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EDITORIAL

Raising the Dead: Mitochondrial Cardiolipin as a Key Target for Post-Cardiac Arrest Resuscitation, Ischaemia-Reperfusion Injury and Cardiomyopathy

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Having the largest energy demand of any mammalian organ, the heart rapidly produces and consumes adenosine triphosphate (ATP) required to fuel the bioenergetic metabolism of myocardial work, performed during blood perfusion of the cardiovascular system. Blocking mitochondrial ATP energy production even for only a few minutes leads to cardiac failure and arrest of function [1,2]. Not surprisingly cardiac myocytes are dense with mitochondria, comprising approximately a third of myocyte volume. Mitochondria are cell organelles that have long been identified as a major power source of cellular life, endowed with the adaptive capacity to support periods of varied increases in energy demand. However, it is now recognised that mitochondria are not just ATP generators but are a network of integrated dynamic regulators of adaptive cellular metabolism, growth, repair and homeostasis critical to cell survival and death [1,2].

In addition to an outer membrane, mitochondria have an inner mitochondrial membrane (IMM) that features numerous cristae, which are invaginations that bunch up to cover a large proportion of mitochondrial volume (Figure 1A,B). Within this IMM, the mitochondrial respiratory protein complexes reside to support oxidative phosphorylation. This process involves sequential transfer of electrons from nicotinamide adenine dinucleotide (NADH) via mitochondrial respiratory complex I to oxygen at complex IV, where oxygen is reduced to water. The electron transfer process is coupled to active pumping of hydrogen ions from the mitochondrial matrix into the space between the outer and inner membranes generating a proton gradient and mitochondrial membrane electrical potential across the IMM. This facilitates the return of protons back through to drive the F_OF_1-ATP synthase to phosphorylate adenosine diphosphate (ADP) to produce ATP (Figure1C). Proton leak from the IMM and inefficient electron transfer, diminish the efficiency of ATP synthesis contributing to incomplete reduction of oxygen to form reactive oxygen species (ROS). ROS exert important intracellular signalling, however unmanaged ROS may accumulate to create oxidative damage of proteins, lipids and DNA [1,2].

Cardiolipin (diphosphatidylglycerol), is a phospholipid unique to mitochondria and the IMM. Unlike other membrane phospholipids, that have two acyl chains, cardiolipin has four (primarily linoleic acid in cardiac cardiolipin), which are attached to glycerol and two anionic phosphate groups (Figure 1D). The physico-chemical structural properties of cardiolipin confer the shape of the IMM: forming the curvature of cristae membranes and stabilising the assembly of oligomeric respiratory protein complexes, ADP/ATP carrier-containing complexes, mitochondrial creatine kinase, nucleoside diphosphate kinase and others [3], (Figure 1E). Cardiolipin interfaces thus enable optimal functional activities of IMM proteins, including membrane anchoring between the

