Ischemia - Reperfusion: A Look from Yeast Mitochondria

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Abstract: The apoptotic phenomena observed in tissues which are subdued to ischemia and then to technical therapeutics of perfusion keep causing serious problems in the patient's clinical recovery. Then, they constitute a challenge to resolve.

The objective of this work is to discuss the intracellular mechanisms that lead cells to apoptosis during the ischemia-reperfusion process, taking into consideration that these phenomena are observable in a simple microorganism as the yeast *Saccharomyces cerevisiae*. Yeast provide an alternative study system in which the effects of certain cytoprotective drugs can be evaluated. The results can then be extrapolated to other types of cells.

Several works have focused on the role of mitochondria in the apoptotic processes of cellular necrosis. One of the main factors responsible for this process is the unregulated opening of the permeability barrier. The inner membrane thus allows the unrestricted passage of ions and the release of apoptotic mediators from the inner membrane space towards the cytosol. Also, there is an increase in the level of reactive oxygen species (ROS) and the uncoupling of oxidative phosphorylation, which lead to the reversal of ATP synthesis to ATP hydrolysis.

The driving cause of this complex process is the opening of an non-specific pore located in the mitochondrial membrane, denominated mammalian permeability transition pore (mPTP), which is also expressed in yeast (yPTP).

From the functional point of view, the yeast pore presents some of the characteristics observed in mammals, and is similar in the defensive response against the deleterious mechanisms caused by oxidative stress.

An increasing body of evidence supports the concept that the pharmacological inhibition of the mPTP is an actual and promising strategy for the protection of tissues in ischemic situations in order to avoid the damage induced by perfusion.

Keywords: Yeast, ischemia–reperfusion, mitochondria, pore, reactive oxygen species, apoptosis.

1. INTRODUCTION

The apoptotic phenomena observed in tissues which are first subdued to ischemia and then to technical therapeutics of perfusion keep causing serious problems in the patient's clinical recovery. These are situations in which there can be ischemic cellular damage, caused mainly by glucose and oxygen reduction. This reduction not only creates a decrease in mitochondrial ATP but also an increase in the generation of reactive oxygen species (ROS). Due to the fact that these molecules are highly reactive, they react with cellular substances like nucleic acids, proteins and lipids, causing irreversible damage in the cellular structure.

It is worth mentioning that mitochondrial proteins are more susceptible due to their physical proximity to the site of ROS production. This cellular damage is accompanied by the constant increase in cytoplasmic Ca^{2+}, which is originated both in cellular structures and in mitochondria [1].

When tissues in ischemic conditions are subdued to surgical and medical techniques of reperfusion to guarantee the oxygen contribution/supply to the tissue, this restoration of the blood irrigation to the ischemic tissue may allow the recovery of the cellular structure if the cells have not been irreversibly damaged. However, depending on the intensity and duration of the ischemic aggression, after the blood irrigation is restored, a variable number of cells may be derived to cell death, through necrosis or apoptosis.

In summary, this ischemic aggresion determines a paradoxical cellular damage generated when using a putative therapeutic technique [1]. Moreover, it is necessary to consider diseases like sleep apnea, in which transient phenomena of hypoxia take place. In this disease, it is possible to observe cellular damage in specific points of the organism that are induced by the same phenomena previously described.

In addition, in unicellular microorganisms like yeast, a similar deleterious effect can be observed when the cells are subjected to hypoxia-reoxygenation conditions. Therefore, we consider that using yeast as a biological model for hypoxia-reoxygenation studies is an interesting and innovative possibility [2]. We propose mainly the use of *Saccharomyces cerevisiae*, which is the most studied yeast and whose genetics and molecular biology have been well established. Moreover, in the case of *S. cerevisiae*, cells can grow not only in aerobic conditions but also in anaerobic conditions, depending on the oxygen present in the culture conditions.

2. ISCHEMIA- REPERFUSION

The role of mitochondria in apoptotic processes related to hypoxia-reoxygenation has been recently well established [2,3,4].

It is well known that whenever a tissue is subjected to periods of ischemic conditions, the supply of oxygen, glucose and many other nutrients is dramatically compromised. All this bears a blockade of phosphorylative oxidation and consequently a decrease in ATP production which lead to: 1) an increase in anaerobic glucolysis with a decrease in the intra-cytoplasmic pH value, 2) a blockade of NaK^+ ATPase, which causes an increase in intracellular Ca^{2+} level, 3) an alteration in protein synthesis, and 4) an increase in the production of ROS.

The mitochondrion is an important target of virtually all kinds of harmful stimuli, including hypoxia and toxins [5]. Frequently, cellular damage is accompanied by morphological changes in mitochondria. These organelles can be injured by an increase in cytosolic Ca^{2+} concentration, by oxidative stress and by phospholipid degradation through the phospholipase A2 pathway and sphingomyelin. They can also be damaged by the products derived from the activity of the mentioned enzymes, such as fatty acids and ceramide.

Often, the mitochondrial damage gives rise to the formation of a channel of high conductivity which results in an increase in the permeability of the mitochondrial membrane (MPT: mitochondrial permeability transition) [6]. This process is associated with a massive entry and exit of substances that generate a depolarization and destabilization of the organelle. Moreover, this circumstance is often accompanied by conditions of an increase in the intra-

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mitochondrial Ca^{2+} level, a decrease in adenine nucleotides, or an increase in ROS generation.

