of hypoxia, glycolysis becomes the main route for ATP production, leading to intracellular acidosis via the production of cytosolic protons which activate the Na⁺-H⁺ exchanger and the Na⁺-Ca²⁺ exchanger causing intracellular calcium overload and contractile dysfunction [6, 7]. Excess of Ca²⁺ ions leads to excessive inflow of water, which is followed by the opening of the mitochondrial permeability transition pore (MPTP), mitochondrial swelling [8], leading to the necrosis and apoptosis of cardiomyocytes [9]. Moreover, the neutrophils induce inflammation mediators which amplify recruitment of neutrophils in the ischemic-reperfused myocardium, thus expanding myocardial damage [10]. During myocardial ischemia, tissue pH declines significantly, only returning to

normal after reperfusion [11]. During reperfusion, the electron transport chain is reactivated, generating reactive oxygen species (ROS), which mediate myocardial reperfusion injury by inducing the opening of the MPTP, acting as a neutrophil chemoattractant [12]. The antioxidant protective enzymes are overwhelmed, followed by membrane lipid peroxidation [13] and loss of intracellular K⁺ [14]. The reperfusion can actually be more damaging than the pre-reperfusion ischemia [15], and may result in serious myocardial damage-induced ventricular remodeling, deterioration of cardiac function, development of congestive heart failure and mortality [16]. During reperfusion, FAO becomes the predominant source of ATP production once again.

Trimetazidine [1-(2,3,4-trimethoxybenzyl) piperazine] dihydrochloride (TMZ) is an anti-ischemic agent efficient in the treatment of various clinical forms of ischemia, including angina pectoris and acute coronary syndromes [17-19]. It inhibits the long-chain mitochondrial 3-ketoacyl coenzyme A thiolase (LC 3-KAT), the enzyme which catalyzes the terminal step of FAO, resulting in the improvement of the mitochondrial metabolism through inhibition of FAO and the consequent stimulation of glucose oxidation by an increase in pyruvate dehydrogenase activity [20]. Although most literature data supports TMZ involvement in the inhibition of FAO, some research [2] suggests that TMZ is not a LC 3-KAT inhibitor - a finding which can however be explained by the high concentration of 3-keto-hexadecanoyl coenzyme A substrate used in their experiments. TMZ exerts its beneficial effects without

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altering the hemodynamic function of the heart [6] and can be combined with conventional pharmacotherapy of the coronary artery disease (CAD), as either add-on therapy, or as substitution therapy when conventional drugs are not tolerated [21,22]. The cardioprotective effects of TMZ have not been fully understood as of yet. Various studies have demonstrated that TMZ exerts cardioprotective effects by various mechanisms: a) conserving intracellular ATP levels, decreasing oxygen consumption during the production of ATP and lowering intracellular acidosis and calcium overload, while maintaining cellular homeostasis [20]; b) reducing the loss of intracellular K⁺ induced by ROS [23]; c) reducing the oxidative damage produced via the activation of antioxidant enzymes and thus decreasing the level of malondialdehyde, the end product of lipid peroxidation [24]; d) inhibiting inflammation response [24-26], neutrophil infiltration [20] and apoptosis pathways [26, 27], thereby protecting the heart from ischemia/reperfusion (I/R) induced injury [27-29]; e) decreasing the production of thromboxane A₂ produced by the free radical-induced stimulation of phospholipase A₂ and thromboxane synthetase [30]; f) binding to a MPTP binding site [2]. Therefore, TMZ preserves the energy potential of the cell, corrects ionic disturbances, favorably affects cell membrane equilibrium, counteracts the harmful effects of free radicals, all of which ensures an optimal metabolism of the myocardial cell during ischemia.

The aim of the current study is to assess the cardioprotective effects of TMZ in myocardial ischemia and the efficiency of TMZ administration as a metabolic modulator, along with the traditional hemodynamic methods of decreasing oxygen consumption by reducing heart rate, blood pressure, and cardiac work in acute myocardial infarction. Moreover, the study emphasizes the importance of administering TMZ before reflow to prevent I/R heart injury and dysfunction.