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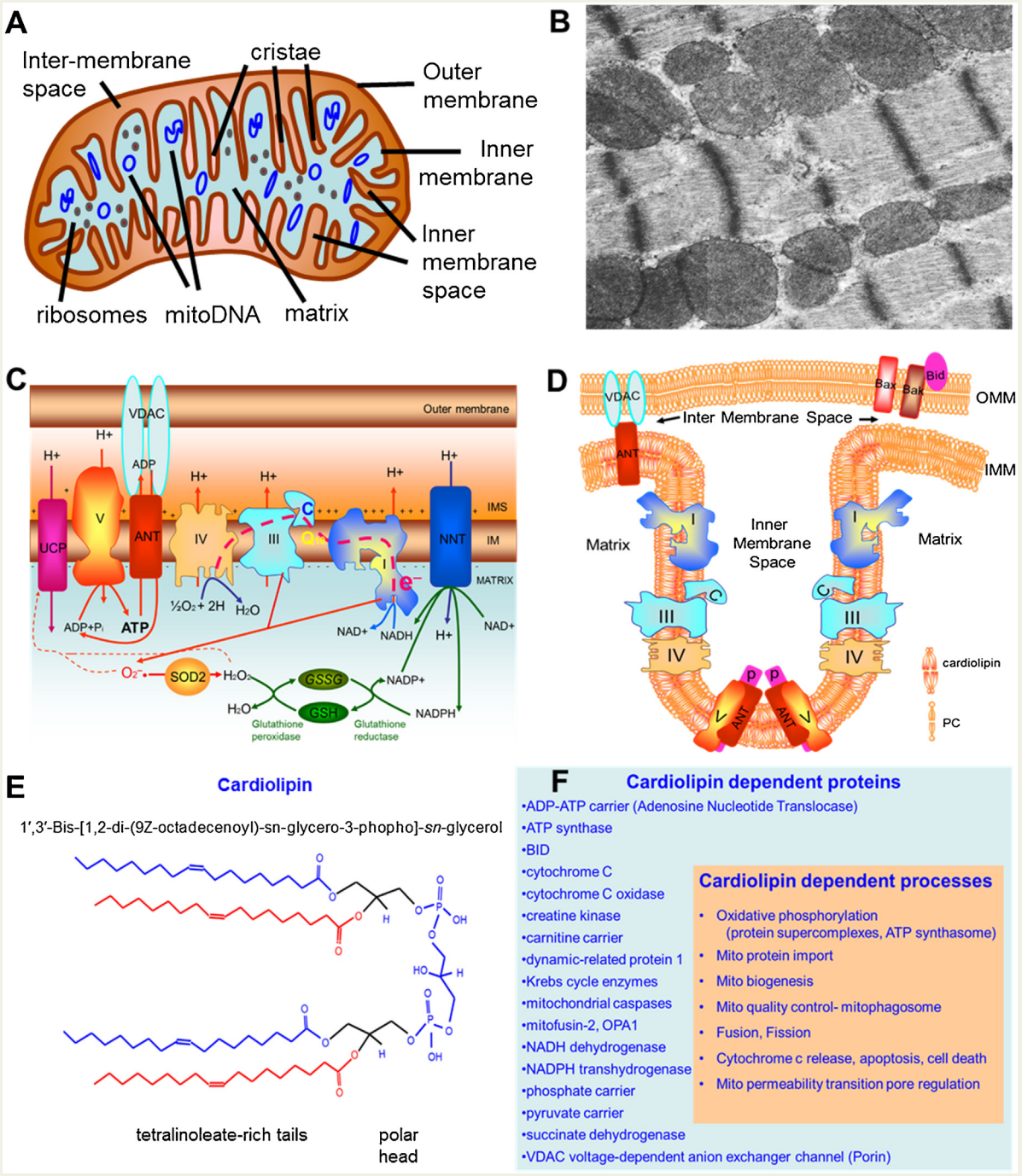

Figure 1 **A.** Scheme of mitochondrial ultrastructure. **B**. Electron micrograph of intermyofibrillar mitochondria showing high density cristae in myocardium. **C.** Major components of inner mitochondrial membrane oxidative phosphorylation and coupled REDOX/reactive oxygen species management (see Text). I, complex I; III, complex III; IV, complex IV; V, complex V – ATP synthase. NNT, NADPH transhydrogenase; GSSG oxidised glutathione, GSH reduced glutathione; SOD, superoxide dismutase; Q10, coenzyme Q_{10}; OMM, outer mitochondrial membrane; IMM inner mitochondrial membrane; ANT, ADP/ATP translocase; VDAC, voltage dependent ion channel (porin). P, phosphate ion carrier; PC, phosphatidylcholine membrane phospholipid; C, cytochrome c. **D.** Scheme illustrating cardiolipin phospholipid rich regions of curvature and support of inner mitochondrial membrane respiratory proteins (especially ATP synthase complexes) and cytochrome C. Excess superoxide production causes cardiolipin oxidation which may trigger cytochrome c release from the IMM into the cytosol through OMM, via MPTP or Bax/Bak oligomerisation. **E.** Cardiolipin (diphosphatidylglycerol) general structural formula of four C:18 (linoleic acid) acyl tails and two phosphate heads. **F.** Summary of key cardiolipin-dependent mitochondrial proteins and processes.

IMM and outer mitochondrial membrane. Cristae junctions and tips are sites of cardiolipin concentration crucial to the dimerisation and localisation of ATP synthase [3]. Cardiolipin is thus a crucial 'glue' that keeps respiratory proteins close together to facilitate efficient electron transfer and minimise electron leak. Loss of cardiolipin has been shown to disrupt respiratory super-complexes and disorganise or dissipate cristae structures, thus preventing ATP synthesis. Being anionic, cardiolipin electrostatically ties down cationic cytochrome c ensuring efficient electron transfer from complex III and IV. Oxidation and cumulative loss of cardiolipin increases proton leak and causes release of cytochrome c which itself precipitates apoptotic signalling and mitochondrial membrane potential loss and mitochondrial membrane pore opening (Figure 1E).

