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Review

The infarcted myocardium: Simply dead tissue, or a lively target for therapeutic interventions

Jack P.M. Cleutjens[a,*], W. Matthijs Blankesteijn[b], Mat J.A.P. Daemen[a], Jos F.M. Smits[b]

[a]*Department of Pathology, Cardiovascular Research Institute Maastricht (CARIM), Universiteit Maastricht, P.O. Box 616, 6200 MD, Maastricht, The Netherlands*

[b]*Department of Pharmacology, Cardiovascular Research Institute Maastricht (CARIM), Universiteit Maastricht, P.O. Box 616, 6200 MD, Maastricht, The Netherlands*

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1. Introduction

It has been known for many years that infarction of the heart induces prominent alterations of cardiac structure. The most apparent is the scarring of the infarct. Structural changes after infarction are, however, not limited to the infarcted area, but also extend into the non-infarcted myocardium. Changes in the non-infarcted myocardium include hypertrophy of the cardiomyocytes, growth of the capillary network, and an increase in interstitial collagen.

Cardiac structure is a major determinant of function, which is depressed after myocardial infarction (MI). After infarction, both short term and long term compensatory or regulatory mechanisms are activated. Often these mechanisms also affect cardiac structure. Although activation of these compensatory mechanisms may be beneficial early after infarction, they may have adverse effects, when activation is continued for a longer time. Indeed, pharmacological treatments that block the long term activation of these compensatory mechanisms, like angiotensin converting enzyme inhibitors (ACEI) that block the renin–angiotensin system (RAS), have been shown to improve cardiac function after infarction.

Although we know that cardiac function and structure are closely related and do indeed both change after infarction, it is largely unknown what the exact structural component is that causes the reduction in cardiac function after infarction. Also it is not clear which structural component should be targeted for effective pharmacotherapy after infarction.

In this review we attempt to clarify the structural alterations after infarction. We and others have focused for many years on the potential importance of changes in the vital non-infarcted myocardium and, indeed, found several alterations in cardiac structure after infarction and effects thereon of drugs that improved cardiac function. However, recent data in animal studies and humans point to the importance of the infarct itself as a potential target for intervention. The infarct appears to be more than just dead tissue and infarct healing turns out to be an active and well controlled process. Also, interventions in infarct healing appear to affect cardiac function. Therefore we hypothesize that changes in the structure of the infarct may be of major importance for the maintenance of cardiac function. Future pharmacological interventions should, for this reason, not only be directed to the non-infarcted myocardium, but also to the mechanisms that control adequate infarct healing.

After describing the components of the normal heart, we will focus on the characteristics of cardiac wound healing and some of the control mechanisms involved, with special

*Corresponding author. Tel.: +31-43-387-6631; fax: +31-43-387-6613.

E-mail address: jcl@lpat.azm.nl (J.P.M. Cleutjens)

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emphasis on the control of architectural adaptations in the infarct. Also changes in the non-infarcted myocardium will be described. Subsequently we will focus on the effects of interventions on both the infarcted and non-infarcted myocardium and the regulation of granulation tissue formation.

2. The structure of normal myocardium

The vast majority of the volume of the normal adult mammalian heart is taken up by cardiomyocytes. Cardiomyocytes are regarded to be terminally differentiated cells, and constitute the core of the contractile unit of the heart. Cardiomyocytes contain several specific contractile proteins and contract in a rhythmic and coordinated fashion. They are surrounded by capillaries, that are lined by endothelial cells, and provide oxygen to the cardiomyocytes. Capillaries surround myocytes in a hexagonal fashion which limits the diffusion distance of oxygen.

The structural backbone of the heart is provided by the extracellular matrix. Extracellular matrix proteins, like type IV collagen, are present in the basement membrane surrounding the cardiomyocyte. Other collagen types, like type I, III and VI collagen, are present in and around the coronary arteries and in the interstitium. In a normal adult heart interstitial collagens make up 1–2% of the volume of the heart. Extracellular matrix proteins are produced in (myo-)fibroblasts, which are also found in the interstitium. Together with the endothelial cells they make up the majority of the cell number in the heart. Cardiomyocytes, extracellular matrix and cardiac vasculature are major determinants of cardiac function. Moreover, remodeling of the heart may constitute one of the most important and powerful long-term adaptive mechanisms to changes in load. Unloading of the heart causes rapid regression of cardiac mass, through myocyte atrophy. Similarly, cardiomyocytes respond within 1 h to an increased load by increasing protein synthesis. The outcome of the latter hypertrophic response depends upon the nature of the imposed load, and may constitute concentric hypertrophy (in which the ratio of wall thickness and diameter of the left ventricular cavity increases) or eccentric hypertrophy (in which wall thickness: cavity diameter decreases).

