Mitochondrial Tolerance to Drugs and Toxic Agents in Ageing and Disease

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Abstract: Better understanding of the effect of ageing on mitochondrial metabolism and of the mechanisms of action of various drugs is required to allow optimization of the treatment of many diseases with minimized risk of dangerous impairment of mitochondrial function. Numerous reports show that efficacy of medical treatment depends on the age of treated subjects. This applies particularly to the effect of drugs on various senescence-prone cellular pathways. In this review, we demonstrate how ageing affects various mitochondria-associated pathways and their response to a variety of factors. These factors include registered drugs and other chemicals, and account for diverse consequences which vary depending on the physiological condition. Pharmacological treatments aimed at improving mitochondrial function should thus have in mind the subject age.

Keywords: Mitochondria, ageing, drugs, toxic agents.

1) MITOCHONDRIAL RESPIRATORY CHAIN STRUCTURE, FUNCTION AND IMPERFECTIONS

Mitochondrial Respiratory Chain Structure and Function

Mitochondria are dynamic, plastic organelles linked to the "cell´s biochemical powerhouse", as they produce the majority of cellular ATP through oxidative phosphorylation (OXPHOS) and carry out several other crucial metabolic processes [1] (Fig. **1**). The tricarboxylic acid cycle, β-oxidation of fatty acids, segments of the urea cycle, and pyruvate oxidation by pyruvate dehydrogenase (PDH) all occur in the mitochondrial matrix. Proteins of the respiratory chain, ATP synthase and enzymes involved in heme biosynthesis are associated with the inner mitochondrial membrane (IMM). The composition and structure of the IMM are critical for the diverse reactions of the OXPHOS [2, 3]. Both mitochondrial membranes are very rich in proteins. Porins in the outer mitochondrial membrane (OMM) allow small molecules (<10 KDa) to be freely exchanged between the cytoplasm and the intermembrane space (IMS). By contrast, the IMM is completely impermeable even to small molecules (with the exception of O_2, CO_2, and H_2O). Numerous transporters in the IMM ensure the import and export of important metabolites. The IMM also contains proteins responsible for the import of newly synthesized proteins, including components of the respiratory chain complexes and ATP synthase, and others [4].

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The OXPHOS is by far the major source of ATP in mammalian cells relying on aerobic energy metabolism. The electron transport chain (ETC) consists of: a) three major protein assemblies: mitochondrial respiratory complex I (NADH:ubiquinone oxidoreductase), complex III (ubiquinol:ferricytochrome *c* oxidoreductase) and complex IV (cytochrome *c* oxidase), which build up transmembrane electrochemical potential ($\Delta\psi$) by coupling their electron transfer activities to H^+-translocation from the matrix (negative) to the outer (positive) side of the inner mitochondrial membrane, and b) two mobile carrier molecules, ubiquinone (Coenzyme Q) and cytochrome *c*. The electrochemical gradient is then utilized for ATP synthesis by complex V (ATP synthase) (Fig. **1A**). Succinate-Q oxidoreductase, which is part of the tricarboxylic acid cycle, is also assigned to the respiratory chain as complex II. All the respiratory chain complexes are made up of numerous polypeptides and contain a series of different protein-bound redox coenzymes [5], including flavins (FMN or FAD in complexes I and II), iron–sulfur clusters (in I, II, and III), and hemes (in II, III, and IV) [6, 7]. Of the more than 80 polypeptides in the respiratory chain, only 13 are encoded by the mitochondrial genome. The others are encoded by nuclear genes and have to be imported into the mitochondria after being synthesized in the cytoplasm.

As described above, the respiratory chain is responsible for the OXPHOS process. Although its component proteins are not organized in a chain-like mode, electrons are transported from reducing equivalents ($NADH+H^+$ and succinate) to molecular oxygen in a chain fashion, driven by the large difference between the redox potentials of the donor and the acceptor [8]. The reaction is strongly exergonic and most of the energy released is used to establish the proton gradient across the IMM [9, 10]. ATP synthesis is ultimately coupled to the return of the protons from the IMS into the matrix [11]. The large number of coenzymes involved in the elec-

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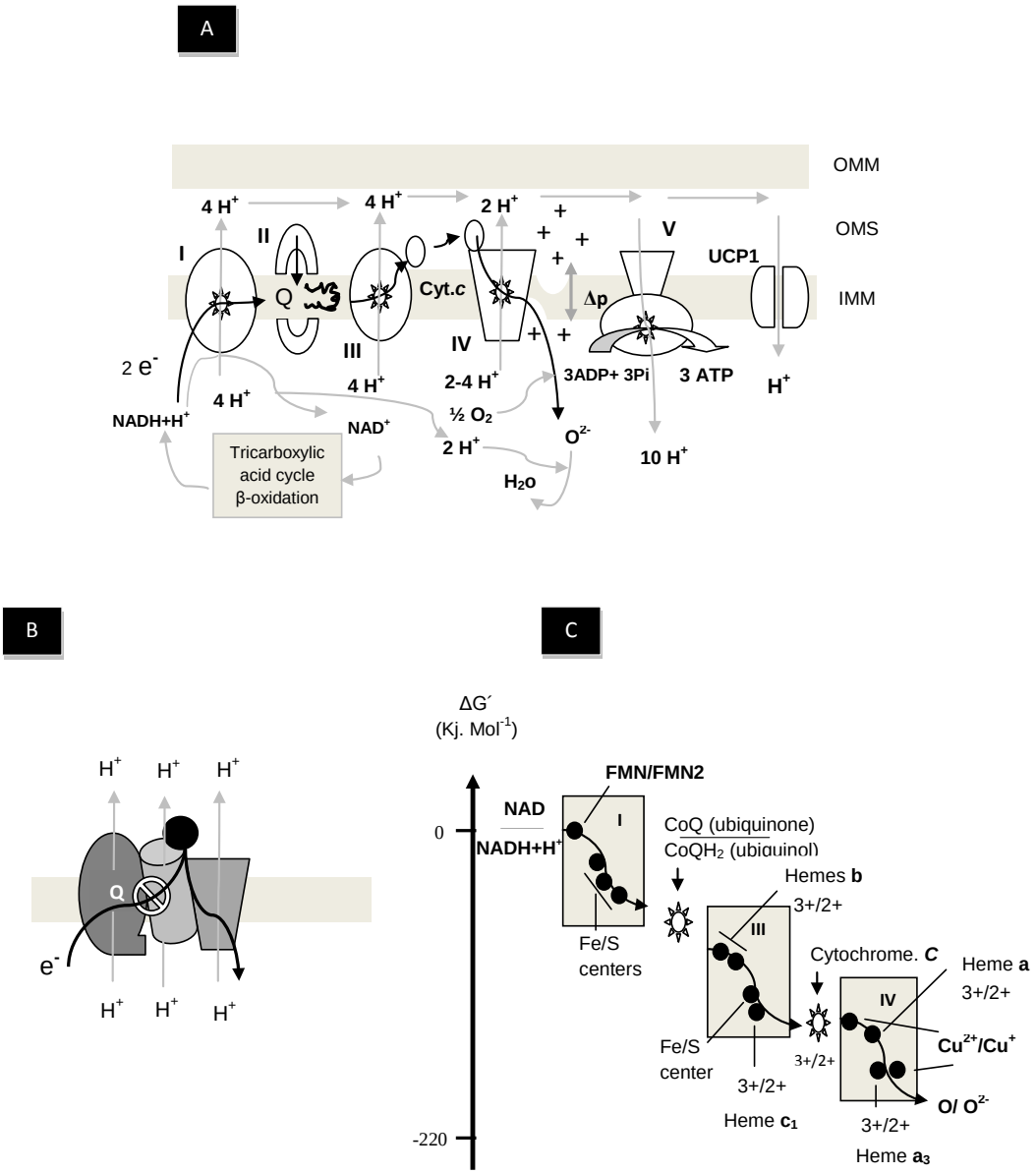

Fig. (1). A schematic drawing of models of the mitochondrial respiratory chain structure and function. (**A**) The mitochondrial respiratory chain. The transmembrane protein complexes of the electron transport chain generate an electrochemical gradient over the IMM. $NADH+H^+$ is oxidized to NAD^+. The electrons are transferred from NADH via complex I and ubiquinone (Q) to complex III. Afterwards they pass through the peripheral electron carrier cytochrome *c* and complex IV to the terminal acceptor, molecular oxygen, which is reduced to water. The electrochemical proton gradient is used by complex V (F1FoATP synthase) to produce ATP and/or by natural uncouplers (Thermogenin, UCP-1); (**B**) The "solid state" organization of the OXPHOS system and hypothetical electron transfer within supercomplex I-III-IV; (**C**) Redox systems involved in mitochondrial electron transport and their approximate redox potentials. These potentials determine the path followed by the electrons if transport is to occur spontaneously. > Black arrows represent electron flow.

tron transport may initially appear surprising. However, the change in free enthalpy ΔG, i.e., the chemical work that is done, depends only on the difference between the redox potentials of the donor and the acceptor [8] (Fig. **1C**). According to the chemiosmotic theory, the proton gradient that is formed between the matrix and the intermembrane space has two components, one being the transmembrane electric potential (Δψ) and the other one being the pH gradient (ΔpH). Mitochondria are unique cellular organelles which can build up a Δψ of up to -180 mV [12, 13]. This large value is probably divided into smaller, more manageable "packages" whose size is determined by the difference between the redox potentials of the respective intermediates. It is assumed that this division is responsible for the astonishingly high energy yield achieved by the respiratory chain. These redox potentials determine the path followed by the electrons, as members of a redox series have to be arranged in order of increasing redox potential if the transport is to occur spontaneously [6, 14] (Fig. **1C**).

Mitochondrial Respiratory Chain Control

The cellular energy demand ranges widely, depending on the function and activity of the cell. Thus, adjustment of

energy production to the physiological demand is essential to all organisms. The first mechanism shown with isolated mitochondria to control OXPHOS is called respiratory control [15].