Experimental part

The study population consisted of 76 patients diagnosed with acute myocardial infarction (group I). Myocardial infarction was diagnosed upon the basis of clinical symptoms, characteristic electrocardiogram changes and enzyme activities (creatin kinase MB (CK MB)) and high sensitivity troponin I [31, 32]. The subjects from group I were treated with TMZ (2x35 mg TMZ/day), beginning on the day of diagnosis, in addition to standard therapy: fibrinolytic agents, double therapy with antiplatelet agents (Aspirin and Clopidogrel or Ticagrelol), beta blockers, angiotensin-converting enzyme inhibitors, statins. Fibrinolytic therapy consist of intravenous alteplase (tPA), administered as follows: a bolus of 15 mg, followed by 0.75 mg/kg over 30 min (up to 50 mg) and then 0.5 mg/kg over 60 min (up to 35 mg). TMZ was administered for a long period of time beginning with the day of diagnosis. The control group (group II) consisted of 70 age and sex matched patients diagnosed with acute myocardial infarction, who received only the standard therapy mentioned above, without TMZ. All enrolled subjects did not suffer from diabetes mellitus, recent infections, recent surgery or any other illnesses including malignant pathologies. Before inclusion, informed written consent was obtained from each patient. The objective was to compare the groups in terms of oxidative stress (SOD activity) and cytolysis (CK, MB and total plasma sialic acid level) markers, which were measured at the enrollment in the study and in each subsequent day during the following 7 days.

Materials and methods

Determination of creatine kinase MB (CK MB)

Serum CK MB was determined spectrophotometrically, using commercially available kits from Abcam (ab15590).

Determination of superoxide dismutase (SOD)

SOD is an index of the antioxidant capacity. SOD enzyme activity was determined spectrophotometrically using the pyrogallol autoxidation method described by [33]. The principle of this method is based on the competition between the pyrogallol autoxidation by O₂^{•-} and the dismutation of this radical by SOD. SOD activities are expressed as units per milliliter (u/mL) and were measured at 420 nm. Its normal value is over 10 u/mL.

Determination of the total plasma sialic acid (TSA)

TSA levels were determined using the thiobarbituric acid assay of Warren with some modifications [34, 35]. First, acidic hydrolysis (0.1N sulfuric acid) was used to liberate free SA from sialoglycoconjugates. Then, periodate is used under strongly acidic conditions (9 M phosphoric acid) to oxidize SA to β-formylpyruvic acid, the reaction being stopped by adding a mixture of sodium arsenite, sodium sulphate and sulfuric acid. After subsequent adding of thiobarbituric acid, a red colored compound was generated, extraction of which into cyclohexanone resulted in an upper red phase. The absorbance of the cyclohexanone layer was measured for the samples and for the standards against the blank at 549 nm. The concentration of TSA was determined for all the samples and expressed as mmol/L. Its normal level in plasma is 1.58-2.22 mmol/L [36].

Statistical Analysis

All data was presented as mean ± standard deviation. Differences between the 2 groups were observed using Student's t-test. A statistically significant difference was considered if p<0.05.

Results and discussions

Creatine kinase MB (CK MB) was used as a diagnostic index for acute myocardial infarction, and its dynamics followed the characteristic aspect of normalization of serum values 48 h after the onset of the infarction. Two patients in the conventional treatment group experienced a new increase of the enzymatic activity at 36 h after the onset of the infarct, corresponding to the extension of the infarct area.

Superoxide dismutase (SOD) decreased in the first few hours after the onset of the infarction in both groups of patients without significant differences. The normalization of the values depended on the restoration of the antioxidant capacity, and on the decrease of free radical production, respectively - residual ischemia, for example, translates enzymatically by low SOD activity. From day 2 onwards, group I had an increase in SOD activity values, which were normalized on the next day. In the group of patients treated conventionally, SOD activity remained at low levels. This significant difference indicates a stabilization of the ischemic process with TMZ therapy, the control of the myocardial lesion with decreased free radical production and the restoration of the antioxidant capacity (p = 0.00782) (fig. 1).

