Review

Myocardial ischemia, stunning, inflammation, and apoptosis during cardiac surgery: a review of evidence

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Received 18 July 2003; received in revised form 18 September 2003; accepted 1 December 2003

Summary

Cardiac surgery (CS), in particular cardiopulmonary bypass and cardioplegia, have been reported to trigger myocardial inflammation and apoptosis. This surgery-related inflammatory reaction appears to be of extreme complexity with regard to its molecular, cellular and tissue mechanisms. Both experimental and clinical studies have ascertained the role of several hormonal mediators, mitochondria, cardioplegia and extracorporeal circulation temperature, apoptosis and even genetic modulators of damage. However, the correlations between these factors in vivo and post-surgery outcome and prognosis have not yet been systematically investigated. In animal models of myocardial cardioplegia and/or ischemia–reperfusion, experimental drugs such as antioxidants have been documented to provide amelioration of post-intervention cardiac performance and reduction of apoptosis suggesting the possibility of new therapeutic strategies. However, these findings have been only partially confirmed in humans. Moreover, markers for the differential detection of early and late phases of apoptosis are subjects of intense investigations. This review will provide an overview of the major studies about the link between ischemia, myocardial inflammation and apoptosis during and after CS, with particular regard to the markers and methods for apoptosis detection.

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Keywords: Cardiac surgery; Apoptosis; Cardiopulmonary bypass; Inflammation

1. Introduction

Several myocardial stresses occurring during cardiac surgery (CS), including ischemia and ischemia–reperfusion (I/R), inflammatory response, operative trauma, cardioplegia and oxidative stress have been reported to trigger myocyte death \(^[1–3]\). These factors are also likely to affect the post-operative course of patients. Both experimental and clinical studies have demonstrated that transient reversible myocardial ischemia in various settings, such as cardioplegia and cardiopulmonary bypass (CPB) during CS, early reperfused internal mammary artery and coronary angioplasty, leads to prolonged depression of cardiac contractility after reperfusion (myocardial stunning) \(^[4–6,42]\). Even though local production of free oxygen radicals and myocyte calcium homeostasis disturbance have been proposed to be the determinants of this phenomenon (the ‘oxy-radical hypothesis’ and the ‘calcium hypothesis’), the pathogenesis of stunning remains to be clarified. Initial activation of the apoptotic cascade, which is probably reversible during its early phases, has been suggested to play a significant role in post-infarction left ventricular remodeling \(^[8,9]\). The link between ischemia and I/R and apoptosis suggests that myocardial apoptosis may be involved in the pathogenesis of stunning and most importantly of persistent myocardial dysfunction after CS. Apoptosis or programmed cell death is a highly regulated and energy-requiring process. In cells subjected to pathologic stresses such as ischemia there is a delicate balance between survival and death. Membrane signaling pathways, mitochondrial release of mediators, balance of pro-apoptotic bax and antiapoptotic bcl-2 proteins expression, and caspase 8, 9 and 3 activation degree are involved in this balance. Estimated duration of apoptotic process is from 12 to 24 h, but cellular morphologic changes are visible in <2 h; detection of
such changes and of previously produced specific biochemical markers are potential methods for early assessment of apoptosis [7]. In contrast to apoptosis, necrosis is a violent, irreversible and non-regulated process of cell killing in consequence of profound disengagement of cell homeostasis due, for example, to prolonged anoxia or strictly impaired environmental conditions, leading to complete and prolonged ATP depletion. Necrosis entails plasma membrane rupture, thus leading to local inflammation, endothelial activation, monocyte chemoattraction and infiltration. It is likely that necrosis-dependent inflammation in the myocardium could be an enhancer for subsequent apoptosis of surviving cells. The necrotic process culminates in aspecific DNA fragmentation, which needs to be differentiated from apoptosis. Schmitt et al. [21] proposed the detection of cytosolic cytochrome c.

It has been reported that serum collected from patients who had received coronary artery bypass grafting (CABG) at 1, 6 and 12 h after weaning from CPB induced apoptosis on cultured endothelial cells, meanwhile serum harvested at 1, 6 and 12 h after weaning from CPB induced apoptosis of surviving cells. The necrotic process culminates in aspecific DNA fragmentation, which needs to be differentiated from apoptosis. Schmitt et al. [21] proposed the detection of cytosolic cytochrome c.

