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Mitochondria-targeted ROS scavenger improves post-ischemic recovery of cardiac function and attenuates mitochondrial abnormalities in aged rats

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ABSTRACT

Mitochondria-generated reactive oxygen species (ROS) play a crucial role in the pathogenesis of aging and age-associated diseases. In this study, we evaluated the effects of XJB-5-131 (XJB), a mitochondria-targeted ROS and electron scavenger, on cardiac resistance to ischemia–reperfusion (IR)-induced oxidative stress in aged rats. Male adult (5-month old, n = 17) and aged (29-month old, n = 19) Fischer Brown Norway (F344/BN) rats were randomly assigned to the following groups: adult (A), adult + XJB (AX), aged (O), and aged + XJB (OX). XJB was administered 3 times per week (3 mg/kg body weight, IP) for four weeks. At the end of the treatment period, cardiac function was continuously monitored in excised hearts using the Langendorff technique for 30 min, followed by 20 min of global ischemia, and 60-min reperfusion. XJB improved post-ischemic recovery of aged hearts, as evidenced by greater left ventricular developed-pressures and rate-pressure products than the untreated, aged-matched group. The state 3 respiration rates at complexes I, II and IV of mitochondria isolated from XJB-treated aged hearts were 57% ($P < 0.05$), 25% ($P < 0.05$) and 28% ($P < 0.05$), respectively, higher than controls. Ca^{2+}-induced swelling, an indicator of permeability transition pore opening, was reduced in the mitochondria of XJB-treated aged rats. In addition, XJB significantly attenuated the H_2O_2-induced depolarization of the mitochondrial inner membrane as well as the total and mitochondrial ROS levels in cultured cardiomyocytes. This study underlines the importance of mitochondrial ROS in aging-induced cardiac dysfunction and suggests that targeting mitochondrial ROS may be an effective therapeutic approach to protect the aged heart against IR injury.

1. Introduction

Aging is associated with increased prevalence of cardiovascular diseases. In particular, myocardial infarction is a major cause of chronic disability and mortality in the elderly [1]. Aged hearts exhibit low tolerance to various forms of stress, including oxidative stress induced by ischemia/reperfusion (IR). Aged animals [2–4] and elderly patients [5,6] are more prone to myocardial damage following IR than their younger adult counterparts, although the precise mechanisms underlying this decreased tolerance are not fully understood. The existing cardioprotective strategies confer minimal or no protection against IR injury in aged hearts. For instance, although ischemic preconditioning with brief periods of IR protects the adult heart from damage induced by a subsequent prolonged ischemia [7], this protection is remarkably reduced in aged rodent [8–10] and human hearts [11]. Likewise, neither pharmacological preconditioning, induced by activation of mitochondrial K_{ATP} channels [12] and stimulation of adenosine receptors [12,13], nor anesthetic preconditioning [14,15] provides protection or reduces infarct size in aged animals. The mechanisms underlying this loss of cardioprotection likely involve 1) inadequate energy metabolism, including impaired oxidative phosphorylation and energy transfer,

Abbreviations: BW, body weight; DMNQ, dimethoxynaphthoquinone; dP/dt_{max}, maximum velocity of contraction; $-dP/dt_{min}$, maximum velocity of relaxation; ETC, electron transport chain; HR, heart rate; HW, heart weight; IMM, inner mitochondrial membrane; IR, ischemia–reperfusion; LDH, lactate dehydrogenase; LVDP, left ventricular developed pressure; LVEDP, left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure; mitoROS, mitochondrial ROS; PTP, permeability transition pore; RCI, respiratory control index; ROS, reactive oxygen species; RPP, rate-pressure product; XJB, XJB-5-131 (L-ornithinamide, 1-[(2*S*,3*E*,5*S*)-5-[[(1,1-dimethylethoxy)carbonyl]amino]-7-methyl-1-oxo-2-(phenylmethyl)-3-octen-1-yl]-L-prolyl-L-valyl-N^5-[(phenylmethoxy)carbonyl]-*N*-(2,2,6,6-tetramethyl-1-oxy-4-piperidinyl)-).

