Anti-inflammatory effects of trimetazidine in patients with ischemic heart disease

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Abstract
Recent studies have demonstrated that alterations in cardiac metabolism occur in ischemic heart disease and heart failure. This suggests that there is an increased utilization of non-carbohydrate substrates for energy production with a resultant reduction in the efficiency of myocardial oxygen consumption. A direct approach to modifying cardiac energy metabolism could involve altering substrate utilization. Trimetazidine, which acts by selectively inhibiting mitochondrial 3-ketoacyl-coenzyme A thiolase, (an enzyme involved in β-oxidation) is an antianginal drug that shifts the preference for energy substrate away from fatty acid metabolism and towards glucose metabolism. It has a reduces ischemia-reperfusion damage and left ventricular function by reducing cell damage, tissue inflammation and left ventricle remodeling. Recent research has demonstrated that the anti-inflammatory action of trimetazidine reduces long-term mortality in patient with ischemic cardiomyopathy.

Key words: trimetazidine, coronary artery disease, inflammation, reperfusion injury, heart metabolism.

Introduction
Ischemic heart disease remains the major cause of mortality in developed countries [1] and, since 1990, has become the most frequent cause of chronic heart failure [2]. There is no doubt that the extended use of coronary angioplasty, thrombolytic therapy and coronary artery bypass surgery has had a fundamental impact on limiting cardiovascular mortality and improving the quality of life. Angiotensin converting enzyme (ACE) inhibitors, β-blockers, nitrates, angiotensin type 1 receptor blockers, anti-platelet and lipid-decreasing agents are currently the keystones of pharmacologic management, supplemented by lifestyle changes [3]. However, side-effects of chronic drug treatment may affect compliance. This reduces the number of fully-treated patients and thus affect their quality-of-life. Despite the range therapeutic options that are available, mortality rates remain high, and many patients continue to have troublesome symptoms. An additional strategy could be to treat the metabolic causes and effects of myocardial ischemia [4].

Heart metabolism
The myocardium depends on oxygen to support high energy phosphate production by oxidative phosphorylation. This is the only metabolic process
that can generate adequate energy for the heart. When the amount of oxygen is unable to meet the requests for mitochondrial respiration, the high energy phosphates production goes down and lactate, the end-product of anaerobic glycolysis, accumulates which encourages the deleterious effects of intracellular acidosis [5]. In the normal heart, adenosine-triphosphate (ATP) is produced by the metabolism of fatty acids and carbohydrates with fatty acids contributing about 60 to 80% of ATP [6]. Fatty acid oxidation is directly related to plasma free fatty acid (FFA) oxidation, while glucose and lactate uptakes are inversely related to plasma FFA concentration. Fatty acids are not as efficient a source of myocardial energy as glucose, requiring 10% more oxygen to produce an equivalent amount of ATP [7]. The oxidation of fatty acids is regulated by:

i) the concentration of FFA in plasma,
ii) the activity of carnitine palmitoyl transferase-I (CPT-I), and
iii) the enzymes that catalyze fatty acid β-oxidation in mitochondria [8-10].

Because high levels of fatty acids inhibit glucose oxidation, a favorable approach to treating ischemic heart disease could be to stimulate glucose oxidation or to inhibit fatty acid oxidation. Unlike other pharmacological approaches there would not be any direct effects on heart rate, arterial blood pressure or coronary flow. Pharmacologic agents that inhibit fatty acid oxidation include β-oxidation inhibitors such as the 3-ketoacyl-coenzyme A thiolase (3-KAT) inhibitor trimetazidine.

Myocardial damage in ischemic heart disease: the pathophysiological relevance of inflammation

Early reperfusion has been shown to be useful to preventing cell death after coronary artery occlusion. It is generally accepted that the prompt reopening of the occluded vessel, either by mechanical (coronary angioplasty or bypass surgery) or pharmacological means (thrombolytic drugs), should be performed as soon as possible in patients with acute coronary syndromes (ACS) [11-13]. The aims of therapeutic management of ACS are plaque stabilization and reduction of reperfusion damage.

