Protection in the aged heart: preventing the heart-break of old age?

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Abstract

The aged heart has a diminished functional and adaptive reserve capacity, an increased susceptibility to incur damage (e.g., as a result of ischemia), and a limited practical ability for repair/regeneration. Thus, there has been considerable interest to harness the heart’s endogenous capacity to resist such damage, known as ischemic preconditioning (IPC), as well as other cardioprotective mechanisms. However, the translation of basic research findings into clinical practice has largely been inadequate because there have been few if any successful implementations in terms of viable therapies activating cardioprotection mechanisms to limit infarct size. Here, we provide an overview of the general mechanisms of cardioprotection, changes in the structure and function of the aged heart, and the current knowledge regarding cardioprotection in aged heart. The problems and opportunities for successful bench-to-bedside translation of cardioprotection in the elderly are discussed.

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1. Introduction

Heart aging is accompanied by changes that are progressive, pervasive, potentially deleterious, and, as far as known, irreversible. Cardiovascular diseases such as hypertension, atherosclerosis, and congestive heart failure are approaching epidemic proportions in the elderly population, and are the leading cause of morbidity and mortality among this group, making these diseases a major focus of interest in internal medicine and geriatrics. Regardless of age, the heart’s well-being is critically dependent on its blood supply, and vascular disease place this in jeopardy. Because physiological aging induces its own modifications in heart and because comorbidities (secondary to hypertension, diabetes, smoking, hypercholesterolemia, etc.) add their own effects to cardiac aging-induced modifications, the heart’s response to ischemia could differ from that of middle-aged populations usually studied, and also from controlled experimental models.

The duration of ischemia is critical for the cellular survival. Indeed, if the ischemic period is short, arrhythmias and reversible ventricular contractile dysfunction appear (i.e., stunning [1]) but few cardiac myocytes die. However, if ischemia is prolonged, irreversible damage resulting in widespread cell death appears, leading to loss of contractility and impairment of cardiac pump function. Cell death can be induced by two different pathways, necrosis and apoptosis which coexist during ischemia/reperfusion. As the apoptotic component of cell death contributes to the extension of infarct size during reperfusion, inhibition of this component contributes to improved contractile function of ischemic heart [2]. Apoptosis involves a mitochondrial permeability transition (MPT) [3]. Indeed, opening of the MPT-pore leads not only to the arrest of oxidative ATP-production but also to the release of pro-apoptotic factors for further completion of apoptotic cascade. As cell death is a
primary factor in the pathogenesis of infarction after ischemia/reperfusion and as survival is strongly correlated to the cardiac myocytes mass lost after ischemia/reperfusion injury [4], inhibition of apoptosis [5] by increasing resistance to MPT-induction [6–8] may confer protection.

Given that the aged heart has a diminished functional and adaptive reserve capacity, and an increased susceptibility to incur damage (e.g., as a result of ischemia), there has been considerable interest to harness the heart’s endogenous capacity to resist such damage, known as ischemic preconditioning (IPC) [9]. Here, we review the current knowledge regarding cardioprotection in aged heart. Firstly, we discuss the general mechanisms of cardioprotection in various model systems, including preconditioning (PC) of human heart. This is followed by a succinct overview of changes in the structure and function of the aged heart. The potential benefits of PC implementation as a therapeutic strategy is discussed with a critical overview of related clinical trials. Finally, we focus on aging-induced modifications in preconditioning response and discuss the consequences for clinical applications and the difficulties in the translation from bench-to-bed-side.

2. Cardioprotection

2.1. Description of the phenomena

IPC is manifested by a reduction of cardiomyocyte injury and death by increasing their resistance to ischemic damage, marked suppression of ventricular arrhythmias, and enhanced recovery of contractile function. There is specific chronology regarding IPC: “the first window” of protection lasts approximately an hour (acute, or early, or classic PC). One to four hours later, protection is no longer evident. However, a degree of protection returns around 24–96 h later (the “second window”, or late or delayed PC [10]).

IPC has been demonstrated to occur in humans [11–15] and several studies suggest that ischemia occurring “naturally” prior to AMI could reduce the infarct size [14,15]. Furthermore, IPC is likely responsible for the warm-up phenomenon [16,17]: patients often describe that after exercising to the point of angina, following a stop and rest, they can continue exercising without further angina. There is less ST-depression and better exercise tolerance and performance during the second period of exercise, similar to that found in experimental IPC models [17]. Exercise-induced ischemia could also trigger late PC [18].