Cardiac hypertrophy has also been distinguished as physiologic or pathologic [1]. Physiologic hypertrophy is considered a benign process that occurs, for instance, in athletes, and which does not result in pump failure. In contrast, pathologic hypertrophy, for instance, as a consequence of hypertension, does ultimately result in heart failure. In pathologic hypertrophy there is excessive deposition of interstitial collagen. This increases cardiac stiffness, thereby hampering both diastolic relaxation and filling, as well as systolic contraction of the heart in humans and animal models. Many factors can be involved the regulation of oxygen supply to the tissue. Collagen deposition is one of these factors. Collagen excess has been suggested to occlude coronary vessels, thereby limiting oxygen supply, and causing a negative energy balance. Oxygen supply may also be limited by inappropriate development of vascular supply, i.e. a decrease in capillary density, in association with the hypertrophic response, such as actually observed in pathologic hypertrophy. The shift in energy balance through processes that occur in the cardiac interstitium may then alter the cardiomyocyte phenotype, which has been proposed to switch to a fetal program, comprising not only a switch in myosin expression to a slower but less energy requiring isoform, but also a shift from lipid to glucose metabolism. In this way, all components of the heart muscle contribute to proper functioning of the heart.

3. The wound healing process after myocardial infarction

To a large extent, the wound healing after myocardial infarction mimics the wound healing processes observed in other tissues, like the skin. However, cardiac wound healing has some unique features. One of those features is that adult cardiomyocytes are terminally differentiated cells that have lost, at least to a great extent, the capacity to divide and regenerate [2]. Adequate cardiac wound healing is, therefore, mainly determined by the factors that control the formation of the granulation tissue. Factors that determine myocyte regeneration or myocyte cell division appear to be of minor importance. A second unique feature of cardiac wound healing is the rhythmic contraction of the non-infarcted myocardium, which puts a cyclic stretch on the healing wound. The tightly controlled architectural changes in the infarct, that serve to maintain cardiac function after infarction, constitute a third unique feature of cardiac wound healing.

In general, four phases can be distinguished during cardiac wound healing; in Fig. 1, this is illustrated with the time course observed in humans. Phase 1 is characterized by death of cardiomyocytes. Release of fatty acid binding protein (FABP), troponin T, and creatine kinase, skeletal–brain hybrid type (CK-MB) and serum glutamic-oxaloacetic transaminase (SGOT) into the blood are hallmarks of myocyte death. Elevated plasma levels of these cardiomyocyte specific components can thus be used for an early detection of cardiomyocyte cell death. Myocyte death, also after coronary artery occlusion, can occur by two, probably interdependent pathways, that is myocyte necrosis, characterized by swelling of the cells, and myocyte apoptosis, characterized by cell shrinkage. The peak of myocyte apoptosis has been reported 6–8 h after infarction in humans and rats. Apoptosis is a major source of myocyte loss after infarction [3], also in human infarcts [4,5]. The fact that myocyte apoptosis is a tightly regulated process may yield possibilities for early interventions