Plaque development and destabilization

Previously considered a cholesterol accretion disease, atherosclerosis is now considered to be a complex inflammatory process. When coronary endothelium encounters certain bacterial products or risk factors such as hyperlipidemia, vasoconstrictive hormones, products of glycoxidation associated with diabetes, or inflammatory cytokines derived from adipose tissue (metabolic syndrome, obesity), these cells increase the expression of adhesion molecules that endorse the adhesion of leukocytes to the inner surface of the artery [14]. Transmigration of the adherent leukocytes depends on the expression of chemoattractant cytokines regulated by signals associated with risk factors for atherosclerosis. Once resident in the arterial intima, leukocytes promote inflammation by interacting with endothelial cells and smooth muscle cells (SMCs) [14]. This results in a localised inflammatory process with cell proliferation and secretion of matrix metalloproteinases (MMPs) [15]. These proteinases modulate numerous functions of vascular cells, including activation, proliferation, migration, and cell death, together with neoangiogenesis and left ventricle and extracellular matrix remodeling. In addition to proliferation, cell death (commonly related to apoptotic processes) commonly occurs in the atherosclerotic lesions. The death of lipid-laden macrophages can lead to extracellular deposition of tissue factor (TF). The extracellular lipid that accumulates in the intima, forms the classic, lipid-rich necrotic core of the atherosclerotic plaque [16]. According to autopsy studies, rupture of the plaque’s protective fibrous cap causes coronary thrombosis and acute coronary syndromes [17-19]. Disrupted plaques provoke thrombosis in several ways. First, contact with collagen in the plaque’s extracellular matrix triggers platelet activation. Second, TF produced by macrophages and SMCs activates the coagulation cascade [20]. The disrupted plaque represents a stimulus to both thrombosis and coagulation. These pathways reinforce each other, as thrombin generation amplifies the activation of platelets and other cells in the plaque. Conversion of fibrinogen to fibrin and release of von Willebrand factor from activated platelets can provide the cross-linking molecular bridges between platelets. Finally, the occurrence of distal embolization explains in part the no-reflow phenomenon that can complicate both spontaneous and iatrogenic (coronary angioplasty, thrombolysis) plaque disruption and prevent the effective reperfusion of distal microcirculation [14]. A variety of biomarkers linked to inflammation could predict plaque destabilization and coronary events recurrence [21]. These markers include acute-phase reactants (C-reactive protein), pro- and anti-inflammatory cytokines, cell adhesion molecules, MMPs, and other markers of activation of platelets and white cells, including soluble CD40 ligand and myeloperoxidase [14]. Furthermore, data obtained from several databases support the importance of the anti-inflammatory and immune system in modulating effects of drugs normally used in patients with CAD (aspirin, statins) [22, 23].

Reperfusion injury

Reperfusion injury may affect various aspects of myocardial and endothelial function, with different and complex pathophysiological consequences.
[24, 25]. The term encompasses several events including:

a) microvascular damage,

b) reperfusion arrhythmias,

c) reversible myocardial mechanical dysfunction (stunning), and

d) cell death (due to apoptosis or necrotic processes).

Oxidative stress, intracellular calcium overload, neutrophil activation, metabolic alterations, and excessive intracellular osmotic load have all been proposed to explain the pathogenesis and the consequences of inflammatory injury in ischemic-reperfused myocardium.

1) **Oxidative stress:** The increase of reactive oxygen species during ischemia-reperfusion and the adverse effects of oxyradicals on myocardium have been well established. Although several experimental studies have demonstrated the cardioprotective effects of antioxidants, larger clinical studies have so far failed to confirm such earlier results. The importance of various endogenous antioxidants in reperfusion injury is evident from the decrease in their activity that occurs at the time of myocardial damage, and from the reduction in cardiac damage during ischemia-reperfusion that has been reported when antioxidants are administered [26].

