Mechanisms of cell death in acute myocardial infarction: pathophysiological implications for treatment

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The purpose of this review is to draw attention to the growing list of pathophysiological phenomena occurring in blood, the vessel wall and cardiac tissue during myocardial infarction. A further aim is to point to the complexity of factors, contributing to cardiac dysfunction and the implications for therapy, aimed at limiting myocardial cell death. Not all pathophysiological mechanisms have been elucidated yet, indicating the necessity for further research in this area. In addition we describe interventions which have shown promise in animal studies, those which may show promise in humans, and those which are accepted as therapies of choice. (Neth Heart J 2001;9:30-44.)

Key words: acute myocardial infarction, cell death, treatment

Since the early 1970s it has become clear that in patients with acute myocardial infarction, the severity of haemodynamic abnormalities,[1] the incidence of shock,[2] the frequency of ventricular arrhythmias[3] and the prognosis, both in hospital[4] and after discharge[5,6] is related to infarct size. Therefore, treatment of acute myocardial infarction is directed towards limitation of infarct size and prevention of complications. Today this goal is best achieved by early reperfusion of the ischaemic myocardium.

Physicians caring for patients with an acute myocardial infarction, receiving reperfusion therapy (thrombolytics, percutaneous transluminal coronary angioplasty or cardiac surgery), should be aware of factors that contribute both to myocardial ischaemia and reperfusion damage. Thanks to experimental studies that have been performed in animal models and in ex-vivo preparations, the amount of new knowledge on basic mechanisms in this field is almost exploding.

This article reviews the current knowledge on anatomy, physiology and pathophysiology, at the cellular level, contributing to myocardial ischaemia and reperfusion injury. Furthermore, the investigative work done in animals and the implications for treatment, which may possibly reduce myocardial damage and infarct size in humans, will be discussed.

Anatomical and pathophysiological considerations

A comprehensive knowledge of myocardial anatomy at the cellular level is an indispensable foundation for the clinician. In addition, ultrastructural studies of the myocardium have greatly contributed to our understanding of cardiac function.

The cardiac muscle is made up of individual fibres, which are the 'building blocks' of the cardiac muscular system. The fibres are composed of fibrils and the fibrils are divisible into filaments. These filaments contain contractile proteins (myosin and actin).

The fibres are arranged in parallel as a series of cells termed myocytes, so that force of contraction of the units is additive. A fibre is surrounded by a membrane, the sarcolemna, which becomes fused to the membrane of a neighbouring fibre. These 'tight junctions' (intercalated disks) provide low electrical resistance between the fibres, enabling rapid spread of electrical activity from one cell to the next. Invaginations of the sarcolemna form transverse tubules (T tubules), which enable extracellular fluid to penetrate deep within the cytoplasm of the myocardial cell.

A muscle fibril is surrounded by a structure made up of a unit membrane, which appears as a vesicle, and a tubule. This structure forms an irregular curtain, the sarcoplasmic reticulum, which is closely adjacent to the T tubules. This is considered the most likely site from which calcium ions are liberated to initiate contraction of the myocardial cell.

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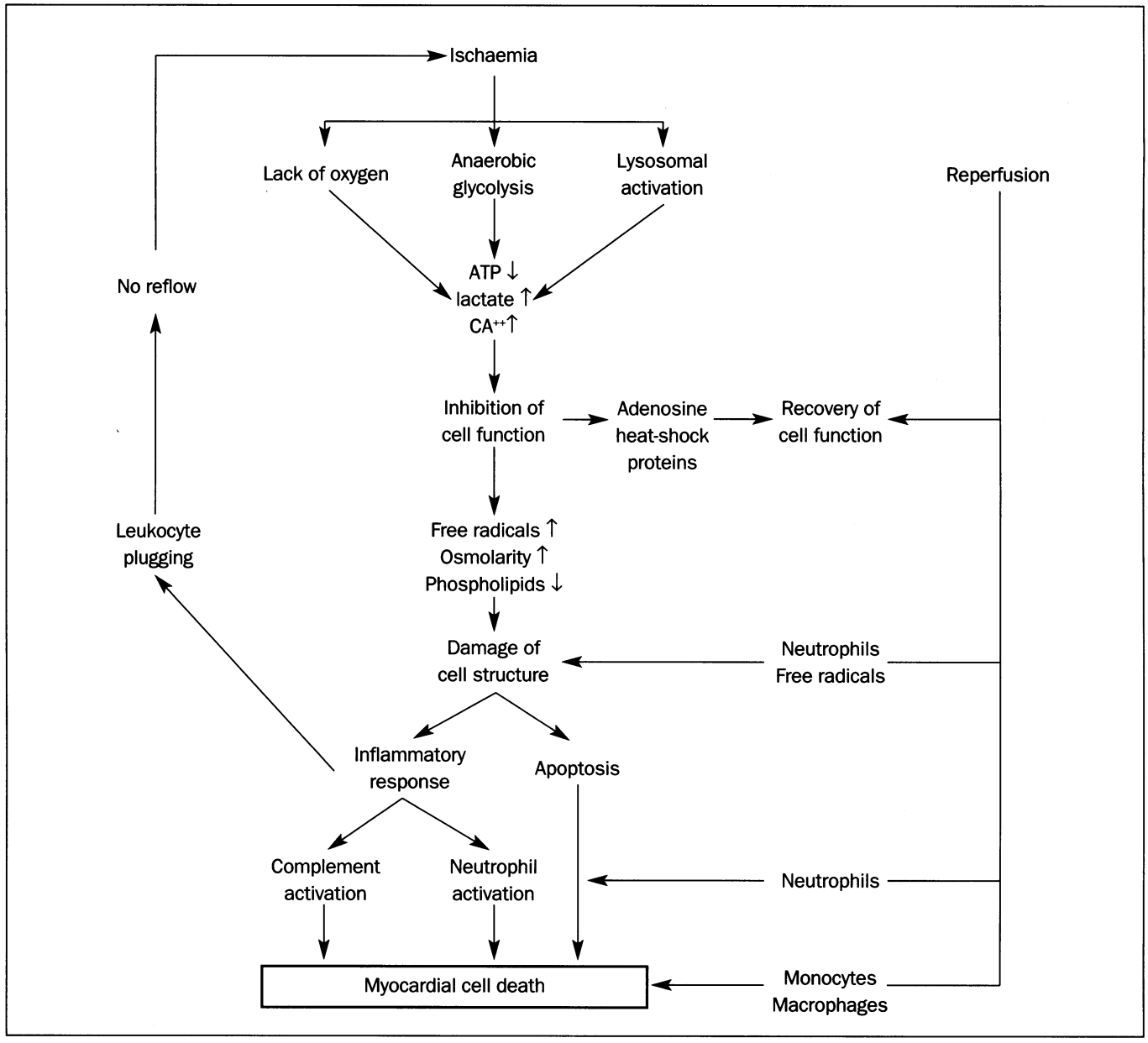

