

Aged rat heart: Modulation of age-related respiratory defects decreases ischemic-reflow injury

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ABSTRACT

Myocardial injury increases in the elderly heart during ischemia and reperfusion. Mitochondria, the key targets and sources of injury during ischemia and reperfusion, sustain ischemic damage to the electron transport chain that is superimposed upon age-related defects. In the adult heart, interventions to activate endogenous cytoprotective signaling systems meet in mitochondria to decrease cardiac injury. Unfortunately, these systems are largely ineffective in the aged heart. Thus, new treatment concepts are needed to reduce injury in the aged heart. Our group chose a strategy to directly treat the effector of cardiac injury in the aged heart, the mitochondria. We further utilized a novel approach to ask if the reversal of aging defects in cardiac mitochondria before ischemia could decrease ischemia-reperfusion injury in the heart. Three hours following treatment with the small molecule, nutraceutical acetylcarnitine (AcCN), oxidative phosphorylation as well as age-induced defects in electron transport chain complexes III and IV was corrected in the heart. When such hearts were then exposed to ischemia and reperfusion, cardiac injury was markedly reduced. Contraction during reperfusion improved and recovery became similar to that in adult hearts. Cardiac cell death was substantially reduced. Thus, age-related defects in electron transport are a key mechanism of the increased myocardial injury in the elderly heart during ischemia and reperfusion. Modulation of aging-induced defects in mitochondrial metabolism reduces cardiac injury from ischemia and reperfusion, and is a novel strategy to protect myocardium in the elderly patient at risk for an acute myocardial infarction.

Keywords: Mitochondria; Cytochrome Oxidase; Complex III; Myocardial Infarction; Aging

1. THE CLINICAL CHALLENGE OF MYOCARDIAL INFARCTION IN THE ELDERLY: INCREASED CARDIAC INJURY CONCOMITANT WITH DECREASED RESPONSIVENESS TO INNATE CYTOPROTECTION

During the course of an acute myocardial infarction, elderly patients experience an increased mortality and sustain greater cardiac damage compared to younger patients despite timely and successful reperfusion [1]. The aged heart suffers greater damage during ischemia and reperfusion in both experimental and clinical settings [1-6]. In a National Institutes on Aging rat model of aging, the Fischer 344 rat (F344), isolated, buffer-perfused hearts from aged rats (24 mo.) exhibit decreased hemodynamic recovery and greater cardiomyocyte death following ischemia and reperfusion compared to hearts from adult (6 mo.) controls [4-7]. Age-enhanced cardiac injury during ischemia and reperfusion has been observed in other rat strains [3] and species [2,8] as well.

Mitochondria are key targets of ischemic damage as well as effectors of cardiac injury during ischemia and reperfusion [2,3,9,10]. Mitochondria-driven effector mechanisms, especially relevant to the aged heart, include oxidative damage [6], calcium accumulation [2], and activation of apoptosis [5]. In the aged heart, ischemic damage to mitochondria is superimposed upon pre-existing age-related alterations in mitochondrial metabolism [10,11]. We posited that intervention to improve aging-induced defects in mitochondrial function would decrease myocardial injury during subsequent ischemia and reperfusion.

Mitochondria-directed strategies to limit cardiac injury during ischemia and reperfusion are highly relevant to the protection of the aged heart and the ultimate im-

provement of clinical outcomes in high-risk elderly patients. Novel therapies are required because classical approaches widely used in experimental studies of ischemia-reperfusion injury in the adult heart increasingly appear to be of limited efficacy when applied to the aged heart. Interventions that utilize activation of cytoprotective signaling cascades that converge on mitochondria include ischemic preconditioning, where preemptive periods of brief ischemia limit injury from a subsequent longer period of ischemia [7,12-15]; and postconditioning where brief ischemia is applied during early reperfusion [16]. Cytoprotective cascades converge to modulate mitochondrial function via mitochondrial K_{ATP} channel via PKC epsilon [17] or to modulate susceptibility to permeability transition via the insulin-signaling related Akt-GSK3beta pathway [18]. These pathways that are highly effective in the adult heart are clearly less effective in the aged heart, as shown by multiple investigators for ischemic preconditioning [19-21] and recently for postconditioning [19,22]. Because ischemic preconditioning and postconditioning share overlapping mechanisms of protection [23] and overlapping extents of cardioprotection [24], it is very likely that observations of the ineffectiveness of ischemic postconditioning in the aged heart will continue to be reinforced as further studies of postconditioning are performed.