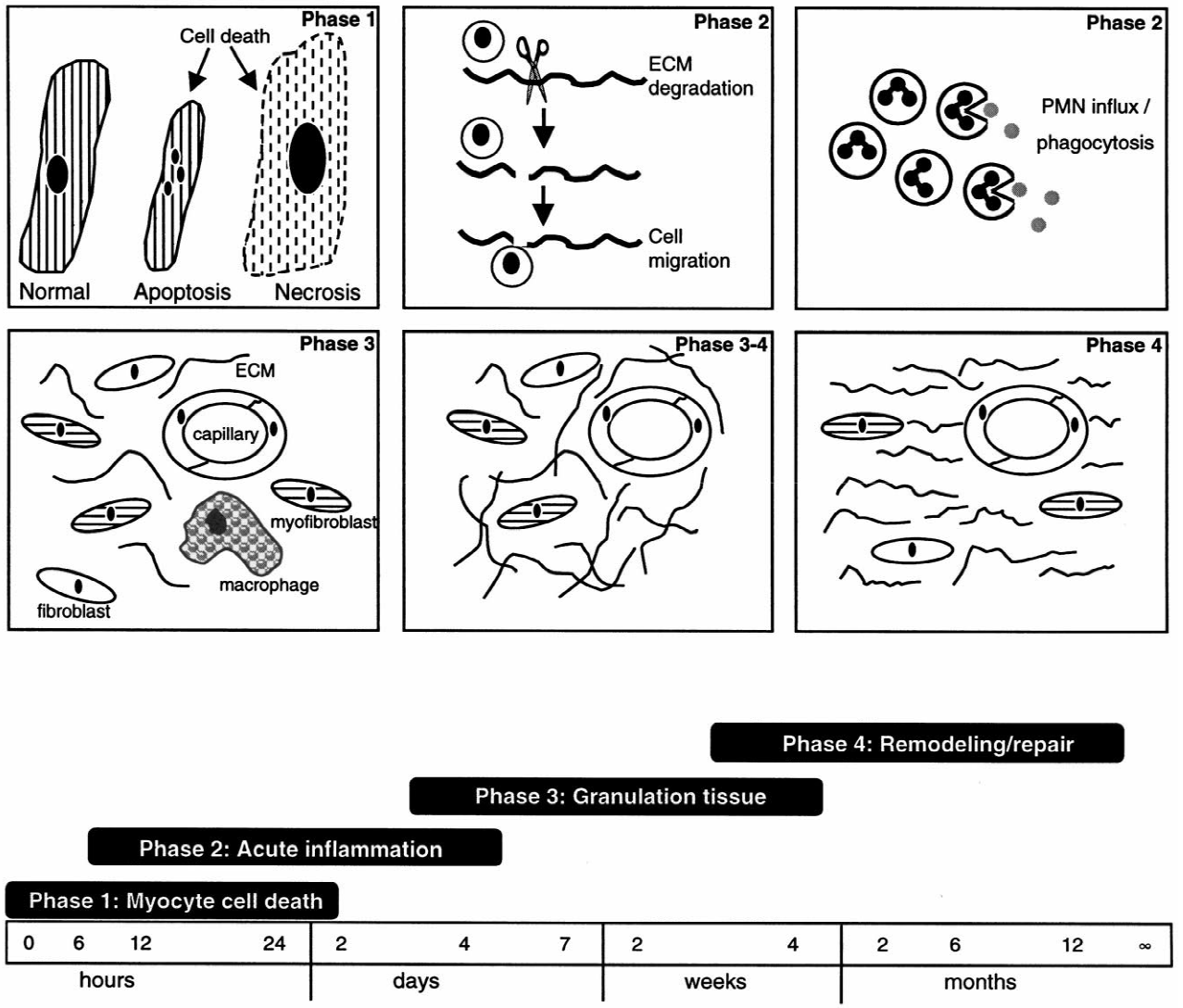

Fig. 1. Phases of cardiac wound healing in humans. (Numbers refer to the different phases of wound healing). Abbreviations: ECM: extracellular matrix; PMN: neutrophilic granulocytes.

directed to limit the amount of myocyte loss after an ischemic event. One example of such interventions in rats is the use of inhibitors of the caspases, proteolytic enzymes involved in the apoptosis pathway [6]. Caspase inhibitors may thus reduce apoptotic cell death after infarction and limit the ischemic damage to the heart.

The majority of the apoptotic cells after infarction cannot be phagocytosed by neighboring cells and secondary necrosis occurs from 12 h till 4 days after infarction [3]. This type of cardiomyocyte death evokes an early inflammatory response (phase 2 of cardiac wound healing). One of the first features of this inflammatory response is activation of the complement system, and release of several cytokines, like the interleukins IL-6 and IL-8, which occurs within 12–16 h after the onset of ischemia in humans [7–9]. Complement activation and the early cytokine release after myocyte death have been suggested to be useful as early markers of cardiomyocyte death and may provide potential targets for early interventions to limit myocyte loss.

Within 6–8 h after the onset of infarction neutrophilic granulocytes (PMN) migrate into the infarcted area. Peak numbers of granulocytes are observed within 24–48 h after infarction. Granulocytes help to remove the dead myocytes. Granulocyte infiltration is followed by influx of other inflammatory cells, like lymphocytes, plasma cells and macrophages.

Two to three days after infarction new extracellular matrix proteins are being deposited, first in the border zone between infarcted and non-infarcted tissue, and later in the central area of the infarct. This marks the onset of phase 3 of the process of cardiac wound healing, the formation of granulation tissue, which increases the tensile strength of the infarct and prevents cardiac rupture.