Figure 1. Proposed pathogenesis of postischaemic myocardial cell injury. This proposal integrates and reconciles different mechanisms into an unifying pathogenetic hypothesis. The ultimate consequence of this complex series of perturbations is initially a reversible depression of cell function and ultimately myocardial cell death. Legends: ATP indicates adenosine triphosphate; Ca^{++}: calcium (overload).

The nucleus of the myocardial cell is centrally positioned, often with a perinuclear lighter zone. The mitochondria are located in between the myofibrils and represent the main energy source for myofibrillar contraction.

An area of total ischaemia has no flow and diffusion is very slow from the centre of the area of ischaemia. All products of ischaemic metabolism are trapped in the region. With small amounts of collateral flow, exchange is very slow but faster than when there is no flow.

Acute myocardial ischaemia may lead to different degrees of myocardial cell injury. If there is a single sudden episode of ischaemia associated with a persistent complete thrombotic occlusion without collaterals, the degree and extent of myocardial cell injury can differ from situations in which the infarct-related artery does not remain occluded and collaterals are present. The extent of myocardial cell injury may vary from small regional injury to a non-transmural (subendocardially located) lesion or to transmural damage of myocardial structure.

In addition, intermittent coronary occlusion, or total occlusion in the face of well-developed collateral

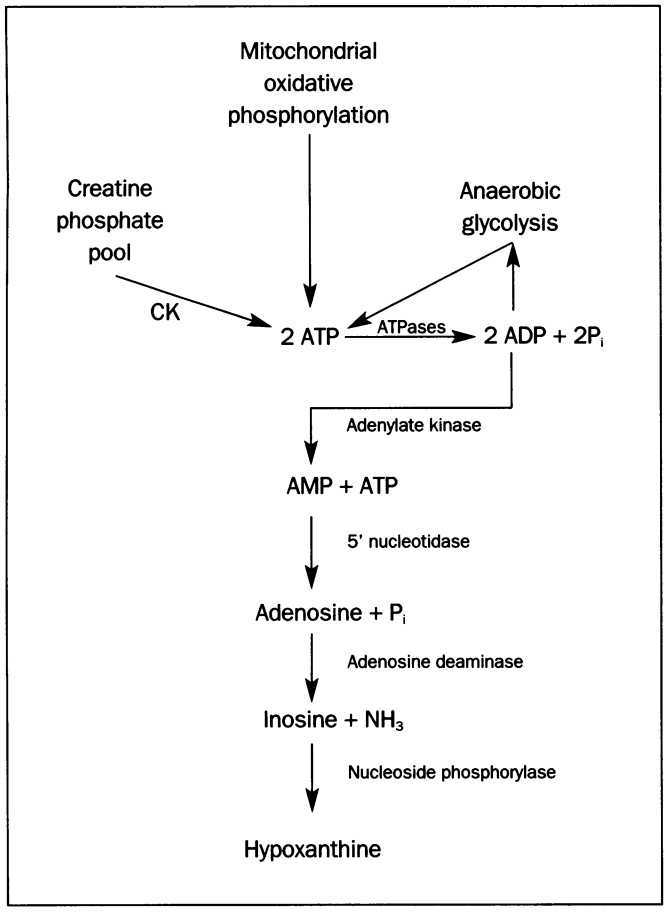

Figure 2. Diagram depicting enzymatic reactions involved in myocardial energy metabolism. During ischaemia breakdown of high-energy phosphates becomes predominant. In severe ischemia the utilisation of ATP results in a proximately 50% early increase in ADP. However, this increase is transient because of the adenylate kinase reaction. After 10 minutes of severe ischaemia, declining ATP content is paralleled by declining ADP. Moreover, the AMP produced by adenylate kinase is catabolised further, primarily to adenosine via the enzyme 5' nucleotidase (located both within the cytosol and in the sarcolemna). Adenosine does escape from the myocyte and is further catabolised to inosine-hypoxanthine by the enzymes adenosine deaminase and nucleoside phosphorylase (localised to endothelial cells or pericytes). Legends: ADP indicates adenosine diphosphate; AMP: adenosine monophosphate; ATP: adenosine triphosphate; CK: creatine kinase.

circulation, may lead to several modes of inhibition of function at the cellular level. Stunning is a form of prolonged contractile dysfunction that occurs after relief of a discrete episode or episodes of ischaemia; hibernation is a form of prolonged contractile dysfunction associated with ongoing low blood flow; preconditioning is a cardioprotective mechanism in which the heart is exposed to a short period of sublethal ischaemia that attenuates cellular damage from a subsequent prolonged lethal episode of ischaemia. These modes of inhibition of myocardial function have significant clinical implications suggesting that myocardial salvage is possible at the cellular level. Why they are so relevant will be illustrated in the section on therapeutic implications.