TMZ has been assessed in several double-blind randomised studies as a treatment of IHD or as an agent given prior to or during percutaneous transluminal coronary angioplasty, coronary artery bypass grafting and thrombolysis to prevent or limit I/R damage in the heart.

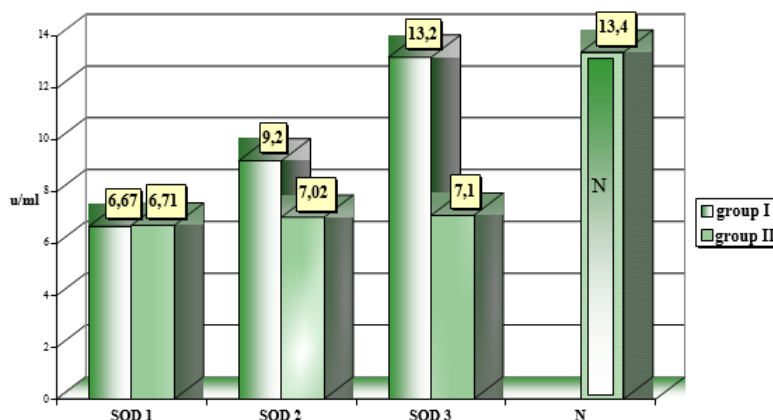


Fig. 1. The temporal variation of SOD activity (u/mL) under TMZ treatment (group I) vs. control (group II).

SOD1 - SOD activity at 24 h after the onset of infarction, SOD2 - SOD activity at 48 h after the onset of infarction, SOD3 - SOD activity at 72 h after the onset of infarction

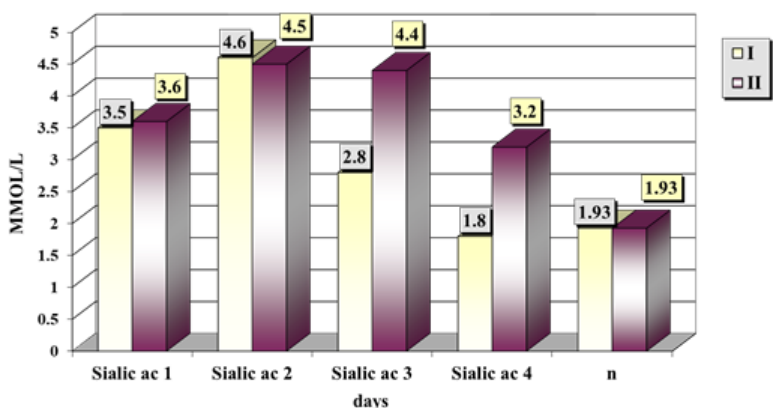


Fig. 2. The temporal variation of TSA concentration in serum under TMZ treatment (group I) vs. control (group II).

Sialic ac 1 - TSA level at 24 h after the onset of infarction (day 1), Sialic ac 2 - TSA level at 48 h after the onset of infarction (day 2), Sialic ac 3 - TSA level at 72 h after the onset of infarction (day 3), Sialic ac 4 - TSA level at 96 h after the onset of infarction (day 4)

