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Metabolic modulation and cellular therapy of cardiac dysfunction and failure

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Abstract

At present the prevalence of heart failure rises along with aging of the population. Current heart failure therapeutic options are directed towards disease prevention *via* neurohormonal antagonism (β-blockers, angiotensin converting enzyme inhibitors and/or angiotensin receptor blockers and aldosterone antagonists), symptomatic treatment with diuretics and digitalis and use of biventricular pacing and defibrillators in a special subset of patients. Despite these therapies and device interventions heart failure remains a progressive disease with high mortality and morbidity rates. The number of patients who survive to develop advanced heart failure is increasing. These patients require new therapeutic strategies. In this review two of emerging therapies in the treatment of heart failure are discussed: metabolic modulation and cellular therapy. Metabolic modulation aims to optimize the myocardial energy utilization *via* shifting the substrate utilization from free fatty acids to glucose. Cellular therapy on the other hand has the goal to achieve true cardiac regeneration. We review the experimental data that support these strategies as well as the available pharmacological agents for metabolic modulation and clinical application of cellular therapy.

Keywords: heart failure • metabolic modulators • trimetazidine • perhexiline • etomoxir • stem cells • cardiac stem cells • endothelial progenitor cells • haematopoietic stem cells • cardiac regeneration

Introduction

We are currently witnessing an increase in prevalence of heart failure as result of population aging and improvement in the therapy of cardiovascular diseases. Heart failure affects 4–5 million people in the United States and its prevalence has a direct relationship to age, ranging from 0.4–2% in the adult population and rising to 5–10% in patients aged more than 65 years [1, 2]. The incidence of heart failure is increasing, as reflected by the statistics of hospital admissions and visits to clinics. The prognosis of heart failure is still poor with mortality similar or even higher than with many common types of cancer, 5-year mortality being 56% for men and 45% for women [3–7].

At present the heart failure dynamic is viewed from the perspective of evolution from subclinical pathological changes towards clinical syndromes. Thus four stages have been identified

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in the American College of Cardiology/American Heart Association guidelines [8]:

Stage A – includes patients who have increased risk for developing heart failure but without structural heart disease or symptoms of heart failure;
Stage B – includes patients who have structural heart disease but without symptoms of heart failure;
Stage C – includes patients who have structural heart disease and symptoms of heart failure;
Stage D – includes patients with refractory heart failure requiring special interventions (*i.e.* transplantation, left ventricular assist device).

Using treatments (β-blockers, angiotensin converting enzyme inhibitor, aldosterone antagonists, internal cardioverter defibrillator) that do not cure but alter the natural history of the disease, we are facing a situation where more patients are surviving to a stage of advanced chronic heart failure (Stage D, above). This condition is defined as a 'state in which patients have significant cardiac dysfunction with marked symptoms of dyspnea, fatigue or symptoms relating to end-organ hypoperfusion at rest or with minimal exertion despite maximal medical therapy' [9]. This definition underscores the existence of a group of patients with poor prognosis, increased risk for clinical events, and most importantly, compromised quality of life despite available treatment. These patients are in desperate need of new effective therapeutic options and should be included in future research initiatives.

In this article we will review two out of many newly emerging strategies in cardiovascular therapy – metabolic modulation and cellular therapy.

Metabolic modulation

A renewed interest in heart failure metabolism has arisen mainly as a result of newly emerging therapies that hold great promise. Combining old theories with new discoveries in myocardial enegetics is challenging because there exist a multitude of data that often appear contradictory. In order to more clearly summarize the current understanding of metabolism in the failing heart, normal cardiac metabolism should first be reviewed.

Metabolism in the normal heart

The metabolism in the cardiomyocyte can be divided into several steps: (*i*) substrate uptake and oxidation; (*ii*) oxidative phosphorylation and adenosine triphosphate (ATP) production and (*iii*) ATP transfer and utilization (Fig. 1).

Substrate uptake and oxidation

In a healthy heart 10–40% of acetyl-coenzyme A (acetyl-CoA) comes from oxidation of pyruvate that is the end product of aerobic glycolysis [10]. Intracellular glucose comes from two sources: extracellular glucose and intracellular glycogen stores. Glucose transport across the sarcolemma is controlled by the transmembrane gradient and by the number of glucose transporters (mainly GLUT-4) present. Insulin and ischemic states stimulate translocation of GLUT-4 into the sarcolema increasing the rate of glucose uptake [11, 12]. Another potent stimulus for GLUT-4 translocation especially during ischemic conditions is activation of adenosine monophosphate activated protein kinase, which regulates the so-called insulin-independent ischemia-induced glucose uptake [13, 14]. Once intracellular, glucose enters the glycolytic pathway that is present in the cytosol in close proximity to the mitochondria. The primary regulator of the glycolytic process is the rate of fatty acid oxidation. High free fatty acid oxidation is translated into an increase in mitochondrial acetyl-CoA/free CoA and NADH/NAD (reduced form of nicotine amide dinucleotide / nicotinamide adenine dinucleotide) ratios that activate the pyruvate dehydrogenase kinase leading to phosphorylation and inactivation of pyruvate dehydrogenase (PDH), which controls the glycolytic pathway. The end product of glycolysis is pyruvate, which can have three fates: (*i*) decarboxylation to acetyl-CoA which enters the Krebs cycle; (*ii*) 'anaplerotic' reaction to maintain the pool of Krebs cycle intermediates by carboxylation to oxaloacetate and malate and (*iii*) conversion to lactate. The myocardium produces excess lactate only in settings of impaired oxidation of pyruvate, as occurs in ischemia, otherwise the healthy heart consumes lactate even under conditions of maximal cardiac work by converting it to pyruvate [15–17].