Patients promptly treated after the onset of symptoms, by restoration of patency of the infarct-related artery, will have the greatest benefit of reperfusion treatment. This time-dependent treatment saves myocardial tissue and therefore results in a better prognosis. However, the benefit of reperfusion treatment three to four hours after onset of symptoms is more or less equal to the positive effect of an intervention later after acute myocardial ischaemia, suggesting time-independent effects and the role of different mechanisms of cell death.

Several mechanisms of myocardial tissue injury, occurring during and after diminution of myocardial perfusion, have been elucidated and will be discussed.

Mechanisms involved in/or responsible for injury (figure I)

1. Oxygen deprivation and depletion of high-energy phosphates

To sustain the continuous contractile function, the myocardium is absolutely dependent on aerobic metabolism for the production of energy in the form of adenosine triphosphate (ATP), because myocytes contain very limited reserve stores of high-energy phosphates. During normoxia, ATP is produced in the mitochondria by oxidative phosphorylation. Under physiological conditions there is hardly any break down of the high-energy phosphates to purines, as the purine-producing enzymes are scarcely active. However, during oxygen and substrate deprivation, breakdown of high-energy phosphates becomes predominant (figure 2), a disorder that is accompanied by cellular and subcellular alterations in the cardiac myocytes.

Furthermore, in response to oxygen deprivation, adenosine can be released from the myocytes. It may enter the extracellular space where it has multiple effects. Its protective role is manifested by vasodilatation and by effects to decrease myocardial oxygen demand (i.e. negative ionotropism, chrono-tropism and dromotropism). During these periods adenosine on hand enhances energy production via increased glycolytic flux. During reperfusion it can act as a substrate for purine salvage (by phosphorylation to AMP) to preserve the adenine nucleotide pool.[7]

2. Osmotic cell stress

The myocardium can utilise a variety of metabolic substrates, including fatty acids, glucose, ketone bodies and amino acids. The myocytes preferentially oxidise fatty acids; furthermore, glucose is converted to glycogen. During severe ischaemia and following the accumulation of ATP catabolites,

the availability of glucose transporters increases[8] and anaerobic glycolysis is activated through enhanced activity of the glycolytic enzymes (e.g. phosphofructokinase and glyceraldehyde-3-P-dehydrogenase).[9] As an endpoint of the glycolytic pathway, pyruvate will be transformed into lactate. Thus, ischaemia induces a change from myocardial lactate extraction to lactate production. The lactate production leads to osmotic cell stress and water accumulation in the cells causing sarcolemmal disruption.

The myocardial interstitium plays an important role in the regulation of cardiac function. Post-ischaemic water accumulation in the myocardium results in structural alterations reducing myocardial function and activating the renin-angiotensin-aldosterone system, which lead to myocardial fibrosis.[10]

3. Lysosomes

In the cytoplasm of the cell, there are large, somewhat irregular structures surrounded by a unit membrane. These organelles are called lysosomes and contain enzymes, which could cause destruction of most cellular components if these enzymes were not separated from the rest of the cell by a unit membrane. During long-lasting ischaemia, the lysosomes are activated and may hydrolyse the cell membrane and cell content by way of exposure to these destructive enzymes.[11] As a result, the development of a severe membrane permeability defect allows the unregulated influx of divalent and trivalent cations, including calcium. Just as with lactate production, the influx of cations leads to osmotic cell stress and water accumulation in the cells causing sarcolemmal disruption.

4. Intracellular calcium

The functions of excitable cells that are fundamental to the cardiovascular system are governed in part by the behaviour of the semipermeable hydrophobic membranes that envelop them. These membranes control the movement of some ions into and out of the cells against their concentration gradients by ion pumps, by ion exchangers, and by ion-carrying channels that can open in response to a transmembrane potential difference.

Calcium flux is controlled by all three mechanisms. The calcium-selective voltage-sensitive channels provide the route for little calcium entry that is ultimately responsible for excitation-contraction coupling. They can be subdivided into four main varieties, designated L, T, N and P types. The calcium that enters myocardial cells acts as the major trigger for release of more calcium from the main internal reservoir in the sarcoplasmic reticulum.

During ischaemia, anaerobic metabolism leads to intracellular acidification and to activation of pH regulation ion transport systems as the Na^+/H^+ exchangers. This causes an increased influx of Na^+ ions that cannot be sufficiently extruded by the energy-depleted cells (via Na^+/K^+ ATPase). The resulting intracellular Na^+ overload leads to an increase of intracellular Ca^{++}, because Na^+ and Ca^{++} are reversed by Na^+/Ca^{++} exchange.

The channels permitting the release of calcium from the sarcoplasmatic reticulum are also influenced by ischaemia. This may increase the probability of their remaining open, initially (in reversibly injured myocytes) resulting in very limited calcium overload with contraction band injury and triggering activation of phospholipases and proteases, and possibly impairment of oxidative phosphorylation.[12] In irreversibly injured myocytes, these processes will eventually lead to damage to the membrane phospholipids and ion channels, and lowering of the ATP production, and will ultimately accelerate cell necrosis.

5. Complement system

Acute myocardial ischaemia also induces activation of the complement system, a cascading series of plasma enzymes and proteins. Complement induction is caused by various pro-inflammatory cytokines, which are released from the inflamed tissue and stimulate the liver to synthesise a number of acute-phase proteins. C-reactive protein (CRP), regarded as a prototype of acute-phase proteins in humans, has the ability to activate complement.[13-15] Through the 'classic' and 'alternative' pathways, cytolytic membrane complexes are produced.[16] Complement complexes are deposited in myocardial fibres, located within the zones of infarction, and form a scroll in the cellular membrane that comprises transmembrane pores.[13]

Furthermore, several experiments provide compelling evidence for another pathological role of complement activation, namely in the chemo-taxis of neutrophils[13,17] associated with reperfusion injury[18] and in the enhancement of the 'late' no-reflow phenomenon.[19] (see under Plugging)

6. Apoptosis

Recently, considerable attention has been directed to another form of cell death, referred to as apoptosis. Cleavage of DNA at linking regions between nucleosomes (to form series of double-stranded DNA fragments), is indicative of cells undergoing 'programmed cell death'.[20] Next to the changes in the nucleus, the apoptotic process involves changes in the composition of the cell membrane, changes leading to nuclear condensation and cellular shrinkage ('oncosis'). The apoptotic processes trigger rapid phagocytosis of apoptotic bodies by polymorphonuclear leukocytes and adjacent myocardial cells.

