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Nonmetabolic effects of trimetazidine and ventricular remodeling: role in regulatory gene expression

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Abstract

Although the management of ischemic heart disease and chronic heart failure (CHF) has made considerable progress over the past years, CHF is still a tremendous medical burden. The metabolic and therapeutic approach might play a significant role in reducing disease progression and mortality rate. The metabolic modulator trimetazidine, a partial inhibitor of long chain 3-ketoacyl CoA thiolase activity, the last enzyme involved in β-oxidation, seems to exert positive effects in the management of left ventricle remodeling in heart failure patients by reducing the progression of disease, and by improving quality of life and prognosis. Recent reports suggest that trimetazidine could also exert nonmetabolic effects useful in preventing left ventricle remodeling by modulating the expression of various regulatory genes (nitric oxide synthase, endothelin-1, tumor necrosis factors, atrial natriuretic peptide, glucose transporters) involved in endothelial and mitochondrial function, myocardial cell death, and tissue fibrosis. ■ *Heart Metab*. 2014;65:26-30

Keywords: heart failure; mRNA; remodeling; trimetazidine

In ischemic heart disease (IHD) and chronic heart failure (CHF) therapeutic strategies have traditionally focused on the modification of hemodynamic alterations (blood pressure, heart rate, wall stress) occurring in the ischemic or failing heart. However, in addition to hemodynamic alterations, deep changes both in systemic and in cardiac metabolism were evidenced.

Trimetazidine (TMZ) has been reported to exert anti-ischemic properties without affecting myocardial oxygen consumption and blood supply. The beneficial effect of this agent has been attributed to the preservation of phosphocreatine and adenosine triphosphate (ATP) intracellular levels, and a reduction in cell acidosis, calcium overload, and free radical–induced injury caused by ischemia. TMZ affects myocardial substrate utilization by partially inhibiting oxidative phosphorylation and by shifting energy production from free fatty acids (FFAs) to glucose oxidation. This effect appears to be predominantly caused by a selective block of long chain 3-ketoacyl coenzyme A (CoA) thiolase activity, the last enzyme involved in β-oxidation. Various studies performed in patients with postischemic left ventricular dysfunction, have shown that TMZ may be beneficial in terms of improvement in left ventricular function, control of

Abbreviations

ANP: atrial natriuretic peptide; **ATP:** adenosine triphosphate; **BNP:** brain natriuretic peptide; **CHF:** chronic heart failure; **CoA:** coenzyme A; **CPT1:** carnitine palmitoyltransferase–1; **eNOS:** endothelial nitric oxide synthase; **FFA:** free fatty acid; **IHD:** ischemic heart failure; **NO:** nitric oxide; **PDH:** pyruvate dehydrogenase; **TMZ:** trimetazidine; **TNF:** tumor necrosis factor

symptoms, and recently, prognosis.[1-3] This pharmacological approach could also be useful in the treatment of patients with heart failure of various etiologies, probably due to the reduction in whole body resting energy expenditure or, recently, to nonmetabolic effects.

Recent reports suggested that TMZ could exert nonmetabolic effects that are useful in preventing left ventricle remodeling by modulating the expression of various regulatory genes involved in endothelial and mitochondrial function, myocardial cell death, and tissue fibrosis, contributing to minimize gene regulations occurring in response to ischemia or wall stress changes.

Metabolic changes in chronic heart failure and ischemic heart disease

The classic mechanism of CHF includes neurohormone (adrenergic nervous system and renin-angiotensin system) and ventricular remodeling. The overexpression of various biologically active molecules contributes to the progression of the disease. More attention has recently been given to subcellular remodeling, including changes in biochemical composition and molecular structure of various subcellular organelles such as the extracellular matrix, sarcolemma, sarcoplasmic reticulum, mitochondria, and energy metabolism. The changes in metabolic remodeling are the availability of the metabolic substrate and the decline in metabolic capability.[4,5]

In the normal heart, the large amounts of ATP essential to maintain contractile function and metabolism are generated mainly by mitochondrial oxidative metabolism, with a small percentage derived from glycolysis. The heart can use many different energy substrates (FFA, glucose, lactate, ketones, amino acids), but mitochondrial ATP is primarily produced by the oxidation of FFAs and pyruvate (derived from either glycolysis or lactate). Approximately 10% to 40% of ATP is produced via pyruvate oxidation, whereas the remaining 60% to 90% is derived from the oxidation of FFA. An important enzyme at the crossing point between carbohydrate oxidation and FFA metabolism is pyruvate dehydrogenase (PDH), which decarboxylates pyruvate to acetyl-CoA. PDH activity is influenced not only by glycolysis, but also by an inhibitory effect exerted through FFA oxidation.