2) **Inflammatory changes:** The inflammatory processes that characterize early and late reperfusion, are factors in the process that lead to tissue damage. Neutrophils feature prominently in the inflammatory component of post-ischemic injury. Ischemia-reperfusion prompts a release of oxygen free radicals, cytokines and other pro-inflammatory mediators that activate both the neutrophils and the coronary vascular endothelium [27, 28]. Activation of these cells promotes the expression of adhesion molecules on both neutrophils and the endothelium which recruit neutrophils on the endothelial surface and initiate a specific cascade of cell-cell interactions. Neutrophils adhere to the vascular endothelium and subsequently migrate across the endothelium, to interact directly with interstitial matrix and myocytes [29-33]. This specific series of events is a prerequisite for the full expression of reperfusion injury, including endothelial dysfunction, microvascular collapse, impairment of blood flow (no-reflow phenomenon), myocardial infarction and apoptosis [34]. Pharmacological therapies can target the different stages in this critical series of events. Effective targets for pharmacologic agents include:

a) inhibiting the release or accumulation of pro-inflammatory mediators;

b) altering neutrophil or endothelial cell activation; and

c) attenuating adhesion molecule expression on the endothelium, neutrophils and myocytes [28].

Both nitric oxide (NO) and adenosine, two fundamental regulators of coronary flow and endothelial function, exhibit a wide-range of effects against neutrophil-mediated events. These agents can therefore be used to tackle several critical points in the ischemia-reperfusion response, and offer greater benefit than agents acting at one single point in the pathogenetic cascade [35-37]. The intense inflammatory response following reperfusion has been implicated as a factor not only in the extension of tissue injury [27] but also in tissue repair. Myocardial injury initiates a cascade of cellular and humoral responses that ultimately facilitate tissue repair. The early generation of complement-derived chemotactic factors does not depend upon reperfusion, but reperfusion of the infarcted myocardium accelerates other cellular and cytokine responses, thus providing the potential for post-reperfusion injury [38].

3) **Endothelial function:** Alterations of endothelial function are pivotal in the development of reperfusion damage and the no-reflow phenomenon. Here the enhanced release or increased bioavailability of nitric oxide (NO) appears to be central. Besides its well known vasodilatory effects, NO reduces microvascular dysfunction [39], platelet adhesion and aggregation [40], and leukocyte adherence or emigration [41, 42]. NO also reacts with superoxide to form peroxynitrite, which is a strong cytotoxic agent. Because of this, the role of NO in ischemia-reperfusion damage and myocardial dysfunction remains controversial. Several investigators have reported that the administration of NO donors prevents reperfusion injury [43]. Removing NO by pharmacologically inhibiting NO synthases (NOS), or by breeding transgenic endothelial and inducible NOS (eNOS and iNOS) knockout mouse models have been shown to exacerbate reperfusion injury [42-44]. It is plausible that the biological role of eNOS and iNOS are different in ischemia-reperfusion conditions. The basal NO production in the picomolar range prevents deterioration and/or restore endothelial function in the coronary microcirculation. Conversely, the burst of NO production in the nanomolar range that occurs during reperfusion by an increase of iNOS activity promotes lipid peroxidation and oxidative cell damage [26].

4) **Metabolic changes:** A metabolic protection of the ischemic myocardium appears to be an important factor in limiting reperfusion damage [45]. Major metabolic changes occurring during the early hours of myocardial infarction include increased secretion of catecholamines and production of circulating FFA. Under normal conditions, the myocardium depends on aerobic metabolism, with FFA as the preferred energy source. During ischemia-
reperfusion, FFA levels are greatly increased, and exert a toxic effect on the myocardium. This results in increased membrane damage, endothelial dysfunction, tissue inflammation and decreased cardiac function [46]. Decreasing plasma FFA levels and cardiac fatty acid oxidation, together with stimulating glucose and lactate uptake might reduce these detrimental effects. This might be achieved by the administering glucose-insulin-potassium (GIK) solutions at the time of reperfusion [47, 48] and inhibiting fatty acid oxidation with 3-KAT inhibitors.