The main cause of this complex process is the opening of a non-specific pore in the internal mitochondrial membrane, known as mammalian permeability transition pore (mPTP) [2,7,8], or its analog in yeast (yPTP) [9]. Patch clamp studies have shown the presence of channels for ions in the internal mitochondrial membrane (IMM), whose conductance range is of 1 nS for both yeast and mammals [6,10].

Although in the first stages of ischemia, this passage through the mitochondrial membrane is reversible, it could become irreversible. This last process prevents the maintenance of the mitochondrial proton gradient and leads to the destabilization of the membrane structure, and even to apoptosis through cytochrome C release.

Taking into consideration that the maintenance of the mitochondrial gradient is essential for oxidative phosporylation, we can see that the activation or opening of the mPTP or yPTP is a lethal blow for cellular functionality. It is worth mentioning that it appears ineludible to consider the relation between ischemia reperfusion and the permeability of the mPTP. This has been clearly demonstrated in works carried out in mammalian cells [2], in which the mPTP keeps closed during ischemic periods but opens when the tissue involved is reperfused.

3. MITOCHONDRIAL PERMEABILITY TRANSITION PORE (PTP)

3.1. Mammalian PTP (mPTP)

The mPTP is a unique molecular entity that allows the passage of any molecule smaller than 1.5 kDa through the IMM of mammalian cells [7], Fig. (**1**). It has been described as a dynamic protein complex that, in these cells, involves an anionic voltage-dependent channel denominated VDAC [11], an adenine nucleotide translocase (ANT) [7], and many cyclophilins, especially cyclophilin D [12], which is directly associated to a molecular chaperone heat shock protein (Hsp60). The latter are considered possible regulators and stimulators of the opening of the mPTP, and thus of mitochondrial destabilization [13].

It is known that ANT could be involved in the edematization and blockade of oxidative phosphorylation, through an increase in the permeability of mitochondrial membranes. It is important to mention that the opening is not only observed in physiological conditions, which, as described below, have the utility to equilibrate the energetic levels of the cell [14,15], but also under certain pathological conditions. However, not only ANT but also VDAC are required for the stability and release of cytochrome C to the cytosol and other apoptotic mediators.

Finally, it must be stressed that the pore is not a stable and static structure that only crosses the internal or external parts of mitochondrial membranes, where VDAC and ANT are coupled, but a dynamic structure subjected to intracellular changes that allow the cell to adapt to different physiological conditions.

In physiological or pathological conditions during which the pore is open, the structure as a whole is probably not assembled. The assembled structure would appear when the pore is closed [7, 16, 17]. In addition, the integral membrane proteins suffer misfolding, and are damaged by oxidative and other stresses. Chaperone-like proteins initially block conductance through these misfolded protein clusters, and when protein clusters exceed chaperones available to block conductance, unregulated pore opening occurs [18,19].

As mentioned above, when the pore is open in a deregulated way, there is a decrease in the impermeability barrier of the IMM, allowing not only the passage of molecules but also the release from the inter-membrane space from the apoptotic mediator to the cytosol. The mPTP allows an unrestricted movement of protons through the internal membrane, which not only uncouples oxidative phosphorylation, but also reverses ATPase function, producing hydrolysis instead of ATP synthesis.

Moreover, the movement of small solutes through the IMM results in the swelling of the organelle due to the osmotic pressure generated [7].

3.2. Yeast PTP (yPTP)

A transitional mitochondrial pore analogous to that of mammalian cells, denominated in diverse bibliographical data as YMUC (Yeast mitochondrial unspecific channel), has been found in the yeast *Saccharomyces cerevisiae* [20, 21]. However, in the present work, we will denominate the pore as yPTP because it is the most used designation by research groups and also because it is the denomination that better describes its function.

The yeast pore shares many similarities with its homologous in mammals, with the exception that it allows the passage of molecules of 1.1 kDa, i.e. a smaller size than its homologous in mammals [22]. This structure is also described as a dynamic protein complex that involves an anionic voltage channel that depends on the external mitochondrial membrane (EMM) denominated POR1. POR1 is a protein with similarities to VDAC from mammals. Also, an adenine nucleotide translocase (ANT), which in yeast is called ATP/ADP carrier (AAC), is present [23]. It must be mentioned that cyclophilins have not been reported in observable quantities [12]. Then, the action of drugs like Cyclosporine A (CsA), whose blockade over these molecules has been identified in mammalian cells [22], is not feasible in yeast. However, studies can be carried out when these proteins are overexpressed in yeast [24].

On the other hand, it has been demonstrated that like ANT, the AAC is involved in the edematization of mitochondria and blockade of oxidative phosporylation both in physiological and pathological conditions.

Regarding apoptosis, its induction in yeast cells is comparable to that observed in mammalian cells. In yeast, POR1 seems to contribute to death resistance, while AAC would induce the release of Cytochrome C and other mediators [23].

4. PTP REGULATION

Under physiological conditions, the pore is regulated by many mitochondrial molecules, such as adenine nucleotides or cations, contrary to what happens under pathological situations when the pore enters an unregulated mode. The knowledge of the molecular regulation of mPTP and yPTP is essential to understand how the cell behaves when facing diverse metabolic conditions either in the presence or the absence of oxygen. This knowledge will allow the design of drugs with specificity over mitochondria to prevent cellular activation of apoptotic cascades.