Mitochondrial energy production can alternate between two steady-states. Respiration is slower during a so-called state 4 respiration as no ATP production occurs, leading to the maintenance of high $\Delta\psi$ value. In turn, state 3 respiration is faster as ATP is generated by the ATP synthase with concomitant use of the $\Delta\psi$. Under physiological conditions, mitochondrial ATP production seems to occur in an intermediate state between states 3 and 4, depending on the metabolic status of the cell [16-18]. The synthesized ATP is then exported by the adenine nucleotide translocase (ANT) to the cytosol in exchange for ADP. Electrical currents associated with the electrogenic ATP/ADP transport can be measured directly [19, 20] using a technique [21] in which the capacity currents across a planar membrane between two bath electrodes are measured. To estimate the number of ATP molecules formed in the aerobic state one needs to know the P/O quotient, i.e., the molar ratio between the synthesized ATP and the oxygen consumed in the process [18]. During transport of two electrons from $NADH+H^+$ to oxygen about ten protons are pumped into the IMS, while for transport from ubiquinol (QH_2) the number is only six. ATP synthase probably requires three protons to synthesize one molecule of ATP, so the maximum P/O quotients possible are around 3 or 2. However, the actual value is much lower since the transport of specific metabolites into the mitochondrial matrix and exchange of ATP for ADP also consume the proton gradient. The P/O quotients generally accepted for the oxidation of $NADH+H^+$ and QH_2 are therefore closer to 2.5 and 1.5, respectively [22], although several well documented studies show other values [23-26]. The electrical nature of the ADP/ATP exchange was not immediately accepted, partially, because it would affect the standard textbook P/O ratios. Mitchell and Moyle believed to have shown [27] that the exchange is neutral, corresponding to ADP/ATP antiport with a parallel H^+ release, or that it is neutralized by Ca^{2+} co-transport [28]. Vignais *et al*. assumed [29] that the energy transduction to the ATP synthesis also drives the ATP export, implying a localized release of newly synthesized ATP.

The amount of nutrient catabolism and ATP synthesis have to be continually adjusted to the changing energy requirements of the cell. This conceptually simple regulatory mechanism which ensures that ATP synthesis is "automatically" coordinated with ATP consumption is known as respiratory control, where the different elements of the OXPHOS are coupled via shared coenzymes and other factors [11]. If a cell is not using any ATP, hardly any ADP will be available to be converted back to ATP in the mitochondria, hence ATP synthase is unable to use the proton gradient across the IMM. This in turn inhibits electron transport in the respiratory chain, which means that the reducing equivalent $NADH+H^+$ can no longer be reoxidized to NAD^+ [30]. Consequently, the resulting high $NADH/NAD^+$ ratio inhibits the tricarboxylic acid cycle, and thus slows down the catabolic conversion of the substrate [11, 31]. Conversely, high rates of ATP utilization stimulate nutrient degradation and the respiratory chain via the same mechanism. If the formation of the proton gradient is prevented by means of short-circuiting the process (e.g. with a protonophore), substrate oxidation and electron transport proceed much more rapidly, however, instead of ATP only heat is produced (Fig. **1A**). A new type of control of mitochondrial $\Delta\psi$ and formation of reactive oxygen species (ROS) has been proposed by Lee *et al*. based on allosteric inhibition of cytochrome *c* oxidase by ATP at high intramitochondrial ATP/ADP ratios [30]. An interesting study [32] described recently in more detail the mitochondrial OXPHOS and concluded that the most important factor in determining the rates of ATP synthesis is not the level of ADP or the proton gradient, but rather the concentration of O_2 and the state of reduction and/or protonation of the inner mitochondrial membrane.

Organization of Mitochondrial Respiratory Chain Complexes

The original model for organization of the respiratory chain complexes assumed that they diffuse freely and independently of one another in the IMM [33]. However, recent data suggest that the complexes form higher-order structures called supercomplexes or "respirasomes" [34, 35] (Fig. **1B**). In this model, the complexes exist as organized sets of interacting enzymes [36] and these associations allow channeling of substrates between the various enzyme complexes, thereby increasing the rate and efficiency of electron transfer and proton translocation and minimizing the formation of free radicals by limiting the direct transfer of electrons to oxygen [37, 38]. Within such supercomplexes some components would be present in higher amounts than others, with some data suggesting a ratio between complexes I/II/III/IV and the ATP synthase of approximately 1:1:3:7:4 in mammalian mitochondria [39]. An inspiring study suggests that dimerization of ATP synthases and supercomplexes formation is essential for cristae morphology [40]. However, the debate over this supercomplex hypothesis is ongoing, as some data do not appear to support it [41]. Besides functional reasons, supercomplex formation seems to be necessary for the assembly and stability of its individual components.

Mitochondrial Respiratory Chain and its Natural Imperfectness

The coupling mechanism between the electron transfer and proton pumping activities of the mitochondrial respiratory complexes has been the aim of intense research for many years. A particularly controversial aspect concerns the possible physiological variability of the energy conservation efficiency of the redox-driven proton pumps. Several models have been proposed to explain the peculiar properties of this process which remains still unsolved [11, 14, 42]. Here we review some of the intrinsic imperfections of mitochondrial energetics, mainly the metabolic consequences of OXPHOS impairment including accumulation of metabolic intermediates, increased generation of ROS, and decreased ATP production. The yield of mitochondrial ATP production by OXPHOS can be influenced by several factors among which uncoupling (leak) and decoupling (slip) play a major role in the modulation of the protonmotive force [43-46]. Several physiological functions have been proposed to justify the energetic expenses of maintaining the mitochondrial proton

leak. These include, but are not restricted to the regulation of OXPHOS efficiency, heat production by dissipation of the proton gradient (thermogenesis), and production of mitochondrial ROS. Uncouplers (such as protonophores) break down the proton gradient by allowing H^+ ions to pass from the intermembrane space back into the mitochondrial matrix without an involvement of ATP synthase. Thermogenin (uncoupling protein-1, UCP-1), an ion channel in mitochondria of brown fat tissue is a naturally occurring uncoupler [47] (Fig. **1A**). Whereas UCP-1 in brown adipose tissue has a well-defined role in thermogenesis, the roles of other UCPs (UCP2-UCP5) are still tentative, such as in the control of immune response, oxygen radical formation, and insulin secretion [48-52]. Therefore, uncoupling manifests itself as a non site-specific decay of the mitochondrial energy conservation efficiency. Decoupling of specific redox-driven proton pumps derives from "slippage" events that could be caused by the activation of intramolecular electron transfer routes not associated with proton translocation, or by mechanistic/kinetic alterations of the coupling pathways/ reactions [24, 31]. Mitochondrial proton leak may also represent a mechanism for the regulation of mitochondrial production of ROS, mediators of oxidative cell damage [53]. In addition to UCPs, adenine nucleotide translocase (ANT) is a second candidate that may modulate mitochondrial energy efficiency. ANT is known to mediate uncoupling by fatty acids and to lower mitochondrial $\Delta\psi$ in the heart and skeletal muscle, and it can also induce proton leak in the presence of activators such as adenosine monophosphate [54]. Like UCP and ANT, activation of the mitochondrial ATP-sensitive K^+ channel (mitoKATP) plays a central role during pathological conditions associated with oxidative stress. During ischemic preconditioning, moderate increments in ROS release activate mitoKATP, which then leads to mild uncoupling and prevents consequences of mitochondrial oxidative stress during reperfusion such as calcium overload, resulting in tissue protection [55].

Mitochondrial ROS are important determinants of cell functioning, participating in many signaling networks and also in a variety of degenerative processes. A small but constant leak of electrons from the mitochondrial respiratory chain induces monoelectronic reduction of molecular oxygen, forming superoxide anion ($O_2^{\bullet-}$). Nearly 2-4% of the total oxygen consumed by mitochondria is not fully reduced to water and results in the formation of ROS, which together with their reaction products, such as the hydroxyl radical, are very harmful to cells as they oxidize proteins and fatty acids and damage DNA. Oxidative modifications of mitochondrial electron transport chain proteins compromise normal activity, bringing about a further increase in ROS production and oxidative damage contributing to the mitochondrial dysfunction [56]. The ROS-mediated cellular damage might contribute to disease and is proposed as a cause of ageing [57]. Besides being traditional targets for ROS effects, mitochondria are also recognized producers of the same species that can destroy them. Currently seven separate sites of ROS production in mammalian mitochondria have been identified. Two of them are well-documented sources of mitochondrial ROS: complexes I and III [58-61]. Despite the absence of ROS formation by Complex II itself, succinate is an important source of ROS in many tissues. This is due to reverse electron transfer from succinate to ubiquinone (via Complex II) and back to Complex I [62, 63]. Other documented ROS sources among mitochondrial enzymes are the flavoproteins acyl-CoA dehydrogenase and glycerol phosphate dehydrogenase, which can also generate ROS in some tissues when oxidizing lipid-derived substrates [58, 64, 65], monoamine oxidase and dihydroorotate dehydrogenase [66, 67]. Pyruvate and α-ketoglutarate dehydrogenase both contain flavoenzyme dihydrolipoyl dehydrogenase subunits [68], which are very important ROS sources in the brain [69-70] and may be involved in ageing, at least in model organisms. Furthermore, not all mitochondria are alike and can present quite diverse ROS release patterns in various tissues and organisms [71-73].

The traditional view of ROS is that they have a negative effect on cell functioning and viability, and therefore antioxidants that inhibit their reactivity must be beneficial. An increasing recognition of the roles of ROS in cell signaling and modulation of gene expression has forced a re-evaluation of this simplistic view. Although ROS production by mitochondria is a continuous process in physiological conditions, mitochondria also have an efficient antioxidant defense network [74]. Mitochondrial superoxide dismutase (SOD2), glutathione peroxidase (GPx), glutathione redutase (GR), α-tocopherol and cytochrome *c* are examples of the mitochondrial antioxidant defences [74]. It is apparent that our understanding of the roles of ROS and antioxidants must be based on improved knowledge of their actions in individual physiological and pathophysiological conditions.

