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Review

Identifying the role of cytochrome c in post-resuscitation pathophysiology☆

Athanasios Chalkias, PhD [a,b,*], Artem Kuzovlev, PhD [c], Antonio Noto, PhD [d], Ernesto d'Aloja, MD [d], Theodoros Xanthos, PhD [b,e]

[a] National and Kapodistrian University of Athens, Medical School, MSc "Cardiopulmonary Resuscitation", Athens, Greece
[b] Hellenic Society of Cardiopulmonary Resuscitation, Athens, Greece
[c] V.A. Negovsky Research Institute of General Reanimatology, Moscow, Russian Federation
[d] Department of Public Health, Clinical and Molecular Medicine, Forensic Science Unit, University of Cagliari, Cagliari, Italy
[e] European University of Cyprus, School of Medicine, Nicosia, Cyprus

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ABSTRACT

Cytochrome c, an electron carrier that normally resides in the mitochondrial intermembrane space, may translocate to the cytosol under ischemic and hypoxic conditions and contribute to mitochondrial permeability transition pore opening. In addition, reperfusion of brain tissue following ischemia initiates a cell death cascade that includes cytochrome c–mediated induction of apoptosis. Further studies are needed to determine the contribution of cytochrome c in the regulation of cell death, as well as its value as an in vivo prognostic marker after cardiac arrest and resuscitation.

1. Introduction

The cytochrome complex (CYTc) is a small heme protein of the inner mitochondrial membrane. Unlike other cytochromes, CYTc is highly soluble and is an essential component of the electron transport chain [1]. Although it undergoes oxidation and reduction, it does not bind oxygen but transfers electrons between complexes III and IV [2,3]. In mitochondrial electron transport chain, the heme group of CYTc accepts electrons from the b-c1 complex and transfers electrons to the cytochrome oxidase complex [3,4].

Immediately after restoration of spontaneous circulation (ROSC), patients enter the post-resuscitation phase during which several factors may affect outcome [5]. Despite the advances in cardiopulmonary resuscitation (CPR) and post-resuscitation care, prognosis of cardiac arrest victims remains dismal. Systemic biomarkers provide little information, whereas many of the accepted predictors of poor outcome in comatose survivors of cardiac arrest are unreliable [6]. Although research has been focused on the mitochondrial permeability transition pore (mtPTP) and its role in post-resuscitation apoptosis, data from various studies indicate that CYTc may play a pivotal role in the pathophysiology of post–cardiac arrest syndrome.

2. Cardiac arrest and post–cardiac arrest syndrome

Cardiac arrest is a major cause of death, as approximately 300,000 arrested people are treated annually by medical personnel. The annual incidence of emergency medical services–treated out-of-hospital ventricular fibrillation arrest is 17 per 100,000, and survival to hospital discharge is 10.7%, whereas the reported incidence of in-hospital cardiac arrest is 1-5 per 1000 admissions, and survival to hospital discharge is 17.6% [5].

Despite the term *post-cardiac*, the pathophysiological cascade of post–cardiac arrest syndrome is activated by the onset of cardiac arrest and results in 4 key components with common pathophysiological origin [7]. These are the post–cardiac arrest myocardial dysfunction, the post–cardiac arrest brain injury, the systemic ischemia/reperfusion (I/R) response, and the persistent precipitating underlying pathology. The severity of these disorders is not uniform and varies in individual patients based on the patient's prearrest state of health, the severity of the ischemic insult, the cause and duration of cardiac arrest, the time to CPR, the time to ROSC, and the quality of post-resuscitation care [7,8].

The time frame of post–cardiac arrest syndrome includes 4 distinct time-defined phases. The *immediate post-arrest phase* is defined as the first 20 minutes after ROSC, the *early post-arrest phase* is the period between 20 minutes and 6-12 hours after ROSC, the *intermediate phase* is between 6-12 and 72 hours, whereas the *recovery period* is the period extending beyond 3 days [6]. Although therapeutic aggressive interventions may be more effective during the early and intermediate phases, specific attention should be given to all 4 phases of post–cardiac arrest syndrome [8]. Causes of death in this setting can be divided into neurological causes, resulting from ischemic-anoxic encephalopathy, and hemodynamic causes, leading to multiple organ failure [5,8].