The present review will discuss current pertinent experimental and clinical evidence and appraise methodological issues related to the study of ischemia and myocardial inflammation and apoptosis injury during CS, surgical-related apoptosis and its role in determining cardiomyocyte loss and post-operative cardiac dysfunction.

2. Serum and tissue markers of ischemia and ischemia–reperfusion injury and apoptosis

Myocardial injury causing stunning after CS has been associated with an enhanced inflammatory response [5,39, 40,45–47,50,52,56]. In fact, indexes of post-operative stunning were reversed by blocking the neutrophil–endothelial cells interactions via monoclonal antibodies against CD18 or ICAM-1 receptors, or leukotriene or thromboxane receptors inhibition. Such findings suggested a pivotal role of acute phase reaction in the pathogenesis of stunning. Indeed, there is a growing body of evidence describing the features of myocardial tissue inflammation associated with CPB. Zahler et al. [2] pointed out in 12 patients undergoing CABG surgery, the presence of a transcardiac veno-arterial difference of plasma levels in interleukin-6 (IL-6) rising from 0.1 to 110 pg/ml after 75 min of reperfusion. IL-6, however, has been reported to be a marker rather than a critical tissue mediator of inflammation [10]. Among the culprit inflammatory cytokines, tumor necrosis factor-α (TNF-α) is the one that is primarily involved. It has been shown that TNF-α actively contributes to depressed myocardial performance after CPB [11]. To date, the exact pathophysiologic mechanism of TNF-α action is unknown, even if it has been suggested to have a possible role in triggering the apoptotic process [12], possibly through myocyte membrane TNF receptor type 1 (TNFR1) and type 2 (TNFR2) [13].

Data reported by Wan et al. [19] have linked inflammation and apoptosis following CPB. They documented that both IL-6 and IL-8 mRNAs are upregulated in human cardiac myocytes following CPB, and that such increase is associated with expression of FHL2 (Four and a Half LIM-only protein 2), whose biological function has not been entirely understood. However, enhanced expression of FHL2 is considered to be proportional to the degree of myocardial injury, and experimental evidence also indicated that upregulation of FHL2 might be involved in the last steps of the apoptotic process [20]. Table 1 summarizes the notions in the literature about the main markers of inflammatory damage after CPB.

A number of serum biochemical markers for myocardial injury during and after CS have been identified [24].

<table>
<thead>
<tr>
<th>Marker</th>
<th>Tissue or soluble</th>
<th>Main features</th>
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<tbody>
<tr>
<td>IL-6</td>
<td>S</td>
<td>peak at 75 min of reperfusion. From &lt;0.1 to 110 pg/ml&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-8</td>
<td>S</td>
<td>peak at 35 min of reperfusion. Increase = 500% of basal&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD11b (blood neutrophils surface)</td>
<td>S</td>
<td>peak at 25 min of reperfusion. increase = 140% of basal&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soluble ICAM</td>
<td>S</td>
<td>peak at 10 min of reperfusion. Increase = 10% of basal&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD62 (or P-selectin; platelet surface)</td>
<td>S</td>
<td>peak at 75 min of reperfusion. Increase = 25% of basal&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-6 mRNA (myocardioocytes)</td>
<td>T</td>
<td>increased in 33% of patients undergoing CPB</td>
</tr>
<tr>
<td>IL-8 mRNA (myocardioocytes)</td>
<td>T</td>
<td>increased in 42% of patients undergoing CPB</td>
</tr>
<tr>
<td>TNF-α</td>
<td>S</td>
<td>increased production by inflammatory cells during post-CPB reperfusion</td>
</tr>
<tr>
<td>ICAM, VCAM, PECAM</td>
<td>T</td>
<td>increased endothelial expression in patients undergoing CPB</td>
</tr>
<tr>
<td>HLA-1, HLA-DR</td>
<td>T</td>
<td>increased endothelial expression in patients undergoing CPB</td>
</tr>
<tr>
<td>NF-κB</td>
<td>T</td>
<td>activation in both EC and cardiomyocytes during CPB-dependent inflammation</td>
</tr>
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Data from Refs. [1,2,9,11,21,58].