In addition to IPC, many pharmacologically induced cardioprotective signaling-pathways have been elucidated. Multiple receptors can work in parallel, affording redundancy to the PC stimulus [6]. Different cell surface receptor activators: adenosine, bradykinin, opioids, insulin, IGF-1, norepinephrine, as well as increased K+ influx into mitochondria via mitochondrial K\(_{ATP}\) channel (mitoK\(_{ATP}\)) activation or Na+/H+ exchanger (NHE) inhibition, and non-receptor activation by short periods of elevated Ca\(^{2+}\), transient periods of hyperthermia, reactive oxygen species (ROS), and NO can trigger similar degrees of cardioprotection [6,10]. Several trials reported the effectiveness of pharmacologic protection in humans, although with some contradictory results [19–24].

2.2. Mechanisms of protection: classical or early IPC

2.2.1. Role of PKC and mitochondrial K\(_{ATP}\) channel

Several studies have demonstrated a role for PKC, and particularly PKC\(_{\epsilon}\), in PC [6,25–29]. PC, NO and ROS, are all involved in activation and translocation of PKC\(_{\epsilon}\) [6,30–33]. PKC\(_{\epsilon}\) plays a role in activation of mitoK\(_{ATP}\) [33] and activated PKC seems to be associated with MPT-pore components [34].

Although it has not been cloned, a functional mitoK\(_{ATP}\) has been isolated, reconstituted, and pharmacologically characterized [35,36]. Considerable data support the important role of mitoK\(_{ATP}\) activation in PC [6,37–39]. Selective activation of the mitoK\(_{ATP}\) is protective whereas inhibitors (e.g., 5-hydroxydecanoate) block the protection afforded by PC [37,38]. Potassium channel openers mimic the effects of endogenous defense mechanisms induced by PC. Activation of mitoK\(_{ATP}\) is assumed to occur as a result of impaired metabolism or in response to stimuli such as NO [40], PKC activation [6,33], ROS [36] and pharmacologically by diazoxide [41].

2.2.2. Central role of GSK-3\(\beta\) on end-effector MPT-pore

Direct measurements of the MPT-ROS threshold recently confirmed that the end-effector in cardiomyocytes is likely the MPT-pore complex, and that phosphorylation and inhibition of glycogen synthase kinase-3\(\beta\) (GSK-3\(\beta\)) activity results in protection of the MPT-pore complex against ROS-induction [6]. Sollott and colleagues [6] found that activation of diverse upstream protective signaling-pathways (i.e., acting through kinases including, PKA, PKB, PKC, or p70s6k) converge on GSK-3\(\beta\), which serves as a requisite point-of-integration and acts on the end-effector, the MPT-pore complex, to limit MPT-induction (Fig. 1). Direct inhibition of GSK-3 reduces infarct size and improves postschismic function [42].

2.2.3. Memory of protection

Since PC can be induced within 10–15 min, the involvement of reversible posttranslational protein modification was suggested [10]. PC can be characterized as protection with a durable “memory” that lasts at least an hour beyond the triggering stimulus (Fig. 1) [6]. It was recently demonstrated that PC memory is “encoded” by regulated, low-amplitude, and reversible mitochondrial swelling that results in enhanced substrate oxidation, mitochondrial electron transport and ROS-production, leading to redox-activation of PKC which in turn inhibits GSK-3\(\beta\). Within such a definition, durable memory can be induced by
transient hypoxia, or pharmacologically (e.g., via mitoKATP activation, NHE inhibition (which may work directly on mitochondria via inhibition of K+H+ exchange), as well as by activation of bradykinin B2 or δ-opioid receptors). Protection without a durable memory is characterized by agents which can increase MPT-ROS threshold during the actual period of exposure, but this protection returns to baseline levels within minutes (formally, <15 min) after washout, and occurs independently of upstream signaling by mitochondria. This protection can be elicited by receptor tyrosine kinase activation by insulin or IGF-1, signaling through PI3K/PKB/Akt and mTOR/p70s6k, or by agents that act directly on their downstream effectors, PKC and GSK3. The latter group does not act by regulation of respiration via mitochondrial swelling and its consequent signaling (Fig. 1). Thus, while affording potentially potent cardioprotection during the period of exposure, nevertheless, this group would but poorly be able to precondition.