Under normal conditions, scavenging mechanisms operate swiftly to remove excess ROS. Superoxide radicals are removed by SOD in cytosol (Cu/Zn-SOD) and in mitochondria (Mn-SOD) [37], and the resultant hydrogen peroxide is removed by catalase, glutathione peroxidase and peroxiredoxin [38, 39]. However, elevated levels of ROS can swamp the protective mechanisms of the cell, and represent a significant cause of I/R damage. The importance of ROS in inducing ischemic damage is shown by a protective effect in perfused hearts of transgenic mice overexpressing Mn-SOD [40] and by a study showing that post-ischemic recovery of contractile function is impaired in Mn-SOD-knockout mice [41]. Determination of SOD levels in ischemic myocardium yielded equivocal results, with Ueta et al. [42, 43] indicating a decrease in SOD in myocardial ischemia and infarction, while others observed increased activity associated with mitochondrial protection and a reduction in apoptosis, possibly through a mechanism involving HSP72 [44]. Moreover, Iskesen et al. [15] showed a marked increase of SOD activity in the study group pretreated and treated with TMZ during coronary artery bypass grafting under cardiopulmonary bypass, comparatively to the control group. SOD levels in the study group were significantly higher than those of the control group at 1 min and 15 min after release of the cross-clamp. These results are in accordance with other studies which showed that TMZ was able to produce the beneficial effect in the reduction of the ROS and the improvement of coronary microcirculation following reperfusion [45]. Our findings are similar and they highlight that this agent is particularly promising clinically, in the treatment of reperfusion injury in combination with reperfusion strategies, during the acute phase of myocardial ischemia and infarction.

Sialic acid (SA) is a component of membrane glycoconjugates and is not specific to myocardial tissue. Its levels can increase in conditions of neoplasia, diabetes mellitus and acute pancreatitis, and so we have excluded the presence of these pathologic conditions from our study.

SA can be used as a marker of the membrane lesion. Literature data did not provide specific results on SA dynamics in acute myocardial infarction, which is why we attempted to establish this dynamics by serially determining TSA level from the onset of myocardial infarction and every 6 hours, in parallel with the known enzymatic dynamics (CK MB and glutamate oxaloacetate transaminase (GOT)) until normalization. TSA level increased in the first two days in parallel in the two groups of patients: it began to increase in the first 12 hours after onset and it reached a peak after 48 hours. Subsequently, however, they followed different profiles: in group I (TMZ-treated group) TSA levels declined significantly on the third day and became normal after another day. In group II, the one used as a control group, normalization occurred more slowly: TSA levels began to decrease on day 4 and progressively reached the normal value only on day 6 ($p = 0.00452$) (Figure 2), however the time of initiation of the increase and of reaching the maximum level is maintained. This may last longer depending on the stabilization of the ischemic process and the permeability of the coronary artery, which is also true for the enzymatic markers. Thus, TSA in the myocardial infarction develops a curve which starts similarly to CK MB, increases in parallel with it, and then normalizes in the rhythm of GOT.

Few earlier reports have investigated the relation between serum TSA concentration and cardiovascular diseases, and a positive association between elevated serum TSA levels and CAD, especially acute myocardial infarction, has been demonstrated [46-50]. Nevertheless, contradictory reports have been published on the possible relation between serum TSA levels and the severity of coronary lesions in patients with CAD. While Allain et al. [51] found a positive correlation between increased serum TSA and the severity of the coronary lesions, others [52, 53] demonstrated that serum TSA concentrations are not associated with the severity of CAD in patients with stable angina, and proposed that the association with serum TSA and cardiovascular mortality revealed by other studies may

involve other pathophysiological mechanisms in addition to the narrowing of the coronary artery. Moreover, factors such as aging, pregnancy, smoking, a number of pathologies may affect sialic acid concentration, thereby limiting its potential clinical usage [46].

Our results regarding TSA concentration for the control group suffering from acute myocardial infarction without TMZ administration are partially comparable with those obtained by Gokmen et al. [54], namely that the TSA level is elevated in patients with acute myocardial infarction treated with classic thrombolytic therapy in the first three days post-infarction, and that there exists a gradual elevation of TSA levels during this period post diagnosis. Other similar data [55-57] also showed a significant increase of the mean TSA levels in people afflicted with myocardial infarction, in comparison with healthy individuals, indicating that TSA is a risk factor for CAD.