2) MITOCHONDRIAL DYSFUNCTION DURING AGEING AND MITOCHONDRIAL DISORDERS – ROLE OF OXIDATIVE STRESS AS A PRIMARY AND SECONDARY CAUSE OF MITOCHONDRIAL DYSFUNCTION

Ageing can be defined as a genetic and physiological process associated with the gradual biological impairment of normal organism functions. On the cellular level, ageing leads either to the loss of cellular homeostasis, decreased proliferation, or even to cell death. Consequently, these changes have a direct impact on the functional ability and physiological performance of tissues and organs [75]. Ageing can be also driven by a decreased capacity to maintain energy homeostasis. The reduced ability to produce ATP in the cells of aged animals emphasizes the importance of the mitochondrial theory of ageing. It is now widely accepted that the primary factor determining the age-dependent dysfunction in the energy state of mitochondria is the efficiency of oxidative phosphorylation. Ample experimental data show that in rodents and human subjects the activities of the respiratory enzyme complexes decrease with age in various tissues and organs such as brain, liver, and skeletal muscles [76-84].

In rodents the age-dependent decrease in electron transfer activity in brain, kidney, heart and liver refers mainly to complexes I and IV of the respiratory chain. The activity of complexes II and III remains virtually unaffected [85-94]. By the mid 1990s Boffoli *et al*. [95] demonstrated that the decrease in the activity of the individual respiratory chain complexes mentioned above can be correlated with the reduction of their content in mitochondria. Additionally, a

decrease of ubiquinone content in skeletal muscles of old mice, reported by Lass *et al*., contributes to the OXPHOS disturbance [96]. In turn, no deterioration in the activity of catalytic subunits of complex III ATPase (F1 subunit) and ATP synthase has been observed, respectively, in ageing humans [95] and mouse liver and brain [89, 92]. This stands in contrast with the assumption that one of the important parameters describing age-dependent alterations in mitochondrial metabolism is the respiratory control ratio (proportion of ADP-stimulated - state 3 to resting - state 4 respiration). Moreover, in contrast to those enzymatic studies, mitochondrial ATP synthesis has been observed to decrease with age to various levels in different human tissues [77, 84] and skin fibroblasts [97]. The data suggest that the impairment of ATP synthesis may be a combined result of age-induced lesions of the respiratory chain, disturbances in mitochondrial membranes and a decreased activity of the adenine nucleotide translocase, which is also known to decline with age [98].

The list of age-dependent alterations in mitochondrial physiology is extensive and includes:

a) Mitochondrial Morphology

A characteristic distinguishing feature of mitochondria in cells of aged individuals is their increased volume [99]. Moreover, mitochondria in cardiac myocytes derived from aged rats have been reported to possess fewer cristae [100, 101], while those in hepatocytes from old humans retain their higher number [102]. However, in both cases the organelles were larger in size. Such an enlargement of mitochondria with age is not fully understood. Nevertheless, oxidative stress and subsequent mitochondrial swelling seems to be responsible for this condition [103].

b) Mitochondrial Mass, mtDNA Content and Expression

Studies on human lung fibroblasts showed increased mitochondrial mass during replicative senescence [104, 105]. This could be a consequence of a decreased mitochondrial turnover controlled by autophagy [106], which is responsible for disposal of mitochondria. An argument in favor of this theory is the increased number of damaged mitochondria in aged and senescent cells [107]. The group of Wei and colleagues from China observed a significant increase in mitochondrial DNA content in lungs of aged human subjects [108]. Other studies have shown that reduced activity of the respiratory chain complexes in old rodents can be correlated with the overexpression of mitochondrial genes encoding subunits of complexes I, III, IV and V. On the other hand, Northern blot analysis indicates that such a relationship is in opposition with the observed decrease in mRNA expression [109] and indicates that the presence of a compensatory mechanism (increasing the number of mtDNA copies) cannot be sufficient for long term protection of the respiratory chain from age-related dysfunctions.

c) Mitochondrial Fusion and Fission

It has been reported that in senescent cells fission is decreased with simultaneous increased fusion of mitochondria, which results in the formation of enlarged, elongated giant mitochondria [110]. This could have impact on the degradation of those organelles because smaller particles undergo autophagy more easily [111]. It seems that such elongated mitochondria enable exchange and distribution of correct copies of mtDNA along a fused mitochondrial network [102, 112, 113].

d) Overproduction of Mitochondrial ROS/Free Radical Theory of Ageing

The age-dependent handicapping of mitochondrial energetics is associated with the accumulation of defective mtDNA and respiratory chain complex copies with enhanced electron leakage due to their dysfunction. These symptoms are believed to be in direct relation with a diminished ATP synthesis and increased ROS generation in organs and tissues of old mice [114] as well as skin fibroblasts from elderly human subjects [115].

Since the free radical theory of ageing was proposed by Harman in his landmark original paper in 1956 [116], mitochondria have been linked to the prooxidative properties of free radicals in the ageing process. Also now it is universally accepted that an age-related accumulation of mutations in mtDNA leading to a decline in respiratory chain functions induces an increase of ROS generation by mitochondria. This in turn causes oxidative damage, further mtDNA mutations and so the merciless vicious circle forms [117, 118]. Apart from damaging DNA, the elevated production of ROS is responsible for oxidation of other fundamental cellular components such as proteins and phospholipids. Another feature conductive to senescence is an age-dependent reduction in the degradation efficiency of oxidized, nitrated and damaged proteins. Lastly antioxidant defences, also decline with age [119, 120].

Independently from the Harman's hypothesis, another theory emerged and is dynamically studied. Based on recent findings that autophagy is diminished in lipofuscin-loaded cells and that cellular lipofuscin content positively correlates with oxidative stress and mitochondrial damage, it has been proposed the mitochondrial-lysosomal axis theory of aging, according to which mitochondrial turnover progressively declines with age, resulting in decreased ATP production and increased oxidative damage [121, 122]. Lysosomes, which are normally responsible for mitochondrial turnover, gradually accumulate an undegradable material, called lipofuscin or age pigment [123]. Finally, new findings point to a slow accumulation of lipofuscin in the lysosomal compartment of long-lived postmitotic cells due to the existence of this mitochondrial-lysosomal cross-talk in which formation of ROS by mitochondria gives rise to peroxidation of autophagocytosed lysosomal contents under degradation [122].

Recent studies have shown that PKC can also play a critical role also in the regulation of autophagy, the dynamic process of protein degradation, typically observed during nutrient deprivation, and occurs when cells need to "self-cannibalize" or degrade their constituents [124]. However, the exact mechanism remains ambiguous [125].

Zhang and co-workers demonstrated that inhibition of PKC reduced significantly autophagy and increased apoptosis markedly, whereas pre-treatment with a PKC activator caused the opposite results [126].

Other recent observations showed that acute hypoxic stress induces autophagy through a process involving PKCδ. Upon stress, a rapid activation of PKCδ occurs, with the release of Beclin-1 from its inhibitor Bcl-2, leading to autophagy induction during the early phase of hypoxia [127].

However, an opposite effect of PKCδ on autophagy was also described [128]. Indeed, in pancreatic ductal carcinoma cells, PKCδ expression constitutively suppressed autophagy through the induction of tissue transglutaminase expression. This apparent contradictory function for PKCδ could be explained by a "dual role" of PKCδ in cell survival and cell death: early induction of PKCδ may contribute to a transient protective response by stimulating autophagy, whereas the delayed and sustained activation of PKCδ by cleavage could lead to an eventual pro-death signal [129], to regulate the cell fate decision inhibiting autophagy. Finally, treatment with the Endoplasmic Reticulum stressors thapsigargin or tunicamycin induced PKCθ phosphorylation and activated autophagy in a mTOR-independent way [130].

Numerous proteins have been proposed to be responsible for lifespan regulation. Recently, one protein in particular, p66Shc and its signaling properties has attracted major interest in ageing research. Less than 12 years ago, Migliaccio and coworkers proposed that the p66Shc protein can control mammalian life span by regulating cellular response to oxidative stress. Studies on transgenic mice lacking p66Shc protein have shown that their life span increased by 30-40% in relation to their wild type counterparts without any pathological consequences. The reason that p66Shc may be recognized as related to the oxygen radical theory of ageing is that the genetically modified animals demonstrated improved resistance to oxidative stress [131].

In agreement with those observations, Mouse Embryo Fibroblast cells (MEFs) from which p66Shc had been completely depleted, were resistant to apoptotic death induced by oxidative stress [131] similarly to $p53^{-/-}$ MEFs [132]. Deletion of p66Shc causes resistance to oxidative stress but p66Shc level can be upregulated only in p53 wild type MEF after H_2O_2 treatment [133], though in cell lines like HeLa or SaOs-2 phosphorylation of crucial for p66Shc apoptotic pathway activation residue-Ser36, occurs independently of p53 presence [134]. In the absence of p53 the stability of p66shc protein does not increase as it happens in p53 wt cells after UV exposure and apoptosis induced by prooxidant agents such as H2O2 connected with p66Shc pathway is possible only in p53 wt cells [133], all of these data suggest that p66Shc acts downstream and independently of p53 and plays regulatory role in apoptotic process. However there is still close relation between p53 and p66shc on the antioxidant defense system activation. p53 induce p66Shc gene transcription. Both these proteins, indirectly-by inhibiting the FOXO transcription factors, down regulate antioxidant enzymes (catalase, superoxide dismutase) expression under oxidative stress condition [135]. Furthermore some data show that p53 gene polymorphism correlates with p66Shc protein level in centenarians [136].

The p66Shc protein is an alternatively spliced isoform of a growth factor adapter which belongs to the ShcA family. The p66Shc protein differs from two other ShcA members (p46Shc and p52Shc) by the presence of an additional N-terminal proline-rich domain (CH2). This domain carries an important serine phosphorylation site, Ser36. Recently we have shown that phosphorylation of this residue plays an important role in the cellular response to oxidative stress and in ageing. This phosphorylation is an important step in the initiation of p66Shc translocation to mitochondria and mitochondria-associated membranes during ageing or upon oxidative stress and can be mediated by one of the serine-threonine kinases (especially by protein kinase Cβ (PKCβ)) [137, 138]. p66Shc translocated to the mitochondria perturbs their structure and functions and, most importantly, accelerates ROS production which in turn propels the vicious cycle responsible for further excessive ROS formation by mitochondria [138].