2.3. Delayed PC

The myocardial protection of IPC occurs with a bimodal time course. Early PC is highly effective but relatively short lived, whereas a delayed form of adaptation is manifested approximately 24 h following PC [43]. The degree of this protection is usually weaker than early PC but its duration is considerably longer. Apart from ischemia, late PC can be induced by e.g., heat stress [43], exercise [44], NO donors [45], adenosine and opioid receptor agonists [10]. The role of ROS also appears to be crucial (serving as a trigger) because administration of antioxidants prevents the protective effects of delayed PC.

The late onset and the prolonged duration of protection suggest that delayed PC is related to changes in transcription factor activity and new protein synthesis. Heat shock protein (HSP72 and HSP70) overexpression in the myocardium are associated with resistance to ischemic damage [43]. Antioxidant enzymes such as MnSOD [46] and inducible enzymes such as iNOS [47] or COX-2 could also play important role in delayed PC [48,49].

2.4. Postconditioning

The concept and applicability of cardioprotection was recently extended by the discovery of “postconditioning”, where ischemia/reperfusion brief episodes occurring at the time of reperfusion were as effective as preconditioning in reducing infarct size [50]. This suggests that a therapeutic intervention performed as late as at the time of reflow may still significantly limit infarct size and opens new possibilities for translation of this form of protection into the clinical setting by the strategic modification of the reperfusion protocol. Mechanisms of postconditioning seem to involve activation of the “pro-survival” kinases, PI3K-Akt, p70s6k, and eNOS [51].

3. Structure and function of the aged heart

The cardiovascular system structure undergoes subtle but progressive changes with age that result in altered function. Anatomical, ultrastructural, mechanical, and biochemical changes may compromise the adaptive responses of the aged heart, and impair the efficacy of the preconditioning response. Aging is accompanied by an increase in arterial systolic pressure, reduced or maintained diastolic pressure, increased pulse pressure and aortic dilatation and wall thickening.

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**Note:** The diagram in the text represents a series of signaling pathways involved in myocardial protection, with various agents and kinases depicted. The text provides a detailed explanation of each pathway and its role in cardioprotection, including the activation of GSK-3, PKC, and other kinases through PI3K/PKB/Akt and mTOR/p70s6k pathways. It also highlights the importance of ROS in memory-associated signaling, which can result in protection lasting several hours after the upstream stimulus is removed.
[52]. The old heart is characterized by an increase in LV mass, myocyte hypertrophy and reduction in their number [52–54]. Although most myocytes are postmitotic, studies on the aged heart suggest that a portion of myocytes retain proliferative capacity, and that cellular hypertrophy is accompanied by hyperplasia [55–57]. Cardiomyocyte death by apoptosis and necrosis can be detected together with a low frequency of nuclear mitotic division [56]. Telomere length measurements support the suggestion that a portion of myocytes may divide repeatedly from birth to senescence, offsetting the continuous cell death in the aging rat heart [58]. On the other hand, although a review of cardiomyocyte DNA synthesis studies supported the existence of a limited proliferative ability in these cells, it cautioned against the significance of their regenerative capacity [59]. Controversy related to the regenerative capacity of the heart seems to continue despite recent evidence that the adult rat heart possesses stem cells that can differentiate into structurally and functionally competent cardiac cells, and it was suggested that these cells can form new muscle tissue following damage [60]. The sufficiency of this intrinsic regenerative capacity to reverse the progression to failure in badly injured hearts remains questioned [61]. The existence of a time-dependent disease state of the human heart, an “aging myopathy”, characterized by an early cellular senescence and death of primitive cells and myocytes, has been proposed [62]. The regenerative capacity of the aging heart could be impaired by this process of cardiac stem cell senescence where the age-affected IGF-1 receptor/PI3K/Akt signaling-pathway may play a role [63].

3.1. Signaling in aged heart

Age-associated impairment in the myocardial contractile response to β-adrenergic receptor (βAR) stimulation, a decreased reactivity of baroreceptors and chemoreceptors, and an increase in circulating catecholamines has been widely demonstrated. The decline in the so-called “fight-or-flight” response is related most likely to a specific deficit in βAR signal-transduction, while the intrinsic properties of the excitation–contraction coupling machinery remains intact at low work load [53,64] but this may not be the case at high work load. The age-associated reduction in maximal heart rate and LV contractility during vigorous exercise are manifestations of reduced β-adrenergic responsivity [65] despite an increase in circulating levels of noradrenaline and endothelin-1.