First, fibrin is being deposited. Fibrin deposition is followed by the deposition of other extracellular matrix

proteins, like fibronectin and tenascin in rats [10–12]. Within a few days after infarction, myofibroblasts have surrounded the infarcted area (Fig. 1). These myofibroblasts produce interstitial collagens, and, in rats, elevated amounts of type III collagen can be found within 2–3 days after ligation of the left coronary artery. The peak of type III collagen production is followed by a lower and slower developing peak of type I collagen. Type I collagen brings tensile strength to the healing wound, but only if multiple fibers are cross-linked [13]. Complete collagen cross-linking may take another few weeks to occur. Collagen turnover in a normal adult non-infarcted heart may take approximately 120 days; it occurs much faster in the early phase of cardiac wound healing [14].

Concomitant with activation of collagen synthesis, collagen degradation is activated. Ultrastructural signs of degradation of collagen fibrils can be observed already 40 min after coronary occlusion in a pig model [15]. Collagenolytic activity, that is activation of specific matrix metalloproteinases that cleave interstitial collagens, is enhanced in the first week after infarction in the rat [16,17]. Increased collagenolytic activity can result in loss of structural support, distortion of tissue architecture, reduction of cardiac stiffness, wall thinning and even rupture of the myocardium. Interestingly, most studies demonstrating enhanced collagenolytic activity after infarction, find it only in the infarcted region and not in the non-infarcted myocardium.

Granulation tissue is also characterized by the presence of many blood vessels. Within a few days after infarction new blood vessels start to appear in the wound. These new blood vessels are derived from pre-existent blood vessels or from endothelial cells that migrate from the border zone into the wound. This process of neovascularization is very efficient. In rats, already within 1 week after infarction, basal coronary flow was normalized. Coronary flow at maximal dilatation was almost normalized at 35 days after surgery for the whole rat left ventricle. At 7 days after MI the center of the infarcted area obtained 25% of the maximal left ventricular flow [18].

A 2–3 week old granulation tissue in an infarcted heart is characterized as a cell rich tissue, containing (partly) cross-linked interstitial collagens, macrophages, blood vessels, and (myo-)fibroblasts. Subsequently cells, except the majority of myofibroblasts [12] start to disappear from the wound, which is the main characteristic of the fourth period of cardiac wound healing, the period of scar tissue formation. In this period, cells do not only disappear from the wound, most probably by apoptosis [19], but also does the collagen become almost completely cross-linked. The so formed scar tissue has a permanent nature because of the lack of cardiomyocyte regeneration. This is in contrast with dermal wound healing and other types of injury, in which parenchymal cells can regenerate and the formed fibrous tissue can be resorbed.

The above described time course of cardiac wound healing after infarction is generalized for humans. Cardiac wound healing is accelerated in smaller animals, like rats and mice, compared to humans.

The healing process of the infarcted myocardium, as discussed above, is a multifactorial event, that requires the contribution or activation of various cell types. The blood supply to the area of infarction has to be restored by remodeling of the vascular tree and angiogenesis, and scar tissue has to be formed to reinforce the injured area. At the cellular level these processes include proliferation, apoptosis and differentiation, with specific roles for a large number of growth factors, mitogenic factors etc. However, apart from changes in cell number or cell differentiation, angiogenesis and scar formation also require cell migration and correct orientation to preserve an optimal cardiac function. The aspects of cell proliferation and degradation in infarct healing have received considerable attention, but the architectural control of the different events, that take place during cardiac remodeling, is only poorly understood. The importance of the control of cardiac architecture for maintaining cardiac function became evident when several groups showed that prevention of ventricular dilatation was important to prevent cardiac failure after infarction in the rat [20].

As we will discuss below, tissue polarity genes may be regulators of the architectural control of cardiac wound healing. Before doing so, we will first describe the changes in the non-infarcted myocardium.

4. Changes in the non-infarcted myocardium after myocardial infarction

At first sight the changes in the non-infarcted myocardium are not as dramatic as the changes in the infarct. Changes in the non-infarcted myocardium, however, affect both the non-infarcted left ventricle and the right ventricle and affect many of the constituents of the myocardium, including the cardiomyocytes, the endothelial cells and the extracellular matrix.