In the acute stage, hypoxia may cause expression of an inhibitory (bcl-2) protein of

Table 1. Myocardial cell death: animal data.

Mechanism		Potential treatment
1.	O_2 and substrate deprivation	β-Blocker: +[79,80,100-103], GIK: +[81,82], vasodilators: +[85,86], Magnesium: +[109], Adenosine: +[7], Monophosphoryl lipid A: +[112], Succinate: +[204]
2.	Osmotic stress	Mannitol: +/- [113-115]
3.	Lysosomal activation	Curcumin: +[11]
4.	Calcium overload	Calcium channel blockers: +[118], Na^+/H^+ exchange inhibitor: +[120], Magnesium: +[109], Nitric oxide: +[121], Carvedilol: +[122], Captopril: +[123]
5.	Complement activation	Cobra venom factor: +[124], C1EINH: +[18,125], Heparin: +[126]
6.	Apoptosis	Bcl-2 expression: [130], ACEI: +[131], β-Blocker: +[131], Nitric oxide synthetase inhibitor: +[133]
7.	PMN infiltration	Corticosteroid: + :87, Prostacyclin: +[134], Adenosine: +[7,135], 'Depletives ': +[46,136,137], 'Anti-adhesives ': +[29,65,138-154]
8.	Free radicals formation	Co-enzyme: +[163], Captopril: +/-[165-167], Scavengers: +/-[169-181]
9.	Plugging	Nifedipine-Nisoldipine: +[185], Fluosol: +[187], Adenosine: +[188]
10.	Heat-shock protein production	Amphetamine: +[51,189], Transcription induction: +[190-192]

Legends: ACEI indicates angiotensin converting enzyme inhibitor; C1EINH: C1 esterase inhibitor; GIK: Glucose-Insulin-Potassium; PMN: Polymorphonuclear leukocytes; (+,-: #): with, without effect: references.

apoptosis in surviving myocytes; however, at the more advanced stage, expression of a promotive (Bax) protein may start the programmed cell death.[21,22] Although these processes of programmed cell death are not typically associated with inflammatory cell infiltration,[23,24] cytokines, as tumour necrosis factor, may play a role in the induction of apoptotic death.[25,26] Furthermore, apoptosis has recently been shown to depend on the activation of a class of proteases ('caspases').[27] Lastly, reactive oxygen species may play a role as mediators of apoptosis by causing mitochondrial alterations.[28]

7. Inflammatory cells

As a reaction to ischaemic tissue injury, by migration and infiltration of (activated) neutrophils, a secondary inflammatory component of injury can be seen.[29] In classic histopathological descriptions, neutrophils are predominantly present during the first 12 to 24 hours, whereas monocytes and macrophages are found in the cardiac tissues two or three days after the ischaemic event.[30,31] Although it is likely that infiltrating neutrophils injure cardiac myocytes, monocytes and macro-phages may have other roles including clearance of debris and promotion of scar tissue formation.[32-34]

8. Oxygen free radicals

Under normal conditions small quantities of oxygen free radicals are produced, but they are quenched by intracellular free radical scavenging enzymes (superoxide dismutase, catalase, glutathione peroxidase) or alpha-tocopherol.[35] However, more reactive oxygen species and free radicals are generated upon the onset of ischaemia reperfusion.[36,37] Since free radicals possess an unpaired electron, they are very reactive and can generate another radical. An example of such behaviour is the hydrogen abstraction mechanism, operated by the hydroxyl radical on the polyunsaturated fatty acids of membrane phospholipids. The hydroxyl radical starts a chain of reactions, which ultimately lead to lipid peroxidation of cell membranes resulting in loss of fluidity and changes in permeability.[38,39]

Furthermore, oxygen free radicals are known to stimulate platelet aggregation after exposure to anoxia-reoxygenation.[40] On the other hand, free radicals may also have a beneficial role. Reactive oxygen metabolites are known to be important in our natural defence against infection, and may well initiate tissue repair by, for example, promoting fibroblast proliferation.[41]

9. Plugging

Brief periods of ischaemia are insufficient to produce local activation of complement, formation of chemotactic factors or activation and infiltration of neutrophils.[42] However, during prolonged ischaemia the endothelial cell activity changes and pro-inflammatory cytokines are released, stimulating neutrophil accumulation.[43,44] Reperfusion markedly increases the number of available neutrophils circulating through the injured area. These reactions may lead to plugging by the leukocytes and, as a result of the 'no-reflow phenomenon' and well beyond coronary occlusion and reflow, to secondary inflammatory injury of potentially viable tissue.[45-47] This phenomenon can occur in ischaemic periods lasting more than three hours and can become obvious several hours after reperfusion, especially at the inner portion of the left ventricular wall.[48]

10. Heat-shock protein

The cardiomyocyte can form stress proteins or heat-

Table 2. Myocardial cell death: human data.