Numerous studies on old animals have attempted to pinpoint the defect in the G-protein-coupled-receptor (GPCR)-adenyl cyclase-cAMP-dependent PKA-signaling cascade that leads to the inadequate activation of contraction in aged heart. Studies showing changes in G-protein, GPCR and adenyl cyclase levels, as well as G2-mediated coupling of βAR to adenyl cyclase, and decreased levels of cAMP, have been published [66,67]. The age-associated reduction in βAR-signaling could be also related to up-regulation of opioid peptide receptor-signaling because of the significant antagonistic effects between stimulation of opioid peptide receptor and βAR-mediated positive contractile response [68]. Cardiac muscarinic receptor density and function is also diminished with increasing age and might contribute to the decrease in baroreflex activity observed in aged subjects [69]. These age-associated changes in receptor density, function or signaling to downstream mediators could, in principle, significantly impair the response of the old heart to activate cardioprotective pathways.

Age-altered adrenergic signaling is closely related to coordinated changes in levels of SR Ca2+-handling proteins and/or their function. In aged hearts, selective down-regulation of SERCA protein levels [70], decreased phospholamban phosphorylation [71], which might be reflected in the desensitized adenylyl cyclase response, and increased levels of Na+/Ca2+ exchanger [72] could lead to the reduction in SR Ca2+-load. It is also suggested that the aged heart utilizes the compensatory increase in the L-type Ca2+ currents [73] and the significant prolongation of action potential duration to preserve SR loading and to keep the amplitude of intracellular Ca2+-transients and contractions in old ventricle similar to those of the young organism [74]. Many aspects of Ca2+-regulation responsible for impaired excitation–contraction coupling, defective Ca2+-signaling with possible effect on cardioprotection in the aged heart are not yet known.

Mitochondria possess unique Ca2+-transport machinery, regulate metabolism under partial control by Ca2+ [75] and may themselves contribute to cell Ca2+-homeostasis. Thus, they play a central role in the reduced Ca2+-tolerance observed in aged animals [76] which can be partially reversed by diets enriched in ω3-polyunsaturated fatty acids (PUFA) [77].

Mitochondria were recently found to possess SERCA [78] and ryanodine receptor [79] proteins suggesting dual mitochondrial and reticular Ca2+-regulation and metabolic control [80]. Ca2+ can regulate the activity of key rate-limiting dehydrogenases that govern mitochondrial metabolic output, i.e., ATP-production [75]. Thus, regulation of mitochondrial Ca2+ level serves to maintain myocardial function by matching ATP-production to metabolic demands. Factors and conditions that cause mitochondrial damage and Ca2+-dysregulation can affect overall cellular metabolism, leading to energy deficits and cardiac dysfunction which may contribute to the aging process [81].

Mitochondria constantly produce ROS as by-product of electron transport. Mitochondrial ROS production seems to increase with age, leading to higher oxidative mitochondrial damage (protein oxidation, lipid peroxidation, mitochondrial DNA defects leading to abnormal phenotypes, and reduced capacity of ATP-synthesis [53]. Recently, a novel point mutation in mitochondrial DNA was identified in
humans that is associated with hypertension, hypercholesterolemia, and hypomagnesemia, a constellation similar to that of the metabolic syndrome [82], which constitute important risk-factors during aging. Furthermore, this study [82] suggests that the age-related loss of mitochondrial function [83] could underlie the age-associated development of hypertension and hypercholesterolemia.

Defects in oxidative metabolism could impair performance in aged heart. The activity of electron transport chain complexes is decreased, leading to oxidative metabolism abnormalities. The decrease in complex IV activity observed in aged heart mitochondria can be reversed by coenzyme Q10 supplementation [84], suggesting that aging alters the inner membrane environment. Indeed, CoQ10 [85] and ω3- to ω6-fatty acids ratio [86] decrease in aged heart mitochondria. Moreover, aging induces a defect in the ubiquinol-binding site of complex III of the electron transport chain [87] resulting in higher ROS production. As a result, the amount of membrane PUFA that are converted to lipid hydroperoxides increases. 4-Hydroxy-2-nonenal (HNE), formed by superoxide reaction with ω3-PUFA, is a reactive and long lived component that can diffuse and gain access to potential targets and produce oxidant stress at sites distant from the initial site of ROS-production [88].