<sup>a</sup> Expressing veno-arterial cardiac difference.
Creatine kinase (CK)-MB heart-specific isoform has been reported to peak within 6–8 h after surgery, and decrease to normal values within 2–3 days. Cardiac troponins T and I isoforms have been strongly associated with myocardial injury (reported to reach up to a 50-fold increase in 2 h after regional ischemia), just as myoglobin (showing a more rapid rise and fall within 1 h from the ischemic injury), even if the latter has low specificity, being detectable also in skeletal muscle. However, these represent markers of necrosis and are insensitive to myocardial apoptosis. In fact apoptosis is an ATP-dependent precisely programmed and regulated pathway of cell suicide, and pursues a genetically encoded protocol culminating with DNA fragmentation. No rupture of plasma membrane occurs and no release of such markers is therefore expected. These markers are useful for detection of necrosis related to perioperative myocardial infarction or to subclinical myocyte injury rather than apoptosis. Searching for more specific and sensitive markers of apoptosis, some authors focused on the early events which have been recognized as the initial steps of the programmed cell death process. Fas and Fas ligand are membrane molecules known to be involved in the induction of the apoptotic cascade. Soluble Fas molecule has also been suggested to correlate positively with the extent of myocardial injury and inflammation after CPB [25]. Indeed soluble Fas showed a remarkably similar temporal profile to IL-6 in the coronary sinus and peripheral blood, and the attenuation by administration of steroids suggests the feasibility of soluble Fas also as a pro-inflammatory marker. This finding strengthens the hypothesis of the link between tissue inflammation and apoptosis. Indeed, serum collected from patients who had received CABG induced apoptosis on cultured endothelial cells [23]. Soluble markers may therefore provide an indication of the pro-apoptotic status of the patients. The assessment of endothelial apoptosis may appear difficult to obtain, therefore surrogate markers of apoptosis need to be used [26]. An attractive hypothesis is to assess pro-apoptotic effects on circulating blood cells, which may be readily available for evaluation.

Nevertheless, the mechanisms leading from inflammation to increased Fas expression remain to be clarified.

Transmission electron microscopy (TEM) could be performed in myocardial specimens searching for outer membrane swelling or disruption, or for cristae derangement.

3. Overview of experimental studies

Several studies have addressed the issue of apoptosis during CPB. Schmitt et al. [21] have studied 11 patients undergoing elective CABG surgery. Cardioplegia was obtained with Kirsch solution and cold Bertocheiner solution (time of arrest 38.3 ± 16.2 min, and total reperfusion time 38.3 ± 7.8 min). Two myocardial biopsies of approximately 300 mm³ were obtained from the right atrial appendage just before the procedure (reference control) and after the extracorporeal circulation, immediately before chest closure. Samples were then analyzed using the in situ end-labeling for DNA fragmentation (terminal deoxynucleotidyl transferase nick-end labeling (TUNEL) assay), electron microscopy, cytochrome c and citrate synthetase release determination. Due to the short interval between induction of myocardial ischemia and tissue sampling, electron microscopy revealed no signs of terminal apoptotic processes (apoptotic bodies), but some nuclei displaying addensation of chromatin, and swollen mitochondria (early ‘apoptotic’ changes) in the same cells were noticed. Surgical procedure indeed determines a short lag time between the first and the second biopsy, which can result in the apoptotic myocytes being only in the initial phases of the apoptotic cascade—ideally, a third specimen taken during post-operative myocardial stunning may be taken to provide additional useful data. Moreover, this may explain the reason why overall the number of cells showing DNA fragmentation (TUNEL positive cells) tripled (1.3 ± 0.4 and 3.2 ± 1.3% after surgery) without reaching statistical significance (P = 0.14). TUNEL in fact marking DNA fragmentation represents a pre-terminal stage of the process. In order to assess the earlier stages of apoptosis Schmitt et al. have also evaluated the release of cytochrome c from mitochondria (which is an early event in the apoptotic cascade) by computing the cytosolic cytochrome c/activity/cytosolic citrate synthetase ratio, the latest indeed reflects the occurrence of inner mitochondrial membrane disruption, which more likely reflects initial necrotic cell death. This index correlated well with clinical parameters in the work by Schmitt et al.; it correlated positively with the time of cardioplegic arrest and reperfusion, and it also correlated with variations in cardiac hemodynamic parameters, positively correlated with variations in pulmonary capillary wedge pressure (PCWP) and negatively with variations in the cardiac index (CI; see Fig. 1). Moreover this index showed a significant 1.7 ± 0.2-fold increase (P < 0.05) during surgery. It is noteworthy that the duration of cardioplegia and reperfusion increased with mitochondrial changes characteristic of apoptosis.

Similarly, Zorc et al. [22] have evaluated apoptotic rate in endomyocardial biopsies from a cohort of patients who underwent CS and correlated it to post-operative short- and long-term mortality. Increased percentage of apoptotic myocyte and decreased expression of bcl-2 were found in the group of patients with early mortality compared to the group with longer survival.