4.1. Energetic Equilibrium

The opening of the regulated mode of the pore responds fast to cellular respiration or ATP level. Homogonously to mPTP, yPTP is inhibited by ADP [25,26]. Unlike the characteristics of mammalian cells, inorganic Phosphate (Pi) promotes the closure of yPTP through the action exerted over the pH of the mitochondrial matrix, acting probably over a pore-specific site from the extra-mitochondrial side.

These facts indicate that, in physiological (pore-regulated) conditions, when a high energetic charge is maintained by ATP levels, the pore will open to block the oxidative phosporylation and to

favor ATP hydrolysis, suggesting the presence of a valve mechanism that dissipates the gradient. [27,28]. In relation with these last properties, "*in vivo*" research studies with intact cells in fluorescence assays have proved the opening-transient closure (flickering) of the mPTP [29].

4.2. Cations and their Relation with the Pore

Calcium ions are important mediators in cellular damage. Free cytosolic Calcium is maintained in extreme low concentrations (nearly 0.1 μM) compared with extra cellular levels (1.3 mM). The main part of intracellular Calcium is contained in mitochondria and the endoplasmic reticulum. These gradients are modulated by an energy-dependent Mg^{+2}, Ca^{2+} ATPase action associated with those membranes.

Ischemic conditions and toxins cause a sudden increase in intracytoplasmic Calcium. Subsequent changes determine that this sudden increase in Ca^{2+} generates an increase in mitochondrial permeability, resulting in a cellular imbalance.

This process is different from that observed in normal physiological conditions when the pore is regulated. It has been proposed that a minimal increase in intracytoplasmic Ca^{2+} levels in mammalian cells will reduce the permeability of the mitochondrial membrane. This is probably related to the mPTP closure (turn off). As mentioned above, this will favor the energy efficiency coupled to oxidative phosphorylation.

In yeast cells, Ca^{2+} regulates the cell cycle. This cation is required during mitosis, a period of high energetic demand. This happens concomitantly with an increase in cytoplasmic Ca^{2+} concentrations. Then, the closed pore will favor energy production. The ion must operate in combination with PO_{3-} in order to close the yPTP, blocking the utilization of energy derived from oxidative metabolism for ATP production. In contrast, a decrease in PO_{3-} concentration, ATP accumulation and Ca^{2+} when the cells enter stasis leads to the opening of the yPTP.

Then, an uncoupling of the oxidative phosphorylation and the dissipation of the membrane energetic gradient in the form of heat take place [26]. The yPTP opens when it is possible for the mitochondrion to consume oxygen during the respiration process or when the organelle is incubated with ATP and Pi is present.

Regarding the sites for interaction of cations in the mPTP, these sites are essential to establish and understand the differences in the pore physiology and its pathological deregulation. The mPTP possesses two sites for divalent cations: one in the internal side of the IMM, where Ca^{2+} induces the opening of the pore and where other divalent cations like Sr^{+2} or Mn^{+2} have the opposite effect, and the other in the external side of the IMM, where all the divalent cations, including Ca^{2+}, lead to the closure (turn off) of the pore. Ca^{2+} and Mn^{+2} act synergistically inducing the closure of the pore, Fig. (**1**).

This indicates that, in contrast to previous ideas, Ca^{2+} levels in the mitochondrial matrix would be modulators of the opening of the pore. Likewise, in yeast, it has been considered that there is only

