Role of apoptosis in reperfusion injury

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Abstract

Many changes occur during reperfusion of the myocardium after ischemic damage. Necrosis and apoptosis appear to be ongoing during ischemia, while apoptosis is boosted by the reperfusion event. In the past 10 years, distinct intracellular pathways important for hypertrophy, apoptosis, cardiac failure, ischemic preconditioning and reperfusion damage have been recognized. The eventual response of the cardiomyocyte will depend on energy and time available as well as changes in pH and ion handling and the delicate balance of activation of signaling molecules and transcription factors. There is agreement on the central role of mitochondria and nitric oxide (NO) in programmed cell death. However, although many groups analyzed the contribution of NO to cell death, still the circumstances and levels required for cardioprotection or death are unclear. Growth factors, cytokines, and downstream signaling molecules have been shown to influence programmed cell death through mechanisms reminiscent of preconditioning. Here, the role of apoptosis in ischemia reperfusion-related cell death is reviewed. Important data have been obtained in isolated cells, intact hearts and intact animals. Both pharmacological as well as genetic interventions are discussed. Proof for apoptosis in man post-myocardial infarction (MI) treated through primary Percutaneous Transluminal Coronary Angioplasty or other reperfusion therapy is reviewed. Finally, the currently available quantification methods for apoptosis post-MI are mentioned.

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Keywords: Apoptosis; Caspase blockers; Reperfusion injury; Cardiomyocyte; Mitochondria; Nitric oxide; Growth factors; Annexin-V

1. Introduction

Before we can bring our knowledge on the role of apoptosis in relation to reperfusion to the clinic we will have to show that reperfusion itself triggers apoptosis, and that pharmacological interventions applied at the onset of reperfusion can reduce cell death. Then we have to examine whether apoptosis during reperfusion is important in the clinical setting, design the tools to quantify post-myocardial infarction (MI) apoptosis and then test various compounds either alone or in combination to try to reduce apoptosis in small sized clinical trials. The main difficulty here is that apoptosis blocking therapy is an adjuvant, add-on therapy.

The anticipated impact of reducing apoptosis is small compared to the beneficial effect of reopening the infarct-related vessel itself [1]. The limited salvage makes it arduous to prove efficacy of additional interventions. In patients suffering from MI, there are many confounding variables that can never be controlled such as age, gender, co-morbidity, duration of chest pain and localization of the occlusion [2]. All of these variables can potentially influence apoptosis levels. Crucial is the development and validation of new imaging techniques to measure infarct size and apoptosis, as these techniques will provide an essential surrogate endpoint for clinical studies [3]. Here we will try to follow a systematic approach to demonstrate the importance of apoptosis in reperfusion injury by looking at both genetic and pharmacological interventions. In addition, the techniques and evidence for post-MI apoptosis in animal models and man will be discussed, and, finally, the available imaging techniques to quantify MI and reperfusion damage are briefly indicated.
2. Definitions and apoptosis assays

The difference between apoptosis and necrosis, the two distinct forms of cell death, is becoming more obscure. Apoptosis indicates cell death and removal without the activation of an inflammatory process, based on DNA and cellular fragmentation. This form of cell death requires caspase activation, as these enzymes can activate the endonucleases responsible for DNA degradation [4]. Necrosis is a faster process with early membrane failure, cell swelling and the release of cellular debris, activating inflammation. In a minority of the dying cells in the setting of ischemia, strict criteria for apoptosis or necrosis can be detected. Therefore, Leist and Jaattella defined in addition to necrosis and apoptosis, two intermediate stages. They labeled them apoptosis-like programmed cell death (PCD) often caspase independent and necrosis-like PCD (aborted PCD) [5]. Here PCD indicates every form of cell death, in which DNA degradation is detected, preceding the loss of cellular integrity. This classification is based on morphological changes, and does not provide information on the different signaling pathways involved (Fig. 1). The importance of this classification is to show that there is a continuum in modes of cell death with apoptosis on one end and necrosis at the other. As all the cell death pathways interact with one another, the pathway

![Diagram of signaling pathways in cardiomyocyte apoptosis](image-url)