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* Corresponding author at: National and Kapodistrian University of Athens, Medical School, MSc "Cardiopulmonary Resuscitation," Hospital "Henry Dunant," 107 Mesogion Ave, 11526, Athens, Greece. Tel.: +30 2110121756; fax: +30 2110121758.
E-mail address: thanoschalkias@yahoo.gr (A. Chalkias).

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3. The cytochrome c

Cytochrome c is a small, very stable hemoprotein containing covalently bound heme c as a prosthetic group and functions as an electron shuttle between complex III and complex IV of the respiratory chain [3,9]. Cytochrome c is synthesized in the cytosol as a single polypeptide chain of 104 amino acid residues (apoprotein), and upon translocation to the mitochondria, it is covalently bound to the heme prosthetic group [10]. According to crystallographic data, CYTc appears roughly as a sphere with the diameter of 3.4 nm [9]. In mitochondria, at least 15% of CYTc is bound to acidic phospholipids of the inner mitochondrial membrane via both electrostatic and hydrophobic interactions [11], whereas the remaining CYTc is loosely attached to the inner mitochondrial membrane, as a result of weak electrostatic interactions, and can be readily mobilized [2,12]. Loosely bound CYTc is implicated in electron transport, inhibition of reactive oxygen species (ROS) formation, and prevention of oxidative stress, whereas tightly bound CYTc is probably bound to cardiolipin which appears to be necessary for the insertion of CYTc into mitochondrial membranes [13]. Cardiolipin-bound CYTc does not participate in electron shuttling of the respiratory chain but may account for the peroxidase activity recently attributed to CYTc. Cytochrome c can catalyze several reactions such as hydroxylation and aromatic oxidation and shows peroxidase activity by oxidation of various electron donors [11].

4. Cytochrome c and programmed cell death

Apoptosis is the process of programmed cell death characterized by specific morphological cellular changes and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation [14]. Upon apoptotic stimuli, CYTc is released into the cytosol where, in the presence of adenosine triphospate (ATP), it mediates the allosteric activation and heptaoligomerization of the adaptor molecule apoptosis-protease activating factor 1 (Apaf-1), generating apoptosomes [15]. Each apoptosome is a complex molecule that can recruit and activate caspase-9, leading to proteolytic self-processing under the regulation of several heat shock proteins [16]. The aforementioned processes result in the catalytic maturation of caspase-9 and other caspases that eventually mediate the biochemical and morphological features of apoptosis. However, the caspase cascade can be activated by other soluble mitochondrial proteins upon apoptosis induction, which enhance the neutralization of the caspase-inhibitory proteins [9]. Active caspase-9 then initiates apoptosis by cleaving and thereby activating executioner caspases [17]. Although it was shown in 2000 that mammalian cells lacking CYTc could not activate caspases in response to mitochondrial pathway stimulation [18], Hao et al [19] reported that the electron transport function of CYTc is independent of its ability to engage Apaf-1 and induce apoptosome formation and caspase activation. Of note, excessive caspase-1 activity can cause pyroptosis, a nonapoptotic type of programmed cell death characterized by plasma membrane rupture and the release of proinflammatory intracellular contents [20,21].

Inhibition of caspases protects cells only transiently against cell death because once mitochondria are irreversibly permeabilized, cell death proceeds regardless of caspase activity [17]. This caspase-independent cell death may result from the loss of essential mitochondrial functions and/or from the apoptogenic function of the flavoprotein apoptosis-inducing factor (AIF) and endonuclease G [22]. Once in the cytosol, both proteins are able to translocate to the nucleus where they promote DNA fragmentation and apoptotic cell death [23,24].

