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

Pericle Di Napoli[1], Antonio Barsotti[2]

[1]Centre for Study and Treatment of Congestive Heart Failure,
 Department of Cardiology Villa Pini d'Abruzzo Clinic, Chieti, Italy
[2]Department of Internal Medicine (DiMI), Cardiology Division, University of Genoa,
 Genoa, Italy

Corresponding author:
Dr. Pericle Di Napoli
Centro per lo Studio ed il
Trattamento dello Scompenso
Cardiaco
Dipartimento di Cardiologia
Casa di Cura Villa Pini d'Abruzzo
66100 Chieti, Italy
Phone: +39 0871 3430
Fax: + 39 0871 331 515
E-mail: dinapoli@unich.it

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, thrombolitic 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 keytstones 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 encourges 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 palmitolyl 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 (thrombolitic 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 metallopro-teinases (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 i 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 administrating 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-tri-methoxybenzil] piperazine dihydrocloride) with anti-ischemic properties. Trimetazidine exerts myocardial anti-ischemic effects independent ofchanges 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 anda 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, trimeta-zidine 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-
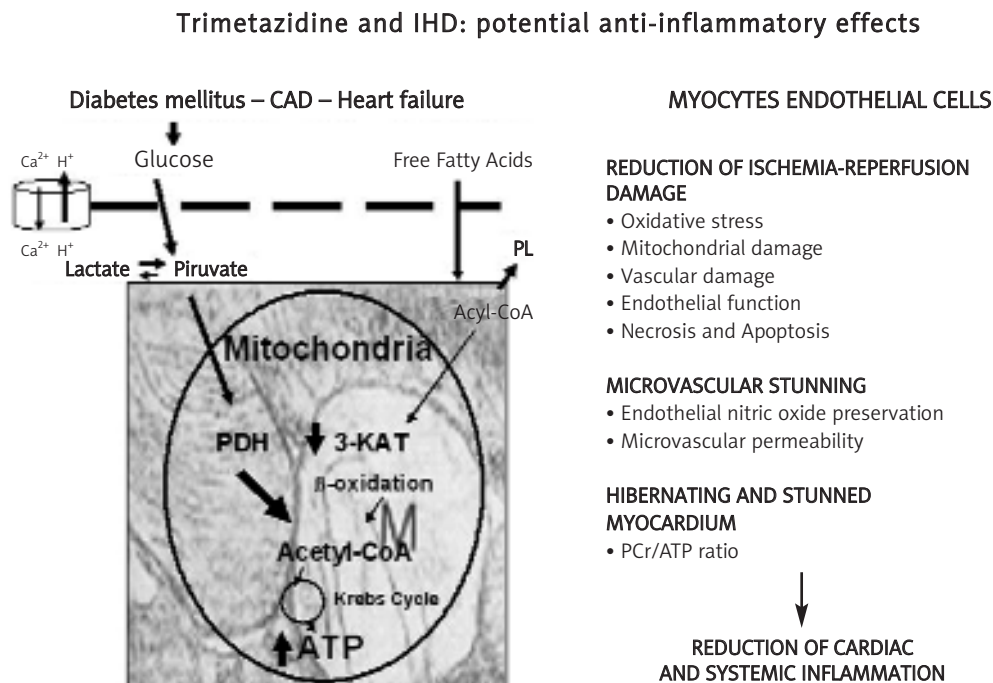
Trimetazidine and IHD: potential anti-inflammatory effects

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 thiolase (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 myocardial cell against necrotic and apoptotic cell death and reduce tissue inflammation and endothelial dysfunction. CoA – coenzyme A, PDH – pyruvate dehydrogenase, PL – phospholipids

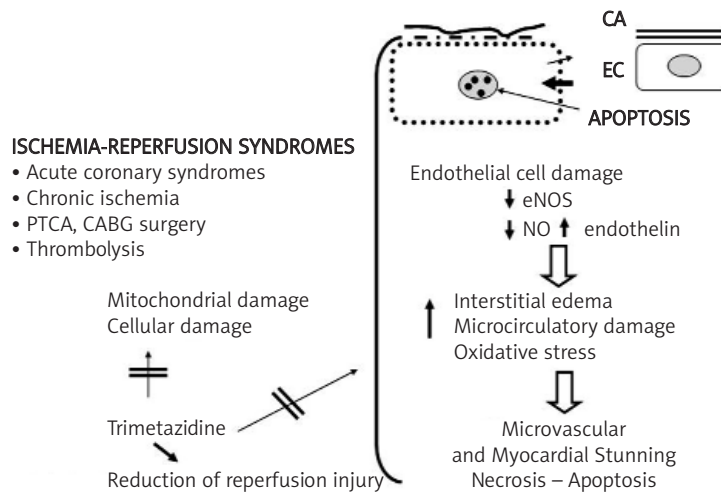## Trimetazidine and reperfusion injury: endothelial protection