**Fig. 1.** Signaling pathways in cardiomyocyte apoptosis. Several intracellular signaling pathways involved in cardiomyocyte apoptosis are depicted in the figure. Activation or inhibition of single molecules are indicated by, respectively, (+) and (−). The figure presents the signaling pathways as discussed in the text.
Table 1
Pharmacological and genetic interventions in animal models to reduce reperfusion injury

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Mechanism</th>
<th>Model</th>
<th>Reperfusion</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) Nitric oxide</strong></td>
<td></td>
<td></td>
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<tr>
<td><em>BH₄</em></td>
<td>Stabilizes NOS → NO production → ROS inactivation →</td>
<td>Rat (in vivo)</td>
<td>Global</td>
<td>Before</td>
<td>Reperfusion-damage ↓, Endothelial-function ↑</td>
</tr>
<tr>
<td>SIN-1 (3-morfolino-sydnonime-1)</td>
<td>NO donor → restores TGF-β activity → NOS ↑ → NO production ↑ → ROS inactivation →</td>
<td>Rat (in vitro)</td>
<td>Cellular</td>
<td>Before</td>
<td>Myocyte injury ↓</td>
</tr>
<tr>
<td>Nitroglycerine</td>
<td>NO donor → NOS ↑ → NO production ↑ → ROS inactivation →</td>
<td>Rat (in vitro)</td>
<td>Cellular</td>
<td>Before</td>
<td>Myocyte injury ↓</td>
</tr>
<tr>
<td><em>S</em>-nitroso-N-acetyl-penicillamine (SNAP)</td>
<td>NO donor → Ca²⁺ uptake mitochondria ↓ → cytosolic Ca²⁺ overload ↓</td>
<td>Rat (in vitro)</td>
<td>Cellular</td>
<td>Before</td>
<td>Mitochondrial depolarisation ↓, cardiomyocyte injury ↓</td>
</tr>
<tr>
<td>Sodium nitroprusside (SNP)</td>
<td>NO donor → Ca²⁺ absorb mitochondria ↓ → cytosolic Ca²⁺</td>
<td>Guinea pig</td>
<td>(ex vivo)</td>
<td>Regional</td>
<td>Histamine release ↓, LDH release ↓, calcium overload ↓, MDA production ↓,</td>
</tr>
<tr>
<td>Glyceryl trinitrate (GTN)</td>
<td>NO donor → Ca²⁺ absorb mitochondria ↓ → cytosolic Ca²⁺</td>
<td>Guinea pig</td>
<td>(ex vivo)</td>
<td>Regional</td>
<td>Histamine release ↓, LDH release ↓, calcium overload ↓, MDA production ↓,</td>
</tr>
<tr>
<td>TCV-309</td>
<td>PAF antagonist → eNOS mRNA ↑ → NO production ↑</td>
<td>Rabbit (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>Myocardial contractility ↑, Myocardial apoptosis ↓ (procaspase-3 cleavage ↓)</td>
</tr>
<tr>
<td>eNOS</td>
<td>eNOS → NO production ↑</td>
<td>eNOS</td>
<td>Tg mice (ex vivo)</td>
<td>Global</td>
<td>Before</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>Estrogen receptors (NOS inhibitors block effect)</td>
<td>Dog (in vivo)</td>
<td></td>
<td>Before</td>
<td>Myocyte injury ↓, incidence ventricular fibrillation ↓</td>
</tr>
<tr>
<td>JTV 519</td>
<td>RyR (NOS inhibitors block effect)</td>
<td>Rabbit (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>ATP levels ↑, LVEDP ↓</td>
</tr>
<tr>
<td>Resveratrol (grapes and wine)</td>
<td>iNOS dependent</td>
<td>Mouse wt and iNOS −/−/− (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>Postischemic ventricular function ↑, infarct size ↓, myocardial apoptosis ↓</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>PKC ↑ → NF-κB → iNOS ↑ → NO ↑ → opening mitoK (ATP) channel</td>
<td>Mouse wt and iNOS −/−/− (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>LVEDP ↓, LDH release ↓, ATP levels ↑</td>
</tr>
<tr>
<td>Bosentan</td>
<td>Endothelin-1 (ET-1) receptor antagonist → iNOS mRNA ↓ Inhibits NOS ↓ NO ↓ → free ROS ↓</td>
<td>Pigs (in vivo)</td>
<td>Global</td>
<td>Before</td>
<td>LV pressure ↓, iNOS mRNA ↓, myocardial apoptosis ↓, function ↑</td>
</tr>
<tr>
<td>NG-nitro-L-arginine (L-NNA)</td>
<td>Inhibits NOS ↓ NO ↓ → free ROS ↓</td>
<td>Dogs (in vivo)</td>
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<td>Before</td>
<td>Free radical concentration ↓</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Inhibits HMG-CoA reductase → eNOS ↑ → NO production ↑</td>
<td>Rat (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>Myocardial injury ↓, vascular hyperpermeability ↓</td>
</tr>
<tr>
<td>Clevidipine</td>
<td>Ca²⁺ channel blocker → NO release ↑</td>
<td>Pigs (in vivo)</td>
<td>Regional</td>
<td>Before and During</td>
<td>Infarct size ↓, coronary endothelial function ↑</td>
</tr>
<tr>
<td>Trandolapril</td>
<td>Bradykinin ↑ → eNOS mRNA ↑</td>
<td>Rat (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>Functional recovery ↑</td>
</tr>
<tr>
<td>MK-954 (losartan potassium)</td>
<td>= AT-1 receptor antagonist → induces eNOS overexpression</td>
<td>Rat (in vitro)</td>
<td>Global</td>
<td>During</td>
<td>Functional recovery ↑, myocardial damage ↓</td>
</tr>
<tr>
<td>Intervention</td>
<td>Mechanism</td>
<td>Model</td>
<td>Reperfusion</td>
<td>Effect</td>
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<tr>
<td><strong>(A) Nitric oxide</strong></td>
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<tr>
<td>IB-MECA (N(6)- (3-iodobenzyl) adenosine-5′-N-methyluronamide)</td>
<td>=A(3)AR agonist → NO production</td>
<td>Mouse (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>Functional recovery ↑, postischemic necrosis ↓</td>
</tr>
<tr>
<td>Sildenafil citrate</td>
<td>Induces NOS → NO ↑</td>
<td>Mouse (ex vivo)</td>
<td>Global</td>
<td>Before</td>
<td>iNOS and eNOS mRNA ↑, infarct size ↓</td>
</tr>
</tbody>
</table>