In situations where the circulating FFA concentrations are high, the oxidation of glucose and pyruvate and the activity of PDH are decreased. Pyruvate is redirected toward lactate production and released from the heart. This produces protons, which the heart must also clear; a process that requires energy and results in redirecting ATP away from contractile function, thereby decreasing cardiac efficiency. On the other hand, decreasing plasma FFA concentrations or directly inhibiting FFA oxidation increases PDH activity and, hence, pyruvate oxidation and cardiac efficiency. A number of membrane transporters and enzymes are involved in transferring the substrates from the cytosol into the mitochondrial matrix. Particularly, the enzyme malonyl-CoA has an inhibitory effect on the enzyme carnitine palmitoyltransferase–1 (CPT1), a key physiological regulator of FFA oxidation in the heart, and acts to suppress FFA oxidation. Increases in malonyl-CoA decrease the rate of FFA oxidation and, conversely, reductions in malonyl-CoA activity will increase the rates of FFA uptake and oxidation.[5,6]

In CHF, complex metabolic changes occur. Various studies document the reversion to the fetal metabolic phenotype, consisting of a shift from FFA metabolism to glucose metabolism, which is analogous to the metabolic behavior of the fetal heart. This reversion involves the expression of some fetal genes and it is useful to improve metabolic efficiency. The main mechanisms involved are: (i) depression of genes encoding the FFA metabolism and downregulation of their transcription factors, including the peroxisome proliferator-activated receptor (PPAR) family, PPARγ coactivator-1 protein family, retinoid X-receptor-α, and CPT1; and (ii) incline of glucose metabolism, including adenosine monophosphate-activated protein kinase, glucose transporters (GLUTs), and phosphofructokinase 2.[5] Other nonfetal metabolic phenotypic changes include the hyperadrenergic states and insulin resistance, leading to an increase in FFA metabolism.

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Several studies show a decrease in tissue ATP content, an increase in adenosine diphosphate, and a decrease in the phosphorylation potential, thus impairing the kinetics for the utilization of ATP for cell contraction. In addition, heart failure impairs the capacity for the creatine kinase system to transfer mitochondrial ATP to the myofibril, and decreases mitochondrial oxidative capacity, in part because of a decrease in electron transport chain activity. The electron transport chain is usually altered in heart failure. The impairment in the electron transport chain reduces the in vivo capacity for myocardial ATP generation and limits cardiac contractile function during high-level work, such as exercise or acute adrenergic stress. Studies in animals and humans suggest increased or normal FFA oxidation in early heart failure and impaired fatty acid oxidation in severe heart failure.[5,6]

Trimetazidine effects on the expression of regulatory genes involved in myocardial remodeling

The main mechanism of action of TMZ can be attributed to the optimization of energy metabolism due to the inhibition of FFA oxidation. However, additional nonmetabolic effects are under investigation and remain to be elucidated.

The potential nonmetabolic TMZ effects on vasculature and endothelial function appear interesting *(Table I)*. TMZ effects on endothelial function have been investigated in humans and animal models.[7-9] TMZ induces an increase in nitric oxide (NO) production, and blunts endothelin-1 (ET1) release in the ischemic rat heart. The beneficial effects of TMZ in the ischemic-reperfused rat heart are mediated by NO because TMZ treatment results in an increased coronary endothelial NO synthase (eNOS) expression at both mRNA and protein levels and in a preservation of coronary microvasculature (ultrastructural damage and microvascular permeability) after ischemia. The protective effects of TMZ against ischemia-reperfusion endothelium damage is strictly related to eNOS expression and NO bioavailability, and it is abolished by inhibiting NO synthase with L-NAME; this effect is due to posttranscriptional mechanisms since the effect is not affected by the transcriptional inhibitor actinomycin-D. In patients with IHD,[7] Fragasso et al evidenced reduced plasma ET-1 levels without alterations in plasma NO.[8] Recently, short-term TMZ therapy was shown to improve the parameters related to heart rate variability and angina, reduce ET-1, and increase NO levels in patients with IHD.[9] TMZ also improved endothelin-dependent relaxation in patients with ischemic cardiomyopathy via antioxidant properties. In a model of renal ischemia-reperfusion, TMZ treatment significantly augmented glycogen synthase kinase 3-β phosphorylation and reduced levels of cytochrome c and voltage-dependent anion channel phosphorylation. It significantly improved the survival rate, attenuated cytolysis, oxidative stress, and improved renal function.[10]