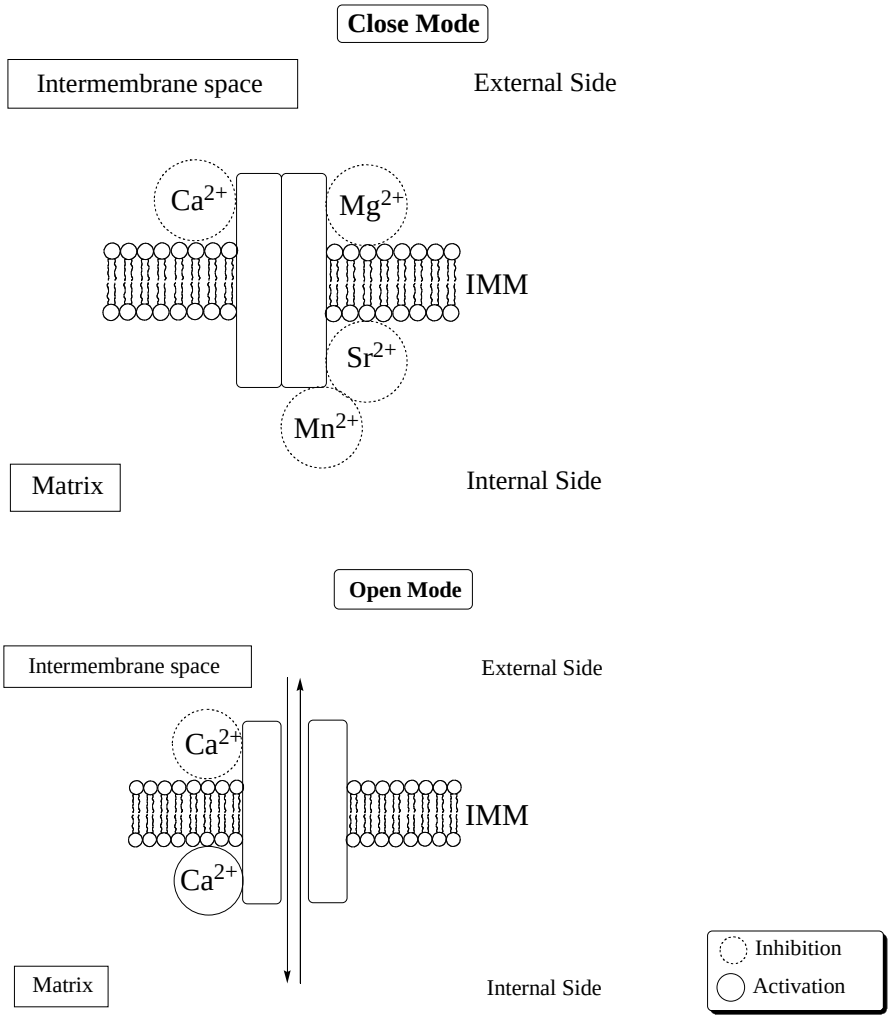


Fig. (1). Effect of divalent cations over the open or close mode of the pore.

one site on the external face of the IMM, and that Ca^{2+} is thus the only cation capable of closing the pore. However, later studies have demonstrated that there are sites on the internal side of the IMM that allow the opening of the pore, with the resulting destabilization of mitochondria and the whole cell [30].

Another important issue to call the attention is the localization of mitochondria inside the cell. If the mitochondrion is near the endoplasmic reticulum, the organelle will be exposed to higher Ca^{2+} levels, a fact that will probably activate the opening/closure of the pore (flickering) and thus result in a transient and physiological and regulated activation of the PTP [31,32]. Recent data suggest that the flickering of the PTP modulates fusion and fission processes of mitochondria [33]. So, if the damage is considerable, the unregulated pore is constantly open, paving the way for mitochondria ulterior degradation [34].

In conclusion, during physiological conditions, the yPTP is regulated through Ca^{2+}, Mg^{+2} and PO_{3-} . These facts seems to lead to an increase in the efficiency of oxidative phosporylation when ATP is highly needed. This is obtained by the closure of the pore. When the energetic requirement diminishes, the pore condition is reversed, i.e., it opens [26]. However, in pathological conditions, the inverse process occurs, causing an unregulated form of the pore, since there is a sudden increase in intracytoplasmic Ca^{2+} levels. As we have previously mentioned, the activation/opening at the internal side of AAC is translated into an activation cascade of pro-apoptotic molecules and a deregulation of the mitochondrial membrane, Fig. (**2**).

5. PTP-MEDIATED APOPTOSIS

The release of pro-apoptotic mediators to the cytosol may result in the degradation of the external mitochondrial membrane (EMM). Since ATP reduction and the increased level of ROS induce alterations in metabolic and ionic homeostasis, they are the main causes of this phenomenon.

As a consequence of that pathological activation of mPTP or yPTP, uncoupling of oxidative phosporylation takes place, and the decrease in ATP level would promote apoptosis activation [35]. Therefore, enzymes like proteases, phospholipases and nucleases

