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Mass Spectrometry Congress 2019: Proteomic analysis of mitochondrial permeability transition pores in relation to cardioprotection induced by metabolic preconditioning - Ferko M1 - Slovak Republic Comenius University, Slovak Republic

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Introduction: Mitochondrial permeability transition pores (mPTPs) are associated with cell death regulation, but also perform physiological role during calcium homeostasis, bioenergetics and redox signalling of cardiac mitochondria. Metabolic preconditioning (MPC) is an experimental cardioprotective model that has demonstrated sufficient protection to compensate for the mitochondrial energy of the heart under pathological conditions.

Aim: The purpose is to clarify regulatory components of the mPTP complex by means of proteomic analysis and using mass spectrometry. We have focused also on mitochondrial creatine kinase (mtCK) as one of the proposed mPTP regulators.

Materials & Methods: Proteomic analysis was performed using nano high performance liquid chromatography and mass spectrometry (ion mass spectrometer configured with electrospray ionization source (ESI)). Mitochondrial proteins were separated by 1D gel electrophoresis and in-gel trypsin digestion. Male Wistar rats were used for this study. Heart mitochondria were isolated by means of differential centrifugation. MPC was induced for 8-days using single dose of streptozotocin (65 mg/kg b. wt.). At the level of using proteomic analysis, we have focused on proteins currently considered as structural and regulatory components of mPTP. The abundance of the investigated proteins as a whole was significantly lower in the MPC affected group ($p = 0.048$), expressions of individual proteins expressed by fold change parameter were maintained (analysed using TREAT (t-tests relative to a threshold) procedure). An important outcome in terms of cardioprotective regulation is that remaining identified mPTP proteins retained expression at the level of healthy mitochondria without significant change. MPC has been able to preserve the activity of mtCK, one of the key enzymes in the energy metabolism.

MPTP is a large, non-specific channel that opens to IMM and is known to form under conditions of mitochondrial stress such as mitochondrial Ca2 + overload and high oxidative stress, which causes the release of huge amounts Ca2 + and proapoptotic proteins from mitochondria, subsequently leading to cell death. Due to the size of its pores, opening the mPTP also results in the equilibration of cofactors and ions through the IMM, including the release of accumulated Ca2 +. This leads to a disruption of the metabolic gradients between the mitochondria and the cytosol, and a concomitant influx of water occurs, causing the mitochondria to swell until the OMM eventually ruptures. OMM disruption releases cyt c and other proapoptotic proteins, potentially leading to apoptotic cell death.

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The mPTP opening plays a significant role in the generation of not only apoptotic cell death but also necrotic cell death, both of which are implicated in the etiology of myocardial infarction. It is now widely recognized that the opening of mPTP is a major cause of reperfusion injury and is an effective target for cardioprotection.

Mitochondria play a critical role in the life and death of cells. In healthy heart myocytes, their main function is the supply of ATP by oxidative phosphorylation to meet the high energy requirements of the beating heart. Glycolysis alone is unable to meet these demands even at rest, and inhibition of oxidative phosphorylation, as occurs in anoxia or ischemia, leads to impairment or arrest of normal heart function. However, latent in the mitochondria, there are mechanisms that, when activated, convert the mitochondria from organelles that support cell life into those that actively induce apoptotic and necrotic cell death. The change in role, similar to the conversion of Dr. Jekyll to Mr. Hyde, is mediated by the opening of a non-specific pore in the mitochondrial inner membrane, known as the Mitochondrial Permeability Transition Pore (MPTP). In this review, we will briefly summarize what is known about the molecular mechanism of MPTP and why it opens upon reperfusion. We will then describe the techniques that were used to measure pore opening in the perfused heart, and how these experiments led to the proposition that the extent of pore opening is a critical determinant of reperfusion injury. Finally, we will describe how inhibiting the opening of MPTP is an effective strategy for protecting hearts against reperfusion injury.

Under normal physiological conditions, the mitochondrial inner membrane is impermeable to all except a few selected metabolites and ions. However, under stressful conditions, a nonspecific pore known as the mitochondrial permeability transition pore can open in the mitochondrial inner membrane, allowing free passage of any <1.5 kDa molecule. When MPTP opens, the permeability barrier of the inner membrane breaks with two major consequences. First, although all low molecular weight solutes move freely across the membrane, proteins do not and, therefore, exert colloidal osmotic pressure which causes mitochondria to swell. Although the deployment of the ridges allows the matrix to expand without disrupting the inner membrane, the outer membrane will break and lead to the release of proteins into the intermembrane space such as cytochrome c and other factors that play a role. critical role in apoptotic cell death. Second, the inner membrane becomes freely permeable to protons. This dissociates oxidative phosphorylation, causing the ATPase translocating the proton to reverse direction and therefore actively hydrolyze ATP, rather than synthesizing it. Under such conditions, intracellular ATP concentrations decrease rapidly, leading to the disruption of ionic and metabolic homeostasis and the activation of degrading enzymes such as phospholipases, nucleases and proteases.

Unless pore closure occurs, these changes will cause irreversible damage to the cell, resulting in necrotic death. Even if closure occurs, mitochondrial swelling and rupture of the outer membrane may be sufficient to set

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in motion the apoptotic cascade. Thus, it is hardly surprising that MPTP is kept firmly closed under normal physiological conditions and is only activated under pathological conditions. The key factor responsible for the opening of MPTP is mitochondrial calcium overload (that is, when the mitochondrial matrix [Ca2 +] is greatly increased), especially when it is accompanied by oxidative stress, depletion of adenine nucleotides, high concentrations of phosphate and mitochondrial depolarization. These are exactly the conditions the heart experiences during post-ischemic reperfusion, and there is growing evidence that opening MPTP is critical in transitioning from reversible to irreversible reperfusion injury.

Results: The results of proteomic analysis under MPC conditions indicate the positive effect of mPTP regulated mechanisms present in the state of increased calcium influx into the mitochondria, thereby contributing to the maintenance of the energy of the pathologically affected myocardium.

Biography:

Ferko M is a researcher who is working at the Department of Biochemistry, Centre of Experimental Medicine SAS as an in charge of the mass spectrometry and fluorescence spectroscopy laboratory. He has 23 publications on the topics like heart mitochondria and cardiac adaptation, endogenous cardioprotection and mitochondrial proteomic analysis published in various journals that have been cited 100 times and his H-index is 11. He is expertise in heart mitochondria proteomic analysis, LC/MS, heart mitochondria function, bioenergetics, heart failure and cardiovascular physiology.

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