Figure 2. 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 trimedazidine had significant metabolic and endothelial protective effects in forearm skeletal muscle in diabetic patients with ischemic cardio-myopathy.

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 patient 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

1. Morrow A, Gersh J, Braunwald E. Chronic coronary heart disease. In: Zipes D, Libby P, Bonow R, Braunwald E (eds). Braunwald's Heart disease – a textbook of cardiovascular medicine. 7th ed. Philadelphia, Elsevier Saunders; 2005. p. 3-118.
2. Felker GM, Shaw LK, O'Connor CM. A standardized definition of ischemic cardiomyopathy for use in clinical research. J Am Coll Cardiol 2002; 39: 210-8.
3. Fox K, Garcia MA, Ardissino D, et al. Guidelines on the management of stable angina pectoris: executive summary: the Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. Eur Heart J 2006; 27: 1341-81.
4. Lee L, Horowitz J, Frenneaux M. Metabolic manipulation in ischemic heart disease, a novel approach to treatment. Eur Heart J 2004; 25: 634-41.
5. Abozguia K, Clarke K, Lee L, Frenneaux M. Modification of myocardial substrate use as a therapy for heart failure. Nature Clin Pract Cardiovasc Med 2006; 3: 490-8.
6. Lopaschuk GD, Belke DD, Gamble J, Itoi T, Schönekess BO. Regulation of fatty acid oxidation in the mammalian heart in health and disease. Biochim Biophys Acta 1994; 1213: 263-76.
7. Wisneski JA, Gertz EW, Neese RA, Mayr M. Myocardial metabolism of free fatty acids. Studies with 14C-labeled substrates in humans. J Clin Invest 1987; 79: 359-66.
8. Wisneski JA, Gertz EW, Neese RA, Gruenke J, Morris DL, Craig JC. Metabolic fate of extracted glucose in normal human myocardium. J Clin Invest 1985; 76: 1819-27.
9. Lopaschuk GD, Stanley WC. Glucose metabolism in the ischemic heart. Circulation 1997; 95: 313-5.
10. Sabbah HN, Stanley WC. Metabolic therapy for heart disease: impact of trimetazidine. Heart Fail Rev 2005; 10: 281-8.
11. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? J Clin Invest 1985; 76: 1713-9.
12. Boyle MP, Weisman HF. Limitation of infarct expansion and ventricular remodeling by late reperfusion. Circulation 1993; 88: 2872-2883.
13. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med 2007; 357: 1121-35.
14. Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation 2005; 111: 3481-8.
15. Libby P, Lee RT. Matrix matters. Circulation 2000; 102: 1874-6.
16. Geng YL, Libby P. Progression of atheroma: a struggle between death and procreation. Arterioscler Thromb Vasc Biol 2002; 22: 1370-80.
17. Davies MJ. Stability and instability: the two faces of coronary atherosclerosis: the Paul Dudley White Lecture 1995. Circulation 1996; 94: 2013-20.
18. Falk E, Shah P, Fuster V. Coronary plaque disruption. Circulation 1995; 92: 657-71.
19. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the unstable plaque. Prog Cardiovasc Dis 2002; 44: 349-56.
20. Toschi V, Gallo R, Lettino M, et al. Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. Circulation 1997; 95: 594-9.
21. Ridker PM, Morrow DA. C-reactive protein, inflammation, and coronary risk. Cardiol Clin 2003; 21: 315-25.
22. Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001; 344: 1959-65.
23. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336: 973-9.
24. Kloner RA, Ellis SG, Lange R, Braunwald E. Studies of experimental coronary artery reperfusion. Effects on infarct size, myocardial function, biochemistry, ultrastructure and microvascular damage. Circulation 1983; 68: I8-15.
25. Tsao PS, Aoki N, Lefer DJ, Johnson G 3rd, Lefer AM. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. Circulation 1990; 82: 1402-12.
26. Barsotti A, Di Napoli P. Trimetazidine and cardioprotection during ischemia-reperfusion. Ital Heart J 2004; 5 (Suppl 2): 29S-36S.
27. Hansen PR. Inflammatory alterations in the myocardial microcirculation. J Mol Cell Cardiol 1998; 30: 2555-9.
28. Jordan JE, Zhao ZQ, Vinten-Johansen J. The role of neutrophils in myocardial ischemia-reperfusion injury. Cardiovasc Res 1999; 43: 860-78.
29. Engler RL, Schmid-Schönbein GW, Pavelec RS. Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. Am J Pathol 1983; 111: 98-111.
30. Engler RL, Dahlgren MD, Morris D, Peterson MA, Schmid-Schönbein GW. Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. Am J Physiol 1986; 251: H314-22.
31. Kukielka GL, Hawkins HK, Michael L, Manning AM. Regulation of intracellular adhesion molecule-1 (ICAM-1) in ischemic and reperfused canine myocardium. J Clin Invest 1993; 92: 1504-16.
32. Davenpeck KL, Gauthier TW, Albertine KH, Lefer AM. Role of P-selectin in microvascular leukocyte-endothelial interaction in splanchnic ischemia-reperfusion. Am J Physiol 1994; 267: H622-30.
33. Moore KL, Patel KD, Bruehl RE i wsp. P-selectin glycoprotein