Mitochondrial p66Shc, by virtue of its association with cytochrome c, interferes with the electron transport chain, inducing accumulation of reducing equivalents upstream of complex III, thus favoring superoxide generation by reduced ubiquinone. Thus, under conditions of acute oxidative stress, p66Shc should inhibit activity of the ETC, in turn reducing mitochondrial electrochemical potential and calcium transport, which would be followed by opening of the permeability transition pore (PTP), swelling of the organelle followed by cytochrome c release and activation of the apoptosome [139].

This molecular pathway extensively studied in our laboratories is probably responsible for the ageing properties of p66Shc. We have proposed that oxidative stress is associated with the activation of p66Shc and thus the recruitment of mitochondria in apoptosis. Understanding of this novel signalling mechanism, operative in pathophysiological conditions of oxidative stress (also in the case of mitochondrial disorders), may open new possibilities for pharmacological slowing down of organ deterioration processes during ageing. That is why, rather than searching for more effective antioxidants we study the possibility of modulating the "p66Shc pathway" by hispidin (a specific inhibitor of p66Shc Ser36 phosphorylation by PKCβ) and the effects of this intervention on mitochondrial metabolism and ROS production. The effects of hispidin and its possible therapeutic usage will be presented below.

e) Mitochondrial Disorders

Mitochondrial disorders (MD) are another excellent example of dysfunctions which are always accompanied by a decreased efficiency of ATP production and many other abnormalities in bioenergetics. Defects of the respiratory chain can be caused by inherited mutations in mitochondrial or nuclear DNA contributing to the appearance of isolated or combined defects in the respiratory chain complexes. Mitochondrial disorders are present in neonates, infants, children and adults with a relatively high (1:5000) frequency. The clinical picture and progress of MD severity depends on many factors, such as heteroplasmy and tissue energy requirements – probably the most important parameter, because the affected mitochondria cannot supply enough energy for proper tissue/organ development and functioning [140]. Muscles, peripheral nerves and the central nervous system all have high basal energy requirements and thus they are most frequently affected in MD. In consequence patients suffer from myopathies, cardiopathies, neuropathies and

retinopathies with different outcomes in each affected tissue [141]. The diversity of mutations and their effects along with the range of symptoms complicate diagnosis, medical intervention as well as scientific investigation of this field.

f) Mitochondrial Disorders and Oxidative Stress

The influence of pathological oxidative stress on the development of mitochondrial disorders and on the overall antioxidant defense balance is an attractive and current issue investigated in our laboratory. We found that fibroblasts derived from patients with different mitochondrial disorders, regardless of the type of genetic defect, have a dramatically higher rate of ROS production and increased level of carbonylated proteins [142]. In such affected fibroblasts the intracellular oxidative stress related to the mitochondrial dysfunction can be a signal for the phosphorylation of p66Shc at Ser36 which activates the vicious cycle described above for ageing. The overproduction of ROS may additionally potentiate the mitochondrial dysfunctions. Hispidin treatment was sufficient in some cases to decrease superoxide production, suggesting that p66Shc is also involved in the cellular response to the endogenous oxidative stress originally initiated by mitochondrial dysfunction. Moreover, in *in vitro* models, administration of hispidin prevents the fragmentation of the mitochondrial network caused by hydrogen peroxide (Fig. **2**).

Recently, a wide range of diseases (cancer, diabetes, Alzheimer, Parkinson's disease) as well as ageing have been proposed to be due to damage generated by ROS. This means that potential therapy or preventive action should be aimed at decreasing oxidative stress and/or protecting from ROS-induced damage, provided that the mitochondrial defect cannot be repaired.

3) PHARMACEUTICAL THERAPY-RELATED AND CHEMICALLY-INDUCED MITOCHONDRIAL DYSFUNCTION. DOES IT DEPEND ON AGE AND INTENSIFY AGE-DEPENDENT ALTERATIONS?

Mitochondria as the center of cellular bioenergetics are a perfect target for drugs and xenobiotics used in the treatment of various pathological conditions (indirectly or directly correlated with mitochondrial dysfunctions like diabetes or inherited mitochondrial disorders). Among the numerous unwanted consequences of medication, some antibiotics and antiviral agents have non-specific effects on mitochondria. That is why, when designing new pharmacotherapies, account must be taken of potential side effects caused by the compound at the molecular level, which in turn may impact the patients' health. Some medical compounds may alter a cell's destiny, which could have repercussions on the whole organism's condition.

An increase in mitochondrial dysfunction with age may account in part for the greater sensitivity to these pharmaceuticals of the old individuals as compared to young ones.

a) Mitochondrial Dysfunction Associated with Administration of Antibiotics and Anticancer Agents

The toxicity of xenobiotics depends on their metabolism and renal clearance. These two parameters undergo profound changes during the first years of human life [143]. Clinical studies have demonstrated that the majority of xenobiotics are metabolized at the fastest rate by children and that this rate gradually declines with age to the levels observed in adults [144]. This renders children more resistant to the cytotoxicity of some drugs. Conversely, children are more susceptible to drugs and chemicals that undergo metabolic activation (conversion to bioactive or cytotoxic metabolites).

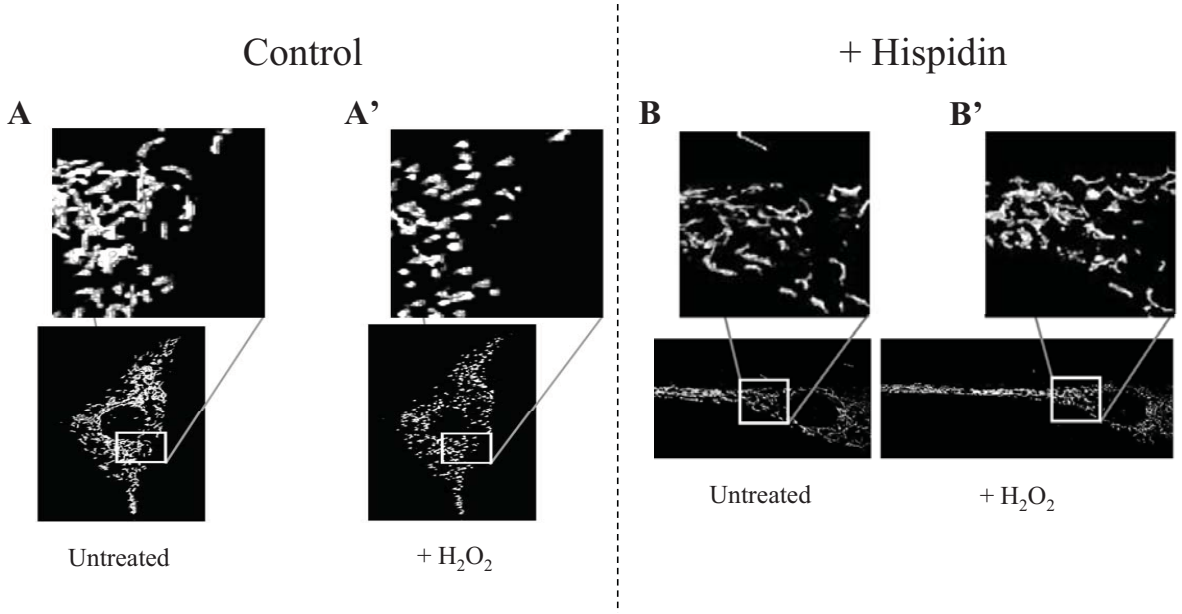

Fig. (2). Protective effect of hispidin on mitochondrial structure. Mitochondrial structure in mouse embryonic fibroblasts (MEFs) was visualized by expressing the mitochondrial targeted fluorescent marker mtGFP and recorded using a digital imaging system based on a Zeiss Axiovert 200 fluorescence microscope equipped with a back-illuminated CCD camera. Induction of oxidative stress by the application of the H_2O_2 (1mM, for 120 minutes) induces morphological changes (a major fragmentation) of the mitochondrial network in control MEFs (A'). Although, pre-treatment of MEFs with hispidin (5μM, for 30 minutes) protected mitochondrial network against fragmentation caused by H_2O_2. In this case no significant alterations in mitochondrial structure were observed (B').

For example, vincristine a drug that is widely used in the treatment of solid tumors is significantly more neuro- and hepatotoxic in infants than in older children [145]. The toxicity of vincristine has been pinpointed to mitochondrial dysfunction [146], including disorganization of mitochondrial cristae structure and induction of apoptosis by activetion of a mitochondria-dependent apoptotic pathway with ROS as an important regulatory factor [147]. Another example of a mitotoxic compound is acetaminophen (Tylenol). This drug employed in relieving pain is known to be hepatotoxic due to induction of mitochondrial dysfunction and increased oxidative stress [148]. However, the hepatotoxicity of acetaminophen metabolites is lower in young children than in adults [149] due to the greater capacity of detoxification and higher glutathione level in the former [150].

The literature offers numerous examples of drugs which cause mitotoxicity connected with the distortion of mitochondrial energy production. One of them, ciprofloxacin, a 4-fluoroquinolone antibiotic, is commonly used in treatment of bacterial infections and in anticancer therapy. The drug inhibits bacterial DNA gyrase. An unwanted side effect is that it also inhibits mammalian topoisomerase II, particularly its mitochondrial isoform. This causes improper mtDNA replication, resulting in mtDNA fragmentation and its gradual loss [151-153]. We studied the effects of long-term exposure to a relatively low concentration (25 μg/ml) of ciprofloxacin on the mtDNA content and mitochondrial metabolism. The decreased mtDNA level led to complete inactivation of complex I of the respiratory chain. At the same time complexes III and IV although reduced were still sufficient for electron transport from complex II and from other NAD^+-independent substrates. This in turn deregulated the mitochondrial energy state, as evidenced by lowered mitochondrial respiration and reduced $mt\Delta\Psi$ and ATP formation [152].