It remains unclear if the major mechanism of the ineffectiveness of signaling-based cytoprotection in the aged heart is at the level of the signaling cascades upstream of the mitochondria or the inability of mitochondria in the aged heart to respond to the cytoprotective modulation. Two lines of evidence suggest that mitochondria in the aged heart retain the capacity to respond to cytoprotective modulation. First, the endogenous protective mechanisms can be restored in aging hearts by treatments including caloric restriction [25], exercise [26], and a pharmacologic strategy to inhibit protein phosphatase 2A activity [27]. Second, the attenuation of preconditioning protection observed in the F344 rat experimental model occurs mainly by reduced activation of signaling systems [13] with mitochondria able to respond to direct pharmacologic agonists of the mitochondrial potassium-ATP channel [28]. Thus, the potential for direct mitochondria-targeted intervention remains.

The aged heart exhibits a major clinical challenge for protective therapy. First, not only myocardial injury increases compared to younger hearts for a similar ischemic stress, but the ability to muster endogenous protective pathways is impaired. This challenge in the aged heart makes a strong case for the consideration of novel, direct mitochondrial-centered treatments to mitigate cardiac damage during ischemia and reperfusion. An innovative approach to decrease cardiac ischemia-reperfusion injury by improvement of aging-induced defects in electron

transport is the subject of this minireview.

2. REVERSAL OF AGE-RELATED DEFECTS IN MITOCHONDRIAL ELECTRON TRANSPORT TO DECREASE ISCHEMIA-REPERFUSION INJURY IN THE AGED HEART

Age-Induced Defects in Mitochondrial Electron Transport

Cardiac mitochondria exist as two distinct populations. Subsarcolemmal mitochondria (SSM) are located beneath the plasma membrane whereas interfibrillar mitochondria (IFM) are located among the myofibrils [29-31]. Aging impairs oxidative metabolism only in IFM, whereas SSM are unaffected [29]. Age-related defects in electron transport involve complex III [10,11,32] and complex IV [29,33]. Aging also decreases the content of IFM in the 24 and 28 months old F344 rat [29]. Using rat cardiac permeabilized fibers, we observed that Fischer 344 had an approximately 45% decrease in coupled mitochondrial oxidation, similar to that seen using isolated mitochondria [34]. This quasi in situ preparation reinforces the findings of decreased content of IFM with decreased oxidative capacity per mitochondrion observed with isolated mitochondria. In effect, these data are complementary.

In the Brown Norway rat, which lives longer than the F344, we saw a small (15%) but not significant loss in oxidation in fibers [34]. In contrast, in the hybrid Fischer 344-Brown-Norway, which lives the longest of these strains, there were no oxidative changes in the fibers [34]. Lujbicic and colleagues assessed respiration in an SSM-like population isolated from hearts of the Brown Norway-F344 hybrid, and with complex I substrates noted to decrease approximately 20%, but IFM were not examined [35]. This finding is consistent with the trend toward a minimal decrease in respiration in SSM in the Fischer 344 rat of 18% in SSM in the Fischer 344 rat [29] that reaches statistical significance when a large number of animal are studied [36]. Despite this modest decrease in respiration with NADH-linked substrates, oxidative phosphorylation using substrates that donate electrons to complex III or complex IV, as well as the activities of complexes III and IV, were unaltered with age in SSM [11,29,32].