| (B) Growth factors | | | | | |
| FGF-2 | activates PKC | Rat (ex vivo) | Global | Before | Cardioprotective effect, sarcolemmal Ca²⁺-independent PKC ↑ | [84] |
| FGF-2 | Through FGFR-1 → activates PKC | Rat (ex vivo) | Global | During | PKC levels ↑, functional recovery ↓ | [53] |
| FGF-2 | Activates FGFR-1 → hypertrophy → improved injury repair | FGF-2 tg mice | Global | Before | 30–45% increase in myocyte viability | [85] |

| Insulin | P1γ, kinase ↑ → Akt ↑ → cell survival + phosphorylation of eNOS↑ | Rat (in vivo) | Regional | After | myocardial apoptosis ↓ | [86] |

| IGF-1 | Stimulates Bcl-2 and Bax↑ → myocyte apoptosis ↓ | Rat (ex vivo) | Global | Before | Myocardial viability ↑, Positive ionotropic effect | [87] |
| IGF-1 | P1γ, kinase ↑ → Akt ↑ → cell survival ↑ | Mouse (in vitro + ex vivo) | Global | Before | Cardiomyocyte survival ↑ | [55, 56] |

| GH | Unclear | Rabbit (ex vivo) | Global | Before | Coronary vascular dilation | [54] |
| CP-424, 391 | GH secretagogue → GH ↑ → damage ↓ | Rabbit (in vivo) | Regional | Before | Infarct size ↓ | [88] |
| FGF-1 | → induces iNOS → NO production | Rat (ex vivo) | Global | During | Functional recovery ↓, myocardial injury ↓, infarct size ↓ | [52] |
| Hexarelin | Stimulates GH-axis → ischemic damage ↓ | Rat (ex vivo) | Global | Before | Myocardial injury ↓, plasma cholesterol ↓ | [89] |