Cells from aged hearts have a lower threshold for ROS-induced ROS release and a higher probability of MPT-induction [6,53,89]. Indeed, MPT-induction could be favored by the decrease of membrane cardiolipin content, or by HNE increase, which could affect adenine nucleotide translocator function [86]. Thus, taken together, the data support that aging results in a vicious cycle of higher oxidative damage followed by lower coupling of electron transport to ATP-synthesis, which could eventually lead to elevated cellular damage and cardiomyocyte loss. It has been suggested that the damaging ROS impacts on mitochondria can be partially attenuated by a ω3-PUFA-rich diet [53,90]. Similarly, lipoic acid supplementation may limit the age- and cardiac reperfusion injury-related deterioration of mitochondrial function [91].

One important issue should be emphasized concerning the need for a degree of caution in the interpretation of results from different experimental models. The obvious discrepancy between the results obtained by different authors using isolated mitochondrial or intact cellular preparations is a case in point (for example, the differences in data from isolated mitochondria showing no age-related changes in a ROS-production, versus that from whole tissue in which there is higher ROS-production with age). There are at least three possible explanations: (1) isolated mitochondria may not be an adequate model system to study these kinds of changes; potentially there are some important factors lost during isolation (and even worse, the loss is disproportional between the young and old models); (2) there are small or barely detectable quantitative changes accumulated with age which non-linearly affect the cell and organ fate, but which cannot be detected in mitochondrial suspension; (3) changes are not at the level of individual mitochondria or cells, but rather at the organ level and only manifest as synchronized behaviors (such as cardiac electric activity) which are not evident when the tissue is dissociated.

In summary, aging induces major changes in heart structure and function which may place the aged heart constantly “on the edge” and at risk including the loss of its adaptive response to stress. Thus, it is even more critical that the aged heart retain the capacity to engage cardioprotection signaling.

4. Cardioprotection in aged heart

The incidence of coronary heart disease increases in the elderly. The increased frequency of acute coronary syndromes may be at least in part due to a loss of PC protection. A range of studies performed in animals and in humans has sought to pinpoint the impairments in protection observed in old patients, and to understand which mechanisms may still remain functional or can be restored.

4.1. Animal studies

Most experimental studies performed in rodents, using infarct size and hemodynamic measures to assess cardioprotection, found that IPC effects are diminished or abolished in old heart (Table 1; [92–96]). However, in in vivo aged rabbit [102] and sheep [103] models it was found that IPC reduces the infarct size. These apparently contradictory results can probably be explained by the absence of standardized IPC protocols and animal models, the different species used, and more importantly the absence of reliable definitions of aging in these models [104]. Experimentally, caloric restriction both reduced posts ischemic dysfunction and restored PC in aged rat heart [96].

4.2. Human studies

Experimentally, myocardium (e.g., isolated atrial samples) obtained from elderly patients is more sensitive to ischemia than myocardium from middle-aged or young patients [105]. In vitro creatine kinase (CK) release from atrial samples exposed to short ischemia episodes mimicking IPC was similar in young, middle-aged and elderly patients (70–90 years) [106], suggesting that IPC could reduce ischemia-induced injury, even in aged myocardium. However, a beneficial effect of IPC was not found by Bartling et al. [107] who showed that improvement of contractile force after prolonged ischemia observed in “young” patients (<55 years) was lost in old patients (>70 years) when a brief ischemia/reperfusion sequence is applied.
Clinical trials have also tried to define if IPC occurs in elderly patients (Table 2). Lee et al. [110], using intermittent balloon inflations (of either 120 or 180 s, followed by 20 min of I and 40 min of R) in the coronary artery to mimic IPC in patients undergoing interventional coronary angiographic procedures, showed that the protection (manifest as less EKG ST-shift and lactate production) observed in adult patients was not seen in elderly patients using 120 s balloon inflation. The elderly patients required an increased PC stimulus (180 s inflation) to manifest IPC [110] suggesting that human aging is associated with an increased threshold to trigger cardioprotection (see Fig. 2A and Conclusion). This beneficial effect of IPC seems to be mediated by mitoK<sub>ATP</sub> mechanisms because mitoK<sub>ATP</sub> blockade abolished this effect. The duration of balloon occlusion seems to play a major role in the successful induction of IPC in humans. Indeed, other studies using a shorter duration of inflation (90 s) failed to induce IPC even in younger patients [113]. This apparent threshold duration of ischemia needed to trigger PC might lie between 90 and 180 s in normal adults and seems also to increase with age [114].