Mechanism	Potential treatment
1. O_2 and substrate deprivation	β-Blocker: +[89,90,104-108,193-195], GIK: +[91,200,201], vasodilators:+[94-97,196-198], IABP: +[202], Magnesium: +[110,111], Adenosine: +,s[206], Monophosphoryl lipid A (s)
2. Osmotic stress	Mannitol: -[116,117]
3. Lysosomal activation	
4. Calcium overload	Calcium channel blockers: -[119], Na+/H+ exchange inhibitor: +,s[207]), Magnesium: +[110,111]
5. Complement activation	C1EINH (s)
6. Apoptosis	
7. PMN infiltration	Corticosteroid: -[98], Adenosine: +,s[206]
8. Free radicals formation	Streptokinase: +[155], Co-enzyme Q10: +[161,164], Captopril: +[168], Allopurinol: +/-[182-184]
9. Plugging	Nitroglycerin: +[186], Adenosine: +:s,[206]
10. Heat-shock protein production	

Legends: ACEI indicates angiotensin converting enzyme inhibitor; C1EINH: C1 esterase inhibitor; GIK: Glucose-Insulin-Potassium; IABP: Intra-aortic balloon pump; PMN: Polymorphonuclear leukocytes; (+, -, s: #): with, without clinical effect, running study: references.

shock proteins. These proteins can reduce infarct size: they have delayed beneficial effects on preconditioning the heart, which enhances cellular tolerance to ischaemia-reperfusion injury[49] and can reduce free radical-mediated reperfusion injury.[50,51] The amount of (induced) proteins most likely correlates with the extent of myocardial salvage.[50,52]

Reaction to injury

As described above, inflammatory cells play an important role 'as a reaction to injury'. Influx of inflammatory cells into the ischaemic myocardium is seen as a secondary phenomenon to injury of myocardium resulting from the previous episode of ischaemia.[53,54] The influx of inflammatory cells occurs late after continuous ischaemia and early after reperfusion.[55]

Chemical substances play a role in attracting circulating neutrophils: chemotaxis.[55] The initial step consists of adhesion of neutrophils to the endothelial cell. This phenomenon is called 'rolling-sticking'. The neutrophil-endothelial cell interaction is caused by at least two classes of adhesion molecules, which are expressed on the surface of neutrophils. These include L-Selectin, which is constitutively functional on non-activated neutrophils during 'rolling' and the β_2-integrins, which are upregulated during 'sticking' when neutrophils are activated.[29,56] The next step of neutrophil-endothelial cell adhesion involves neutrophil 'trapping' in the microvasculature, specifically in capillaries,[57] which is induced by several adhesion molecules on the vascular endothelial cells, like P-Selectin and intercellular adhesion molecule or ICAM.[58,59] If occlusion of an artery is followed by reperfusion, neutrophil activation, by generation of platelet-activating factor (PAF),[29,60,61] and intercellular adhesion, by adhesion molecules on neutrophils (ICAM),[62] lead to neutrophil 'plugging'. This step is followed by transendothelial migration of the neutrophils into the extravascular compartment, which is induced by factors like platelet-activating factor,[29,60,61,63] and associated with adhesion molecules on the neutrophil (ICAM),[57] in the interstitial fluid (C5a)[64] and on the surface of the myocyte (ICAM).[62,65] The release of monocyte cytokines can stimulate the neutrophil-myocyte adherence.[59,66,67]

Furthermore, during ischaemia, neutrophils can release several products like autocoids, such as thromboxane A2 and leukotriene B4, which induce platelet aggregation and vasoconstriction.[63,68,69] During reperfusion, neutrophils are able to generate oxygen-derived free radicals,[29] suggesting that neutrophils may directly injure parenchymal myocardial cells.

Lastly, neutrophils may secrete growth factors, such as transforming growth factor. TGF-β stimulates fibroblast growth and neovascularisation[70] and inhibits acute inflammatory responses following ischaemia reperfusion.[71] One might speculate that reperfusion of the myocardium at a later time (the open vessel hypothesis) would accelerate neutrophil cell influx and thereby promote healing.

All the biological processes described above will determine the extent of infarct size, and will influence the course, consequences and ultimately the prognosis of the patient. When these systems are activated under extraordinary conditions, they can potentially become deleterious.

Therapeutic implications

Certain factors can predict death in patients admitted to hospital with acute myocardial infarction. These include age (over 65 years of age), previous medical history (like diabetes, or previous infarction), infarct size, site of infarction (anterior vs. inferior), low initial blood pressure (systolic pressure <100 mmHg), presence of pulmonary congestion, time-dependent restoration of patency of the infarct-related artery and extent of additional ischaemia.[72-76] Other factors are associated with a higher mortality in the subacute phase in Q-wave and non-Q-wave infarction.[77,78] The importance of these factors is attested by positive

correlations between the incidence of these factors and the size of myocardial injury.

Myocardial cell death inhibition

The extent of myocardial necrosis, developing during the course of a myocardial infarction, can be both dependent and independent of the underlying coronary pathology. This concept has been the focus of extensive investigation (tables 1 and 2). In the late 1960s, studies in laboratory animals showed that the extent and severity of myocardial ischaemic injury, consequent to coronary occlusion, could be altered substantially by a number of pharmacological interventions to preserve energy stores: β-blockers,[79,80] glucose-insulin-potassium infusion,[81,82] hyaluronidase,[83,84] trimethaphan,[85] nitrates.[86] The pharmacological interventions could also retard disintegration of necrotic myocytes and delay the inflammatory process.[87]

In the 1970s 'the time for testing in humans to reduce infarct size had come', according to Braunwald. [88] In patients there were no particular difficulties in applying most of the 'early' interventions that had been demonstrated to exert positive actions in animal models: β-blockers,[89,90] glucose-insulin-potassium-infusion,[91] hyaluronidase,[92,93] trimethaphan[94] and nitrates.[95-97] The demonstration of a possible beneficial effect was based on the use of a variety of techniques, including electrocardiographic findings, biochemical methods and radionuclide imaging. However, experience with the anti-inflammatory strategy of methylprednisolone, which appeared to influence myocardial injury favourably in experimental models, resulted in an increased incidence of ventricular aneurysm and rup-ture when applied clinically.[98]

During the last decades of last century, there came a better understanding of the many mechanisms by which tissue injury during myocardial ischaemia and after reperfusion occur. Although we have to realise that not all the described mechanisms will ultimately determine the extent of infarct size, and although concepts of mechanisms of myocardial injury continue to be complex and controversial,[99] by intervening in some of these mechanisms, deleterious effects could be diminished while retaining the positive reparative effects. Several studies identified from the literature will be discussed.