| (C) Gene deficiency models | | | | | |
| MMP-9 | induces neutrophil migration | MMP-9 def. mice | Regional | Before | infarct size ↓ | [90] |
| p38α | kinase → activates unidentified pathways | P38α (+/-) mice | Before | infarct size ↓ | [91] |
| AK1 | catalyzes adenine nucleotide exchange → impairs ATP formation | AK1 def mice | Global | Before | nucleotide salvage ↓, postischemic recovery ↓ | [92] |
| eNOS | NO production ↓ → ROS inactivated ↓ | eNOS def mice | Global | Before | myocardial necrosis ↑, reperfusion injury ↓, iNOS mRNA ↑ infarct size ↓, arrhythmia frequency ↓, functional recovery ↑ neutrophil infiltration ↓, myocardial injury unchanged | [48] |
| TNF-α | Blocked leukocyte infiltration | TNF-α def. mice | Global | Before | myocardial injury ↓ | [93] |
| CD18 | Impaired binding of neutrophils to α4-integrines or VCAM-1 | CD18 def mice | Global | Before | Neutrophil infiltration ↓, myocardial injury unchanged | [94] |
| A(3)AR | (A)AR undefined compensatory mechanisms | A(3)AR def. mice | Global | Before | post-ischemic recovery ↑, energy metabolism unaltered | [95] |
| MEKK1 | Blocked c-Jun kinase (JNK) activation in hypoxia → formation of proapoptotic cytokines → apoptosis | MEKK1 def ES cell-derived cardiomyocytes | Global | Before | Increased susceptibility to H₂O₂, and apoptosis | [96] |

| poly (ADP-ribose) synthetase (PARS) | Reduced oxidant-mediated DNA-injury | PARS def. mice | Global | Before | neutrophil infiltration ↓, reperfusion injury ↓, ventricular recovery ↓, infarct size ↑ | [97] |
| Heme oxygenase-1 (HO1) | Reduced intracellular antioxidant activity | H(mox-1)(+/−) mice | Global | Before | | [98] |
activation is not very helpful in distinguishing various modes of PCD.

Comparison of apoptosis blocking studies is hampered by the use of different apoptosis detection assays [6]. Apoptosis assays include electron microscopy to detect changes in chromatin and mitochondrial integrity. The fragmentation of DNA can be detected by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) or in situ end labeling (ISEL); these techniques allow one to determine which cell types are involved. Through gel electrophoresis, the appearance of DNA laddering can be demonstrated in tissue extracts [7]. Annexin-V staining is considered to be a relatively early apoptosis assay and is based on the expression of phosphatidylserine groups on the extracellular surface [6,8,9]. Alternatively, activation of death pathways can be used through the immunodetection of activated caspase 3 or the cleavage of poly(ADP-ribose) polymerase [9]. Fluorescent probes have been used successfully to detect the loss of mitochondrial membrane potential indicating apoptosis [10,11].

For the evaluation of studies, technical and experimental difficulties have to be taken into account, since overestimation of the number of apoptotic cardiomyocytes (false-positives) and misinterpretation of electron microscopic photos are relevant pitfalls. Early papers on post-MI apoptosis reported levels up to 10% where others reported a maximum of 1% in the infarct borderzone [12,13].

### 3. Ischemia-induced apoptosis

The importance of apoptosis in cell death following ischemia and reperfusion has been demonstrated in vivo rodent models. Prolonged periods of myocardial ischemia are related to an increase in the rate of necrosis, whereas, paradoxically, reperfusion leads to an enhancement in apoptosis [14–18]. Essentials for the survival of viable cells are provided as reperfusion restores oxygen and glucose supply. However, reperfusion also restores energy required for the completion of apoptosis and can accelerate the apoptotic process [15–17].