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<th>Table 1: Preconditioning response in aged animals</th>
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<td>[95]</td>
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<tr>
<td>-2 age groups: 6 and 24 mo old</td>
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<tr>
<td>-IPC protocol: 2 min of I, 10 min of R followed by 20 min of I and 40 min of R</td>
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<tr>
<td>-norepinephrine (NE) PC</td>
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<td>[97]</td>
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<td>-2 age groups: Y 12 wk, M 50 wk</td>
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<td>-IPC protocol: 3×5 min of I before 20 min of I (Y) or 15 min (M) of I and 30 min of R</td>
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<tr>
<td>-3 age groups: Y 2 mo; M 6 mo; O 20 mo</td>
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<tr>
<td>-IPC protocol: 5 min of I, 5 min of R before 30 min of I and 40 min of R</td>
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<td>[98]</td>
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<td>-IPC protocol as in [113]</td>
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<td>-PC impaired in O</td>
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<td>-2 age groups: 6 and 24 mo old, sedentary vs. trained</td>
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<td>-2 groups: 6 and 24 mo, CR vs. Ad Lib</td>
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<td>-2 groups: 6 and 24 mo old and O 18–20 mo</td>
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<td>-IPC protocol: 5 min of I or 3×5 min of I followed by 35 min of I and 2 h of R</td>
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<td>-3 groups: Y 2–4 mo, M 10–12 mo and O 18–20 mo</td>
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<td>-3 groups: Y 2–4 mo, M 10–12 mo and O 18–20 mo</td>
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<td>[101]</td>
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<tr>
<td>-2 groups: Y 2–4 mo and O 18–20 mo</td>
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<td>-IPC protocol: sevoflurane 0.4 mM during 10 min, 5 min washout, 25 min of global I, 60 min of R</td>
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<td>[102]</td>
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<tr>
<td>-3 groups: Y 4–6 mo, M 24–27 mo, O 42–60 mo</td>
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<tr>
<td>-In vivo model: 5 min of coronary artery (CA) occlusion, 10 min reflow and 30 min CA occlusion and 3 h reperfusion</td>
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<tr>
<td>-Sheep</td>
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<tr>
<td>-2 groups: Y 0.5–1 yr and O 5.7–8 yr</td>
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<td>-In vivo model: 3×5 min transient CA occlusions, before 60 min of I and 150 min of R</td>
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Abbreviations: PC (preconditioning), DP (developed pressure), LVDP (left ventricular diastolic pressure), LVP (left ventricular pressure), EDP (end-diastolic pressure), LVSP (left ventricular systolic pressure), Y (young), M (middle-aged), O old, wk (week), mo (month), yr (year), I (ischemia), R (reperfusion), IS (infarct size), CR (caloric restriction), Ad Lib (Ad libitum), NE (norepinephrine).
Abete et al. [108] described that old patients (>65 years) having an angina episode 48 h before their AMI had an increased mortality rate, a greater CK peak, an increased incidence of CHF, ventricular arrhythmias and LV dysfunction compared to patients under 65 years, suggesting that the benefit of repeated ischemic periods before AMI is lost in old (vs. younger) patients. However, the time course of angina is highly important because if angina occurred one week before AMI in patients >65 years, the proportion of patients with LVEF <40% decreased, the incidence of arrhythmia, CHF and cardiogenic shock was reduced and the CK peak was decreased [109]. This study confirmed data obtained from a TIMI-4 substudy where older patients with preinfarct angina had a lower rate of death, heart failure, shock and/or reinfarction versus older patients without angina [115]. This suggests that delayed PC could still exist in elderly, whereas early PC is deficient. Recently, by studying the “warm-up” phenomenon in elderly, the early phase of IPC is found deficient in these patients. Indeed, the improvement in exercise tolerance was not found, and the time to onset 1 mm ST-depression was higher in the elderly population [112].