1. **Oxygen deprivation and depletion of high-energy phosphates**

   Immediate administration of β-blockers limited infarct size in experimental models. Early administration of these agents preserved mitochondrial function during periods of hypoxic substrate-free perfusion,[100] increased subendocardial blood flow and improved segmental wall function in the ischaemic region of partially occluded vessels.[101,102] Early intravenous administration of metoprolol plus rt-PA enhanced the effects of thrombolysis on infarct size and left ventricular function in experimental myocardial infarction.[103] In humans the demonstrated reduction in enzyme levels and the electrocardiographic benefits strongly suggest a true positive effect produced by early intravenous β-blockade in patients with definite myocardial infarction at entry.[104,105] Reduction in cumulative enzyme output appeared to be around 20%, at least for patients who were treated within the first few hours of the onset of pain.[104] However, the concept that immediate administration of β-blockers alone, or in combination with thrombolytic therapy, does indeed limit infarct size in humans has not been definitely proven.[106-108]

   Magnesium protected animals against ischaemic injury by preservation of intracellular ATP and creatine phosphate reserves.[109] Patients with acute myocardial infarction treated with magnesium chloride or sulphate showed significantly less heart failure than those who received placebo and had equal peak creatine kinase.[110,111]

   Adenosine limited the degree of vascular injury during ischaemia and reperfusion by en-hancement of energy production via increased glycolytic flux and by acting as a substrate for purine salvage in the animal model.[7] At the same time it inhibited both oxygen radical release from activated neutrophils, thereby preventing endothelial cell damage, and platelet aggregation, (thereby pre-serving microvascular perfusion).[7]

   Monophosphoryl lipid A had cardioprotective properties in various animal models, both during ischaemia (associated with preservation of ATP) as well as during reperfusion (with induction of 5-nucleotidase, which removes the phosphate group from AMP thus forming adenosine, and enhancement of calcium re-uptake by sarcoplasmic reticulum).[112] At present this drug is undergoing clinical investigation.

2. **Osmotic cell stress**

   Administration of the hyperosmotic agent mannitol could diminish tissue oedema and attenuate the rise in NMR relaxation parameters and ultrastructural myocyte injury in ischaemia-reperfused myocardium in an animal model.[113,114] However, in freshly isolated adult rat myocytes,[115] as in patients,[116,117] beneficial effects of mannitol could not be demonstrated.

3. **Lysosomes**

   Oral treatment with a natural product of plants, called curcumin (the major yellow pigment in turmeric and the Indian food curry), had a protective effect against the damage caused by myocardial ischaemia in rats. Curcumin inhibited the disintegration of cell membrane polyunsaturated fatty acids by reducing the release of beta-glucuronidase from e.g. lysosomes.[11]

4. Intracellular calcium

Calcium antagonists have the potential to prevent or mitigate some of the processes leading to calcium overload. They are coronary vasodilators. As with β-blockers, their negative inotropic effects reduce metabolic demand, they protect mitochondrial function during ischaemia, but afterwards they reduce calcium flux through calcium channels thereby possibly aggravating stunning. Several types of drugs have shown protective effects experimentally when the drug was used prophylactically.[118] However, in patients most results to date have been disappointing.[119]

Activation of Na^+/H^+ exchange in myocardial ischaemia and/or reperfusion leads to calcium overload and myocardial injury. Experimental studies have shown that Na^+/H^+ exchange inhibitors can attenuate Ca^{++} influx into cardiomyocytes.[120] Clinical trials are running but so far have not produced evidence of the benefit of this type of treatment (see section on future considerations.)

Magnesium has also been described as a physiological calcium antagonist, because it inhibits mitochondrial calcium overload.[109] As described above,[110,111] treatment with magnesium in acute myocardial infarction resulted in significantly less patients with cardiac insufficiency than placebo, suggesting a cardioprotective effect.

Nitric oxide, derived from organic nitrate esters, stimulates soluble guanylate cyclase. The guanylate cyclase produces cyclic-GMP and acts via a cyclic-GMP dependent protein kinase. Ultimately this protein kinase lowers intracellular calcium,[121] which results in dilatation of vessels and inhibition of platelet aggregation.

Carvedilol reduced infarct size by 90% in a pig model. The cardioprotective effect may result both from the combined effects of β-adrenoreceptor blockade and vasodilatation and from inhibition of intracellular calcium.[122]

Captopril partially normalised the defect in excitation-contraction coupling in rats with post-infarction heart failure, among other ways by partial normalisation of intracellular Ca^{2+} handling.[123]

Thus, by various processes it seems to be possible to lower intracellular calcium and thereby improve prognosis.

5. Complement system

Studies in the late 1970s showed that inactivation of the third component of the complement system in vivo with cobra venom factors resulted in a reduction of the inflammatory response subsequent to ischaemic damage and caused a significant reduction in myocardial damage.[124] Blocking of the classic complement pathway by a C1 inhibitor in a feline or pig model appeared to be an effective way of preserving the ischaemic myocardium from reperfusion injury. The cardioprotective effect is caused by an inhibition of a polymorphonuclear leukocyte-endothelium interaction.[18,125] At present a C1 inhibitor is undergoing clinical investigation in patients with myocardial infarction.