The rate of fatty acid uptake on the other hand is directly proportional to the concentration in plasma [18, 19]. Plasma fatty acid concentration is determined by the activity of hormone-sensitive lipase in adipocytes that releases them from triglycerides. In plasma, fatty acids are bound to albumin, triglycerides or are transported within chylomicrons or very low density lipoprotein. The hormone-sensitive lipase is activated by catecholamines and inhibited by insulin, thus assuring that during the hyperadrenergic state of fasting, the high level of fatty acids available will cover the energetic needs of the cells. At the level of the cardiomyocyte the fatty acids are released from chylomicrons and very low density lipoprotein by lipoprotein lipase [20, 21]. Fatty acids are transported into the cytosol by either passive diffusion or by protein mediated transport performed by fatty acid translocase or plasma membrane fatty acid binding protein [22, 23]. In cytosol, fatty acids are esterified to long chain fatty acyl-CoA that eventually has two fates: (*i*) esterification to triglycerides and storage or (*ii*) conversion to long-chain fatty acylcarnitine by carnitine palmitoyltransferase I (CPT-I) and transportation into mitochondria for oxidation. Also in the cytosol, long chain fatty acids bind to nuclear receptor transcription factors known as peroxisome proliferator-activated receptors (PPAR) [24]. The PPAR family in heart is primarily represented by PPAR-α that controls the expression of enzymes directly involved in fatty acid oxidation. PPAR-α first forms a heterodimer with the retinoid X receptor and PPAR-γ coactivator-1 (PGC-1) and then binds to specific response elements (peroxisome proliferator response element) within promoter regions

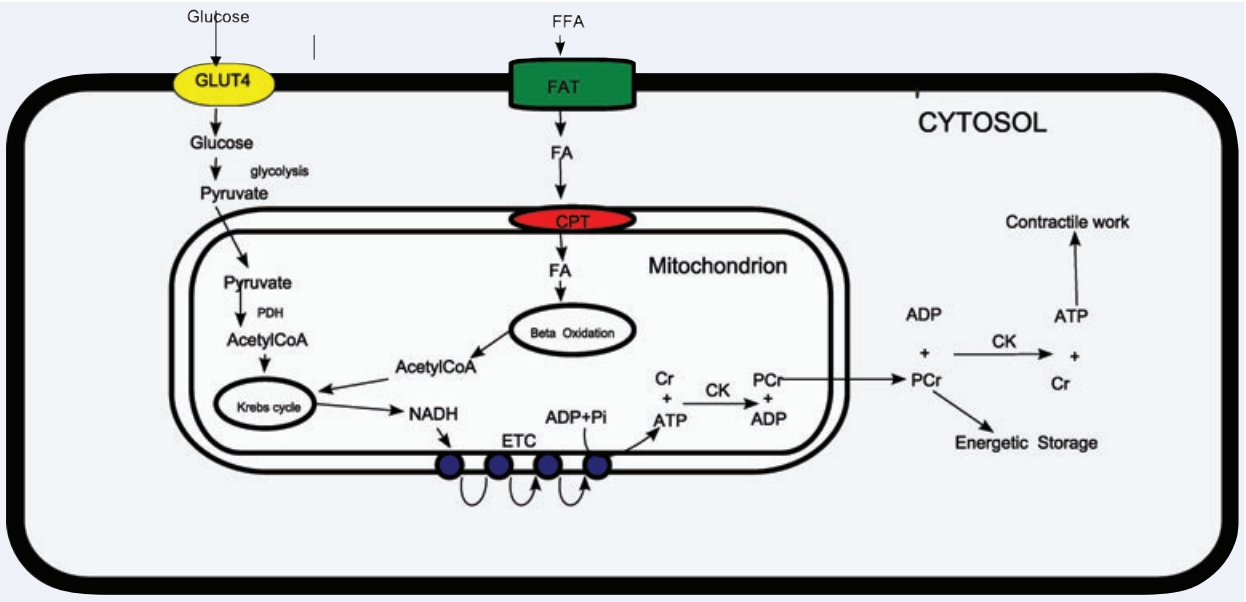

Fig. 1 Basic steps of cardiac metabolism. GLUT4 = glucose transporter; FAT = fatty acid transporter; FA = fatty acid; PDH = pyruvate dehydrogenase; CPT = carnitine palmitoyltransferase; Pi = inorganic phosphate; Cr = creatine; PCr = phosphocreatine; ETC = electron transport chain and CK = creatine kinase.

of genes encoding metabolic enzymes, increasing the rate of transcription of fatty acid oxidation genes and pyruvate kinase-4, thus promoting inhibition of PDH and glycolysis [25–27].

Oxidation of fatty acids occurs in mitochondria. The fatty acids are transported across the impermeable mitochondrial membrane by carnitine-dependent transport system [19]. This system is controlled by the CPT-I. CPT-I is inhibited by the key regulator of fatty acid oxidation, malonyl CoA, which is formed as result of acetyl-CoA carboxylation by acetyl-CoA carboxylase [28, 29]. Once in mitochondria, fatty acids are oxidized by repeated cleavage of acetyl-CoA units producing NADH and reduced form of flavin adenine dinucleotide. The final step of β-oxidation is catalysed by 3-ketoacyl CoA thiolase, which provides one acetyl-CoA for another round of β-oxidation and second one to enter the Krebs cycle.