Another example of an antibiotic with a broad spectrum of activities, effective against a variety of Gram-positive and Gram-negative bacteria and believed to cause mitotoxicity is chloramphenicol. Studies by Weiss *et al*. [154] on the biological half-life of chloramphenicol in the blood of infants, few-day-old, and 5 year old children showed a substantial acceleration of chloramphenicol metabolism during the first days of life, however later its metabolism is gradually reduced. Interestingly, in combination with hexachlorophene [155] or diazepam [156], the metabolism of chloramphenicol slows down, enhancing its toxicity in newborns and neonates. Other data in HepG2 and H1299 cells show that chloramphenicol induces down-regulation of mtDNA-encoded COX I subunit without any alterations in nuclear-encoded proteins. Futhermore, this was accompanied by the appearance of resistance to mitomycin-induced apoptosis probably due to mitochondrial stress. Similar effects have been observed for other antibiotics affecting mitochondrial translation such as minocycline, doxycycline, and clindamycin [157]. Also daunorubicin (DNR) is believed to be mitotoxic. This anthracycline antibiotic is a major antitumor agent used in the treatment of a variety of malignancies, soft-tissue sarcomas, non-Hodgkin's lymphoma and primarily in the treatment of acute myeloid leukemia and acute lymphocytic leukemia [158-160]. The mechanism of DNR action, although still not fully understood, involves high affinity sequence-specific DNA intercalation [161, 162] and its mitotoxicity is connected with ROS generation [163-167]. Studies performed by Paul *et al.* examined also an immediate effect of DNR on mitochondrial parameters [167]. Interestingly, low concentrations of DNR (2-10 mM) increased mitochondrial respiration, while higher doses of DNR (>10 mM) inhibit complex I-dependent respiration. However, further analysis showed that DNR can be accumulated in the inner mitochondrial membrane where it enhances electron deviation from the regular respiratory chain pathway. This leads to increased ROS generation both by complex I and III and additionally amplifies mitochondrial dysfunction [163, 167].

The anticancer agent adriamycin (ADR) has long been recognized to induce a dose-dependent cardiotoxicity [168-171]. Although the exact mechanism of this cardiotoxicity remains unsolved, it is accepted that it is correlated with the induction of mitochondrial ROS production via redox cycling of the drug by complex I [172]. Further studies have shown high accumulation of ADR within mitochondria [173] and also in the nucleus [173, 174].

A greater tolerance (resulting in lower mitotoxicity) towards the drugs described above as well as many other anticancer drugs, observed in children seems to result from a higher metabolism rate or renal clearance. This increased tolerance in children found confirmation in a work published by Glaubiger *et al*. [175] who evaluated the maximal tolerated doses (MTD) of anticancer drugs in children and adults. However, even though the MTD for daunomycin is approximately 20% higher in children than in adults, they seem to be more prone to congestive heart failure [176].

b) Antiretroviral Therapy

Antiretroviral therapy may also be burdened with the risk of mitochondrial failure. Prolonged antiretroviral therapy may sometimes abate its beneficial outcome and results in a loss of mtDNA content and thus handicapped mitochondrial functions [177]. *In vitro* experiments on Human Aortic cell lines (HAECs) have shown that long term treatment with some nucleotide reverse transcriptase inhibitors (NRTIs) which suppress viral replication, such as zidovudine (AZT), may cause increased oxidative stress, augmentation of cellular and mitochondrial superoxide production and a decrease of mitochondrial membrane potential but without alterations in the mtDNA level [178]. Interestingly, it has been observed that the incidence of side effects caused by AZT is similar in adults and children. However, based on the average AZT therapy duration, it seems that children tolerate it somewhat better compared to adults [176].

4) STRATEGIES OF MITOCHONDRIAL PROTECTION IN AGEING AND IN MITOCHONDRIA-ASSOCIATED DISEASES – GOOD AND BAD THERAPEUTIC APPROACHES

Modern therapeutics are in many aspects based on traditional medicine. Natural compounds are often used to cure common diseases. From the mitochondrial point of view the most interesting are those compounds which contribute to the improvement of mitochondrial bioenergetics, decrease ROS production, stimulate antioxidant defense

system and prevent damage associated with mitochondrial disorders. Mitochondrial membrane phospholipids can be protected from peroxidation by flavonoids such as kaempferol, luteolin, myricetin and quercetin present in the diet or polyphenols from grapes [179]. Additional protection can be provided by ω-3 fatty acids [180]. Dietary habits of populations are characterized by lowering cancer incidence and extending lifespan with a simultaneous low rate of age-associated diseases. Such features are typical for Mediterranean populations: Greek, Italian, French, Spanish and Portuguese [181], whose diet is composed of foods rich in antioxidants, as well as substances inducing apoptosis of cancer cells and improving mitochondrial metabolism. Such compounds are described by The American Dietetic Association as 'functional foods' and 'nutraceuticals' [182]. According to the definition 'functional food' must contain compounds with beneficial biochemical and physiological properties. This includes chemoprotective compounds protecting from carcinogenesis (resveratrol, curcumin, isoflavones), phytochemicals modulating metabolism and preventing diseases (lycopene, quercetin, ally-sulphides) and nutraceuticals (vitamins, minerals, plant extracts, animal extracts like chitosan) [183].

a) Polyphenols and Flavonoids

Curcumin is a widely used polyphenolic compound present in a popular spice – turmeric. It has been widely demonstrated on animal models as well as human cell lines that curcumin has strong chemopreventive properties against colon cancer [184, 185]. It has been proposed that the induction of apoptosis by curcumin is mediated by the mitochondria-dependent pathway [186, 187]. Interestingly, studies performed by Volate *et al*. and confirmed by Kwon *et al*. showed that the level of active caspase-9 is much higher (a higher ratio of active caspase-9 to procaspase-9) only in young, but not old, rats fed curcumin [188, 189].

Resveratrol, another natural polyphenolic compound demonstrating strong antioxidant properties, is present in grape skin, so has been present in the human diet for ages. This compound was tested for positive action in many disease models offering hope of finding a safe pharmacological strategy for age-related and neurodegenerative disorders [190]. Resveratrol decreases oxidative stress and activates molecular pathways increasing the level of antioxidant enzymes [191]. The senescence-accelerated mouse (SAMP1) fed a resveratrol-supplemented diet combined with physical exercise had better muscle condition. Mice were also protected from an age-associated decline in physical strength through activation of mitochondrial genes related to bioenergetic functions like oxidative phosphorylation [192]. Hence, it is plausible that a similar mechanism may help to improve survival time and reduce effects of aging in human subjects. In a study of 123 Finnish adults, those born with certain increased variations of the SIRT1 gene had faster metabolism, helping them to burn energy more efficiently—indicating that the same pathway shown in laboratory animals works in humans [193]. Futhermore, resveratrol protects against age-induced osteoarthritis disease by preventing IL-1β-induced catabolic effects and chondrocyte apoptosis via its inhibition of mitochondrial membrane depolarization and ATP depletion [194]. In some epidemiological studies, consumption of resveratrol has also been also associated with a reduced risk of heart disease and improved cardiovascular health. Four weeks of treatment with lyophilized grape powder containing resveratrol reduced risk factors for coronary heart diseases in pre- and post-menopausal women [195]. The numerous documented properties of resveratrol make it potentially useful in cancer therapy, neuroprotection, obesity prevention, therapy of immune disorders, as well as a simple dietary supplement that may improve overall body condition and preventing ageing-associated health problems which depend on mitochondrial functions [196-199]. However, some scientists, e.g. Galati and coworkers [200] have pointed out that flavonoids and polyphenols may also display pro-oxidant activities because they can undergo oxidation by various peroxidases with superoxide as a product. Curcumin and resveratrol, famous for their antioxidant activities, have been found to act as pro-oxidative agents for NADH, GSH and ascorbate. In any case, when recommending or prescribing such dietary supplements, clinicians should exercise special care, in particular when dealing with pediatric patients [201].

b) Herbals

Traditional Chinese medicine has also brought successful strategies to potentially resolve some major health problems. One of them is the metabolic syndrome associated with impaired glucose and lipid tolerance as well as hypertension, which may lead to a serious threat of cardiovascular disorders and increased mortality. Chinese herbs like ginseng, berberine or yang-tonic herbs activate AMPK, the kinase responsible for stimulation of mitochondrial energy metabolism or accelerate energy utilization through enhanced thermogenesis. All of them contribute to mitochondrial redox stabilization, prevent disease progression, improve health and lifespan, and effectively sustain mitochondrial ATP production [202, 203]. 4-hydroxybenzyl alcohol (4HBA), a compound found in a Chinese herb, *Gastroida elata,* has been used for centuries in concussive disorders. It has recently been described as an antioxidant which effectively scavenges free radicals and restores a decreased level of SOD2 [204]. *Gingko biloba* extract (EGb) may have a protective effect upon chemotherapy. While doxorubicin induces apoptosis in cancer cells, normal cells are protected by EGb [205]. In another study, protection of rat liver and heart mitochondria from swelling and oxidative stress was achieved by propolis extract supplementation [206].

Various *in vivo* and *in vitro* models have been used to study the anti-ageing effects as well as improvement of mental and physiological parameters thanks to the intake of polyphenolic and catechin compounds [207-209]. Studies on humans have shown a beneficial effect of black and oolong teas. Regular tea drinking may delay the cognitive impairment in elderly people [210]. The beneficial properties of black tea are connected with the activation of FOXO1a transcription factor responsible for transcription of genes encoding antioxidant defense enzymes [211]. Green tea extract has also been tested for its impact on lifespan in fast ageing mouse models (SAMP10). Moreover, the antioxidant properties of catechins from green tea prevented memory regression due to neuron protection [212] owing to an improved glutathione antioxidant system [213]. In aged rat

hearts, green tea extract has been demonstrated to lower lipid peroxidation, improve redox homeostasis and elevate levels of non-enzymatic free radical scavengers [214].