Cardiomyocyte hypertrophy occurs within several days after infarction and cardiomyocytes may increase their volume up to 112% in humans [21]. In cardiomyocytes DNA synthesis is a limited process and involves less than 1% of the DNA synthetizing cells in rats [22,23]. Cardiomyocyte apoptosis has also been described in the non-infarcted myocardium, and may contribute to the remodeling process after infarction [24], and to the induction or progression of cardiac failure after infarction [25].

DNA synthesis is more prominent in the endothelial cells lining the capillaries. In fact approximately one third of the total DNA synthesis that takes place in the non-infarcted myocardium is localized in endothelial cells in the rat [26]. Endothelial cell proliferation is, however, not high enough to fully compensate for the amount of cardiomyocyte hypertrophy. The result is a decrease of the

capillary to myocyte fiber ratio and, hence, an increase of the oxygen diffusion distance. The relative oxygen deficit is associated with a shift of the myocyte phenotype to a fetal phenotype in the rat, and enables the myocyte to function at a lower energy consumption level [27].

Rats and humans share many pathophysiological characteristics after myocardial infarction, like left ventricular remodeling and function, neurohormonal and molecular changes. But unlike human heart failure, left ventricular dysfunction in rats is associated with alterations in the myosin heavy chain (MHC) isoforms from the high ATP activity V1 (α,α) to the low ATPase activity V3 (β,β) isoform [28].

Furthermore, in rats the growth-hormone and its local effector Insulin-like Growth Factor I (IGF-I) seem to be involved in myocyte growth without affecting capillary density, collagen deposition and improvement of cardiac function [29]. Growth hormone downregulates α-MHC and reinduces β-MHC in rats [28]. Humans with growth hormone deficiency (GHD) have a reduced left ventricular mass and cardiac function. Growth hormone replacement therapy increased cardiac mass and improved hemodynamics, myocardial energy metabolism and clinical status [30].

Metabolic changes take place in the infarcted myocardium, where mitochondrial activity and high energy metabolites decline are rapidly [31]. In the non-infarcted myocardium of dogs and rats the energy metabolism was impaired [32,33]. The decrease in these metabolites was accompanied by a decreased myocardial function (decreased dp/dt and stroke work) [32].

Besides changes in the cardiomyocyte and endothelial cell components, changes in the content of the interstitial collagens are apparent in the non-infarcted left and right ventricle. As mentioned above, interstitial collagens constitute approximately 1–2% of the volume of the normal mammalian heart. Within 1 week after infarction, the amount of interstitial collagens at least doubles in humans and rats [13,34]. As in the infarct, type I collagen deposition is preceded by type III collagen. An increase in interstitial collagens may be beneficial to the heart in that it may help to prevent dilatation. Increased amounts of interstitial collagens will, on the other hand, increase the stiffness of the heart and result in a reduced cardiac function [35].

5. Effects of interventions on cardiac remodeling after myocardial infarction

Observations on the effects of angiotensin converting enzyme inhibition (ACEI) in rats following MI [36,37], followed by similar clinical studies [38] have not only stressed the importance of cardiac remodeling in determining the clinical outcome, but also triggered numerous follow-up studies addressing the role of the renin–angiotensin system in the control of remodeling.

Increased cardiac stiffness in rat hearts following MI could be prevented by treatment with captopril [36]. Observations on increased interstitial collagen deposition and the inhibitory effect of ACEI thereon resulted in the hypothesis that the renin–angiotensin system (RAS) might be involved in the control of collagen synthesis in the cardiac interstitium [23,39]. In several studies a coincidence of increased interstitial cell proliferation and collagen deposition was observed. ACEI-induced inhibition of both effects made it logical to assume a causal relationship between the two responses. Both (AII) and aldosterone have been considered as modulator molecules [40]. Recent studies using selective angiotensin (AT)-receptor antagonists, however, shed a different light on these phenomena (Table 1). Early angiotensin type 1 receptor (AT_1)-blockade in rats, significantly reduced interstitial collagen following MI, without affecting cell proliferation. In contrast, early angiotensin type 2 receptor (AT_2)-blockade in rats abolished the increased cell proliferation [41], but completely lacked an effect on collagen deposition [26,42]. More importantly, using the same experimental setup late but not early captopril treatment restored cardiac function following MI. Starting the treatment with ACE inhibitors, 7 or 14 days after infarction, improves cardiac function and the 1 year survival rate of MI rats [37,43]. AT_2- but not AT_1-blockade had similar effects [26]. Since this improvement coincided with effects on cell proliferation and not with effects on collagen, this implies that proliferative phenomena, rather than interstitial collagen, determine cardiac function following MI. Moreover, since AT_2-blockade does not affect aldosterone, the role for AII seems to dominate.