Although certain aspects of the endogenous protection deriving from IPC seems to be lost during aging, nevertheless, it might be possible to (partially) restore it. Indeed, practicing sustained physical exercise in old patients decreased mortality rate, CK peak, incidence of arrhythmias and cardiogenic shock, when AMI occurred [111]. However, this benefit of sustained activity was only seen in aged patients with preinfarction angina. This suggests that exercise, while not sufficient by itself to produce protection in the elderly, is still necessary for preinfarction angina to be protective (at least partially restoring cardioprotective mechanisms). A training-induced increase in myocardial MnSOD activity could in part explain this observation [1].

In vitro pre-treatment of human trabeculae with CoQ10 overcomes the reduced capacity of aged cardiac muscle to recover contractile function after ischemia and more importantly, pre-operative supplementation with CoQ10 in

![Fig. 2. Changes in the injury and protection thresholds in aging heart. (A) Aging diminishes the heart’s threshold to sustain injury (e.g., from ischemia/reperfusion, etc.). Lifestyle modifications, including exercise and possibly caloric restriction, may partially diminish the aging effect. Comorbidities (such as diabetes) have negative influence. (B) Aging increases the heart’s threshold to activate protection-signaling mechanisms. Various pharmacologic agents (e.g., sulfonylureas, antioxidants, partial fatty acid oxidation (PFAO) inhibitors, and COX-2 inhibitors) that can interfere with cardioprotective signaling-pathways can exacerbate this trend and further increase the protection threshold. Exercise and caloric restriction might attenuate the age-dependent trends.](https://academic.oup.com/cardiovascres/article-abstract/66/2/233/269826)
patients undergoing cardiac surgery reduces myocardial damage, and improves pump function and decreases the in-hospital stay [85].

4.3. Mechanisms impaired with age

Compared to young adult myocardium, the senescent myocardium is more sensitive to ischemia [97,116], suggesting that protective pathways existing in adult myocardium are modified or impaired by aging. For example, diminished HSP70 expression has been reported [117]. NO, produced by eNOS or iNOS, plays a role in cardioprotection [118]. However, iNOS and eNOS expression and activity are modified in senescent myocardium [119]. Changes in PKC translocation have been described in aged myocardium [120,121], leading to certain age-associated changes in the specific sites targeted by each PKC isoform.

As with the rest of the cell, mitochondria undergo aging-related changes which may enhance their sensitivity and compromise their response to stress (such as ischemia), but can also result in the loss of the ability to trigger cardioprotection mechanisms. These changes can obviously have deleterious effects on the fate of the mitochondrion, cell and organism.

5. Conclusion and where we go from here

This review focused attention on specific issues and needs of protection in the aged heart. Overall, despite some inconsistency, the literature suggests that: (1) Aging impairs the intrinsic capacity of the heart to resist potentially damaging stress (such as IR), which makes the heart more likely to sustain a greater degree of injury. This can be portrayed as a decreased “threshold for injury” with aging (Fig. 2A). (2) Changes in lifestyle, specifically with regard to exercise levels and diet (e.g., caloric restriction, consumption of fish oil-reach diet, or possibly CoQ10 supplementation), can potentially restore some of the aging related defects and increase (improve) the injury threshold. On the other hand, confounding effects such as the presence of comorbidities (e.g., diabetes, etc.) can have a substantially negative impact on the injury threshold (Fig. 2A). (3) Endogenous mechanisms, which must be successfully triggered to afford significant protection above the level of the heart’s intrinsic, basal capacity to resist stress, apparently decline during aging. The evidence suggests that the stimulus-threshold for triggering protection increases with age, and that a certain degree of protection can still be achieved with a sufficiently enhanced trigger (Fig. 2B). Importantly, this protection threshold can probably also be modulated. Exercise, and possibly caloric restriction, can partially reverse the aging trend, whereas certain pharmacologic agents, by an (initially) unanticipated interference with cardioprotection signaling mechanisms, could accentuate the age-related impairments and result in an apparent increase in the protection threshold (Fig. 2B).

It is probable that a certain degree of the age-related impairment in cardiac protection-signaling could remain unresponsive to these lifestyle/diet measures, but pharmacologic approaches aimed at downstream targets (such as GSK-3 and the MPT-pore) might provide a rational strategy to further restore these mechanisms. Since most of these approaches have shown relatively little protection efficacy when given during or after reperfusion, there seems to be little feasibility for their use (alone) in patients presenting with AMI. However, postconditioning protocols could feasibly resolve this timing problem, and thus could show great promise if efficacy could be demonstrated in the elderly.