In rats, ischemia-induced apoptosis going into necrosis has been demonstrated [14]. Anversa quantified the level of apoptosis and necrosis and reported much higher levels of apoptosis (30 ×) after 2 h of ongoing ischemia. Eventually 86% of cell death was based on apoptosis versus 14% on necrosis. Although the onset of cell death is different, transitions from apoptosis to necrosis were observed. Others described apoptosis to be induced following 2 h of coronary occlusion and showed acceleration after 45 min of ischemia followed by 1 h of reperfusion [19,20]. There is more supportive evidence for ischemia-related apoptosis as mitochondrial dysfunction has been demonstrated with leakage of cytochrome C, and caspase activation following global ischemia in the isolated rat heart [21]. Borutaite et al. showed that caspase activation was dependent on the time of ischemia. The release of cytochrome C following mitochondrial-permeability transition (MPT) activation seems to be the apoptosis inducing mechanism. The level of apoptosis was shown to depend on the duration of reperfusion [15]. These studies confirm that ischemia by itself can trigger apoptosis. Reperfusion accelerates the process.

The results by Gottlieb et al. [17] were different. They reported apoptosis in the rabbit myocardium after 30 min of ischemia and 4 h of reperfusion, but not in the permanent ischemic area. This would suggest that apoptosis is initiated by reperfusion only. In a more recent study in dogs, apoptosis was detected in myocardium subjected to a brief period of ischemia followed by reperfusion, and not in ischemic tissue without reperfusion [22]. Whether these findings are related to species or differences in the methods used remains unclear. In general, the most important differences in apoptosis detection and frequency can be explained by variation in the interpretation of the specific apoptosis assays.

### 4. Blocking apoptosis to reduce reperfusion injury

The importance of apoptosis in cell death following reperfusion has been demonstrated in vivo rodent models, allowing also the evaluation of pharmacological (Table 1A), growth factor mediated (Table 1B) and genetic (Table 1C) interventions [23]. Many strategies have been developed that were successful in animal studies to reduce reperfusion damage and reperfusion-induced apoptosis. However, many approaches have little relevance for the

### Table 1 (continued)

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Mechanism</th>
<th>Model</th>
<th>Reperfusion Effect</th>
<th>Reference</th>
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<tbody>
<tr>
<td>(C) Gene deficiency models</td>
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</tr>
<tr>
<td>ICAM</td>
<td>Reduced neutrophil infiltration</td>
<td>ICAM def mice</td>
<td>Before initial reduction reperfusion injury</td>
<td>[99]</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD)</td>
<td>Less inactivation of ROS</td>
<td>SOD1 def mice</td>
<td>Before Functional recovery</td>
<td>[100]</td>
</tr>
<tr>
<td>Glutathione peroxidase knockout</td>
<td>Reduced antioxidant capacity</td>
<td>GSHPx-1 def mice</td>
<td>Before Functional recovery</td>
<td>[101]</td>
</tr>
</tbody>
</table>

clinical setting and the results in clinical trials have been quite disappointing [24,25]. The negative findings in clinical studies could be based on essential differences between animal models and man, or the formulation of too ambitious primary endpoints.

4.1. Ion channels

Going from the cellular membrane via the mitochondria to the nucleus we encounter several potential targets for pharmacological (and genetic) interventions. Well recognized and studied is the role of ion channels. Ion channels play a role in calcium overload and hypercontracture phenomena, restoration of intracellular pH, and also energy metabolism [26,27]. Both acute calcium contracture phenomena, restoration of intracellular pH, channels play a role in calcium overload and hyperfor pharmacological (and genetic) interventions. Well dria to the nucleus we encounter several potential targets ambition primary endpoints.

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A logical approach is to block calcium fluxes through the L-type calcium channel or sodium–calcium exchanger.

The short acting calcium antagonist Clevidipine was shown to reduce infarct size in pig studies. Here however drug administration was initiated during the last minutes of ischemia and continued during reperfusion. The effect was shown to be NO dependent [28]. Similar results were reported for nicardipine in dogs [29]. Studies in rat brain showed a synergistic effect of combination treatment with nimodipine and citicholine. Nimodipine (Ca channel blocker) was injected acutely during reperfusion while citicholine (involved in the biosynthesis of phosphatidylicholine) was administered in the post-ischemic phase [30]. In treated brain, Bcl-2 expression went up and the percentage of TUNEL-positive cells went down. Cardioprotective effects of citicholine have also been shown in cultured cardiomyocytes [31].