Although cell proliferation in the first 3 weeks after MI in rats is increased throughout the myocardium, it is most prominent in the border zone between the infarct and the

Table 1
Effects of early intervention on cardiac structure and function following MI in rats

	Interstitial DNA-synthesis	Interstitial collagen	Cardiac function
ACE-inhibition	↓	↓	↓
AT_1-inhibition	↔	↓	↔
AT_2-inhibition	↓	↔	↓

surviving tissue. As noted before approximately one third of proliferating cells are endothelial cells [26]. This suggested to us that the anti-proliferative effect of captopril might represent an inhibition of vascular outgrowth following MI. We confirmed that hypothesis in an experiment in which captopril treatment during the period of cell proliferation following MI (0–3 weeks) not only inhibited endothelial cell proliferation, but also blocked the restoration of maximal coronary blood flow [44]. Moreover, early treatment was associated with a reduction of cardiac function as opposed to an improvement during late treatment. Again, this effect, as expected, was mimicked by AT_2- but not AT_1-blockade [26]. Although others found improvement in left ventricular end-diastolic pressure (LVEDP) and right ventricular systolic pressure (RVSP) in infarcted rat hearts, cardiac output was not restored after AT-1 receptor blockade [45,46].

Stoll et al. [47] presented evidence for inhibition of endothelial cell proliferation in cultured cells by AII, mediated by the AT_2-receptor. However, in vivo studies in tissues like retina, kidney and skeletal muscle in mammals and chick chorio-allantoic membrane all point to a stimulatory effect of AII on vascular growth [48].

Although the observation that the interstitium of the whole heart responds to MI might be interpreted as indicative for involvement of a circulating factor (i.e. plasma AII, aldosterone), also the heart itself may provide a source where diffusion of locally produced factors may occur by the cardiac lymphatics. It is noteworthy that all components of the renin–angiotensin system have been identified in the heart, both at the protein and mRNA levels. There is still discussion about the potential cardiac production of renin since the mRNA levels for this enzyme are close to undetectable under physiological conditions [49]. However, following infarction in rats cardiac renin mRNA levels are as high as those in the primary renin source, the kidney [50,51].

AT-receptors have been observed throughout the heart. AT_1-receptors have been found in association with cardiomyocytes, vascular smooth muscle cells and fibroblasts [52]. Localisation of AT_2-receptors is less clear, although there is some evidence for a predominant fibroblast expression in heart failure [53]. In a study from our own group we were able to show abundant expression of AT_2-receptors in the border zone of the rat infarct, but were not able to co-localize the expression with a specific cell type [54].

One of the functions that have been proposed for AT_2-receptors is induction of apoptosis. Indeed, we did observe apoptosis in the border zone of the infarct, where it peaked together with the activation of the RAS, i.e. at 4–7 days post-MI. In the surviving myocardium we could not detect apoptosis (by Terminal transferase-dUTP-Nick End Labeling (TUNEL)). A recent study by Goussev et al. indicated that captopril treatment inhibited apoptosis in a canine model of myocardial infarction [55]. The nature of the receptor involved is not yet clear. Although, as indicated above, this effect may be mediated by AT_2-receptors, AT_1-receptors have also been shown to be able to induce apoptosis in isolated cardiomyocytes [56]. Since this effect occurred in response to stretch, afterload reduction by captopril may also be considered a causal factor for the captopril response on apoptosis.

Intervention in the renin–angiotensin system with ACEI or AT_1-antagonists also inhibits the hypertrophic response. Although, again, afterload reduction may contribute to this effect, studies with AT-receptor antagonists indicate a specific AT_1-receptor mediated hypertrophic response in cardiomyocytes [57].

The beneficial effects of ACE inhibition can be partially explained by kinin breakdown. Experiments with bradykinin infusion or ACE-inhibition combined with B2 kinin receptor blockade demonstrated that kinins can act as mediators of cardioprotective mechanisms, thereby improving myocardial energy metabolism, reducing infarct size and having an inhibitory effect on interstitial collagen deposition [58,59].