Rajesh et al. [27] performed a study in which isolated rat hearts undergoing cold cardioplegia and subsequent reperfusion were treated with lonidamide, an agonist of mitochondrial inner membrane permeability transition pore (PTP). A significant depression of cardiac post-reperfusion performance was shown, supporting the concept that PTP opening is involved in the first steps of apoptosis.
The authors hypothesized that pore opening is likely to be a mechanism for apoptotic process triggering. PTP have been proposed to act as a cell death switch, determining not only whether a cell lives or dies, but also the pathway by which the death occurs, necrosis or apoptosis [28]. Use of propofol, known to enhance PTP closure, improved cardioprotection against global normothermic ischemia and during cold cardioplegic arrest. Stronger evidence regarding the early role of mitochondria has been collected [29]. These authors tested diazoxide, a selective opener of cardiac mitochondrial ATP-dependent potassium channels, in an animal model of cold cardioplegic ischemia and reperfusion-undergoing heart, as a possible cardioprotective agent. In fact, closure of these channels in consequence of ischemia-related lowering of ATP concentration has been reported to enhance the first steps of apoptosis. Hearts treated with diazoxide showed a significantly lower amount of TUNEL-positive nuclei, and a decrease of caspase-3 and pro-apoptotic bax protein cleavage. Similar results about the mitochondrial-initiated apoptotic cascade in consequence of cardioplegia is provided by Wakiyama et al. [30] regarding the same mitochondrial ATP-sensitive potassium channels, proposed to be cardioprotective [31]. These channels open at higher ATP concentrations. During ischemia ATP production is severely reduced and mitochondrial channels tend to close, thus determining a signal for initiating apoptotic cascade. Wakiyama et al. placed 19 pigs on total CPB and on 30 min of normothermic heart ischemia, followed by a 120 min reperfusion. Diazoxide was added to cardioplegic solution administered to a group of animals. Harvested myocardial samples were analyzed with TUNEL assay. In the control group 120.3 ± 48.8 positive nuclei per 3000 myocytes were detected, and a significantly reduced number (21.4 ± 5.3) in the diazoxide group. Nevertheless, it was pointed out that treatment with diazoxide did not ameliorate the functional recovery in the post-operative.
Although the exact mechanism by which the opening of the ATP-sensitive channels enhances cardioprotection remains to be elucidated. Thereafter, administration during reperfusion of a selective inhibitor of apoptosis-related endonucleases (aurintricarboxylic acid, ATA) was associated with improvements in regional contractile and vascular endothelial functions [63]. Interestingly, increased apoptotic myocytes death was noticed in a clinically relevant lamb model of cardioplectic arrest in neonatal patients; it was suggested that the neonatal myocardium could be in a ‘pro-apoptotic state’. Therefore, clinical impacts are to be investigated [48,65].

Addition of antioxidants (such as ebselen or deferoxamine) to cardioplectic solution reduced significantly the incidence of myocyte apoptosis in animal models or isolated hearts [14–18]. Further studies should be performed in patients comparing different cardioplectic solutions containing antioxidants or other antiapoptotic drugs; correlations with clinical conditions (duration of stunning, hemodynamics) after CS could be investigated methodically [38,49,53,55,59,61,62]. Ebselen is a selenium-containing heterocycle working as a glutathione peroxidase mimic. It has been suggested that ebselen reduces the severity of oxygen-free radicals injury in I/R, thus limiting cardiomyocyte loss for apoptotic processes [32]. Isolated swine hearts were used as a surgical model. Hearts were subjected to 15 min of normothermic regional ischemia (LAD ligation) followed by 30 min of normothermic cardioplectic arrest and 3 h of reperfusion. Hearts were randomized into three groups, each one being pre-perfused with three different doses of ebselen (5, 10 and 25 nM). Two other groups of hearts were subjected to LAD ligation and arrest without reperfusion, one last group performed as control. None of the ischemic and non-reperfused hearts showed signs of apoptosis, while apoptosis was reduced with ebselen at 10 and 25 nM in I/R group. These results confirm that myocardial oxidative stress is actually developed during CS with CPB, and provide further suggestions that it is a trigger for apoptosis. A similar study was performed using deferoxamine, an iron chelator known to interfere with reactive oxygen species production catalyzed by transition metals such as iron and copper [33]. Similarly, the cardioprotective potential of deferoxamine has been confirmed, but clinical correlations are still to be investigated. Even pyruvate has been proposed to afford cardioprotection working as a radical scavenger. In isolated working rat hearts subjected to cardioplegia and reperfusion pretreatment with pyruvate showed dose-dependent reduction of apoptotic cells detection and improvements of cardiac function [44]. Moreover, in myocardial biopsies taken before and after aortic cross-clamping, a depletion of antioxidant potential (reduction of plasma glutathione peroxidase activity) was proved which virtually reflects the development of oxidative stress. Therefore, metabolic pathways of purines have also been suggested as possible targets for routine cardioprotectons during surgery [43].