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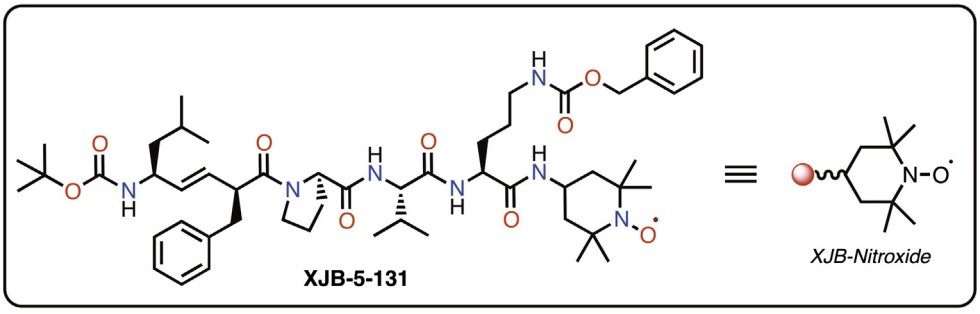
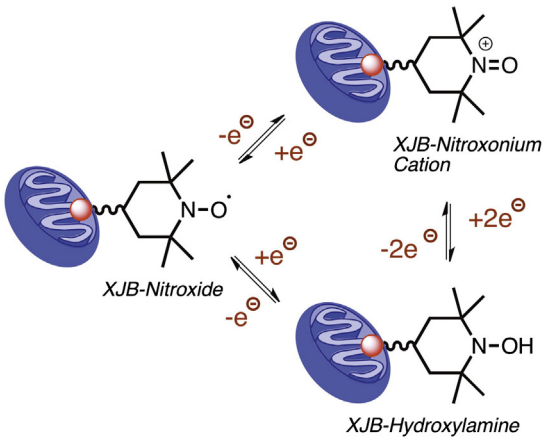
2) disruption of redox status and generation of highly reactive oxygen species (ROS), and 3) alterations of protective signaling pathways in cardiac cells. Thus, existing studies highlight the importance of understanding the molecular mechanisms underlying the loss of cardioprotection and identifying new intracellular targets for cardioprotection in the aged heart.

A major contributing factor in the pathogenesis of an aged heart is mitochondrial dysfunction. Indeed, the mitochondria are common targets and effectors in cardiac injury of aging hearts [16–18]. The free radical theory of aging implicates ROS as a major factor in senescent-induced cell damage [19]. Many studies have established the mitochondria as predominant sources of ROS by demonstrating that diminished electron transport chain (ETC) activity and reduced antioxidant capacity enhance ROS production in aged hearts (*reviewed in* [17]). Accordingly, high ROS levels alter mitochondrial function and integrity, and increase the number of giant mitochondria in aged hearts due to the reduced effectiveness of mitophagy. Giant mitochondria contain mutated DNA that encodes mutated proteins for ETC complexes, thereby leading to further amplification of mitochondrial ROS production [20,21]. The role of mitochondrial DNA (mtDNA) mutations in cardiac aging was confirmed in transgenic mice that expressed a proof-reading-deficient version of mtDNA polymerase with a high load of mtDNA mutations and deletions [20]. This increase in mtDNA mutations was associated with a reduced lifespan and the development of aging-related symptoms between 25 and 40 weeks of age. On the other hand, mice with overexpression of mitochondrial matrix-targeted catalase demonstrated less cardiac mtDNA mutations and protein carbonylation than age-matched wild-type controls. These mice were characterized by an increased lifespan and delayed cardiac aging, suggesting that aging-related mitochondrial and cardiac abnormalities are partially rescued by catalase overexpression in mitochondria [22]. In addition to elevated ROS, aged hearts exhibit diminished mitochondrial Ca^{2+} handling and increased Ca^{2+}-induced mitochondrial damage, associated with increased calcium vulnerability of senescent cardiac mitochondria [23]. Apparently, the decreased ability to accumulate and retain Ca^{2+} reduces tolerance of the aged myocardium to IR injury. The increased mitochondrial ROS generation, along with altered Ca^{2+} handling and energy metabolism (ATP synthesis and energy transfer), predisposes the aging heart to the induction of mitochondrial permeability transition pore (PTP) opening and, thereby, stimulates mitochondria-mediated cell death [24].

Thus, mitochondrial ROS (mitoROS) play a central role in the pathogenesis of cardiac IR injury and senescence. Moreover, cardioprotective signaling pathways that converge on mitochondria are less effective in aged hearts. These observations emphasize the importance of developing and testing new compounds that directly target mitochondria to delay the aging process and increase the tolerance of the aged heart to IR injury. mitoROS scavengers are the most promising pharmacological agents to protect the organelle against ROS induced damage. A recently developed analog of the antibiotic gramicidin S, XJB-5-131 (XJB) [25], has been shown to target mitochondria and provide mitoROS and electron scavenging capacity by virtue of its conjugation to a 4-amino-2,2,6,6-tetramethylpiperidinooxy (4-Amino-TEMPO) moiety [26]. Previous in vitro studies demonstrated that XJB reduced apoptosis and enhanced cell survival in mouse embryonic cells [25]. Further, it





- Nitroxide radical + $O_2^{-\bullet}$ + H^+ ⟶ Hydroxylamine + O_2 (a)
- Hydroxylamine + $O_2^{-\bullet}$ + H^+ ⟶ Nitroxide Radical + H_2O_2 (b)
- Nitroxide radical + e^- + H^+ ⟶ Hydroxylamine (c)
- R-H + $O_2^{-\bullet}$ + H^+ ⟶ $R^\bullet$ + H_2O_2 (d)
- Hydroxylamine + $R^\bullet$ ⟶ Nitroxide Radical + R-H (e)
- Nitroxide radical + $R^\bullet$ ⟶ *O*-Alkyl Hydroxylamine (f)

Fig. 1. Structure of XJB-5-131, equilibria of nitroxide oxidation states, and pertinent reactions with reactive oxygen species, electrons, and radicals. See Introduction for details.