Another possible candidate for the regulation of cardiac remodeling post-MI is endothelin. Endothelin-1 (ET-1) is produced by endothelial cells and cardiomyocytes in the heart. ET-1 increases contractility of vascular smooth muscle and cardiac muscle cells and leads to hypertrophy and cellular injury in cardiomyocytes [60]. ET_A receptor blockade for 12 weeks starting at 10 days after surgery in the rat improved survival, prevented myocyte hypertrophy and improved cardiac function [61]. Treatment of rats directly after MI with an ET_A receptor antagonist demonstrated impaired scar healing, left ventricular dilatation and cardiac dysfunction [61], suggesting that endothelin inhibitors are only beneficial when started as the scar has healed.

New insights in other regulatory pathways of cardiomyocyte hypertrophy, and the growth factors and cytokines involved, are now emerging. Calcineurin, a calcium dependent phosphatase, dephosphorylates the transcription factor NF-AT3. Together with the cardiac transcription factor GATA4 it synergistically activates cardiac transcription and leads to cardiac hypertrophy and heart failure [62]. Pharmacological inhibition of calcineurin activity blocks hypertrophy [62,63]. Cardiotrophin-1 (CT-1), an interleukin-6 related cytokine, induces tyrosine phosphorylation of the signal transducer gp130 and Leukemia Inhibitory Factor receptor (LIFR), giving rise to cardiomyocyte hypertrophy by increasing cell length due to assembly of sarcomeric units in series [64,65]. The IGF-1-IGF-1 receptor (IGF-1R) autocrine system is involved in cardiomyocyte DNA synthesis, myocyte and fibroblast hyperplasia and cell death [66]. Overexpression of IGF-1 prevents cardiomyocyte cell death after myocardial infarction, thereby limiting ventricular dilatation,

myocardial load and cardiac hypertrophy within the first 2–3 days after infarction [67].

These latter data indicate that intervention in the hypertrophic response of surviving cardiomyocytes after infarction may be beneficial for cardiac function, and may become a target for future treatment.

6. Potential regulators of the formation of granulation tissue after infarction

We have recently identified the elevated expression of a mammalian homologue of the Drosophila tissue polarity gene 'frizzled', frizzled2 (fz2), during cardiac remodeling [68]. This gene is highly conserved during evolution, and eight mammalian homologues have been identified so far [69]. The frizzled gene encodes a protein with a predicted 7-transmembrane-receptor structure, for which a role in the architectural control has been shown in the developing Drosophila. Recently, Wnt proteins, which also act as architectural control genes in Drosophila, have been identified as ligands for frizzled receptors. In situ hybridisation revealed that the fz2 gene is highly expressed in myofibroblasts during their migration into the area of infarction [70]. When the cells become stationary in the newly formed scar, the expression of the fz2 gene is decreased. Moreover, the dishevelled 1 (dvl1) gene, known to function as a signal transduction molecule of frizzled receptors, shows a similar pattern of expression as the fz2 gene [71]. These findings suggest the presence of a frizzled signal transduction cascade, which involves dvl1, during the migration of myofibroblasts into the area of infarction.

For the signal transduction of frizzled receptors several different pathways have been proposed (Fig. 2). The first pathway, which is the best studied so far, makes use of β-catenin as the intracellular signal transduction molecule [72]. This molecule can, in a complex with a number of other proteins, interact with E-cadherin, thereby linking the cytoskeleton to the extracellular environment (Fig. 2). This would explain the action of the frizzled receptors as polarity genes, since tissue polarity can be regarded as an alignment of the cytoskeleton of a cell with its neighboring cells or with the extracellular matrix. Moreover, β-catenin can also induce proliferation of cells by interaction with the T-cell factor (TCF) family of transcription factors. It is interesting to note that β-catenin has recently drawn a lot of attention, because of its association with malignities. Mutations in the Adenomatous Polyposis Coli (APC) protein, impairing the degradation of β-catenin, have been observed in colorectal cancer and melanoma as well as other malignities [73]. These observations seem to be in agreement with the oncogenic action of some of the Wnt proteins.

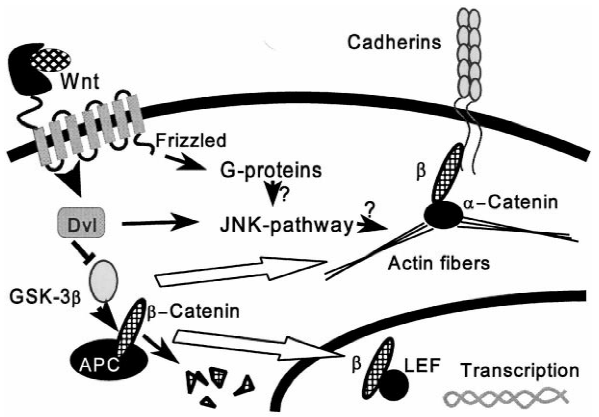

Fig. 2. Signal transduction pathways of frizzled receptors.