Advanced age is believed to be one of the causes of an increased susceptibility to drugs as a consequence of alterations in the antioxidant defense system [215, 216]. In a large-scale experiment Mahesh *et al*. examined the influence of *Terminalia chebula*, an Indian-native plant used in traditional medicine, on a series of antioxidant defense mechanisms and enzyme activities in aged (22-24 months) and young (3-4 months) rats [217]. Supplementation with *T. chebula* reduced the levels of lipid peroxidation indicators such as malonaldehyde (MDA) and of protein carbonylation in the liver and kidney of aged rats. In the young rats this effect could only be seen in the liver. The same was true for xanthine oxidase level, where *T. chebula* treatment increased the manganese superoxide dismutase (Mn-SOD, SOD2) level only in aged rodents. The levels of hydrogen peroxide-scavenging enzymes, catalase (CAT) and glutathione peroxidase (GPx), were lower in aged than in the young rats because *T. chebula* controls the level of lipid peroxides as well as other free radicals. Additionally, *T. chebula* increased the GSH level in aged and young rodents in both liver and kidney. Hence, the analyzed substance is believed to assist aged individuals in reducing oxidative stress by means of lowering lipid peroxidation via free radical scavenging as well as increasing the activity of antioxidants. These effects are not necessarily observed in young animals [217].

In a study aimed at the young and ageing brain, Srividhya *et al*. highlighted the potential of epigallocatechin gallate (EGCG) as an agent capable of counteracting the effects of mitochondrial oxidative damage. EGCG supplementation led to a decrease in hydroxynonenal (HNE), a lipid peroxidation product, in the aged brain. Furthermore, EGCG stimulated the antioxidant system and increased the activity of several Krebs cycle enzymes. A significant increase was also observed in the activities of respiratory chain complexes. These effects were not observed in young rodents [218].

All the presented reports demonstrating the beneficial properties of certain compounds in animal models and the fact that humanity has eagerly used their properties for ages, indicate that their intake can be harmless. On the other hand there is no clear evidence that these compounds do not co-interact with some medical agents taken simultaneously, which could make them a potential hazard.

c) Vitamins

Many scientific reports conclude that a decrease in the level of free radicals improves the whole body's condition, such that people believe that administration of antioxidants will help them live longer and in good health. This point of view has become especially popular following the announcement of Harman's free radical theory of ageing [116, 219] and for many years the concept of vitamins as the best antioxidants has prevailed. Vitamin E (VE) (α - tocopherol) is responsible for keeping balance between antioxidant and prooxidant reactions [220]. Tocopherols are responsible for detoxification of peroxyl radicals which attack membrane phospholipids [221]. It is believed that owing to high consumption of products rich in VE such as vegetable oils and nuts Mediterranean populations generally have low rates of colon cancer [222]. International cancer prevention studies have shown that VE in combination with carotenoids and selenium compounds decreases the incidence of some types of cancers [222].

Our improved understanding of the multifarious implications of mitochondrial malfunctioning on redox and energy balance has led to the development of a novel type of drugs called Mitocans. Mitocans are believed to induce the mitochondrial pathway of cell death, but what is particularly interesting, mitochondrial disruption is initiated only in tumor cells. Among the seven classes of mitocans reported to date we find an analog of vitamin E - α-tocopheryl succinate (α-TOS) - which induces apoptotic cell death in different cancer cell lines by ROS accumulation [223], but in normal cells acts as an antioxidant [224]. Several recent reports have highlighted the role of mitocans (like e.g. tamoxifen which acts on the mitochondrial electron transport chain) as novel anti-cancer drugs [225, 226]. Many of these compounds are under phase I clinical trials, giving hope for a safer anticancer therapy in the future [227].

Vitamin C (VC, ascorbic acid), in contrast to tocopherols, is a water-soluble, mostly extracellular potent antioxidant. It plays an important role in DNA protection and superoxide scavenging [221]. Vitamin C is also important for VE regeneration, so its everyday administration is an important factor maintaining antioxidant defense [228].

The role of vitamins from the B group essential for mitochondrial metabolism has been reviewed thoroughly in the work of Depeint and colleagues [229]. Thiamin in its active form -TPP is a cofactor of many crucial enzymes, e.g., mitochondrial alpha-ketoglutarate dehydrogenase and branched-chain α-keto acid dehydrogenase (deficiency of this enzyme is associated with serious pathologies, such as beriberi disease, colon and breast cancer, diabetes) [229]. Riboflavin and niacin are precursors of, respectively, flavin nucleotides and nicotinamide adenine nucleotides. Biotin is included in lipid metabolism and disturbances in its content are observed in diabetes. In other words, all these compounds are important in energy and redox homeostasis. Regular dietary intake of these substances is important as their deficiency may upset energetic homeostasis and result in serious diseases [229]. Regardless the data indicating the positive effects of vitamins in physiological and various pathological states, some reports claim that their intake may not be associated with any overall benefit or risk [230].

d) Hormones

The mitochondrial theory of ageing offers one explanation why females tend to live longer than males. Females are protected against oxidative stress damage by specific hormones, and this more efficient protection results in an extended lifespan [231]. Basing on this, one can envisage lifespan extension by genetic modulation of antioxidant defense response. In fact, recent experiments on natural soy phytoestrogens, especially genistein, show promising results in postmenopausal women [183]. These natural compounds actively stimulate mitochondrial antioxidant defense by upregulating SOD2 and mtGPx [232]. However, a note of

caution is warranted, as the latest reports suggest that phytoestroegen treatment may also increase the risk of hormone-induced cancer [233].

Numerous studies identify melatonin, a hormone secreted by the pineal gland, as a potent mitochondria-targeted antioxidant with many positive effects, e.g., on the immune system [234, 235]. In fact, its activity has been correlated with antioxidant defense in several oxidative stress-induced pathologies [234]. Melatonin is believed to be an important factor in cardiac ischemia/reperfusion injuries and contributes in the adaptation to disrupted redox balance [236]. Moreover, it has been demonstrated that melatonin protects against ROS-induced mt$\Delta\psi$ collapse and PTP opening in rat brain astrocytes [237]. Studies on senescence-accelerated mouse (SAMP8) have also revealed melatonin anti-ageing properties. All tested oxidative stress markers in brain mitochondria show that melatonin administration protects against age-related decreases in ATP production and lipid peroxidation and increases the levels of antioxidant enzymes. Interestingly, females respond better to this kind of antioxidant therapy than males [238]. It has also been indicated that melatonin is an efficient antioxidant in the therapy of various mitochondrial disorders and can limit ROS production causing mtDNA mutations [239]. However, melatonin is not the only example of a hormone possessing antioxidant properties. Dehydroepiandrosterone (DHEA) and its sulfated conjugate (DHEA-S) also demonstrate similar properties. DHEA is a commercially available diet supplement which is believed to have a rejuvenating impact on humans. It is the so called 'youth hormone' with a well documented positive impact on mitochondrial energy metabolism. Studies by Patel and colleagues [240] on dehydroepiandrosterone-stimulated mitochondrial respiration in rat liver and brain have indicated that DHEA can help balance the cellular metabolism in the liver of old and young rats. The activity of dehydrogenases was up-regulated only in old animals supporting the idea of DHEA anti-ageing effect and its role in progression of some age-related and neurodegenerative diseases [241-243]. Positive trials involving this compound, already widely available in pharmacies, enabled it's recognition as an effective vitality-improving agent and even won it the name "wonder drug". However clinical studies on 144 elderly representatives of both sexes undermined it's beneficial effects [244].

e) Lipoic Acid and Acetyl-L-Carnitine

Lipoic acid (LA), an organosulfur compound is an essential cofactor for many enzyme complexes. Dietary supplementation with lipoic acid has a rejuvenatory impact on hepatic mitochondria of old rats by restoring their membrane potential and lowering the rate of respiration, but has no effect on young animals [245]. Positive effects were also observed in the heart and muscle of old rats, where the mitochondrial potential and cardiolipin content were restored to the levels found in young animals and respiration improved [246]. The protective and anti-ageing properties of lipoic acid are connected with p38 MAP kinase activation and increased activity of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) which is a transcriptional coactivator that regulates genes involved in energy metabolism. This in turn decreases oxidative stress in mitochondria by AMPK activation, which may have impact on glucose metabolism [247].

A similar age-dependent drug efficacy was observed by Long and coworkers for acetyl-L-carnitine (ALC) as well [248]. Ample data document positive effects of LA and ALC on a variety of age-related mitochondrial dysfunctions [249], including their repair after age-induced ultrastructural decay [250] as well as cognitive disorders in rats [249, 251]. Among other reported benefits of LA and ALC treatment are reduced symptoms of neurodegeneration [252], modest efficacy towards neuropatic deficits in diabetes [253] and even some effectiveness in hypertension therapy [254]. Treatment of old rats with combination of LA and ALC leads to a partial recovery of activity of complexes I and IV and a decrease of MDA and protein carbonyl levels. Interestingly, this effect concerns only the activity of complexes I and IV, because mitochondrial protein expression is unaffected, except for complex V whose level in old rats increased. In conclusion, LA/ALC treatment allows full or partial recovery of mitochondrial functions to a level observed in young rats [248].

f) CoQ and its Derivatives

As was already mentioned, diverse chemical compounds either synthetic or naturally found in plants and fungi affect mitochondrial structure and/or metabolism. However, not many of them penetrate mitochondria as well as the mitochondria-targeted ubiquinones do. In this group of compounds, apart from research tools one also finds potential therapeutic agents with strong antioxidant properties. Their structure is based on the endogenous ubiquinone coenzyme Q10 (CoQ) which is a redox-active molecule. Its hydrophobic nature keeps it associated with phospholipids in the inner mitochondrial membrane where it is involved in the electron transport through the respiratory chain [255]. Diverse CoQ analogs interact differently with respiratory chain complexes and have an ability to scavenge ROS directly in the site of their production [256] preventing lipid peroxidation and ROS-induced oxidative damage [257]. The discovery of MitoQ, a ubiquinone derivative with good antioxidant properties and positive effects on mitochondria, has resolved the problem of ubiquinone delivery to mitochondria [256, 258]. Recent experimental data document the therapeutic applicability of MitoQ in endotoxin-induced cardiac damage [259]. It has also been reported that MitoQ can be very effective in protecting vascular tissue and cardiac muscle against hypertension-induced hyperthrophy in rats, which opens up a perspective for its application also in cardiovascular therapy in humans [260]. MitoQ, apart from its antioxidant activity may play an important role in maintaining calcium homeostasis [261]. MitoQ shows protective antioxidant properties not only in humans and rodents but also in *Drosophila melanogaster,* where it increases the lifespan even in the absence of superoxide dismutase [262]. Other studies show that in astrocytes MitoQ contributes to neuroprotection by attenuating mitochondrial ROS formation [263] and can prevent ROS-dependent amyloid formation in patients with Alzheimer's disease [264].