Trimetazidine: anti-inflammatory effects in patients with coronary artery disease

Trimetazidine is a piperazine derivative (1-[2,3,4-trimethoxybenzil] piperazine dihydrocloride) with anti-ischemic properties. Trimetazidine exerts myocardial anti-ischemic effects independent of changes in oxygen supply-to-demand ratio. In conditions of ischemia or hypoxia, trimetazidine maintains cellular functions by selectively inhibiting mitochondrial long-chain 3-KAT [49]. As a consequence, fatty acid β-oxidation is reduced and glucose utilization is stimulated. There is a consequent improvement in mitochondrial function and a reduction in calcium overload and intracellular acidosis. Trimetazidine also has antioxidant effects, protects the endothelium, preserves high-energy phosphates and has an anti-inflammatory action (Figure 1) [50].

A proinflammatory state is recognized in coronary artery disease and chronic heart failure. The degree of immune activation corresponds to disease severity and prognosis. In patients with heart failure and ischemic heart disease, greater concentrations of C-reactive protein have been related to higher rates of mortality, cardiovascular events and hospitalization rate [14, 51, 52]. In diabetics or in patients with insulin-resistance the inflammatory state is increased with a consequential worsened prognostic outlook [53].

In clinical and experimental conditions, trimetazidine reduces inflammation and improves endothelial function in acute (ischemia-reperfusion damage, coronary angioplasty, thrombolysis) and chronic conditions (ischemic cardiomyopathy, stable angina) (Figure 2). In experimental models of cardiac ischemia-

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**Trimetazidine and IHD: potential anti-inflammatory effects**

**MYOCYTES ENDOTHELIAL CELLS**

- Oxidative stress
- Mitochondrial damage
- Vascular damage
- Endothelial function
- Necrosis and Apoptosis

**MICROVASCULAR STUNNING**

- Endothelial nitric oxide preservation
- Microvascular permeability

**HIBERNATING AND STUNNED MYOCARDIUM**

- PCr/ATP ratio

**REDUCTION OF CARDiac AND SYSTEMIC INFLAMMATION**

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**Figure 1.** Potential anti-inflammatory effects of trimetazidine in patients with ischemic heart disease (IHD). Various clinical conditions (i.e. diabetes, ischemic heart disease and heart failure) are characterized by an intensive inflammation of myocyte and extracellular matrix. Trimetazidine reduces fatty acid oxidation and stimulates glucose utilization by selective inhibition of mitochondrial long-chain 3-ketoacyl coenzyme A thioloise (3-KAT). The coupling of glycolysis with glucose oxidation is improved, and production of adenosine triphosphate (ATP) is increased. The deleterious effects of acidosis and intracellular calcium overload in ischemic, hypoxic, and overstretched cells are limited or abolished. Trimetazidine exerts an anti-inflammatory effect by rapidly restoring the phosphorylation processes, preserving the phosphocreatine (PCr)/ATP ratio, protecting cardiac cells against intracellular acidosis, preventing intracellular accumulation of sodium and calcium ions, and, finally, by reducing oxidative damage. All these properties protect the myocaridal cell against necrotic and apoptotic cell death and reduce tissue inflammation and endothelial dysfunction. CoA – coenzyme A, PDH – pyruvate dehydrogenase, PL – phospholipids
Trimetazidine and reperfusion injury: endothelial protection

Potential endothelial protective effects of trimetazidine during ischemia-reperfusion. During ischemia and reperfusion the endothelial cell is early damaged; a morphological (cell swelling) and functional deterioration occurs. Apoptosis processes are responsible for the death of numerous cells damaged during ischemia. Microvascular permeability increases determining interstitial edema and alteration in coronary microcirculation (no-reflow phenomenon). There alterations are partly reversible (microvascular stunning). Trimetazidine exerts a direct effect of endothelial function by increasing endothelial nitric oxide production and availability and by reducing endothelin-1 release. M – mitochondrium, C – coronary artery, EC – endothelial cell, NO – nitric oxide, eNOS – endothelial nitric oxide