ligand-1 mediates rolling of human neutrophils on P-selectin. J Cell Biol 1995; 128: 661-71.

34. Ambrosio G, Tritto I. Reperfusion injury: experimental evidence and clinical implications. Am Heart J 1999; 138: S69-75.

35. Cronstein BN, Levin RI, Belanoff J, Weissmann G, Hirschhorn R. Adenosine. an endogenous inhibitor of neutrophil-mediated injury to endothelial cells. J Clin Invest 1986; 78: 760-70.

36. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991; 88: 4651-5.

37. Lefer AM. Attenuation of myocardial ischemia-reperfusion injury with nitric oxide replacement therapy. Ann Thorac Surg 1995; 60: 847-51.

38. Frangogiannis NG, Youker KA, Rossen RD, Gwechenberger M, Lindsey MH, Mendoza LH, Michael LH, Ballantyne CM, Smith CW, Entman ML. Cytokines and the microcirculation in ischemia and reperfusion. J Mol Cell Cardiol 1998; 30: 2567-76.

39. Kubes P, Granger DN. Nitric oxide modulates microvascular permeability. Am J Physiol 1992; 262: H611-5.

40. Noel AA, Fallek SR, Hobson RW 2nd, Duran WN. Inhibition of nitric oxide synthase attenuates primed microvascular permeability in the in vivo microcirculation. J Vasc Surg 1995; 22: 661-70.

41. Radomski MW, Palmer RM, Moncada S. Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. Br J Pharmacol 1987; 92: 181-7.

42. Conger JD, Weil JV. Abnormal vascular function following ischemia-reperfusion injury. J Investig Med 1995; 43: 431-42.

43. Ronson RS, Nakamura M, Vinten-Johansen J. The cardiovascular effects and implications of peroxynitrite. Cardiovasc Res 1999; 44: 47-59.

44. Weyrich AS, Ma XL, Lefer AM. The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. Circulation 1992; 86: 279-88.

45. Lopaschuk GD, Saddik M. The relative contribution of glucose and fatty acids to ATP production in hearts reperfused following ischemia. Mol Cell Biochem 1992; 116: 111-6.

46. Oliver MF, Opie LH. Effects of glucose and fatty acids on myocardial ischemia and arrhythmias. Lancet 1994; 343: 155-8.

47. Sodi-Pallares D, Testelli MR, Fishleder BL, et al. Effects of intravenous infusion of a potassium-glucose-insulin solution on the electrocardiographic signs of myocardial infarction. A preliminary clinical report. Am J Cardiol 1962; 9: 166-81.

48. Díaz R, Paolasso EA, Piegas LS, et al. Metabolic modulation of acute myocardial infarction. The ECLA (Estudios Cardiológicos Latinoamérica) Collaborative Group. Circulation 1998; 98: 2227-34.

49. Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long chain 3-ketoacyl coenzyme A thiolase. Circ Res 2000; 86: 580-8.