Mitochondria-targeted cations conjugated with antioxidant compounds have attracted keen interest of the renowned

bioenergeticist Vladimir P. Skulachev, who has recently founded a consortium investigating a novel antioxidant molecule called SkQ [265]. SkQ and its derivatives, similarly to MitoQ, can penetrate the mitochondrial membrane with high efficiency and act directly in the site of ROS production. Similarly to MitoQ it also demonstrates concentration-dependent, potent anti- and prooxidant properties [266]. While high doses of SkQ can induce apoptosis and necrosis, low concentrations (0.2 nM) block both these processes in human fibroblasts treated with H_2O_2. In his recent work, Skulachev concluded that SkQ is more efficient in $OH^{\bullet}$ radical scavenging than MitoQ and it penetrates the mitochondrial membrane two times faster. All these properties were also observed in animal studies. SkQ administration seems to be effective in fighting age-associated dysfunctions of the heart, brain and kidney. The most amazing results concern retina healing by SkQ supplementation. This compound was able to return vision to blind OXYS rats suffering from retinopathies and cataract associated with increased ROS level and ageing. Additional data about inhibition of tumorigenesis and the ability to prolong the life span while keeping youthful performance strengthen the initiative to use SkQ and similar compounds in medical practice [267].

g) PKCβ as a New Pharmacological Target in the Control of Mitochondrial-Dependent Ageing

PKCs are serine-threonine protein kinases that participate in the transduction of extracellular signals into the cell. They form a heterogenous group differing in activation mechanisms (the classical PKC is activated by Ca^{2+} and diacylglycerol, the novel by diacylglycerol alone, and the atypical are insensitive to either), substrate specificity and cellular distribution. This allows them to have very different functions, and indeed as far as apoptosis is concerned different PKC isoforms have been demonstrated to play opposite roles [268]. H_2O_2 and other oxidizing agents cause the translocation of different PKC isoforms to membranes [269], an early and necessary event in PKC activation. Mitochondria and their Ca^{2+} signaling events are modulated by PKC activity in a very diversified manner. Some isoforms such as PKCε do not affect Ca^{2+} homeostasis at all, while at the other extreme PKCα induces a global alteration by reducing Ca^{2+} release from the endoplasmic reticulum (ER), and thus affecting Ca^{2+} signals detected both in the cytosol and in mitochondria. Some isoforms display a "pure" mitochondrial effect: they do not alter Ca^{2+} release from the ER (and thus the Ca^{2+} rise in the cytosol), but increase (PKCζ) or decrease (PKCβ) the mitochondrial Ca^{2+} rise [270]. Thus, they probably act on a mitochondrial effector that either stimulates the Ca^{2+} uptake machinery and/or affects the thermodynamic driving force for cation accumulation in the matrix.

Recent evidence indicates that p66Shc protein is the pro-apoptotic (i.e. "ageing") effector system. As was mentioned before phosphorylation of p66Shc on Ser36 is required for its pro-apoptotic function [131]. This phosphorylation can be mediated by several protein kinases and different pieces of evidence suggest that a protein kinase C (PKC)-mediated signalling route plays an important role in linking ROS production to the ageing effects of p66Shc [138]. In particular, we have recently shown that silencing of PKCβ protects cells against H_2O_2 challenge. Furthermore, over-expression of PKCβ reproduces a Ca^{2+} signaling defect only in cells expressing p66Shc. Thus, there is a strong dependence between PKCβ and p66Shc activity at the mitochondrial level, which can be explained by alterations in mitochondrial Ca^{2+} homeostasis. Activation of PKCβ by oxidative stress leads not only to phosphorylation of p66Shc but also promotes binding of p66Shc to Pin1, a peptidyl-prolyl isomerase that induces cis-trans isomerization of phosphorylated Ser-Pro bonds [271]. Moreover, it has been shown that the phosphorylated sites once isomerized by Pin1, become targets for dephosphorylation by PP2A [271]. At this point, the p66Shc translocated into the appropriate cell domain (mitochondria) can exercise its oxidoreductase activity, thereby generating H_2O_2 and inducing the opening of PTP and in turn apoptosis (and ageing).

In the light of these considerations, we investigated the ability of hispidin, a specific PKCβ inhibitor, to interfere with the p66Shc-dependent regulation of the mitochondrial pathway controlling lifespan. Hispidin (6-(3,4-dihydroxy-styryl)-4-hydroxy-2-pyrone) has been isolated from *Phellinus pomaceus* and studied as a potential anticancer agent due to being a selective PKC-βs inhibitor [272].

Our groups analyzed the effect of hispidin on mitochondrial morphology (Fig. **2**) and Ca^{2+} dynamics [270]. The results obtained when analyzing mitochondrial Ca^{2+} homeostasis matched those obtained by overexpressing PKCβ isoform. The inhibition of PKCβ caused a significant increase of the mitochondrial calcium ($[Ca^{2+}]m$). Moreover, all the p66Shc-dependent mitochondrial consequences of hydrogen peroxide (in terms of mitochondrial morphology and Ca^{2+} homeostasis) were also blocked by hispidin [138].

Overall, these results identify and clarify a novel signaling mechanism, operative in the pathophysiological condition of oxidative stress, and may open new possibilities for pharmacologically limiting organ deterioration during ageing. This prospective is particularly intriguing considering that a novel, highly selective inhibitor of PKCβ, ruboxistaurin (LY333531) mesylate, is currently undergoing phase III clinical studies in patients with type 1 or 2 diabetes to investigate prevention and/or reduction in clinical symptoms of diabetic microvascular complications.

h) Pharmacological Therapy of Glucose Metabolism Dysfunction

The long list of mitochondria-associated diseases starts with diabetes mellitus (DM), a global problem. The battle against diabetes and its complications involves a confrontation with high levels of ROS produced in cells exposed to high levels of glucose [273]. The resulting high $NADH+H^+/NAD^+$ ratio promotes electron leakage from complexes I and III of the respiratory chain. The high, non-physiological level of ROS can contribute to serious complications of DM like atherosclerosis, cardiac failure and endothelial dysfunction [274]. All these events are accompanied by up-regulated senescence markers, like p66Shc and Ser36-phosphorylated p66Shc [275, 276]. Experiments carried out both by Menini *et al*. and Rota *et al*. showed that ablation of this redox-sensing protein improves bioenergetic parameters and antioxidant defences in a cellular model of insulin-dependent

diabetes mellitus [277, 278]. Additional evidence for an involvement of elevated ROS in diabetes is that pancreatic beta cells cultured for a long time in high-glucose medium undergo oxidative stress-induced apoptosis [279]. Many anti-diabetic drugs are co-aimed at overcoming mtDNA mutations by means of stopping the enhanced ROS formation [274]. One of them, metformin, acts on complex I and stimulates glycolysis [280]. Recently two other positive effects of metformin have been discovered: the ability to inhibit PTP opening and activation of the p53 pathway promoting lower rate of tumorigenesis in diabetic patients [281]. A possible way to avoid islets' damage and diabetes progression may be the application of mitochondria-targeted antioxidants. For example, an anti-inflammatory and cyto-protective agent, gallic acid, promotes insulin secretion and protects cells against glucolipotoxicity [282]. Moreover, the effects of metformin, may be enhanced by baicalin, a flavonoid well known for its ROS scavenging abilities. It seems that such pharmacological intervention protects mitochondrial membrane phospholipids especially from the destructive action of superoxide [283]. Mitochondria can also be a target of other commonly used anti-diabetic agents like sulphonylureas (SUR). Receptors for SUR, present in the plasma membrane, can bind drugs like glibenclamide or glipizide, which results in insulin release from beta cells. Similar channels are found in the mitochondrial membrane and sulphonylureas have been shown to bind them as well, thus rendering mitochondria their pharmacological target. Additionally, sulphonylureas affect mitochondrial fatty acid metabolism due to their ability to inhibit carnitine palmitoyl-transferases and pyruvate carboxylase [284, 285]. In a ten times higher concentration, sulphonylurea and its derivatives can slow the tumorigenesis by induction of mitochondrial damage, OXPHOS uncoupling and ATP collapse in tumor cells [286]. However, it is suspected that accumulation of these compounds in the liver and kidneys affects mitochondrial energetics, thus inducing undesirable side-effects. On the another hand, the ability of sulfonylureas to uncouple mitochondria may have a positive effect on the inhibition of ROS production [287]. It has been reported that mild uncoupling may lower the rate of mitochondrial ROS production and thus may help in the battle against diabetes. This is the reason why so many scientists investigate UCPs in order to find a way to regulate their level and functioning in diabetes. Large-scale studies are being conducted in order to find safe agents able to imitate UCPs action [288].