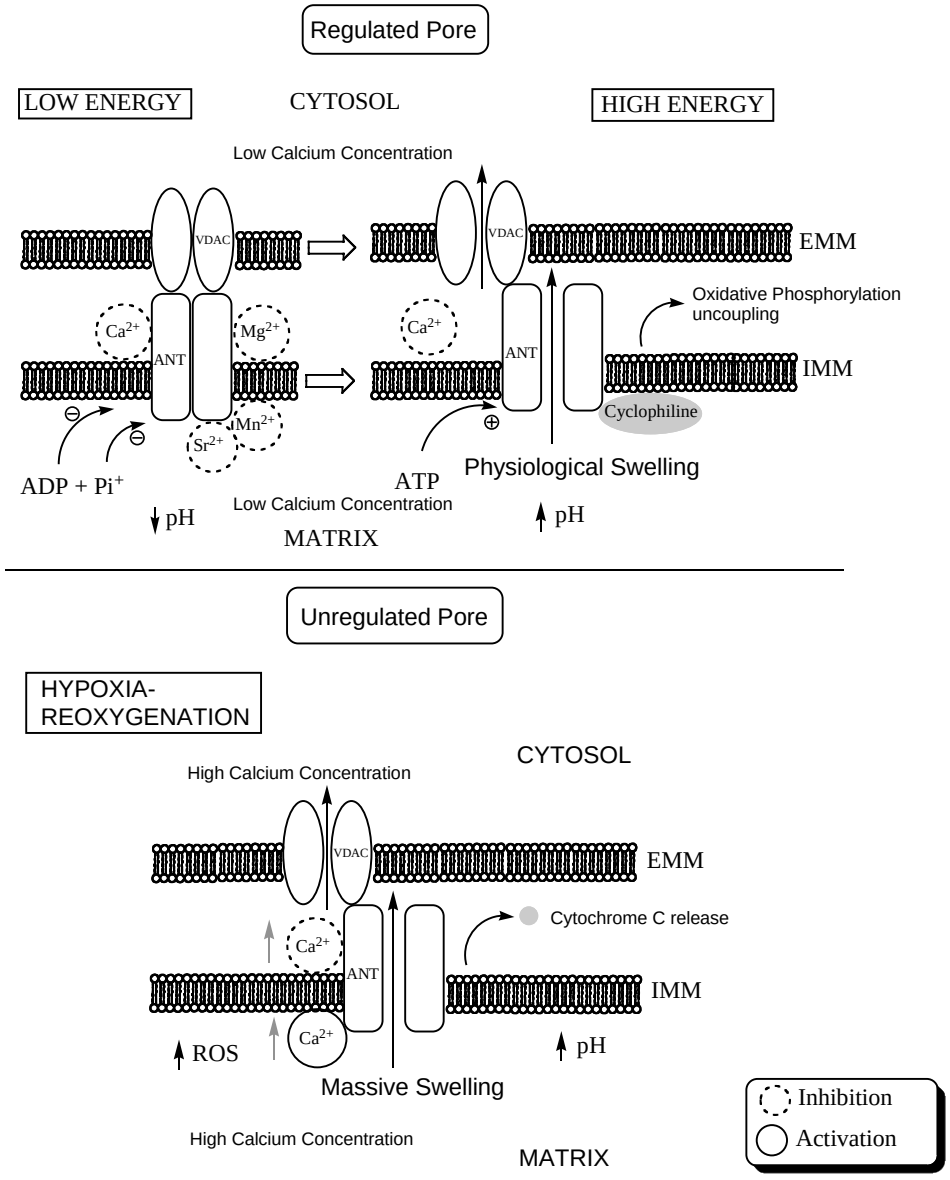


Fig. (2). The figure shows the PTP under physiological and pathological conditions and how divalent cations such as Ca^{2+} interact with the external or internal face of ANT. Under the unregulated mode, apoptotic molecules like Cytochrome C are released.

are released to the cytoplasm, causing the degradation of structural molecules. However, prolonged pore activation can end in cell death by necrosis, Fig. (**3**) [36].

This imbalance finally leads to the rupture of the EMM, which in turn leads to the release of apoptotic proteins, including cytochrome C, Bax proteins and other factors. It is worth mentioning that all these proteins are also possible regulators of yPTP [1,7,23, 26]. Moreover, ROS levels induce the expression of pro-apoptotic ligands like CD95, which later activate the caspase pathway, ending in an apoptotic phenotype. Then, in the case of ROS its pathological mechanism, not only is produced from a direct action over the membranes and cellular proteins but also through genetic induction. For example, this is observed in the induction of Bax genes [37]. Bax genes induce the release of cytochrome C, but at the same time are regulated by bcl-2/bcl-xl [38].

Then, two pathways are involved in the release of cytochrome C: 1) an increase in yPTP permeability through AAC proteins, and 2) the activation of pro-apoptotic genes like Bax, which are induced by ROS [37].

The similarity of the protection responses against the deleterious mechanism of oxidative stress between prokaryotic and eukaryotic cells has been established in many studies [39,40,41]. Diverse proteins related to apoptosis have been identified in *Saccharomyces cerevisiae*. These proteins are members of bax/bcl-2, caspases, Apaf-1/CED and the p53 family. This is something in common with mammalian cells, since it has been shown that these proteins can induce cell death in yeast through the expression of Bax genes [38]. The Bcl-2 family includes two pro-apoptotic proteins (Bax and Bak) and two anti-apoptotic molecules (Bcl-2 and Bcl-XI). The regulation by BCL-2 members seems to depend on their ability to modulate mitochondrial function, which includes the regulation of yPTP. Bax causes hyper-polarization of mitochondria and increases ROS levels, thus decreasing the global functioning of the cell and ending in cell death. The cell death mediated by BAX requires the activation of the mitochondrial respiratory chain, in order to generate ROS or modify ATP levels. Finally, it is important to point out that Bax induces cell death by exerting its effect over mitochondria [42].

In contrast, BCL-XI co-expression partly prevents the actions of BAX. Hence, Bax induces two different cellular processes that can be blocked by BCL-XI: the arrest of the cell cycle, which does not require mitochondrial biochemistry functionality, and cell death, which does [43].

In *Saccharomyces cerevisiae*, the exposure to low doses of H_2O_2 or oxidative stress conditions through glutathione depletion has been shown to induce apoptosis [38]. Diverse pathways in yeast have been associated with molecules like caspase 1 (YCA1). Growth culture conditions also have an effect on the activity of the pore. The sudden opening of the pore takes place with ethanol addition to the growth culture, and also when NADH is used [2].