Blocking the sodium–calcium exchanger only during reperfusion turned out to be a more powerful strategy to reduce infarct size compared with blockade of the sodium proton exchanger. The concept behind this intervention is that a reduction of sodium influx is important, apparently even more important than the efflux of calcium [32]. In addition, beta blockers (propranolol) have been used successfully to reduce sodium influx and cytochrome C release in a rat model [33].

The potassium channel blocker tetraethylammonium (TEA) selectively blocks $I_K$ and thereby prevents potassium efflux, without affecting the voltage-gated $Ca^{2+}$ currents. In addition to TEA, a lipophilic potassium channel blocker, clofilium, was shown to attenuate apoptosis induced by hypoxia in vitro and infarct volume induced by ischemia in vivo [34]. A different way to block potassium channels was shown by overexpression of Apoptotic Repressor with Caspase recruitment domain (ARC), an antiapoptotic protein. ARC inhibits apoptotic cell shrinkage and attenuates apoptosis by reducing potassium efflux through voltage-gated $K^+$ ($Kv$) channels [35].

For an extensive analysis of pharmacological interventions in reperfusion injury the paper by Wang et al. [36] is instructive. Additional approaches in animal and man are outlined in Tables 1 and 2.

4.2. Nitric oxide

NO appears to have a dualistic effect on cardiomyocytes as it can be protective or induce apoptosis. NO has been shown to induce apoptosis in isolated cardiomyocytes from chick and rat in embryonic neonatal and adult cardiomyocytes [37–40]. Short treatment with the NO donor S-nitroso-N-acetylpenicillamine (SNAP) was shown to depolarize mitochondria and influence $Ca^{2+}$ uptake in mitochondria providing a potential mechanism [41]. An important role in reperfusion injury through activation of cGMP (Table 1A) has been proposed.

Various pharmacological NO donors and eNOS overexpression have been demonstrated to reduce reperfusion injury [41–46]. In addition, in isolated guinea pig hearts eNOS inhibition was shown to activate Bax protein and to induce apoptosis [47]. Potentially, the level of available NO for single cells versus the levels reached in the intact organ could be a key factor to explain the reported differences.

Confusing results were reported using different eNOS-deficient strains [48]. Sharp et al. compared mice generated at Harvard with University of North Carolina (UNC) eNOS-deficient mice. In the Harvard mice, a marked increase was reported in MI size, without induction of the iNOS gene. UNC mice had smaller infarcts, increased iNOS expression and blocking iNOS activity increased MI size (Fig. 2). These findings would imply a protective effect of high NO levels, but apparently also genetic background plays a role in the response to injury.

Additional support for the beneficial effects of NO on reperfusion damage comes from pharmacological studies using various statins, including simvastatin, atorvastatin (heart) and rosuvastatin (brain) [49–51]. Statins promote eNOS transcription and enzyme activity. These protective effects of statins were independent of any effect on cholesterol lowering through HMG-CoA-reductase inhibition. Part of the beneficial effects however involves the activation of an anti-apoptotic or pro-survival pathway. This cellular protective signaling pathway involves activation of phosphatidylinositol trisphosphate kinase (PI3K) and Akt [49].
4.3. Growth factors

An important role for Akt has been shown in mediating responses to growth factor treatment (Fig. 1, Table 1B) [52–55]. Important here is the study published by Fujio et al. [56] showing activation of the PI3K/Akt pathway and beneficial effects of IGF-1 administration. Dominant negative Akt constructs completely abolished the protective effects of IGF-1 in a mouse model of ischemia–reperfusion. In addition, this study showed a marked reduction on the level of apoptosis upon Akt activation. Comparable results were reported for IGF-1 in MI and ventricular remodeling (Table 1B). In the heterozygous IGF1 knock out animals, higher apoptosis levels were reported. In contrast in IGF1 transgenic mice, cardioprotection was observed [57]. Constitutive overexpression of IGF-1 prevented activation of cell death in the viable myocardium after ischemia. Growth factors reduce infarct size also in the absence of reperfusion in combination with reduced apoptosis rates.