In human plasma SA is found in various glycoproteins: orosomucoid, α 1-antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, complement proteins, and transferrin [46, 58]. Some of these sialylated glycoproteins are called acute phase reactants, because their concentrations rapidly increase after the onset of an inflammation reaction or injury [46]. SA is also present as constituent of the membrane glycoproteins of erythrocytes, leucocytes and platelets [46]. The increase of the SA level was observed in plasma during CAD progression to myocardial infarction, but the mechanistic aspect of the increase of its concentration in myocardial infarction is still yet not very well understood, although it could be related to:

- increased sialylation of glycoproteins or glycolipids due to increased sialyltransferase activity [59], an enzyme which is situated in the membranes of smooth endoplasmic reticulum and mitochondria and in the plasma membranes of cells, including blood platelets. Stimulation of sialyltransferase activity by colchicine has been observed in blood platelets by platelet-aggregating compound in rat serum and tissues [60];

- increased secretion of SA from cell membranes due to elevated sialidase (neuraminidase) activity [59], which leads to the detachment of sialic groups positioned at the non-reducing end of complex carbohydrates in erythrocyte membranes, decreased red cell SA and to the consequent increase of free SA levels in plasma. Thus, the negative charge of the cell surface decreases, eliminating forces of mutual repulsion of similarly charged membranes resulting in clumps of desialylated erythrocytes that may alter flow in the microcirculation [54, 61]. Silencing neuraminidase-1, the enzyme which regulates SA generation, ameliorated oxygen-glucose deprivation-induced injury in cardiomyocytes and ligation/isoprenaline-induced myocardial ischemia injury in rats. Pharmacological inhibition of neuraminidase by anti-influenza drugs, oseltamivir and zanamivir, also protected cardiomyocytes and the heart from myocardial injury; this may represent an unrecognized therapeutic intervention for CAD [62].

Thus, either the shedding or secreting of SA from the cell (the increased output of SA-rich inflammation-sensitive proteins) or cell membrane surface may be partly responsible for an increased serum TSA concentration following acute myocardial infarction [54, 56].

To our knowledge, there is no literature data investigating the influence of TMZ administration on serum TSA concentration in patients suffering from CAD. Our results indicate a faster decline of TSA levels in group I (treated with TMZ), but it is difficult to say whether this more favorable dynamic of TSA in patients receiving TMZ corresponds to a reduction in the necrotic or ischemic surrounding tissue area or not. However, the faster normalization of TSA levels

indicates a restoration of the membrane lesions and this alteration may be partially related to the TMZ's role in modulating cardiac energy metabolism, by shifting the energy production pathway from free fatty acids to glucose, resulting in a better performance of the heart without altering the hemodynamic function of the myocardium. Much remains to be understood about the effect of TMZ in protecting against myocardial ischemia, and most of its beneficial effects are revealed in clinical studies showing that TMZ administration may result in less myocardial damage, earlier successful reperfusion, improvement of left ventricular ejection fraction, substantially improved effort tolerance, decreased angina episodes and less cardiac adverse events such as reduced hospitalization for heart failure and re-infarction [63, 64]. Moreover, because this agent does not suppress heart rate, blood pressure, or contractility, it is effective as add-on therapy to Ca^{2+} channel and β -adrenergic receptor antagonists.

Conclusions

Our study demonstrated an increase in SOD activity from day 2 onwards, after the diagnosis of acute myocardial infarction, in the TMZ treatment group compared to the control group, in accordance with other studies which showed that TMZ caused beneficial effects in scavenging ROS and that it may reduce I/R damage. The faster decline of TSA levels in the TMZ treatment group demonstrates a positive effect in the restoration of membrane lesions and underlines the cardioprotective role of TMZ. Nevertheless, since there are many factors which influence TSA levels, its potential clinical usability may be limited, with the elucidation of the mechanism of alterations in TSA concentration being paramount in this regard. More research is therefore needed to determine whether this biochemical metabolite can be useful in routine cardiovascular risk assessment and in monitoring new pharmacologic therapies, after acute myocardial infarction.

The obtained results indicate that the administration of TMZ in therapeutic doses exerts a cytoprotective effect, as it limits I/R damage produced by ROS and alleviates myocardial inflammation. Therefore, metabolic modulators such as TMZ demonstrate promising therapeutic potential in patients with myocardial infarction, and deserve to be regarded as an early addition to the conventional *hemodynamic* agents.

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