Oxidative phosphorylation

Acetyl-CoA, the common end product of β-oxidation, and pyruvate dehydrogenation enters the Krebs cycle (in mitochondria), that generates NADH and CO_2. NADH enters the electron transport chain (ETC) in the inner membrane of mitochondria. The ETC complexes I–IV transfer electrons from NADH to oxygen and create a proton electrochemical gradient across the inner mitochondrial membrane [30]. The created gradient activates ATP synthase that produces ATP.

ATP transport and utilization

ATP transport from mitochondria to cytosol is performed by the phosphocreatine shuttle [31]. The mitochondrial creatine kinase facilitates the transfer of the high energy phosphate bonds from ATP to creatine forming phosphocreatine and adenosine diphosphate. Phosphocreatine being smaller than ATP diffuses towards myofibrils where myofibrilar creatine kinase transforms it back to ATP and creatine. Phosphocreatine forms the energetic reserve of the cardiomyocyte. The phosphocreatine level decreases when the energetic demand outweighs the supply.

Metabolism in heart failure

Once heart failure develops major metabolic derangements occur (Table 1). The data available today regarding metabolism in heart failure are enormous and conflicting. One concept is brought to attention through this multitude of studies – the failed heart is an 'engine out of fuel'. This old concept is reinvestigated again and again especially in the view of evidence that medications that influence metabolism show clinical benefits whereas agents that increase metabolic demands (*i.e.* positive inotropic agents) failed to show such benefits.

During evolution, nature endowed the heart with the ability to extract energy from any carbon substrate. At various stages of human development the myocardial metabolic phenotype is different and depends on the general body metabolic milieu and haemodynamic conditions [32]. It is known that during foetal and immediate newborn stages the primary substrate for energy production is glucose as well as lactate [33, 34]. This condition changes to favour fatty acid oxidation within days after birth [35]. Of interest, the volume-overloaded newborn heart has lower expression of enzymes involved in the regulation of fatty acid metabolism suggesting stagnation in the 'foetal state' [36]. In the

Table 1 Major metabolic changes in heart failure

(1) Early stages
Increased levels of free fatty acid
Normal or increased rate of fatty acid oxidation
Local insulin resistance
Uncoupled oxidative phosphorylation
Decreased phosphocreatine levels
Normal ATP level
(2) Late stages
Decreased utilization of fatty acid
Switch to glucose utilization (foetal shift)
Decreased phosphocreatine and ATP levels

mature heart, 60–70% of acetyl-CoA is derived from fatty acid oxidation and only 10–40% is produced from pyruvate [37, 38]. Other minor substrates for myocardium in normal conditions that become increasingly important during starvation or with poorly controlled diabetes are ketone bodies. In the ketotic state myocardial fatty acid and glucose uptake and oxidation are inhibited through poorly clarified mechanisms [18, 39].

Changes that affect cardiac metabolism in heart failure involve all steps in the process:

Changes in substrate utilization

The results of the studies on substrate utilization in heart failure are diverse. The majority supports the concept that in early heart failure there is a normal or slightly increased rate of fatty acid oxidation with down-regulation and actually switch to glucose utilization during late stages of heart failure [32, 39, 40]. When during the course of heart failure this switch occurs, is not completely established. Studies with the canine microembolization model or canine rapid pacing model of heart failure suggest that the changes in substrate utilization are late phenomena [41, 42].

Heart failure creates a hyperadrenergic state that favours an increased plasma level of fatty acids. The abundance of fatty acids creates a state of local insulin resistance by activating protein kinase C-β that phosphorylates the insulin receptor making it inactive [43]. Insulin resistance appears to promote the development of heart failure or it can be a result of heart failure as suggested by a study of canine model of cardiomyopathy that developed myocardial insulin resistance [44–47]. The role of insulin resistance in heart failure pathogenesis requires more attention, especially in the setting of available data that show improvement of heart failure in patients treated with glucagon-like peptide-1 infusion [48]. Also the role of diuretic-induced insulin resistance should be more thoroughly investigated given present evidence of increased mortality associated with chronic diuretic use among patients with heart failure [49, 50].

The state of increased fatty acids and impaired glucose utilization due to induced insulin resistance favours utilization of fatty acids as the primary energy source. During fatty acid oxidation a greater amount of oxygen is used for a given amount of ATP produced than during glycolysis. In association with induced uncoupling of oxidative phosphorylation, this shift creates a perpetual metabolic inefficiency in heart failure [51, 52].

Effects on oxidative phosphorylation

Metabolic inefficiency in heart failure becomes manifest as consumption of a greater amount of oxygen with no translation into greater cardiac work due to increased production of heat rather than ATP. In a failing heart the mitochondria are unable to efficiently perform their major task of oxidative phophorylation due to disruption of the membrane, defective activity of ETC complexes and low capacity of oxidative phosphorilation [53, 54].

Defective structure of mitochondria in heart failure was reported in canine as well as in human hearts [53, 55]. The degree of injury manifested as matrix depletion and membrane disruption appears to have a direct relationship with the levels of plasma norepinephrine and the severity of left ventricular dysfunction [53].

The data concerning defective ETC complexes in heart failure are conflicting with regard to activity of the specific complex affected or whether there is a general dysfunction of ETC rather than one attributable to a single complex. Measurements of respiratory complex activity in heart failure patients showed decreased activity at the level of complexes I, III and IV [53, 56]. In the canine rapid pacing model of heart failure a decreased level of activity was found in complexes III, IV and I [57, 58]. In animals models the defects in ETC complexes III, IV were shown to correlate linearly with the concentration of TNF-α in serum and administration of anti- tumor necrosis factor-α agent partially prevented the defects [32].