Heparin and N-acetyl heparin could significantly reduce the extent of myocardial injury associated with (90 minutes of) regional ischaemia and (six hours of) reperfusion in the canine heart. The mechanism of cytoprotection is not related to alterations in the coagulation cascade but may involve inhibition of complement activation in response to tissue injury.[126]

The activated complement system also affects the size of myocardial necrosis and cardiac function in humans. Treatment with thrombolytic agents produces abrupt activation of the complement system, caused by increased levels of anaphylatoxin C4a, C3a and membrane attack complexes C 5b-9,[127,128] an effect probably mediated by plasmin, by activated factor XII.[128,129] As a result, the abrupt complement activation is associated with plugging of cells in the microcirculation,[19] thus diminishing the positive beneficial effect of thrombolytic treatment. By giving patients with acute myocardial infarction thrombolytic agents together with inhibitors of complement dependent activation, one may be able to decrease both the ischaemic and the reperfusion damage.

6. Apoptosis

Many studies have demonstrated how signal transduction can influence the apoptotic pathways in different types of cells. Only a very limited number of these studies have been performed in an animal model with ischaemia-reperfusion injury to the heart.

Experiments on the expression of the cell death inhibitory gene bcl-2 have started, trying to prevent apoptotic changes and/or to delay programmed cell death.[130] Up to now only the clinically used converting enzyme inhibitors and β-blockers have been shown to have inhibitory effects on the production of transcription factors and thereby on genes involved in the apoptotic programme.[131]

An increased inducible nitric oxide synthetase activity appeared to be related to the induction of apoptosis in infiltrating macrophages and cardiomyocytes.[132] Preferential inhibition of nitric oxide synthetase (by S-methylisothiourea sulphate) resulted in a significant improvement of left ventricular performance and increased regional myocardial blood flow in rabbits.[133]

7. Inflammatory cells

It is not within the scope of this article to assess the extensive literature on inflammatory cells and myocardial infarct size. Many animal studies were performed, where pharmacological interventions altered the function or the influx of inflammatory

cells and thereby influenced the microvascular injury during ischaemia and after reperfusion. These findings illustrate that the neutrophil is a potent pathological mediator of the endangered cardiac tissue. Studies were performed with agents, such as prostacyclin analogues[134] and adenosine,[135] which alter neutrophil functions. Different strategies were designed to reduce neutrophil numbers, such as neutrophil antibodies,[136] antimetabolites[137] and neutrophil filters.[46]

Furthermore, the administration of antibodies or antagonists to and blockers of several adhesion molecules, such as selectin, CD 18 integrin, ICAM and PECAM, could reduce neutrophil accumulation[138-144] and sometimes significantly limit myocardial infarct size, by up to 50%.[65,142-151] When the neutrophils were pretreated with platelet-activating factor receptor antagonist, plasma-mediated neutrophil stimulation was prevented[29] and myocardial infarct size was reduced.[152-154]

Streptokinase modulates human neutrophil function and reduces superoxide production by polymorphonuclear leukocytes.[155] However, so far no other pharmacological interventions aimed at reducing injury during ischaemia and reperfusion are under clinical investigation.

8. Oxygen free radicals

The hypothesis that antioxidants may play a role in ischaemic heart disease was tested in the early 1990s. For instance, patients with angina pectoris or with a coronary event during follow-up, showed a low plasma concentration of vitamins with antioxidant properties (adjusted for plasma cholesterol, age, blood pressure, weight and smoking status).[156,157] Additionally, in contrast to early ischaemic preconditioning,[158] patients with an acute myocardial infarction[159,160] or with stunned myocardium[161,162] have an increased oxidative stress, measured by indices of free-radical activity.

Studies to test whether drugs can inhibit oxidative stress and limit myocardial infarct size have produced conflicting results. In experimental models pretreatment with a lipid soluble membrane antioxidant could oppose propagation of the chain reaction to the neighbouring fatty acids.[163] Clinical studies in patients during heart surgery, with stable angina or after myocardial infarction, also showed myocardial protective effects of this lipid-soluble membrane antioxidant.[161,164]

In animal studies hydroperoxide, one of the products of the chain reaction, could be reduced by glutathione peroxidase or by glutathione 'suppletion', frequently leading to myocardial protection in situations of ischaemia-reperfusion damage and infarction.[165-167] These results were illustrated in 84 patients with an anterior wall infarction who received 6.25 mg captopril orally about 15 minutes before iv administration of urokinase. This sulphydryl-containing drug attenuated the formation of oxygen-free radicals, protecting the lysosomal membranes, and resulted in significantly less reperfusion ventricular arrhythmias, lower CK release and less late arrhythmias.[168]

In different animal species free radical scavengers, cell-activation inhibitors (and metal chelators), preventing the formation of oxygen-derived free radicals by inflammatory cells, were at times successful in reducing ischaemia-related injury[169-175] but not in limiting cell death.[173,176-181]

Also in human beings there have been conflicting results. Allopurinol, an inhibitor of xanthine oxidase, had myocardial protective effects against reperfusion injury in aorta coronary bypass patients,[182,183] but increased the extent of disease in patients with myocardial infarction.[184]

9. Plugging

Neutrophils can be activated during myocardial ischaemia causing capillary plugging by cell aggregates and may thus exacerbate ischaemic myocardial injury. According to animal studies, nifedipine and nisoldipine are able to reduce the number of adherent leukocytes in post-capillary venules and capillaries of the repeatedly ischaemic myocardium.[185] Furthermore, in patients with ischaemic heart disease, intravenous isosorbide dinitrate, acting as nitric oxide donor, inhibited both plasma-mediated stimulation of neutrophil superoxide anion production and neutrophil aggregation.[186] For this reason intravenous isosorbide dinitrate may reduce myocardial injury during ischaemia.