Other reports suggest a coupling between frizzled receptors and GTPases, either of the Gi subtype of the G-protein family [74] or with members of the Rho family [75,76]. It is not clear whether the different members of the frizzled family can couple in a selective way to one of the proposed signal transduction pathways or whether they can cross-talk. However, based on the interaction with the cytoskeleton, both β-catenin and rho-proteins are primary candidates for mediating the architectural control as exerted by frizzled receptors.

The Wnt protein/frizzled receptor system, however, is not the only regulator of the formation of granulation tissue. Other more classical regulators like the renin angiotensin system and the plasminogen/plasmin system also contribute to the control of cardiac wound healing.

In parallel with its function in vascular and dermal wound healing, where the plasminogen/plasmin system has been shown to play a key role in the control of cell migration [77,78], the plasminogen/plasmin system may also be an important regulator of the control of cardiac wound healing. There are several arguments in favor of this hypothesis. Plasmin is a key regulator of fibrinolysis and extracellular matrix turnover. It degrades a variety of extracellular matrix proteins, either directly or indirectly through activation of matrix metalloproteinases. As discussed earlier, matrix metalloproteinase activity is known to be upregulated after infarction [16,17,79].

The conversion of plasminogen to plasmin by plasminogen activators is controlled by a specific inhibitor, plasminogen activator inhibitor type 1 (PAI-1). Another important regulator of cardiac remodeling, the renin angiotensin system, seems to affect the activity of the plasminogen/plasmin system. Indeed AII, increases the expression and activity of PAI-1 [80], which will result in an enhancement of collagen deposition. Moreover, the DD polymorphism of the human ACE gene is associated with enhanced levels of PAI-1 [81] and ACE inhibitors decrease PAI-1 levels in patients with acute myocardial infarction [82]. This effect seems to be mediated by the hexapeptide Angiotensin IV (Ang II, [3–8]) and the AT4 receptor subtype [83] and not by TGFβ1 [84]. We have recently tried to test the hypothesis that the plasminogen/plasmin system is one of the regulators of cardiac wound healing

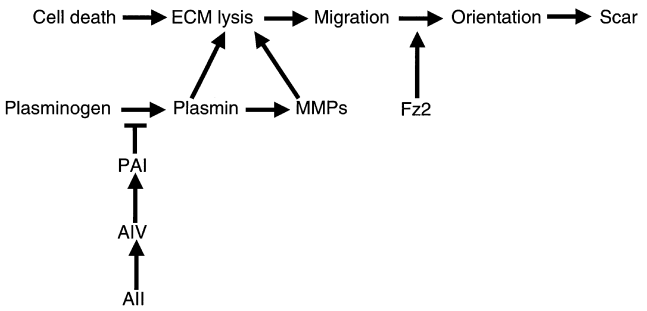

Fig. 3. Factors in cardiac structure that determine cardiac function after infarction.

after infarction, by inducing a myocardial infarction in mice that lack a functional plasminogen gene. The first, preliminary, results do indeed show that cardiac wound healing is severely impaired in these mice [85].

7. Conclusions

In this review we have tried to identify those factors in the cardiac structure that determine cardiac function after infarction (Fig. 3). Knowledge of those factors may eventually lead to new targets for future interventions.

In the non-infarcted left and right ventricle the surviving myocytes appear to be such targets. Prevention or reduction of myocyte apoptosis, enhancement of myocyte DNA synthesis and myocyte differentiation may all improve cardiac function after infarction. This may occur by direct activation of the transcriptional activity of the myocytes [62], or indirectly through effects of growth-factor/receptor systems [66,86] on the cardiomyocytes or on the surrounding capillary network [26,87].

From our review it is evident that infarct healing is an active and tightly controlled process. Potential new targets that arise from this recent knowledge can already be identified. Reduction of ventricular dilatation by interventions in the control of the formation of the scar after infarction may be such a target. Interventions in the Wnt protein/frizzled receptor or in the plasminogen/plasmin system, may thus become candidates to reduce ventricular dilatation after infarction.

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