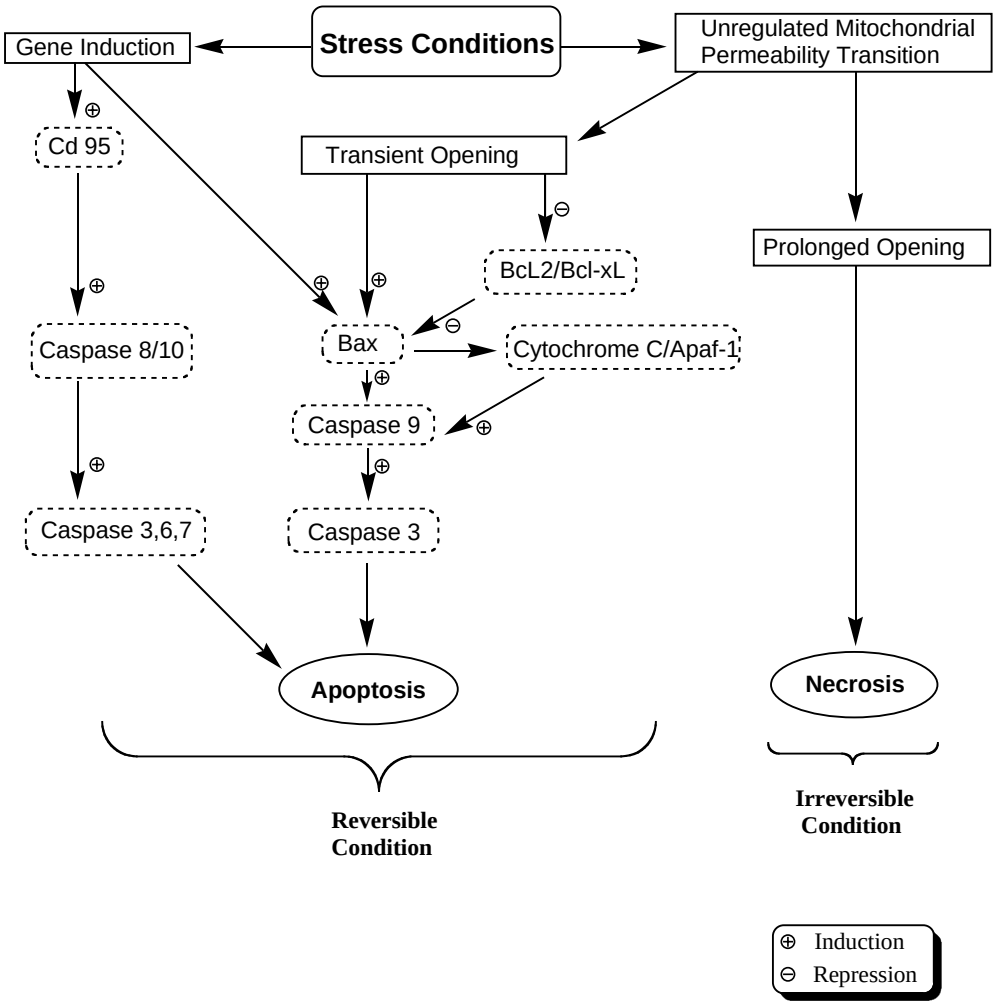


Fig. (3). The figure shows that stress conditions during hypoxia/reoxygenation can be followed by reversible/irreversible cell death depending on the time during which the pore is open.

6. ISCHEMIA- REPERFUSION: TREATMENT WITH MITOCHONDRIAL DRUGS

Although great efforts have been made in the last years to obtain a better treatment of patients with sharp ischemic processes, in many cases the expected results have not yet been obtained. Today, we are aware that when facing severe tissue ischemic conditions, the faster the intervention, the faster the possibility to define the prognosis of the patient and the tissue to be treated. However, in many cases, cells do not respond in the expected way, and among the different reasons for this are the diverse pathways previously described (items 2 and 4). As previously mentioned, the relation between ischemia reperfusion and the permeability of mPTP must be considered.

An increasing number of works hold the concept that the pharmacological inhibition of the mPTP is an effective and promising strategy for protection of tissues under ischemia and damaged by reperfusion.

In the final part of this work we will describe the use of drugs with mitochondrial specificity that can be studied not only in mammalian mitochondria but also in yeast ones. In this way, we will establish a useful comparison for the study of drugs with effects over the functionality of the pore. For a better understanding, we categorized the drugs into two classes: a) one being direct PTP-binding/blocking drugs, like Octylguanidine, Cyclosporine A, N-methyl-Val-4-cyclosporine A [39, 44] and b) ROS scavengers, such as L- Carnitine, Lipoic acid and N- acetyl cysteine. However there are other drugs which have both activities, like melatonin and ubiquinone analogs.

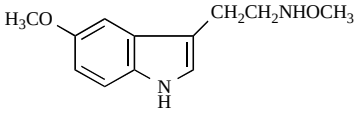
6.1. Melatonin

Melatonin (5-methoxy, N-Acetyltryptamine), a hormone synthesized from tryptophan by the pineal gland, is in charge of the synchronization of endocrine and hormonal mechanisms with the different hours of the day. This synchronization is carried out by the pineal gland since its anatomic conformation receives nervous afferents of the optical nerve. This allows the gland to be sensitized with day light, increasing or decreasing its secretion. Also, melatonin has a direct activity over ROS, playing a role through the up-regulation of antioxidant enzymes like glutathione peroxidase and the down-regulation of pro-oxidant enzymes such as lipoxygenases. In this way, melatonin decreases the damage caused by ROS. Melatonin improves the mitochondrial metabolism through the electron transfer at complex I and IV of the mitochondrial chain.

It has been demonstrated that melatonin enhances the antioxidant effects of ascorbate and Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid). Under physiological conditions, melatonin decreases only a small part of the reactive species, but increases the efficiency of other vitamins.