Complex III activity was decreased in IFM from aged hearts **Figure 1** [10,11,32]. Complex III catalyzes electron transfer from ubiquinol to cytochrome *c* that is coupled to proton translocation [37-39]. Electron transfer within complex III occurs in a bifurcated fashion to cytochromes *b* and *c*₁ via movement of the iron-sulfur protein during the redox cycle [38-40]. When quinol binds to the oxidation site of cytochrome *b* (the Q_o site), a con-

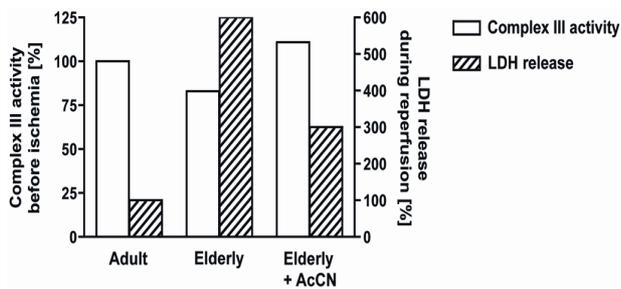


Figure 1. Aging leads to a decrease in the activity of electron transport chain complex III in interfibrillar mitochondria (Open bars; left axis). Treatment three hours before ischemia with acetylcarnitine restored age-related decreases in complex III activity [55]. When elderly hearts underwent ischemia and reperfusion in the presence of restored complex III activity, cardiac injury was decreased following ischemia and reperfusion (Hatched bars; right axis) [55].

certed oxidation of quinol by iron-sulfur protein and cytochrome *b* occurs [37,40]. Functional studies using partial reactions within complex III localized the site of the age-related defect [11] to the Q_o site of cytochrome *b* in IFM at 24 and 28 months of age in the Fischer 344 rat [11].

The aging phenotype of IFM is a combination of decreased maximal enzyme activity [32], preserved electron paramagnetic resonance signal of the iron-sulfur protein [32], increased myxothiazol-resistant cytochrome *b* reduction, and preserved inhibition by stigmatellin [11]. Comparison to the functional cytochrome *b* phenotypes that result from site-directed mutagenesis in the bacterium *Rhodobacter sphaeroides* [37] suggests that modification of a tyrosine residue may be the mechanism of the complex III phenotype observed in IFM from aged F344 rat hearts [41]. As recently shown by the study of the bacterial cytochrome bc_1 complex, this tyrosine residue controls interactions between molecular oxygen and the reduced quinone and attenuates the production of reactive oxygen species by complex III in the baseline state [42].

Complex III is the primary site for the net release of reactive oxygen species from mitochondria [43-46]. The age-related alteration of complex III at the Q_o site of cytochrome *b* in complex III in IFM [11] should augment the net production and release of reactive oxygen species from IFM of elderly hearts, not only in the baseline state, but also during ischemic stress. The aging defect at the Q_o site of complex III in IFM increases the production of reactive oxygen species [11]. In contrast, the production of H_2O_2 was not increased by age in SSM. These findings were supported at the level of intact mitochondria with increased production of reactive oxygen species observed from IFM, but not from SSM [47]. Mitochondria in the aged heart are a source of the increased oxidant production in the baseline resting state and are

poised to amplify cell injury during the stress of ischemia and reperfusion. Ischemia decreased the maximal rate of oxidative phosphorylation due to damage to complex I [48], complex III [10], and complex IV [9,30,49]. In contrast to the selectivity of the aging defect to IFM, ischemia-induced damage occurred in both SSM and IFM [10]. The decrease in the rate of respiration due to ischemia was similar in adult and aged hearts [10]. In IFM from the aged heart, ischemia-induced decreases in respiration were additive to the pre-existing aging defects [10].

3. ACETYLCARNITINE THERAPY: REDUCTION OF ISCHEMIA-REPERFUSION INJURY BY THE RESTORATION OF AGE-INDUCED ELECTRON TRANSPORT DEFECTS

If the aging defect in mitochondrial respiration could be diminished or removed, would the aged heart now sustain less injury during ischemia and reperfusion? Acetylcarnitine treatment improves aging-induced decreases in complex IV activity [33,50-54]. Our group used acetylcarnitine treatment to address the question if age-induced decreases in mitochondrial respiration could be improved. Acetylcarnitine treatment improved rates of oxidative phosphorylation and uncoupled respiration in mitochondria from aged hearts to those observed in adult hearts accompanied by a restoration of the activity of complex III in IFM **Figure 1** [55]. The content of cytochrome *b* in IFM from aged hearts was increased [55]. These data suggested that the newly synthesized cytochrome *b* compensated for the age-damaged cytochrome.