In human acidic fibroblast growth factor (hFGF-1) overexpressing mice, Buehler et al. [58] showed a spectacular delay in myocardial damage development. However, prolonged ischemia resulted in an infarct size comparable to wild type mice. In the transgenic mice, elevated levels of ERK1 and 2 were reported indicating that activated intracellular signaling pathways postpone cell death, but fail to prevent it. No differences were reported with respect to the apoptosis levels. These studies clearly show the alternative effects of selective growth factor receptor activation.

4.4. Signal transduction pathways

Growth factors have more effects than Akt activation. For instance, we know that FGFs mimic preconditioning through PKC-dependent pathways [52,59]. Recent data show that preconditioning activates various anti-apoptotic or pro-survival pathways and inhibits inflammatory cell activation. For instance, increased Bel-2 expression and reduced apoptosis after myocardial preconditioning was reported in rats [60]. Especially in preconditioning the mitochondria play an essential role [61]. Opening of the mitochondrial potassium channel K(ATP) blocks cardio-myocyte apoptosis via activation of PKC-epsilon in chick [62]. Apoptosis was also reduced by adding the mitochondrial K(ATP) opening compound Diazoxxine. A direct interaction between the PKC epsilon protein with components of the mitochondrial MTP (a multiprotein complex) was recently demonstrated [63]. Furthermore, cardiac-
specific over expression of active PKC epsilon in mice, which is cardioprotective, greatly increased interaction of PKC epsilon with the pore components and inhibited Ca2+ -induced pore opening [64]. These data show that the pathways involved in ischemic preconditioning, growth factor mediated protection and apoptosis merge at the site of the mitochondria.

Like PKC, calcineurin also mediates effects through the MAPKs including JNK, ERK and p38 [65,66] The importance of the various growth factors and MAPKs in determining infarct size and reperfusion damage has been evaluated in gene-targeted mice [8,58,67]. Deficiency for the calcineurin Aβ gene was shown to increase myocardial damage in the in vivo model of ischemia and reperfusion. In the calcineurin knock out mice, a marked increase of apoptosis could be demonstrated by DNA laddering and TUNEL staining [7,23].

Through comparative studies in various mouse models, the importance of MEK1 (activating ERK1 and 2) and ERK 2 for cardioprotection during in vivo ischemia and reperfusion was shown [9]. The studies listed here indicate the close interaction of pathways previously identified as specific for hypertrophy and failure to the ischemic and reperfusion-dependent triggering mechanisms. Thus, hypertrophic stimuli can activate pro-survival pathways.

5. Apoptosis after myocardial infarction in patients

5.1. General imaging

Left ventricular remodeling after MI refers to changes in shape and function of both the infarcted area as well as the uninfarcted myocardium that begins minutes after acute MI and may continue for months or years [68,69]. Infarct expansion occurs soon after the onset of coronary occlusion. It is reversible if coronary flow is re-established rapidly; however, it may progress in a time-dependent manner if flow is not re-established or is re-established late [70]. Infarct expansion involves no additional myocardial necrosis but refers to an increment of the functional infarct size with a greater percentage of the LV wall being composed of scar. In this remodelig process, apoptosis has been shown to play an important role [13]. Early reperfusion therapy, either medically with thrombolytics or mechanically with angioplasty and stenting, can achieve epicardial reperfusion in 60% (thrombolytics) to 95%
(angioplasty) of patients. However, despite patency of the epicardial vessel, microvascular perfusion might still be impaired [1]. This so-called no-reflow phenomenon is probably caused by myocardial oedema, intense vasoconstriction of the microvasculature and plugging of the microvasculature with thrombus and macrophages. The severity of the microvascular underperfusion can be assessed by grading the speed of contrast passage through the epicardial coronary artery (TIMI flow grade, TIMI frame count), by grading the density of contrast in the myocardium after contrast injection in the infarct-related artery (blush grade) and finally by serial ST-T analysis of the electrocardiogram [2]. All these measures of microvascular perfusion correlate well with global infarct size and clinical outcome.