In addition, it has been shown that melatonin directly inhibits the activity of the mPTP, operating at different metabolic points: a) it substantially reduces the increase in Ca^{2+} levels, b) it prevents the loss of mitochondrial membrane potential, c) it directly inhibits the mPTP, d) it prevents cytochrome C release, e) it prevents caspase activation, and f) it reduces DNA fragmentation [45, 46].

Regarding yeast cells, it has been observed that melatonin improves the response to oxidative stress and extends the life span of the microorganism, although the mechanisms have not been studied in detail [47] Fig. (**4**).



MELATONIN

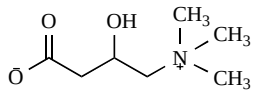
Fig. (4). Melatonin.

6.2. L-Carnitine

L-carnitine or 4-trimethylamine-3-hydroxybutyrate (also known as levocarnitine) is a nutrient synthesized by the liver, kidney and brain from two essential amino acids: lysine and methionine. L-carnitine is responsible for fatty acid transport to the inner part of the mitochondrion, where its metabolization renders energy although it also participates in many metabolic pathways [48].

In a previous work, we used cells from *S. cerevisiae* to study the effect of L-carnitine on Fe^{+2} inhibition over the growth of the microorganism [49]. Also, the effect using wild type cells differs from that using yeast mutants without mitochondrial respiration activity.

Our results demonstrate that the addition of L-carnitine to the growth medium at a 3 mM concentration decreases not only the Fe+ effect but also modifies the mitochondrial electric potential [49] Fig. (**5**).



DL- CARNITINE

Fig. (5). DL-Carnitine.

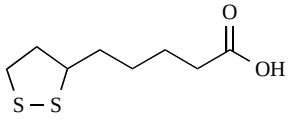
6.3. Lipoic Acid

Lipoic acid (acid 1,2-dithiolane-3-pentanoic), a thiol derived from octanoic acid, is a necessary cofactor which functions as a prosthetic group for the mitochondrial keto-acid dehydrogenases, playing a significant role in energy metabolism [50, 51]. Its function as an antioxidant is given by the interaction with diverse multienzymatic complexes localized in the mitochondria in order to decrease ROS levels.

The acid is covalently bound to the E2 subunit from pyruvate dehydrogenase (PDH) and α-keto glutarate dehydrogenase among other enzymes. In rats as a model system, the administration of lipoic acid has been shown to diminish the decrease in mitochondrial function due to aging. The acetyl-carnitine and lipoic acid administration increases the velocity of the reaction of the acetyl carnitine transferase and mitochondrial function.

In yeast, lipoic acid synthesis involves four different genes through only one pathway: Lip2, Lip3, Lip5, and Gcv3 [50].

In *S. cerevisiae*, the protective effect of lipoic acid has been established in oxidative stress conditions. It has also been found that lipoic acid diminishes the mutagenic effect of hydrogen peroxide, probably through its antioxidant effect Fig. (**6**).



LIPOIC ACID

Fig. (6). Lipoic Acid.

6.4. Ubiquinone Analogs

The effects of ubiquinine analogs have been observed and studied over mPTP activity. Among these analogs, idebenone (2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1,4-benzoquinone) ubiquinone 0, decylubiquinone and ubiquinone 10, plainly known as anti-oxidant agents, have been used [52].

Decylubiquinone and ubiquinone have been found to result in a strong inhibition of the mPTP activity [52]. Idebenone is a synthetic analog of uquiquinone or Coenzyme Q. Coenzyme Q is an essential component of the electronic transport chain and an antioxidant of

the mitochondrial cell membrane. In addition, it improves the electron flow through the electron transport chain, thus decreasing the production of ROS. The compound has been used in patients with Alzeheimer, liver oxidative stress, brain-vascular diseases, and Friedreich ataxia. In all these conditions, the main cause of the pathology is related to a decrease in mitochondrial function [53].

We have previously found that the presence of Idebenone in the medium improves the growth of the yeast strain S288c in low oxygen supply conditions and increases the mitochondrial potential, as measured by the rhodamine 123 signal [54] Fig. (**7**).

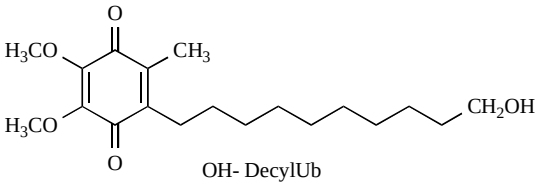


Fig. (7). Idebenone.

6.5. N-Acetyl Cysteine

N-acetyl cysteine (NAC) (Acetylcysteine; mercapturic acid) has diverse cytoprotective effects, but its main effect is the anti-oxidant effect accomplished through three basic mechanisms: 1) a direct connection and the removal of ROS by a redox reaction (cysteine - cysteine), 2) the bisulfite production through the reaction of sulfhydryl groups from N-acetyl cysteine, and 3) as a glutathione precursor.

The compound is extensively used in humans as a treatment in paracetamol intoxication, where depletion in glutathione levels is produced.

In *S. cerevisiae* cells, N-acetyl cysteine works as an antioxidant molecule in the process of removal of mitochondria from yeast grown on different cytoplasmic carbon sources [55] Fig. (**8**).

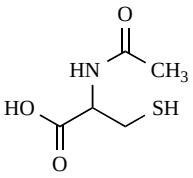


Fig. (8). N-Acetyl Cysteine.