The existing evidence brings to light the concept that in heart failure a significant defect is created at the level of ETC.

The low capacity of oxidative phosphorylation in heart failure is multifactorial, but is clearly related to the substrate that is predominantly used in this deficient state. The mechanical power of the heart is less at a given rate of oxygen consumption when fatty acids are oxidized rather than glucose [59]. The mechanisms behind this process are unclear but several findings suggest a partial explanation. It is becoming clear that in heart failure the differential expression of enzymes of the fatty acid oxidation pathway is higher relative to the ones for glycolysis [60]. A potential role in this is attributed to nuclear-receptor transcription factors, of which the most studied is the PPAR family. As mentioned previously, PPAR-α activates transcription of genes encoding enzymes for β-oxidation of fatty acids. PPAR's role in the pathogenetic process of heart failure is not completely understood. It may facilitate switching of substrate utilization. In the rabbit model of volume overload hypertrophic heart failure there was no down-regulation in PPAR-α protein expression or in the expression of enzymes of fatty acid oxidation, but the uptake and oxidation of fatty acids was increased [61]. In a rat infarction model of heart failure a significant decrease in mRNA expression of PPAR-α was

found with simultaneous decrease in fatty acid oxidation enzymes [62]. It is likely that PPAR-α is down-regulated in late stages of heart failure and in hypertrophied hearts when a switch to glucose metabolism occurs [63, 64]. PPAR-α was shown to regulate the expression of uncoupling protein-3 (UCP-3) and mitochondrial thioesterase-1 and therefore could have a role in regulation of extrusion of fatty acyl-CoA from mitochondria [12, 65, 66]. Extrusion of fatty acyl-CoA from mitochondria is one of the proposed mechanisms for ATP wastage by fatty acid oxidation. The high content of intramitochondrial fatty acyl-CoA would result in higher production of free fatty acids by thioesterase-1. These negatively charged free fatty acids are translocated by UCP-3 to the intermembranous space of the mitochondria where they can associate with a proton [67, 68]. This neutral fatty acid can 'flip-flop' back into the mitochondrial matrix. This results in a leak of protons as with classic uncoupling [67]. It is hypothesized that high rate of fatty acid oxidation wastes ATP *via* this UCP-3 mediated futile cycle as well.

Changes in ATP transport and utilization

The energetic state of the heart is not determined by ATP concentration *per se*. The primary energy reserve in myocardium is phosphocreatine. In situations when ATP utilization exceeds its production the utilization of phosphocreatine is a way to maintain a steady level of ATP.

In advanced heart failure the levels of ATP and phosphocreatine are both decreased [69, 70]. The previously proposed sequence of events most likely is as follows: initially there is a decrease in phosphocreatine levels indicative of a mismatch in ATP production and use. Afterwards the decrease in ATP and creatine levels follows [71]. The phosphocreatine-to-ATP ratio seems to be better predictor of overall and cardiovascular mortality than New York Heart Association (NYHA) class and left ventricular function [72].

In conclusion, at present myocardial metabolism in heart failure has been extensively studied but major controversies persist. It is not clear whether the alterations are adaptive or part of pathological pathway. The existing data are at once conflicting, complementary and contradictory, but one message is clearly delivered that fatty acid oxidation is not the most efficient way to utilize O2 in a failing heart. This process apparently changes in favour of glucose but only during late stage of heart failure. The main question to answer is would it be favourable to switch the substrate in early stages and if yes how to determine the moment when to do so?

Medications that influence metabolism in heart failure (Table 2)

Nearly all the most common heart failure medications such as angiotensin converting enzyme inhibitors, angiotensin receptor blockers and β-blockers reduce metabolic demand and improve outcome in heart failure.

Table 2 Potential metabolic modulators in heart failure

(1) Long-chain 3-ketoacyl coenzyme A thiolase inhibitor
Trimetazidine
(2) CPT I inhibitors
Perhexiline
Etomoxir
Oxfenicin
(3) PPAR-γ activators
Rosiglitazone
Pioglitazone

Another promising strategy for heart failure patients is modulation of substrate utilization, using the concept that stimulating myocardial carbohydrate oxidation and inhibiting fatty acid oxidation would improve mechanical efficiency. Drugs that modulate metabolism exert their action at different levels through different mechanisms and usually do not affect haemodynamics.

Trimetazidine is a well-known anti-anginal drug extensively used in Europe. It appears to selectively inhibit the long-chain 3-ketoacyl CoA thiolase although not all the studies confirm this mechanism [73, 74]. By decreasing the rate of fatty acid oxidation it indirectly stimulates PDH activity and flux through glycolytic pathway. Trimetazidine effects on ischemic cardiomyopathy were explored for more than a decade. It improved the ejection fraction and functional status in few open label randomized studies [75, 76]. Recent studies identified certain groups of patients that would particularly benefit from trimetazidine administration. In elderly patients with ischemic cardiomyopathy and with functional class II-III adding trimetazidine to standard therapy improves systolic and diastolic functions, exercise ability and improves angina control [77]. In diabetic patients with ischemic cardiomyopathy trimetazidine added to standard therapy had beneficial effects on left ventricular systolic function [78]. In a recent open label randomized trial in patients with ischemic cardiomyopathy that were randomized to either receive trimetazidine in addition to conventional therapy, trimetazidine significantly reduced all cause mortality, heart failure hospitalizations and improved ejection fraction as well as functional status [79]. Trimetazidine has a satisfactory safety profile and has been shown to improve left ventricular remodelling as well as to decrease mortality without adversely affecting the haemodynamics. The available evidence is based on small open label studies but these data warrant more investigation on this apparently promising drug.