Regional and global left ventricular function and morphology can be assessed and quantified with magnetic resonance imaging (MRI). The method is safe, non-invasive, well validated and has become a routine investigation. It is therefore an ideal tool to assess changes in left ventricular shape and function after MI. The most accurate method to assess regional myocardial function is to quantify regional myocardial deformation in different parts of the ventricular wall. In addition, the very low short- and long-term variability of cardiac volume measurements allows demonstration of significant differences in small patient populations [71]. It is therefore a suitable tool for the clinical evaluation of different interventions aimed at inhibition of post-reperfusion apoptosis.

With the injection of a small amount of Gadolinium-chelate, a paramagnetic MRI contrast agent, the exact amount of necrotic tissue can be determined and distinguished from non-contracting viable myocardium (stunned or hibernating myocardium) [72]. Both acute and chronic MI show late hyperenhancement after Gadolinium injection and therefore the infarct size can be followed in time. It might well be that the amount of viable myocardium as assessed soon after MI is a strong negative predictor of later remodeling. Although these imaging techniques provide important clinical tools, they provide indirect information on potentially ongoing apoptosis. Thus, far most intervention studies were performed in surgical trials in patients undergoing coronary artery bypass grafting (Table 2).

5.2. Apoptosis imaging

Gradually, molecular and clinical medicine are merging in the field of molecular imaging. If the relevance of changes in gene expression to identify new targets for interventions has to be determined, we will have to validate findings from animal models in man. To intervene in early disease, histological analysis is insufficient. Therefore, we will have to develop clinical tools for dynamic molecular imaging. For most molecular and cellular mechanisms, no techniques are available. In the field of apoptosis, remarkable progress has been made. Many studies showed the exposure of phosphatidylserine groups as an early change in the composition of the extracellular membrane. The presence of phosphatidylserine groups can be detected through binding of endogenous annexin-V. The binding of annexin-V in the in vivo mouse model of ischemia–reperfusion was used to quantify apoptosis [15]. In these experiments, biotinylated annexin-V and annexin-V–Oregon green were used for visualisation of apoptosis. These experiments also showed the importance of the duration of reperfusion for the level of apoptosis and the possibility to reduce apoptosis during reperfusion by blocking caspase activation and through blocking of the sodium hydrogen exchanger. In subsequent experiments, apoptosis was imaged in the in vivo beating mouse heart [16]. For this microscopy-based imaging technique using fluorescent labeled annexin-V, the chest had to be opened and only a small section of the area at risk could be visualized and assessed for apoptotic events. From these studies, we learned that the first signs of apoptosis can be detected after 30 min of ischemia.

The challenge was to bring the imaging based on annexin-V to the clinical setting. This was performed successfully in patients treated for acute MI through primary coronary angioplasty [18]. In six out of seven patients, increased uptake of Technetium-99m-labelled annexin-V was seen in the infarct area of the heart on early and late SPECT images, suggesting that PCD occurs in that area. No increased uptake was seen in the heart outside the infarct area. All patients with increased Tc-99m-labelled annexin-V uptake in the infarct area showed a matching perfusion defect. In a control individual, no increased uptake in the heart was seen. Molecular imaging with annexin-V allows us to study the dynamics of reperfusion-induced cell death in the area at risk and can be used to assess interventions that inhibit cell death in patients with an acute MI [73]. This technique provides a unique tool in cardiology by imaging with specific labeling of apoptotic cells. These studies also reveal that many cells in the infarct area at an early stage have membrane changes indicative of apoptosis. Gradually, we are getting the clinical tools to study reperfusion damage in man, which is crucial for testing additional interventions.

6. Conclusion

Apoptosis plays an important role in lethal reperfusion injury as indicated by numerous studies in various animal models. The pathways involved in preconditioning, growth factor induced myocardial protection, apoptosis, and also hypertrophy are crucial for reperfusion-induced cell death. A central role is suggested by the overwhelming literature for NO and the mitochondria. The sometimes opposing results
reported for the role of NO could depend on cellular NO levels. The central role of the mitochondria is well established. Apoptosis has been demonstrated in patients by isotope labeled annexin-V. Currently, clinicians have limited tools to image and quantify apoptosis post-MI in man. This is the major limitation for small clinical trials aimed at the reduction of clinical reperfusion damage. When the interventional cardiologist is opening the infarct-related vessel, he would prefer to have tools to protect the myocardium that will be reperfused.

References


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