However, we must be aware of drugs that can potentially interfere with endogenous protection-signaling, diminishing the protective response already altered by “physiological” aging. Indeed, COX-2 inhibitors can (at least theoretically) inhibit the protective effects of delayed PC, and the recent recall of the selective COX-2 inhibitor, rofecoxib, based on results from APPROVe study [122], is possibly related in part to PC inhibitory actions. Certain sulfonylureas commonly used to treat type 2 diabetes mellitus, a prevalent affliction in the elderly, are known to inhibit the mitoK\textsubscript{ATP}. Glibenclamide (one of the most commonly used sulfonylureas) can abolish the MPT-protection afforded by diazoxide [6,123], which may explain the unexpected increase in cardiovascular mortality observed in patients receiving these drugs [124,125]. Preventing ROS-signaling (which plays an obligatory role as a trigger of PC) by ROS-scavengers can abolish PC [6,32,33]. Consequently, the currently popular and widespread use of pharmacologic levels of antioxidants, specifically in elderly people, should be reconsidered and reevaluated on the basis of the potential interference with endogenous PC-signaling mechanisms. Partial fatty acid oxidation inhibitors (e.g., trimetazidine), which are used to treat angina [126], were found to prevent endogenous MPT-protection mechanisms in rat cardiomyocytes [6] and PC in isolated rat heart [127], which would be deleterious in patients at risk of vascular complications. In these patients in which the endogenous capacity for protection-signaling might be impaired by aging and/or the untoward action of certain drugs, cardioprotection potentially could still be induced by activation of cell surface receptors by insulin, IGF-1, erythropoietin, GLP-1, etc [6]. However, activation of cell surface receptors and their downstream pathways still could be impaired by aging, per se. Pharmacological agents acting proximally to, or at, the end effector(s) of protection, could serve to bypass upstream age-related signaling defects. For example, direct inhibitors of GSK-3 (e.g., Li\textsuperscript{+} and SB 216763 or SB 415286) could potentially reverse aging-related loss of PC mechanisms. Thus, it might be reasonable to consider adding Li\textsuperscript{+} or another GSK-3 inhibitor to GIK in the treatment of acute ischemic syndromes, myocardial infarction, and stroke, especially in elderly.
For the first time, major age-related cardiovascular risk factors, hypertension and hypercholesterolemia, have been correlated with mitochondrial dysfunction. Since mitochondrial function can decline with aging, future research efforts should examine whether (and how) a causative link exists, and by what mechanisms [82]. Thus, prevention of the deterioration of mitochondrial function could become central in the prevention and treatment of hypertension and hypercholesterolemia.

Although considerable investigative efforts have been directed towards defining the essential cardioprotection signaling-pathways with the implicit goal being the implementation as a therapy, the translation of basic research findings into clinical practice has largely been inadequate. There have been few, if any, successful implementations in terms of viable therapies activating cardioprotection mechanisms to limit infarct size. Unfortunately, most studies have been conducted on healthy young adult animals where cardioprotection signaling is relatively robust and easy to trigger, and hence easy to study, whereas it may require a stronger triggering stimulus in the aged heart. Thus, there is an urgent need for more research conducted on senescent animals, and for carefully designed clinical trials involving aged human subjects. There also needs to be better coordinated efforts between basic science investigators, clinical trial managers and physicians [128]. In studies on elderly subjects, this effort is partially hampered by the added complications arising due to the fact that cardioprotection signaling is impaired in old heart and the design of clinical trials must take this into consideration.

It seems likely that specific reperfusion therapies (i.e. postconditioning) together with adjunct pharmacologic approaches (e.g., GSK-3 inhibition, etc.) hold the greatest promise that must be realized. Furthermore, in light of the progressively limited repair and regenerative capacity of the aged heart, the development of therapeutic techniques to better mobilize (and/or overcome senescence of) cardiac stem cells to replace and offset the loss of cardiomyocytes with the goal to minimize an “aging myopathy,” and particularly to improve cardiac regeneration after injury, is also an obvious priority. All of this argues for clinical trials designed to examine these questions, taking into account the specific age-related issues, to realize the successful translation from bench-to-bedside.

References