Another drug that could reduce neutrophil plugging in a (closed-chest) canine model was the perfluorochemical Fluosol. Administration of this drug resulted in significantly reduced infarct size.[187] Lastly, intravenous adenosine given to a closed-chest dog model reduced neutrophil and erythrocyte plugging of capillaries, which was accompanied by a normal transmural blood flow during reperfusion and a significantly less extensive infarct size.[188]

10. Heat-shock protein

Amphetamine can elevate the body temperature as a result of enhanced endogenous lipolysis and thereby induce whole-body heat shock, associated with the induction of transcription m-RNA for heat-shock proteins. These heat-shock proteins have shown to be able to precondition and protect the heart in an animal model, by enhancing cellular tolerance to ischaemia-reperfusion injury and reduction of free radical-mediated reperfusion injury.[51,189]

Recent advances in molecular genetics have allowed further elucidation of the protective role of heat-shock proteins against myocardial infarction. The recent generation of myogenic cell lines and

transgenic mice that overexpress heat-shock proteins demonstrated a decrease of infarct size and an improvement of functional recovery.[190-192]

Future considerations

Some of the studies we have cited have demonstrated results that are in conflict with other trials. Others are limited because the results have not been considered in a general way.

In the pre-thrombolytic era several interventions appeared to limit infarct size in animals, but only a few had been documented in humans. Among them are, as quoted earlier, β-blockers,[89,90,104-108] glucose-insulin-potassium infusion,[91] magnesium,[110,111] hyaluronidase,[92,93] trimethaphan,[94] and nitrates.[95-97]

Since the thrombolytic era in the late 1980s, the above-cited drugs have mainly been tested in clinical trials, documenting events like mortality. The efficacy in reducing early mortality of myocardial infarction was at times comparable to and independent of thrombolytic therapy (β-blocker[193-195] and magnesium[111]), then again 'modified' by thrombolytic therapy (nitrate[196-198] and hyaluronidase[199]).

In this century there will be an intensification of efforts designed to identify the perfect treatment to protect the ischaemic myocardium. The recent remarkable results of the ECLA study affirm the great potential of glucose-insulin-potassium infusion to reduce myocardial cell death and mortality in acute myocardial infarction when it is added to acute reperfusion therapy.[200,201] Furthermore, it seems likely that patients will be subdivided according to clinical, electrocardiographic and haemodynamic findings, and that the intervention will be tailored appropriately. For example, in hypertensive patients, afterload reduction,by trimethaphan, nitrate or ACE inhibition, may be effective to limit infarct size. In patients without any evidence of myocardial depression, β-blockade, magnesium, or hyaluronidase might be appropriate. In hypotensive patients with pump failure, circulatory support[202] might be the treatment of choice to protect the ischaemic myocardium and to limit myocardial injury. Support of a patient selection process is illustrated by a well-conducted trial in unselected patients receiving early ACE inhibition, where deaths were more frequently allocated to active therapy.[203] In addition, patients who were defined as being at low risk and who received immediate β-blocker therapy showed significantly less deaths at six weeks than those treated later.[107]

It is important to determine whether or not the more recent positive observations with 'new' agents in animals, such as nitric oxide synthetase inhibitors, inhibitors of components of the complement system, prostacyclins, anti-adhesives, scavengers and succinate,[204] are also relevant and promising to the patient with an acute coronary occlusion and merit evaluation in future clinical trials. To date, there have been no particular difficulties in applying to patients the pharmacological interventions that have been demonstrated to exert beneficial actions in the animal, such as monophosphoryl lipid A,[112] Na^+/H^+ exchange inhibitor[120] or C1 esterase inhibitor.[18,125] Recent publications illustrated that infusion of adenosine was well tolerated by patients and that this agent could render the myocardium resistant to ischaemia during coronary angioplasty[205] and was effective as an adjunct to thrombolytic therapy for acute myocardial infarction.[206] However, the remarkable results of small pilot studies, for instance cardioprotective effects of a Na^+/H^+ exchange inhibitor in 50 patients with acute anterior myocardial infarction undergoing direct PTCA,[207] have to be replicated in larger clinical trials before drugs are added to the therapeutic armamentarium.

Research efforts have not yet provided a clear understanding of all mechanisms of myocardial cell dysfunction and cell death. It is still not certain what mechanisms are central to the process of myocyte death and which are minor contributory mechanisms, or only epiphenomena. It seems sensible to concentrate future effort on mechanisms and biology of phenomena of inhibition of cell function that have been found to be related to myocardial ischaemia reperfusion, like myocardial stunning, hibernation and preconditioning.[208] Studies are needed that look at myocardial perfusion, function and viability at multiple time points to answer these questions of whether and why segments of the ventricle show inhibition of function. A better understanding of these ischaemic conditions will lead to new therapies for a variety of ischaemic syndromes and for acute myocardial infarction. For example, if the final effector(s) of endogenous cardioprotection are identified, a more directed approach to designing effective drug therapy to limit infarct size might become possible.

Lastly, we have to understand why there might be problems with animal models in predicting human clinical response. It will be important to analyse why several historically identified interventions worked in animals, but failed in humans. In the hypoxic or ischaemic myocardium, variations in tissue heterogeneity, otherwise phrased in tissue components, e.g. myocytes, endothelial cells, fibroblasts and white cells, are likely to play a prominent part in the differences in efficacy of therapy between experimental and clinical studies. Testing a new and innovative treatment remains necessary to find out whether animal models can predict human clinical response in acute myocardial infarction.

Conclusion

The purpose of this review was to draw attention to the growing list of pathophysiological phenomena occurring in blood, the vessel wall and cardiac tissue during myocardial infarction. A further aim was to point to the complexity of factors contributing to cardiac dysfunction and the implications for therapy, aimed at limiting myocardial cell death. Not all physiopathological mechanisms have been elucidated yet,

indicating the necessity for further research in this area.

In addition, we have described interventions that have shown promise in animal studies, those that may show promise in humans, and those that are accepted as therapies of choice. ■

Acknowledgement

We are indebted to H.J.J. Wellens for his critical insights and review, to P.A.F.M. Doevendans, L. Hofstra, J. Maessen, A.J.M. Oude Ophuis and G. Ramsay for their comments on the manuscript and editorial assistance, and to Vivian Schellings for secretarial assistance.

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