Another class of medications that promotes glucose oxidation includes the CPT-1 inhibitors. They exert their action by decreasing fatty acids entry into mitochondria and thus decrease the substrate available for fatty acid oxidation. A reversible inhibitor of CPT-1, perhexiline, initially introduced as an anti-anginal agent, showed improvement in systolic function among patients with

heart failure [80]. However, its use diminished after high plasma levels of this drug caused cases of unexplained hepatic failure and neuropathy.

An irreversible CPT-1 inhibitor, etomoxir demonstrated attenuation in the transition from compensated to decompensated cardiac hypertrophy of a rat model [81]. A clinical study where patients with ischemic cardiomyopathy received 3 months of treatment with etomoxir showed improvement in left ventricular ejection fraction (LVEF) [82]. However the clinical utilization of etomoxir is limited by a narrow therapeutic window due to its potential to cause phospholipidosis [83]. Oxfenicine through inhibition of CPT-1 prevented left ventricular remodelling in a rapid-pacing canine model of heart failure [84]. The effects of CPT-1 inhibitors were investigated only in one open label pilot study mentioned above with etomoxir. Results of controlled trials with CPT-1 inhibitors are not available currently to our knowledge.

PPAR-γ activators were investigated in animal models, but the results were conflicting. There is evidence that these agents could have beneficial, adverse or no effects on remodelling and mortality [85, 86]. The role of PPAR-γ in heart failure needs to be more clearly delineated before larger trials can be considered.

Metabolic modulation therapy appears to have therapeutic potential as suggested by preliminary studies. However this therapeutic approach is not as popular as neurohormonal antagonism. Possibly this is due to lack of reliable assessment tools to identify the phenotype of metabolic derangements and as well as due to lack of large-scale clinical trials.

Cell therapy

Currently there is a newly emerging therapeutic field in cardiology, so-called cell therapy or cardiomyoplasty.

The idea of regeneration and renewal of the heart is extremely appealing. To date the only form of biological renewal of the heart that gives mortality benefits is total organ transplantation. The increasing shortage of donor organs has stimulated interest in the field of artificial heart development. Randomized evaluation of mechanical assistance in treatment of chronic heart failure (REMATCH), a recent clinical trial of a left ventricular assist device, showed an increased median survival of 7.4 months and improved functional status in comparison with medical management in end-stage heart failure. But this survival benefit came at the expense of a high device failure rate, infections and bleeding as a result of necessary anticoagulation. Basic research is currently oriented towards identifying a way to restore the integrity of the failing heart without paying the price of artificial devices complications. One alternative is cell therapy.

'The enlargement of the heart in hypertrophy is due to principally to a hypertrophy of the muscle fibres without an increase in the number of fibres. There is then no hyperplasia of the fibres and the process is one of pure hypertrophy' [87]. This cited paper is considered to be the basis for the theory that the heart is a post-mitotic organ unable to regenerate. This theory was not challenged for almost seven decades. Over the years the paradigm that cardiomyocytes are cells withdrawn from the cell cycle was supported by an inability to find mitotic nuclei with light microscopy and by negligible DNA synthesis in these nuclei [88]. In the early 1990s this theory was challenged by morphometric studies that showed increased number of myocytes in hypertrophied hearts, identified actual mitotic spindles within cardiomyocyte and found an operative telomere–telomerase system in the adult heart of animals and human beings [89–94]. This evidence suggests that the heart is not a terminally differentiated organ and a fraction of cardiomyocytes re-enter the cell-cycle pathway. Currently, the heart is viewed as an organ with low but present capacity to regenerate.

One step forward was performed once the studies on the cardiac chimerism following sex-mismatched human cardiac transplants were performed. The identification of the host Y-chromosome in the cardiomyocyte of sex-mismatched human cardiac transplants indicates that the host's circulating stem cells can migrate and home into the transplanted heart forming myofibrils [95]. The magnitude of this process varies among different reports, ranging from 18% to none [95–97]. Moreover the mobilization of host cells into myocardium is enhanced after sustaining myocardial infarction [98]. Anversa's group succeeded in demonstrating that in addition to differentiated cardiomyocytes there are committed cardiac stem cells (CSCs) that reside in myocardium and give rise to small developing myocytes [99, 100]. It is not clear yet if these cells are resident cells with potential to replicate or these are migrant bone marrow-derived stem cells. This work was authenticated by identification of a resident population of cardiogenic precursor cells in rats, mice and human beings that express isl1+ gene that was initially described in embryonic mesodermal cells that were committed to myocardial lineage [101]. A new concept developed currently is that the heart is a dynamic self-renewing organ. This revolutionizes our understanding of heart pathology as well as the potential for therapy.