A pivotal step in the evolution of chronic heart disease is the remodeling process. The main factors involved are the progressive cell loss due to apoptotic or necrotic cell death and subsequent tissue architecture rearrangement with the substitution of contractile mass with noncontractile fibrous tissue.[1] Inflammation plays an important role in the pathogenesis of ventricular remodeling. It has been demonstrated that inflammatory cytokines, including interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α), remarkably increase after myocardial infarction and are involved in the subsequent left ventricular remodeling. Studies revealed that pathophysiologically relevant concentrations of TNF-α promote progressive left ventricular dysfunction and remodeling in rats. Gene expression and serum levels of inflammatory markers, including interleukins (IL) and TNF-α, are usually detected to evaluate the extent of systemic

- Preservation of endothelial nitric oxide expression in ischemia/reperfusion
- Reduction in gene expression and serum levels of interleukin 1β, interleukin 6, and tumor necrosis factor-α
- Reduction in endothelin-1 expression and plasma levels
- Reduction in atrial natriuretic peptide mRNA levels and brain natriuretic peptide plasma levels
- Inhibition of myocardial fibrosis through the NADPH oxidase-ROS-CGF signaling pathway
- Glycogen synthase kinase 3-β phosphorylation, reduced levels of cytochrome c and voltage-dependent anion channel phosphorylation
- Direct antiapoptotic effect by regulating mRNA expression
- Modulation of glucose transporter–4 expression

Table I *Main effects of trimetazidine on the expression of regulatory genes involved in myocardial remodeling.*

inflammation. In a model of smoking-induced inflammation, TMZ significantly reduced gene expression and serum levels of IL-1β, IL-6, and TNF-α, which might be an important protective mechanism against left ventricular remodeling via attenuating oxidative stress, apoptosis, and inflammation.[11]

Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) have important physiological roles in fluid homeostasis, vascular tone, and cardiac pathology, including myocardial ischemia and left ventricular dysfunction and remodeling. BNP and the N-terminal precursor fragment (NT-proBNP) have been regarded as the biomarkers of choice when obtaining diagnostic and prognostic information in patients with acute and chronic heart failure. These peptides are commonly involved in the remodeling process via antifibrotic actions, inhibition of aldosterone secretion, and cell proliferation (smooth muscle cell, mesangial cell, fibroblasts). Studies have also assessed a possible connection between natriuretic peptide concentrations and the risk of mortality in random populations, suggesting an association between plasma concentrations and mortality, independently of other risk factors.[12,13]

In a rat model of infarct-induced heart failure, short-term TMZ treatment significantly reduced ANP mRNA levels, but did not significantly affect BNP mRNA levels or genes involved in fatty acid metabolism.[14] In long-term studies performed in patients with ischemic cardiomyopathy, TMZ's beneficial effect on BNP plasma levels were also reported.[15] These effects could have a relevant role in reducing ventricular remodeling and the decline of cardiac function in patients with IHD and heart failure.

Liu et al also reported that TMZ inhibits myocardial fibrosis through the signaling pathway involving NADPH oxidase, reactive oxygen species, and connective tissue growth factor.[16,17] Ruixing et al reported that, in a rabbit model of ischemia-reperfusion, trimetazidine also reduced cardiomyocyte apoptosis and ischemia-reperfusion injury via antioxidant properties.[18]

TMZ also exerts a direct antiapoptotic effect by regulating microRNA (miRNA) expression. These are small, noncoding RNAs that regulate gene expression and exert an antiapoptotic role protecting muscle cells from wall stresses, hypoxia, and H_2O_2-induced apoptosis.[18]

In addition to antifibrotic and antiapoptotic effects, glucose metabolism modulation has been suggested to positively affect cardiac function in heart failure and IHD. Metabolic modulation is linked to the fact that glucose is a more energy-efficient fuel than FFAs. A shift from predominant long-chain FA utilization to glucose utilization will result in an increase in ATP production per unit of oxygen utilization. Impaired glucose metabolism and insulin resistance are commonly considered as maladaptive responses involved in the progression of ventricular function decline. Insulin resistance in heart muscle was recently shown to be related to reduced GLUT4 protein content.[19] TMZ could also act, at a metabolic level, by promoting glucose oxidation, increasing insulin sensitivity and modulating GLUT4 expression. In diabetic rats after myocardial infarction, Zhang et al reported that TMZ improves left ventricular diastolic function and the remodeling process by increasing expression of GLUT4 mRNA and protein and inhibits myocardial fibrosis.[20]

Conclusions

Metabolic therapy could have a relevant role in IHD and CHF treatment. In these clinical conditions, trimetazidine exerts positive effects on ventricular function, quality of life, and prognosis. Although the main mechanism of action is the improvement in cardiac energetic efficiency, emerging reports suggest a potential role of its nonmetabolic effects, such as regulation of gene expression involved in the remodeling process. Future studies should be performed to understand these novel aspects and their clinical relevance. ■

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