In this issue of *Heart Lung and Circulation*, Zhang et al. remind us of the limited pharmaceutical treatment options available for targeted, potent efficacy in patients experiencing cardiac arrest [4]. In a proof of principle study, the authors employed an asphyxial rat cardiopulmonary bypass/extracorporeal membrane oxygenation model of resuscitation to demonstrate that by directly targeting mitochondria — by specifically protecting cardiolipin with the peptide SS-31 — rat survival after 25 minutes of prolonged cardiac arrest was markedly increased. In this very severe model assessing survival in the short term (5 hours), all animals survived for a minimum of 3 hours following resuscitation from cardiac arrest and half the animals survived to the end of the study period. In contrast, 7 of 10 control animals died in less than 2 hours and only one rat survived for 5 hours. Although the model is extreme and impacts multiple organs, the sustained injury and its progression is well characterised and uniformly applied, thus serving a useful first step towards further application in large animal studies before designing studies in patients in intensive care units.

SS-31 (also called MTP-131, Elamipretide, Bendavia) is a cell-permeable peptide that has been demonstrated to rapidly localise to cardiolipin in the inner mitochondrial membrane [5]. Recent preclinical studies using a large number of animal models have reported that SS-31 reduces myocardial and renal ischaemia–reperfusion injury, and restores skeletal muscle function in primary mitochondrial myopathy [5–8]. Although SS-31 attenuates the impact of excess ROS formation it does not act by scavenging ROS. Rather, SS-31 binds with cardiolipin to prevent ROS-induced cardiolipin oxidation and the subsequent loss of mitochondrial membrane potential that triggers apoptotic signalling [5–8].

Decreased levels of myocardial cardiolipin have been reported for paediatric and adult patients with heart failure [5,9,10]. Thus SS-31 has also been studied in experimental animal heart failure studies (including canine heart failure with reduced ejection fraction (HFrEF) [9,10], porcine heart failure with preserved ejection fraction (HFpEF) [11]) and in a series (about 12 to date) of clinical trials Clinicaltrials.gov, National Clinical Trial numbers: 01572909, 01755858, 02245620, 02388464, 02367014, 02805790, 03323749, 02788747, 02814097, 02914665, 03098797, 02653391, 02693119, 02848313), with some currently in progress (see [6] for a detailed summary table), and also focussed on macular degeneration and Leber's hereditary optic neuropathy. Notably, SS-31 markedly impacts mitochondria in the acute myocardial injury and chronic myopathy setting, resulting in improved ventricular ejection fraction, diminished maladaptive hypertrophy, improved ATP energy production and expression of mitochondrial complexes I, IV, and V, and cardiolipin levels. Although very preliminary, a striking feature of SS-31 clinical use to date, has also been its clinical safety. The additional marked impact on skeletal muscle and renal function, and diabetic vascular dysfunction, highlights the systemic benefits and extended potential of SS-31 [8–14].

In summary, to date there is mounting preliminary *in vitro*, cellular and preclinical data that supports a number of important mechanistic actions (as reviewed in detail recently [5,6]) which well justifies the current translational effort to clinic development. Thus, SS-31:

- promotes oxidative phosphorylation;
- limits electron leak and improves management of excess ROS;
- inhibits cardiolipin peroxidation;
- remodels and restores mitochondrial cristae structure that is abnormally modified by senescence, ischemic injury and adaptive heart failure;
- upregulates enzymes required for cardiolipin biosynthesis and repair;
- promotes mitochondrial biogenesis and restores mitochondrial dynamic process; and,
- limits proinflammatory cytokines, NF-κB, inflammatory cells infiltration, and NLRP3 inflammasome activation.

In recent times, it has been rare to see the emergence of a novel, rapidly acting agent with widely pervasive systemic reach, that to-date appears to exert potent benefits. Given the major underpinning of mitochondria in all tissue functions during acute and chronic disease survival and death, it is anticipated that SS-31 and the development of other highly specific, mitochondrially targeted agents are set to transform intensive and chronic disease management.

Disclosures

Author has no financial conflict disclosures.

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