In a healthy heart there is a continuous turnover of parenchymal cells that is supported by the stem cell compartment. This process is sufficient to maintain cellular homeostasis and normal pump function under physiologic conditions. Unfortunately this mechanism is overwhelmed by powerful injury stimuli as ischemia, pressure or volume overload. With increased pressure or volume load the heart remodels through a combination of mechanisms including myocyte hypertrophy and proliferation, myocyte apoptosis and necrosis [102, 103]. In hypertrophic cardiomyopathy the number of cardiomyocytes often exceeds the number of cells in a normal heart [102]. But with further evolution of the disease there is a modest reduction in myocyte number that cannot explain the degree of deterioration in ventricular function. Possibly this deterioration is due to the accumulation of old poorly contracting cells and formation of scars [103, 104]. On the other hand after an ischemic injury there is a severe reduction in cell number. This type of injury inevitably results in scar formation and loss of physiologic geometry of the ventricular cavity. Unfortunately the heart is less well equipped to deal with these acute dramatic injuries. As opposed to mammals, zebra fish fully

regenerate hearts within 2 months of 20% ventricular resection and inhibition of this process would lead to scarring [105]. This indicates that zebra fish possess molecular mechanisms that can overcome scar formation and elucidating the steps of this process could explain why evolutionarily our hearts lack this ability.

At present, in order to prevent scar formation new strategies have been developed including the replacement of dead cells with viable ones a process called cardiomyoplasty. Ideally through cardiomyoplasty the functional electrical and morphological structure would be restored. Initially cell-based cardiac repair aimed the goal to replace lost myocardial tissue by contractile elements [106]. Because the cardiomyocytes in the vicinity of the scar are in a hibernating state due to insufficient myocardial perfusion, the promotion of blood vessels formation is another goal of cell therapy. Stem cells due to their ability to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells become an attractive tool for cardiomyoplasty [107]. Adult stem cell implantation for myocardial recovery was initially performed in animals and quickly translated in human beings [108]. Basically a variety of stem and progenitor cell populations could theoretically be used for cardiomyoplasty.

Skeletal muscle myoblasts

The first cells used for cardiomyoplasty were the skeletal muscle myoblasts. Skeletal myoblasts are also known as satellite cells localized under the basal membrane of mature muscular fibres and are able to differentiate into myotubes with a phenotypic switch towards slow-twitch fibres when transplanted into an infarct scar [109]. Despite the fact that the myotubes do not beat in synchrony with the rest of the heart due to inability to couple electromechanically with the cardiomyocytes, studies in animal models of myocardial infarction have reported beneficial effects on both systolic and diastolic performance [110–112]. Major concern regarding skeletal myoblasts implantation is the possible occurrence of arrhythmias. This occurs through several mechanisms including electrical heterogeneity of action potentials and electrotonic stimulation of cardiac cells [113]. Despite incomplete knowledge regarding their engraftment skeletal myoblasts were the first cells used clinically for cardiac repair in a patient with severe ischemic heart failure with resultant evidence of viability and contraction of the graft (on positron emission tomography and echocardiography respectively) as well as symptomatic improvement [114]. Afterwards a series of non-randomized studies showed improvement in LVEF and symptoms [115]. But as mentioned earlier regarding animal studies, the concern regarding arrhythmias was raised in human studies as well after 4 out 10 patients in one trial experienced ventricular arrhythmias requiring placement of an implantable cardioverter [116]. Another disadvantage of skeletal myoblasts is the delay of more than 3–4 weeks between harvests of skeletal muscle from patients to the culture and preparation of cells for transplantation. In clinical trials skeletal myoblasts have not succeeded. The first randomized placebo-controlled trial, Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC), was ended prematurely because the treatment group was not superior to placebo on the primary end-points of improvement in regional contractility and global function.

Human embryonic stem cells

Embryonic stem cells (ESCs) due to their capacity to be grown *in vitro* and be propagated indefinitely in an undifferentiated state as well as due to the property of multilineage commitment are expected to have broad therapeutic potential. At present however therapeutic use of these cells would likely introduce ethical and legal dilemmas.

ESCs are derived from inner cell mass of the blastocyst. Human embryonic cells cultured in suspension form cellular aggregates so-called embryoid bodies. Embryoid bodies contain cells derived from three germ layers and lack critical features of embryonic patterning [117]. *In vivo* administration of human ESC may give rise to teratomas or other unacceptable cardiac complications [118–120]. Due to risks associated with the broad differentiation potential of ESC only a few studies used these cells in an uncommitted state to repair myocardial infarction [121]. In an attempt to avoid teratoma formation ESC have been partially differentiated *in vitro* before their implantation into the injured heart [122]. ESCs appear to differentiate into immature cardiomyocytes but whether or not these immature cells can reach adult characteristics is not clear [123,124]. Despite greater plasticity of ESCs compared to the adult stem cells their use is not as popular. This is due to ethical issues and potential for teratoma formation. Another limitation is immunorejection of the allogeneic ESC or their differentiated progeny, because even in their undifferentiated state human ESC express HLA class I antigens [125]. As result, immunosuppressive therapy is required and this severely impairs patient's quality of life. However all these limitations do not discourage research in ESC because understanding the mechanisms of differentiation into cardiomyocyte could shed light on the processes of cardiac repair as well.

Bone marrow derived adult stem cells

The fact that stem cells exist in postnatal period was described in the 1960s when Till and McCulloch discovered clonogenic bone marrow cells [126]. The proof of origin of these cells was obtained when a single murine haematopoietic stem cell (HSC) reconstituted all blood cell types following transplantation into lethally irradiated animals [127]. Once it was discovered that bone marrow derived stem cells are able to transverse boundaries of lineage and transdifferentiate into hepatocytes, endothelial cells, skeletal muscle cells and neurons if appropriately stimulated, the question of whether it is possible to use them for heart repair arouse [128, 129]. The majority of studies in regenerative cardiovascular research were performed with the following bone marrow derived stem cells populations: (*i*) HSCs (*ii*) mesenchymal stem cells and (*iii*) endothelial stem cells.