reperfusion, trimetazidine reduces neutrophil accumulation in reperfused myocardium [54]. Tritto et al. [55] reported that trimetazidine inhibits neutrophil activation in vitro and reduced cardiac oxygen radical production at reflow, independent of direct scavenger effects. Thus, trimetazidine can protect post-ischemic hearts from neutrophil-mediated injury. Recently, in ischemic-reperfused rat hearts, we demonstrated that trimetazidine reduced cellular damage and preserved endothelial function and the expression of eNOS [56]. This effect could partially explain the anti-inflammatory effects of the drug. In a rabbit model of ischemia-reperfusion, Ruixing et al. reported that trimetazidine also prevented cardiomyocyte apoptosis and ischemia-reperfusion injury via its’ antioxidant properties [57].

These anti-inflammatory effects are also evident in patients with long-standing ischemic cardiomyopathy. In these patients long-term trimetazidine treatment reduces the systemic inflammation as evaluated by plasma C-reactive protein [58] determination. It has been also observed that trimetazidine is able to reduce the release of endothelin-1 in patients with ischemic cardiomyopathy and heart failure [59]. Growth factors, vasoactive substances, and mechanical stress result in increased levels of endothelin-1. Despite the recognized adaptive advantage of endothelin-1 in supporting the contractility of the failing heart, persistent increases in its expression in the failing heart are associated with an increased severity of myocardial dysfunction [60]. The preservation of eNOS production and its bioavailability appears to be a critical factor in the decrease inendothelin-1 release and the preservation of endothelial function. Recently, Belardinelli et al. reported that trimetazidine improved endothelium-dependent relaxation in patients with ischaemic cardiomyopathy. This effect was associated with antioxidant properties as measured by a reduction in plasma malondialdehyde and lipid hydroperoxide levels [61]. Also, Monti et al. [62] reported that trimetazidine had significant metabolic and endothelial protective effects in forearm skeletal muscle in diabetic patients with ischemic cardiomyopathy.

Kuralay et al., reported that trimetazidine suppressed inflammatory markers (tumor necrosis factor-α, NO products, C-reactive protein) before and after percutaneous transluminal coronary angioplasty (PTCA). This anti-inflammatory effect was also associated with an improvement of global and regional wall motion after PTCA [63]. Administration of trimetazidine limited the harmful effects of reperfusion and protected myocytes and endothelial cells by optimizing their metabolism during the PTCA procedures. Trimetazidine maintains the integrity of cell membranes as well as mitochondrial structure and ensures the protection of myocardial cells that are at risk. Furthermore, it is known that myocardial cells exposed to chronic reduction in blood flow have structural and metabolic alterations (hibernation). More specifically, ATP resynthesis is reduced, glycogen accumulates, and a loss of contractile function takes place. Therefore it is possible that trimetazidine helps chronic post-ischemic stunned cells to normalize their
metabolism and function [63]. It is also possible that hibernating myocardial cells improve their energy metabolism after trimetazidine administration because of a more efficient utilization of glucose with reduced oxygen availability [64].

Similar results were also obtained in patients during coronary artery bypass surgery where pre-treatment with trimetazidine alleviated malondialdehyde production and preserved endogenous antioxidant capacity [65].

The question as to whether the anti-inflammatory effects of trimetazidine improve prognosis is still under investigation. In patients with ischemic left ventricular dysfunction and multivessel coronary artery disease, El-Kady et al. [66] reported that survival at 2 years was 92% among patients treated with trimetazidine and 62% among those treated with placebo. In a recent post-hoc analysis obtained from the Villa Pini d’Abruzzo trimetazidine trial, we reported that trimetazidine treatment could reduce all-cause mortality (17% compared with 39% in controls) and hospitalization (47% vs. controls) [67].

In conclusions, recent findings have demonstrated that trimetazidine exerts a significant anti-inflammatory effect in patients with ischemic heart disease. This effect, related to the metabolic, anti-oxidative, and endothelial protective effects of the drug, probably improve prognosis and quality of life.

References
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