6.6. Octylguanidine

Octylguanidine (OG) (2-octylguanidine hydrochloride) is a hydrophobic cation that belongs to the alkylguanidines group, whose main characteristic is to bind to the mPTP. In this way, it prevents the opening of the pore and therefore the cellular destabilization which ends in cell death. Probably the pore opening takes place through the interaction between OG and the Ca^{2+} binding sites localized in the internal section of the IMM [56]. These studies have been performed in different mammalian models. In heart tissue, it has been observed that OG binding efficiently prevents the increase in mitochondrial permeability and the ulterior unchaining which leads to cell death [57,58].

The yPTP and the mPTP are closed by the strong OG interaction with the pore. This property is explained through a binding with the hydrophobic portion of the IMM [26].

In yeast, the OG molecule presents an inhibitory effect over K+ transport [46]. This inhibition may induce a change in the electrochemical gradient of the mitochondrial membrane.

It has been reported that the site of interaction of alkylguanidines in mammalian mitochondria could be a cavity from ANT constituted by residue 15 of glutamate and 6 of aspartate [7, 57].

Moreover, in yeast, it has been observed that addition of hexylguanidine or OG to the growth medium effectively prevents the increase in transitional permeability [26, 57]. It has also been verified that the inhibitory effect of OG addition can be obtained with a concentration smaller than that needed for mammalian cells [26, 57] Fig. (**9**).

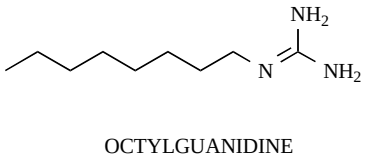


Fig. (9). Octylguanidine.

6.7. Cyclosporine A

In ischemic conditions, the activation of different pathways that interact with the mPTP leads to cytochrome C release and other pro-apoptotic molecules. It has been reported that these pathways could be effectively blocked by the action of Cyclosporine A [15]. Cyclosporine A ({R-[R * ,R*-(E)]}-cyclic-(L-alanyl-D-alanyl-N-methyl -L -leucyl-N-methyl-L-leucyl-Nmethyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenoyl-L-α-amino-butyric-N-methyl-glycinyl-N-methyl- L-leucyl-L-valyl-N-methyl-leucyl) is a drug able to bind to cyclophylin molecules (Cyp), which are proteins that bind to cyclosporines. Also, Cyp molecules present a peptidyl isomerase enzymatic activity. This means that cyclophylins isomerize the peptide bond from a trans to a cis form, which facilitates protein folding and the acquisition of a functional structure [59].

There are two kinds of Cyp: Cyclophylin A (Cyp A), which is an enzyme localized in the cytosol with a beta barrel structure with two alpha helixes and one beta sheet. The binding of Cyp A to Cyclosporine A inhibits calcineurine, a phosphatase Ca^{2+} /calmodulin-dependent protein [22]; and Cyclophylin D, which is located in mitochondria and in involved in the induction of mPTP opening. It must be noted that there are other molecules, like N-methyl-Val-4-cyclosporine A, which have an activity a similar to that of Cyp A [42,44].

However this class of experiment in yeast requires to consider that the microorganism is not sensitive to Cyclosporine A effect [7,22], although it has been recently demonstrated that the overexpression of cyclophylin A in yeast increases the response to oxidative stress [24] Fig. (**10**).

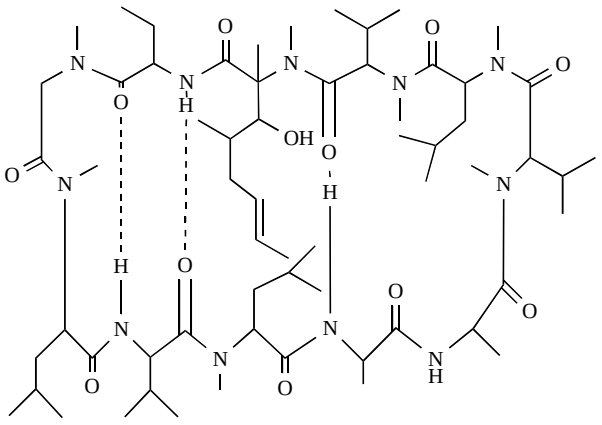


Fig. (10). Cyclosporine A.

7. CONCLUSIONS

Perspectives and Sequels

This work contributes to a better understanding of the permanent increase in the knowledge about the identification and characterization of molecules involved in PTP regulation.

The results discussed considering yeast represent a significant advance since they offer an interesting perspective for the future development of better drugs with the mitochondrion as a target. yPTP and mPTP show many similarities in their structure and functions. The diameter of the pore and the depolarization that generates its opening and mitochondrial edema are similar. It is important to note that this last factor leads to the dysfunction of the cell [28, 60]. Moreover, in yeast, the activation of apoptotic pathways through pro-caspases presents a pattern similar to that of mammalian cells [61, 62].

An interesting strategy can be built when considering the activity of the drugs presented in this work. As an example, it is worth mentioning that the chemical modification of a basic structure like ubiquinone can generate a family of new drugs with potential use in therapeutics. In ubiquinone analogs, the incorporation of carbon chains into the isoprenoide skeleton is able to generate new compounds to evaluate their activity on PTP function [52].

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