Haematopoietic stem cells

HSCs are isolated from bone marrow through selective sorting for particular sets of surface receptors including Lin-, ckit^{+}, Sca-1^{+}, CD34lo, CD38hi, etc. Currently there is no known specific epitope to describe the true bone marrow derived stem cell [130]. The efficacy of adult HSCs for myocardial regeneration was demonstrated when enriched Lin-ckit^{+} cells were implanted in the border zone of an infarcted mouse heart and these cells were shown to colonize the scar area and give rise to contractile myocardium [108]. Afterwards, successful attempts were made to mobilize HSCs from bone marrow *via* systemic administration of stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF). After their mobilization HSCs were shown to form cardiomyocytes and capillaries [131]. Another series of studies documented the ability of HSCs to migrate towards infarcted areas and transdifferentiate into vessels or cardyomyocytes as well, but the degree of engraftment was shown to be low and the formation of endothelial cells and smooth muscle cells exceeded the cardiomyocyte formation [132–135]. Despite low numbers of formed cardiomyocytes in almost all animal studies implantation of HSCs is associated with improved ventricular function.

Mesenchymal stem cells (MSC)

In the stroma of bone marrow there is a subset of non-HSCs that has the potential to differentiate into cells of mesenchymal origin [136]. So far there is no clear definition of MSC, and the fact that these cells were shown to differentiate into tissues other than those of mesenchymal origin raises the question about the appropriateness of their given name [137]. In the majority of studies on MSC these cells are isolated based on lack of typical haematopoietic antigens (CD45, CD34, CD14) and presence of specific adhesion molecules (ALCAM/CD44) and antigens (SH2/SH3/SH4/STRO-1) [138]. Some authors do not agree with only antigenic isolation and recommend the use of functional assays to demonstrate their multipotent growth and differentiation as well [139]. These demonstrate that currently there are no clear criteria for isolation of MSC.

MSC are known to acquire multiple phenotypes (osteoblast, endothelial cells, neuronal-like cells, adipocytes, chondrocytes) when stimulated by appropriate growth factors and cytokines [140]. Reports in animals demonstrate that MSC home to the heart as well [141]. Their fate in the heart is not completely elucidated and varies in different reports ranging from formation of cardiomyocytes and coronary vessels to differentiation into fibroblasts [142, 143]. Interestingly, MSC engraft in the normal heart and remain quiescent but viable and do not participate in the physiological turnover of myocytes. This raises the possibility of trafficking of MSC from bone marrow *via* the blood stream to the heart and their storage for possible future activation in response to injury.

Endothelial progenitor cells (EPCs)

EPCs are a subset of bone marrow derived stem cells that are able to acquire endothelial phenotype [144]. These cells are identified through the presence of HSCs markers CD133, CD34 and the endothelial marker Flk-1 (vascular endothelial growth factor receptor [VEGFR]-2). They can be isolated from bone marrow as well as from peripheral circulation [145]. The fate of these cells after implantation into infracted myocardium is also variable. Mostly EPCs differentiate into mature endothelial cells thus promoting vasculogenesis and angiogenesis. It is possible that their angiogenic potential is conditioned by secretion of growth factors that trigger the development of new vessels [146, 147]. This is a double-edged sword because there are reports linking angiogenesis and atherogenesis; moreover, CD34 cells are found in atherectomy specimens retrieved from in-stent restenosis [148]. This risk must be weighed against the possible beneficial effect *via* angiogenesis when EPCs are delivered through intracoronary infusion. On the other hand reports regarding differentiation into cardiomyocytes are contradictory. Some studies demonstrate the differentiation of EPCs into cardiomyocytes whereas others do not confirm this possibility [149, 150].

Currently available variable data regarding differentiation of bone marrow derived cells into cardiomyocytes is still contradictory but the majority of the evidence supports the beneficial effect that implantation of these cells has on cardiac function. This observation raises the question of whether improvement in cardiac performance regardless of the underlying mechanism should be the primary objective of cell therapy?

Cardiac stem cells

In the last years a distinct population of stem cells has been identified as being resident in the heart – the CSCs.

CSCs are Lin-cells that express c-Kit, MDR1 or Sca-1 antigens [99]. These cells are multipotent *in vitro* and give rise to cardiomyocyte, endothelial cells and smooth muscle cells *in vivo* [151]. CSCs participate in physiologic cardiomyocyte turnover and maintain the cellular homeostasis of the heart. Myocardial aging and heart failure develops once replicative senescence of CSCs becomes apparent [152]. Intuitively it seems that CSCs should be more effective in making new myocardium than progenitor cells from other organs. Given the lack of complete understanding of mechanisms of differentiation and migration of these cells, as well as difficulties of their isolation, their therapeutic use is not settled yet. Nonetheless the demonstration of their existence revolutionized our understanding of cardiac cellular organization and physiology.

Mechanisms of stem cell mediated myocardial regeneration

Existing evidence supports the existence of CSCs as an endogenous regenerative resource. This restoring capacity is insufficient in face of acute major injuries. In order to be able to recover from cell loss, myocardium has to recruit extra-CSCs. The process of recruitment is not completely elucidated. Most likely three major

compartments are involved in this process: the myocardium, the circulating blood and the bone marrow. The injured myocardium releases a set of cytokines that activate local and mobilize distant stem cells from their major reservoir in the bone marrow. The bone marrow stem cells exit into circulation and home into the injured sites in order to initiate repair. The precise timing and factors involved in bone marrow mobilization are still not clearly identified as well as the process of homing and engraftment of these mobilized stem cells. Currently several factors have been studied and shown to promote mobilization of bone marrow stem cells: G-CSF, granulocyte macrophage (GM)-CSF, SCF, vascular endothelial growth factor (VEGF), hepatocyte growth factor, stromal cell derived factor and epogen [153].