Antiapoptotic B-cell lymphoma-2 (Bcl-2) family proteins prevent the release of both CYTc and AIF apoptotic protease, whereas in spite of their stabilization effect on the mitochondrial outer membrane, Bcl-2 proteins may also be involved in the direct binding of apoptotic protease activating molecules as regulatory elements further downstream from the mitochondrial apoptotic signals. Interestingly, acute stress seems to suppress the mitochondrial apoptotic pathway of neutrophils consequent to downregulation of proapoptotic Bcl-2 proteins [25], whereas the increased plasma levels of CYTc likely originate from organs that suffer I/R injury during cardiac arrest and resuscitation [26].

An important proapoptotic stimulus is the sustained elevation in calcium levels [27]. The release of small amounts of CYTc leads to an interaction with the IP3 receptor on the endoplasmic reticulum (ER), causing ER calcium release. The overall increase in calcium triggers a massive release of CYTc, which then acts in the positive feedback loop to maintain ER calcium release through the IP3 receptors [24]. Of note, these calcium events are linked to the coordinate release of CYTc from all mitochondria, identifying a feed-forward mechanism resulting in augmented CYTc release that amplifies the apoptotic signal [24].

Oxidative damage to the mitochondria can occur on proteins involved in respiration as well as lipids critical for respiratory protein function. The inner mitochondrial membrane lipid cardiolipin can become oxidized, resulting in failure of oxidative phosphorylation [28]. In addition, ROS production during the reperfusion phase and subsequent mitochondrial dysfunction activate the intrinsic apoptotic pathway. Cytochrome c is actively targeted by stress signaling during I/R, and phosphorylation of CYTc partially inhibits respiration [29]. In contrast to phosphorylated CYTc, this dephosphorylated CYTc may have the full capability to bind to Apaf-1 and trigger downstream caspase activation [30]. In the progression of brain reperfusion injury, mitochondrial respiration begins to diminish, and mitochondrial dysfunction eventually culminates in cell death possibly due to peroxidation of cardiolipin by CYTc [31–33]. Cytochrome c binds to cardiolipin in the inner mitochondrial membrane, thus anchoring its presence and keeping it from releasing out of the mitochondria and initiating apoptosis [29]. Extensive peroxidation of cardiolipin has been demonstrated during brain reperfusion [29], whereas multiple studies have demonstrated the neuroprotective and antiapoptotic effect of therapies designed to activate cell survival signaling and prevent apoptotic release of CYTc [34,35]. Whereas the initial attraction between cardiolipin and CYTc is electrostatic because of to the extreme positive charge on the latter, the final interaction is hydrophobic, where a hydrophobic tail from cardiolipin inserts itself into the hydrophobic portion of cytochrome.

On the other hand, the data regarding the effects of CYTc on necrosis or paraptosis are scarce. Necrosis is a form of cell injury that results in the premature death of cells in living tissue by autolysis [14]. Necrosis is caused by factors external to the cell or tissue—such as infection, toxins, or trauma—that result in the unregulated digestion of cell components [22]. Cells that die because of necrosis do not follow the apoptotic signal transduction pathway, but rather various receptors are activated that result in the loss of cell membrane integrity and an uncontrolled release of cell products into the extracellular space [22]. Necrosis may occur because of external or internal factors, as well as by components of the immune system [23].

Paraptosis was originally identified in 2000 and often exists in parallel with apoptosis [36,37]. Paraptosis is a type of programmed cell death, morphologically distinct from apoptosis and necrosis [24]. The defining features of paraptosis are cytoplasmic vacuolation, independent of caspase activation and inhibition, and lack of apoptotic morphology [36,37]. Paraptosis lacks several of the hallmark characteristics of apoptosis, such as membrane blebbing, chromatin condensation, and nuclear fragmentation [38]. Like apoptosis and other types of programmed cell death, the cell is involved in causing its own death, and gene expression is required. This is in contrast to necrosis, which is nonprogrammed cell death that results from injury to the cell. Paraptosis can be triggered by the tumor necrosis factor receptor family member TAJ/TROY, the apoptotic protein Bcl-2–associated X (Bax), and human insulin–like growth factor I receptor and takes place in neurodegenerative conditions such as the post–cardiac arrest brain injury [5,39,40].

5. Cytochrome c and cardiac arrest

At the beginning of cardiac arrest, the abrupt loss of effective blood flow is followed by a temporal increase in blood flow due to the