In any case, most of these findings are still waiting for clinical testing; that is, a quantification of the impact of the oxidative stress on myocardial recovery after reperfusion, onset of complications and clinical outcome is to be pursued through randomized trials. Taken together, the above results are enough to demonstrate that oxidative stress developed during the surgically induced reperfusion itself of ischemic myocardium is a trigger for apoptosis [34], probably through the mitochondrial pathway after activation of the membrane β-adrenergic receptor-associated kinases [54]. New cardioprotective strategies based on the hypothesis of the link inflammation–apoptosis–stunning are summarized in Fig. 1.

Vazquez-Jimenez et al. [35] evaluated the effect of cardioplegia temperature on myocardial apoptotic rate in pigs. In this model, intramyocardial TNF-α and IL-10 mRNA, and levels of cardiac troponin I in cardiac lymph and venous blood were assayed; TUNEL probe, electron microscopy and necrosis detection test were performed on tissue probes taken during surgery and postmortem. Higher IL-10 synthesis, lower TNF-α synthesis and lower troponin I release were proved in animals treated with moderately hypothermic cardioplegia (18 °C), and a lower troponin I concentration in cardiac lymph, suggesting a more effective cardioprotection by hypothermia. However, the same authors pointed out that while the percentage of apoptotic nuclei did not differ between the two groups, the ratio apoptosis/necrosis tended to be higher in animals subjected to hypothermia. The hypotheses by Vazquez-Jimenez et al. was substantially supported by data provided by Qing et al. [36]. In a similar experimental protocol, they showed in animals subjected to CPB with moderate hypothermic cardioplegia a higher gene expression and synthesis of heat shock protein (HSP)-72, considered to be cardioprotective towards necrotic process of cardiomyocytes—in fact, a lower percentage of necrotic cells was detected in these probes. Nevertheless, induction of apoptosis regulatory proteins and percentage of apoptotic nuclei did not differ between the two groups. HSP-70, known to interfere with apoptotic process development, has been reported to be induced by cold [37], but no in vivo studies are available to assess this hypothesis [64].

Early and reliable assessment of apoptosis is pivotal in clinical studies investigating the occurrence of programmed cell death in consequence of CS [57,60]. While detection of apoptotic bodies and TUNEL assay provide good sensitivity and specificity, they are almost useless in biopsies harvested during surgery. Immunostaining for cleaved caspase 3 and the apoptotic index could be the best choice in clinical settings. TEM research for early mitochondrial derangements has probably a low sensitivity and its reliability is controversial (see Table 2). The final goal of investigation about apoptosis detection could be the identification of a stable correlation between an early serum marker and the AR in specimens. Soluble Fas and Fas ligand could be tested in further studies in this perspective. Exclusion criteria of
patients should include recognized systemic pro-inflammatory status, which could bias the apoptotic balance in the myocytes. Also steroids administration for any reason should be considered an important variable.

Nevertheless, the reasons for permanently depressed cardiac performance in the post-operative, being the acute phase reaction sloped down at this time, remain to be clarified. Myocardial apoptosis, hypothetically triggered during the acute phase, could subsequently determine a significant cell loss, hence leading to long-lasting cardiac dysfunction, providing an attracting answer to this issue [41,51]. Mechanisms for in-hospital recovery from stunning might include hypertrophy of surviving myocytes, thus reducing the cardiac contractility reserve and affecting the medium- and long-term outcome. Evidence of correlations between pre- and intraoperative antiapoptotic treatment and improved cardiac performance in the post-operative could support this idea. Similar conclusions could be drawn after administration of free oxygen radicals antagonists and scavengers. These hypotheses need to be adequately tested. The role of oxyradicals in the pathogenesis of stunning as additional promoters of apoptosis rather than as direct contractility depressors in surviving myocytes is to be discriminated.