It is also not clear how the transplanted cells contribute to improved functional capacity of the heart. Using genetic markers and labelled fluorescent dyes several studies showed differentiation of bone marrow derived stem cells into cardiomyocytes whereas other attempts to show this process failed [131, 150, 154]. Many reports demonstrated another possible mechanism that of cell fusion being responsible for the observed phenotypic changes [155]. Because the number of new cardiomyocytes derived from exogenously delivered bone marrow stem cells is extremely low in order to produce the frequently reported functional improvement another proposed mechanism is stem cell mediated paracrine effect. This effect may trigger vasculogenesis, activation of resident CSC, inhibition of native cardiomyocyte apoptosis and changes in extracellular matrix composition [156]. This hypothesis is indirectly supported by the finding that transplanted human MSC into the brain of mice increased the expression of trophic factors that stimulate the proliferation of endogenous neural stem cells [157].

To date there is an increasing pool of preclinical evidence for efficacy of stem cell therapy but paradoxically we still do not really understand the underlying mechanism of its action.

Clinical applications of stem cells

Stem cells have been introduced into clinical studies despite the many gaps in our knowledge about their physiology. Currently it is not known what cell is best for cell therapy. However, the majority of clinical studies are done using cells isolated from bone marrow aspirate. This is mostly conditioned due to easy accessibility. The methods of delivery of these cells have been variable and include:

(1) Direct intramyocardial injection (either to endocardium *via* intracardiac catheters or to epicardium *via* surgical or thoracoscopic approach);
(2) Percutaneous intracoronary injection;
(3) Peripheral intravenous injection and
(4) Indirect mobilization with peripheral delivery of cytokines.

The first clinical randomized trial of intracoronary injection of bone marrow derived stem cells in ST-elevation myocardial infarction (STEMI) was the Bone Marrow Transfer to Enhance ST-elevation infarct regeneration (BOOST) trial. In this trial the initial relative improvement in LVEF after infusion of bone marrow derived cells at 6 months as compared to no infusion was no longer present at 18 months follow-up. This was explained by continuous improvement in the control group [158, 159].

To date the largest trial on cardiac cell therapy is Reinfusion of Enriched Progenitor Cells and Infarct Remodelling in Acute Myocardial Infarction (REPAIR-AMI). In this study 204 patients were randomized to receive intracoronary infusions of bone marrow derived progenitor cells or placebo into the infarct artery 3–7 days after reperfusion therapy [160]. At 4 months patients in the treatment group had improved LVEF (measured by quantitative left ventricular angiography) comparative to placebo group (5.5% *versus* 3% $P = 0.01$). At 1-year follow-up patients treated with bone marrow derived cells had improved clinical outcome reported as lower incidence or recurring MI, less frequent revascularizations and rehospitalizations [161]. In contrast to REPAIR-AMI another controlled trial Autologous Stem Cell Transplantation in Acute Myocardial Infarction (ASTAMI) without blinded placebo did not report significant improvement in LVEF 6 months after bone marrow derived cells intracoronary infusion after acute anterior MI [162]. There are many potential explanations for these differences including different timing of administration, methods of cell isolation and number of delivered cells.

Another aspect of stem cell delivery was studied in Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony Stimulating Factor (FIRSTLINE-AMI) trial [163]. This trial demonstrated the safety and feasibility of bone marrow mobilization using G-CSF in patients with STEMI after they had the revascularization procedure. Importantly this trial showed that treatment with G-CSF did not increase the rate of restenosis in treated patients. Subsequent trials Regenerate Vital Myocardium by Vigorous Activation of Bone Marrow Stem Cells (REVIVAL) II and Stem Cell Mobilization Induced by Subcutaneous Granulocyte-Colony Stimulating Factor to Improve Cardiac Regeneration after Acute ST-elevation Myocardial Infarction (STEMMI) failed to reproduce the mobilization effect seen in FIRSTLINE-AMI [164]. All these above mentioned trials were performed in patients in perinfarct period. Studies of similar dimensions on cell therapy in patients with advanced heart failure are not available. TOPCARE-CHD trial evaluated the effects of bone marrow derived cells or progenitor cells derived from circulating blood in patients with chronic ventricular dysfunction due to ischemic cardiomyopathy [165]. In this randomized crossover trial the benefit observed after cell infusion was modest (increase in LVEF by 2.9%). Whether repeated infusions of cells are necessary or infusion with certain chemical factors are necessary to see more significant effect is not clear. But definitely this trial suggests that cell therapy can have effects beyond healing effect after myocardial infarction. Transplantation of Progenitor Cells and Recovery of Left Ventricular Function in Patients with Chronic Ischemic Heart Disease (TOPCARE-CHD) comes to underscore prior reported data that injection of bone

marrow derived cells is not only safe but also could contribute to increased exercise capacity in patients with ischemic cardiomyopathy who were heart transplant candidates [166].

Currently there are no randomized trials investigating the therapeutic role of cell therapy in non-ischemic cardiomyopathy. However stem cell therapy has been explored in this area as well. These attempts in single patients with non-ischemic cardiomyopathy demonstrated improved NYHA functional class and LVEF. Despite being in early stages of its applications stem cell therapy appears to promise new horizons for the heart failure patients and further studies are required to elucidate possible mechanisms and the true potential of this therapeutic option.

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