50. Di Napoli P, Di Giovanni P, Gaeta MA, D'Apolito G, Barsotti A. Beneficial effects of trimetazidine treatment on exercise tolerance and B-type natriuretic peptide and troponin T plasma levels in patients with stable ischemic cardiomyopathy. Am Heart J 2007: 154: 602.e1-e5.

51. Berton G, Cordiano R, Palmieri R, Pianca S, Pagliara V, Palatini P. C-reactive protein in acute myocardial infarction: association with heart failure. Am Heart J 2003; 145: 1094-101.

52. Alonso-Martínez JL, Llorente-Diez B, Echegaray-Agara M, Olaz-Preciado F, Urbieta-Echezarreta M, González-Arencibia C. C-reactive protein as a predictor of improvement and readmission in heart failure. Eur J Heart Fail 2002; 4: 331-6.

53. Ashrafian H, Frenneaux MP, Opie LH. Metabolic mechanisms in heart failure. Circulation 2007; 116: 434-48.

54. Williams FM, Tanda K, Kus M, Willians TJ. Trimetazidine inhibits neutrophil accumulation after myocardial ischemia and reperfusion in rabbits. J Cardiovasc Pharmacol 1993; 22: 828-33.

55. Tritto I, Wang P, Kuppusamy P, Giraldez R, Zweier JL, Ambrosio G. The anti-anginal drug trimetazidine reduces neutrophil-mediated cardiac reperfusion injury. J Cardiovasc Pharmacol 2005; 46: 89-98.

56. Di Napoli P, Chierchia S, Taccardi AA, Grilli A, Felaco M, De Caterina R, Barsotti A. Trimetazidine improves post-ischemic recovery by preserving endothelial nitric oxide synthase expression in isolated working rat hearts. Nitric Oxide 2007; 16: 228-36.

57. Ruixing Y, Wenwu L, Al-Ghazali R. Trimetazidine inhibits cardiomyocyte apoptosis in a rabbit model of ischemia-reperfusion. Transl Res 2007; 149: 152-60.

58. Di Napoli P, Taccardi AA, Barsotti A. Long-term cardioprotective action of trimetazidine and potential effect on the inflammatory process in patients with ischaemic dilated cardiomyopathy. Heart 2005; 91: 161-5.

59. Fragasso G, Piatti Md PM, Monti L, et al. Short- and long-term beneficial effects of trimetazidine in patients with diabetes and ischemic cardiomyopathy. Am Heart J 2003; 146: e18-22.

60. Fragasso G. Inhibition of free fatty acids metabolism as a therapeutic target in patients with heart failure. Int J Clin Pract 2007; 61: 603-10.

61. Belardinelli R, Solenghi M, Volpe L, Purcaro A. Trimetazidine improves endothelial dysfunction in chronic heart failure: an antioxidant effect. Eur Heart J 2007; 28: 1102-8.

62. Monti LD, Setola E, Fragasso G, et al. Metabolic and endothelial effects of trimetazidine on forearm skeletal muscle in patients with type 2 diabetes and ischemic cardiomyopathy. Am J Physiol Endocrinol Metab 2006; 290: E54-9.

63. Kuralay F, Altekin E, Yazlar AS, Onvural B, Goldeli O. Suppression of angioplasty-related inflammation by pre-procedural treatment with trimetazidine. Tohoku J Exp Med 2006; 208: 203-12.

64. Belardinelli R, Purcaro A. Effects of trimetazidine on the contractile response of chronically dysfunctional myocardium to low-dose dobutamine in ischaemic cardiomyopathy. Eur Heart J 2001; 22: 2164-70.

65. Iskesen I, Saribulbul O, Cerrahoglu M, Var A, Nazli Y, Sirin H. Trimetazidine reduces oxidative stress in cardiac surgery. Circ J 2006; 70: 1169-73.

66. El-Kady T, El-Sabban K, Gabaly M, Sabry A, Abdel-Hady S. Effects of trimetazidine on myocardial perfusion and the contractile response of chronically dysfunctional myocardium in ischemic cardiomyopathy: a 24-month study. Am J Cardiovasc Drugs 2005; 5: 271-8.

67. Di Napoli P, Di Giovanni P, Gaeta MA, Taccardi AA, Barsotti A. Trimetazidine and reduction in mortality and hospitalization in patients with ischemic dilated cardiomyopathy: a post hoc analysis of the Villa Pini D'Abruzzo Trimetazidine Trial. J Cardiovasc Pharmacol 2007 (in press).