From a mitochondrial perspective, the age-related defect in complex III thus was effaced, accompanied by normalization of oxidative phosphorylation and integrated mitochondrial function. To determine if the mitochondrial improvement is relevant to the heart *in toto*, we looked at myocardial injury in treated hearts during ischemia and reperfusion. Adult and aged rats were treated with acetylcarnitine followed by removal of the heart three hours later for the study of ischemia and reperfusion *in vitro* without any further treatment [55]. In the aged heart, acetylcarnitine enhanced the recovery of contractile function following ischemia and reperfusion, whereas recovery in adult hearts was unchanged by treatment [55]. In hearts from acetylcarnitine-treated aged rats, recovery was now similar to the recovery occurring in treated or untreated adults. Acetylcarnitine treatment substantially decreased the release of lactate dehydrogenase from the aged heart during reperfusion, indicating less myocyte necrosis **Figure 1** [55], without any protection observed in the adult heart. Acetylcarnitine did not alter mechanisms of ischemia-reperfusion damage com-

mon to both adult and aged hearts. These data from acetylcarnitine provide convincing and compelling evidence that the age-related defects in mitochondrial oxidative metabolism contribute to enhanced cardiac injury that follows ischemia and reperfusion in the elderly heart. Thus, these studies provide strong support for the concept developed herein.

Acetylcarnitine has been postulated to improve aging defects in electron transport by several possible mechanisms. First, acetylcarnitine was proposed to increase the content of cardiolipin [50-54,56] a phospholipid present in mitochondria that is required for optimal function of the electron transport chain [56]. Although aging leads to a functional membrane defect [29,41], aging does not decrease the content or composition of cardiolipin in SSM or in IFM in the aged F344 rat heart [57], rendering cardiolipin an unlikely target of acetylcarnitine therapy in the F344 model. Second, and of much greater interest, treatment with acetylcarnitine results in increased transcription of mitochondrial DNA in the aged F344 heart with increased mtRNA synthesis [58,59]. The optimal response to acetylcarnitine occurs at 3 hours at a dose of 300 mg/kg, the treatment regimen used in our ischemia-reperfusion study [55]. The increased transcription is in turn linked to a greater content of mitochondrial-encoded electron transport subunits [58]. The increase in cytochrome content observed in F344 heart mitochondria following acetylcarnitine treatment is in line with this observation.

Thus, stimulation of mitochondrial protein synthesis may contribute to acetylcarnitine-mediated improvement in respiration in the aged heart and the concomitant decrease in susceptibility to ischemia-reperfusion injury [60]. Potential mechanisms of increased transcriptional responses may occur via acetylation of mitochondrial proteins with subsequent activation of mitochondrial sirtuins [61,62]. Additionally, acetylcarnitine, via increases in intracellular acetyl-CoA, may impact nuclear transcription via acetylation of nuclear proteins or an increase in mitochondrial biogenesis [60].

Despite successful and sustained reperfusion of the infarct zone, the mortality of acute myocardial infarction in elderly patients remains markedly elevated [1]. Although pharmacologic treatment at the onset of reperfusion can reduce myocardial injury [63,64] the option of utilizing chronic pretreatment of high risk elders in a preemptive fashion before ischemia represents a novel cardio protective strategy for the aged heart. The *modulation of aging-induced defects* in mitochondrial metabolism in order to *reduce cardiac injury from ischemia and reperfusion* is an attractive potential strategy to protect myocardium in the high-risk elderly patient suffering an acute myocardial infarction. We are suggesting that elderly individuals take this compound on a regular basis in

order to lessen the effect of a heart attack, in the event that such a happenstance occurs. Furthermore, a problem with other cardio protective strategies is that efficacy is observed in young adult models of experimental ischemia [22], but not in the presence of aging [16,19-21] or other comorbid diseases [65] that impact the elderly who suffer acute coronary syndromes. In contrast, acetylcarnitine therapy exerts a therapeutic specificity for the elderly heart [55]. The approach to rejuvenate mitochondrial function in order to directly address age-enhanced ischemic injury [55] remains an attractive and potentially clinically useful strategy [66].

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