4. Conclusions

The role and clinical impact of apoptosis in CS need to be further assessed. The results of such investigation have probably the potential to lead to the development of more sensitive prognostic indexes and new cardioprotective strategies during CS. Several technical issues need to be addressed; an easy and reliable early marker of apoptosis is still to be found. To date, integration of TUNEL with biochemical assays (i.e. cytosolic cytochrome c release or activated caspase 3 determination) provide a useful method of detection of initial and completed apoptotic cascade. Moreover, this approach may be extended to the investigation of several different clinical conditions, and different cardioprotective solutions may be compared. The molecular and cellular pathophysiological steps linking surgery-dependent myocardial inflammation to post-operative depressed contractility and apoptosis need to be investigated systematically and in detail.

References


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<th>Marker</th>
<th>Assay for detection</th>
<th>Timing</th>
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<tbody>
<tr>
<td>Apoptotic index (cytosolic cytochrome c/cytosolic citrate synthetase activity)</td>
<td>Biochemical/ immunostaining</td>
<td>Early</td>
</tr>
<tr>
<td>Bax protein homodimers, activated caspase 9 and 3</td>
<td>Biochemical/ immunostaining</td>
<td>Early</td>
</tr>
<tr>
<td>Mitochondrial derangements</td>
<td>TEM</td>
<td>Early</td>
</tr>
<tr>
<td>Chromatin condensation/ DNA fragmentation</td>
<td>TUNEL</td>
<td>Late</td>
</tr>
<tr>
<td>Apoptotic bodies</td>
<td>TEM</td>
<td>Late</td>
</tr>
</tbody>
</table>

TEM, transmission electron microscopy; TUNEL, terminal deoxy-nucleotidyl transferase nick-end labeling.


Braun SL, Meissner H. Sodium nitroprusside in patients with 
compromised left ventricular function undergoing coronary bypass: 
reduction of cardiac proinflammatory substances. J Thorac Cardio-

[53] Pruefer D, Buerke U, Khalil M, Dahm M, Darius H, Oelert H, Buerke 
M. Cardioprotective effect of the serine protease aprotinin after 
regional ischemia and reperfusion in the beating heart. J Thorac Cardio-

[54] Remondino A, Kwon SH, Communal C, Pimentel DR, Sawyer DB, 
Singh K, Colucci WS. Beta-adrenergic receptor-stimulated apoptosis 
in cardiac myocytes is mediated by reactive oxygen species/c-Jun 
NH2-terminal kinase-dependent activation of the mitochondrial 

M. Inhibition of caspase-3 improves contractile recovery of stunned 
myocardium, independent of apoptosis-inhibitory effects. J Am Coll 
Cardiol 2001;38:2063–70.

[56] Sato H, Zhao ZQ, Jordan JE, Todd JC, Riley RD, Taft CS, Hammon 
Jr. JW, Li P, Ma X, Vinten-Johansen J. Basal nitric oxide expresses 
endogenous cardioprotection during reperfusion by inhibition of 
neutrophil-mediated damage after surgical revascularization. J Thorac 

[57] Scheubel RJ, Bartling B, Simm A, Silber RE, Drogaris K, Darmer D, 
Holtz J. Apoptotic pathway activation from mitochondria and death 
receptors without caspase-3 cleavage in failing human myocardium. 

shock protein 70 synthesis in the human heart after cold cardioplegic 

JD. Adenosine-enhanced ischemic preconditioning modulates necro-
isis and apoptosis: effects of stunning and ischemia-reperfusion. Ann 

W, Schmid RA. Apoptosis induced by ischemia and reperfusion 
1532–6.

[61] Suleiman MS, Halestrap AT, Griffiths EJ. Mitochondria: a target for 

AO, Svennevig JL. Heparin-coated cardiopulmonary bypass equip-
ment. II. Mechanisms for reduced complement activation in vivo. 

[63] Vinten-Johansen J, Thoumani VH, Ronson RS, Jordan JE, Zhao ZQ, 
Nakamura M, Velez D, Guyton RA. Broad-spectrum cardioprotection 

[64] Yeh CH, Wang YC, Wu YC, Chu JJ, Lin PJ. Continuous tepid blood 
cardioplegia can preserve coronary endothelium and ameliorate the 

[65] Zhao ZQ, Morris CD, Boodle JM, Wang NP, Muraki S, Sun HY, 
Guyton RA. Inhibition of myocardial apoptosis reduces infarct size 
and improves regional contractile dysfunction during reperfusion. 