More recent studies showed that such beneficial uncoupling may also be achieved by curcumin administration [289] as well as dinitrophenol (DNP). The beneficial or harmful effect of DNP is dose dependent [290]. Its uncoupling properties have been employed in obese treatment by increasing oxygen consumption and stimulating mitochondrial metabolism leading to weight loss after dietary intake [290, 291]. Such activity leads to lowering of ROS production in *Drosophila* [292] and in mammals [293]. However, an overdose of DNP results in serious adverse effects like cataract, gastrointestinal, and cardiorespiratory problems [290]. Despite the fact that many serious medical cases associated with DNP intake in high doses have determined this compound as hazardous, people concerned about their body shape, particularly body-builders, include DNP as a dietary supplement during special diets. It must be said that such action is irresponsible and may lead to life-threatening repercussions. Despite toxic effects of high doses of DNP its reasonable administration tested on animal models, seems to be neuroprotective in the case of increased mitochondrial ROS production or intracellular Ca^{2+} induced excitoxicity [294].

i) Pharmacological Therapy of Respiratory Chain Dysfunctions

Currently available therapies for mitochondrial disorders are based on prevention of the worsening symptoms by proper nutrition of the patients [295]. Pharmacological treatment includes mitochondria-targeted ubiquinon and ubiquinol, vitamins (riboflavin, vit C, vit E, vit B such as folic acid), lipoic acid, creatine, L-arginine and L-carnitine [296]. In fact the best strategy to improve health of patients with mitochondrial disorders relies on so called 'mitochondrial cocktails' which contain a combination of various antioxidants. A mitochondrial cocktail is created to decrease the production of free radicals either by quenching ROS (vitamin C, tocopherols, lipoic acid) or bypassing and using alternatives to the affected pathways (creatine, CoQ10, riboflavin). This approach minimizes the pathological consequences of mitochondrial cytopathies [297].

Though the bioenergetic parameters seem to be improved in laboratory studies, they are not necessarily directly proportional to the overall condition of patients. As was reported in case of a one-year-long therapy of 12 patients, CoQ10 was able to improve ATP synthesis but not the overall health condition. Thus it revealed a need to perform more detailed studies on dosage and therapy time as well as the combination of treatment components for each mitochondrial dysfunction. Additionaly therapy may be successful only when the patient's condition is stable. That is why one of the standard recommendations is a precise control of patient's parameters as any infectious disease or biochemical disturbance may change the pharmacokinetics of applied compounds.

Dichloroacetate (DCA), an analog of pyruvate, has been exploited for over 50 years in treatment of mitochondrial disorders. Its basic application concerns pyruvate dehydrogenase deficiency, however, nowadays it is part of a combined metabolic therapy. Though DCA therapy has been reported to be safe, some toxic, age-related neurotoxicities have been observed in rats and humans. DCA has recently become less popular despite ample data about its effectiveness [298].

CoQ10 due to its antioxidant properties, decreases the rate of free radicals-induced DNA damage. CoQ10 administration improves physical performance of healthy subjects and patients with MELAS and MERRF syndromes. Moreover, some pathologies like Leigh syndrome are connected with CoQ10 deficiency [299], so the administration of ubiquinones is reasonable and effective. In the case of Friedreich's ataxia (a serious mitochondrial disorder associated with high production of the very reactive hydroxyl radical), therapy with the CoQ10 analog idebenone results in reduction of cardiac muscles hypertrophy [300]. Moreover, idebenone administration improves mitochondrial metabolism in the brain and muscles of patients with Leber hereditary optic neuropathy [301, 302]. Combination of

CoQ10 with lipoic acid and creatine appears to be effective in an experimental trial involving 16 patients with diverse mitochondrial cytopathies. Such treatment improves energetic metabolism and decreases the level of plasma lactate and urinary 8-isoprostanes [303]. Dietary supplementation with riboflavin has been reported to improve muscle condition in some cases of complex I deficiency. Though there are not many reports on thiamin therapeutic trials, it has been applied in PDH deficiency syndromes. Vitamin K as a phylloquinone or menadione derivative acts as an electron carrier and improves clinical and biochemical parameters of patients with complex III deficiency [304].

The normal requirement for creatine, an essential compound in energy production, is fulfilled by dietary intake. Creatine monohydrate administration decreases free radical production and formation of paracrystalline inclusions in patients with mitochondrial DNA mutations [296, 305, 306]. Creatine supplementation can also be beneficial in other disorders with a mitochondrial background, like muscular dystrophy (Duchenne and Becker's dystrophy), McArdle's disease [307] and in Parkinson's disease. Combined creatine and CoQ10 therapy results in decreased lipid peroxidation and DNA damage and improved glutathione homeostasis [308]. A relevant energy state is necessary to maintain proper functioning of synapses, in which mitochondria are the main source of ATP. Appropriate mitochondrial bioenergetic characteristics are a buffer for calcium homeostasis, which is essential in many fundamental cellular processes. The evident improvement of mitochondrial bioenergetics offers hope for future application of these compounds in neurodegenerative disorders, like Alzheimer's and Parkinson's disease as well as amyotrophic lateral sclerosis (ALS) and Huntington's disease [309].

L-carnitine, another ingredient of the 'mitochondrial cocktail', is not associated with mitochondrial bioenergetics as such, but rather with proper lipid metabolism [296]. L-carnitine protects the brain from consequences of methamphetamine (MPTP) intoxication [310]. Administration of acetyl-L-carnitine in combination with α-lipoic acid had a positive effect in cases of mood disorders [311].

Cytochrome c oxidase (COX) deficiency is one of the most frequent biochemical diagnoses in lethal neonathal and infantile respiratory chain deficiency disorders. It is thought to occur in approximately one in 7000 - 10000 newborns [312, 313]. COX deficiency can be associated with mutations in the *SCO2* gene encoding a mitochondrial copper-binding protein. *SCO2* mutations cause abnormal ^{64}Cu uptake in fibroblasts and increased basal copper concentrations in myoblasts. Interestingly, COX activity was rescued after copper-histidine supplementation to *sco2* mutant myoblasts [312]. Several reports have also indicated good efficacy of this compound in early treatment of Menkes patients [314-316]. Although the exact mechanism of rescue of the biochemical defect remains unclear, the results of the copper supplementation experiments suggest a possible therapy [312].

In a recently published paper on bipolar disorder Maurer and coworkers examined the stimulating effect of lithium (Li^+) on the respiratory chain [317]. Studies of brain energy metabolism in bipolar disorder suggest an impairment of energy generation by mitochondrial oxidative phosphorylation. Although Li^+ is an effective drug widely used in this disorder its mechanism of action remains uncertain. These provided evidence that the activities of complexes I and III as well as II are dose-dependently increased by Li^+. The activity of succinate dehydrogenase remains unchanged at low Li^+ concentrations, but increases when higher doses are used. In contrast, the activity of COX is not significantly affected [317].

Beneficial properties of the trace element selenium (Se) were first demonstrated several decades ago. These properties have been correlated both with low-molecular selenium compounds as well as selenoproteins in which it is present in the form of the amino acid selenocysteine (Sec) [318]. Some properties of selenium compounds have not yet been described in full. However, among their characterized functions is an antioxidant activity due to the presence of selenocysteine residues in ROS-detoxifying selenoenzymes like GPx, thioredoxin reductases (TrxR) and possibly selenoprotein P (SeP). Basing on published data that selenium supplementation has a cytoprotective effect towards different cell types such as neurons, astrocytes and endothelial cells [319-321], it has been proposed that maintenance of an appropriate level of selenium could help in preventing neurological and cardiovascular disorders [322].

The take-home message from all clinical studies with antioxidant compounds or diet supplements indicates that potentially we can create successful therapies merged with our everyday habits. More and more specialists share the opinion that the observed improvement of health conditions and prolongation of lifespan is their effect. As long as long term studies on potential serious side effects do not exclude these compounds we can enrich our diet in available antioxidants.

j) Carvedilol

A recent study highlights carvedilol (CAR) as a possible agent for prevention of age-induced behavioral, biochemical, and mitochondrial dysfunctions [323]. CAR is a nonselective β-adrenoreceptor blocker with multiple pleiotropic antioxidant-like actions useful for the treatment of several ageing-related diseases such as neurodegenerative diseases and dementia [324], diabetes mellitus [325] and cancer [326]. Among other functions at cellular level CAR has also been reported to have anti-inflammatory activity [327], block calcium channels, non-competitively inhibit NMDA recaptors [328], and act as a mitochondrial protective agent of mitochondrial structural integrity and [329]. Earlier studies show that CAR acts as a neuroprotective compound in some models of transient focal [330] and tardive diskinesia [331]. It has also been demonstrated to have nephroprotective activity [332] and a cardioprotective effect in different types and models of cardiovascular ischemia and reperfusion [333, 334]. Administration of CAR in older patients who have high levels of oxidative stress in the myocardium due to heart failure also resulted in a decrease of malondialdehyde (MDA) levels, without changes in anti-oxidant enzyme activities [335] together with amelioration of cardiac function [336].

Kumar with coworkers [323] found good neuroprotective potential of CAR against D-galactose-induced oxidative

damage, mitochondrial dysfunction and cognitive impairment in mice. Antioxidant effect of carvedilol on cardiac mitochondria was extensively studied by Oliveira and coworkers. They described many examples for the mitoprotective effect of carvedilol upon oxidative stress [337-340]. The mechanism, although still to be solved is likely to involve antioxidant and mitochondrial pathways [341-344].

k) Helium Preconditioning

Preconditioning has been long believed to be an effective method of preventing tissue damage during ischemia-reperfusion interventions due to incidents such as acute myocardial infarction, heart failure, sudden cardiac death and arrhythmias in humans and animals [345-347]. In an experiment conducted by Heinen *et al*. it has been established that anti-ischemic cardioprotection can be obtained with helium-induced preconditioning. However, this treatment proved to be effective only in young individuals [348]. The authors concluded that helium induces cardioprotection by activating mitochondrial Ca^{2+}-sensitive potassium channels. At the same time mild uncoupling of mitochondria occurs. This is believed to be a typical characteristic of "preconditioned" state mitochondria [349-351]. It is also proposed that the helium-induced preconditioning is initiated at the level of several pro-survival signaling kinases. The results obtained by Heinen *et al*., in accordance with those of other authors, indicate that the cardioprotective properties of helium are lost in the aged rat and do not reduce the infarct size [348, 352, 353]. The reason behind the loss in the treatment aptitude in senescent rats remains unknown. The authors propose that it is either due to defects of the mitochondrial K_{Ca} channels or somewhere upstream in its signaling cascade [348].

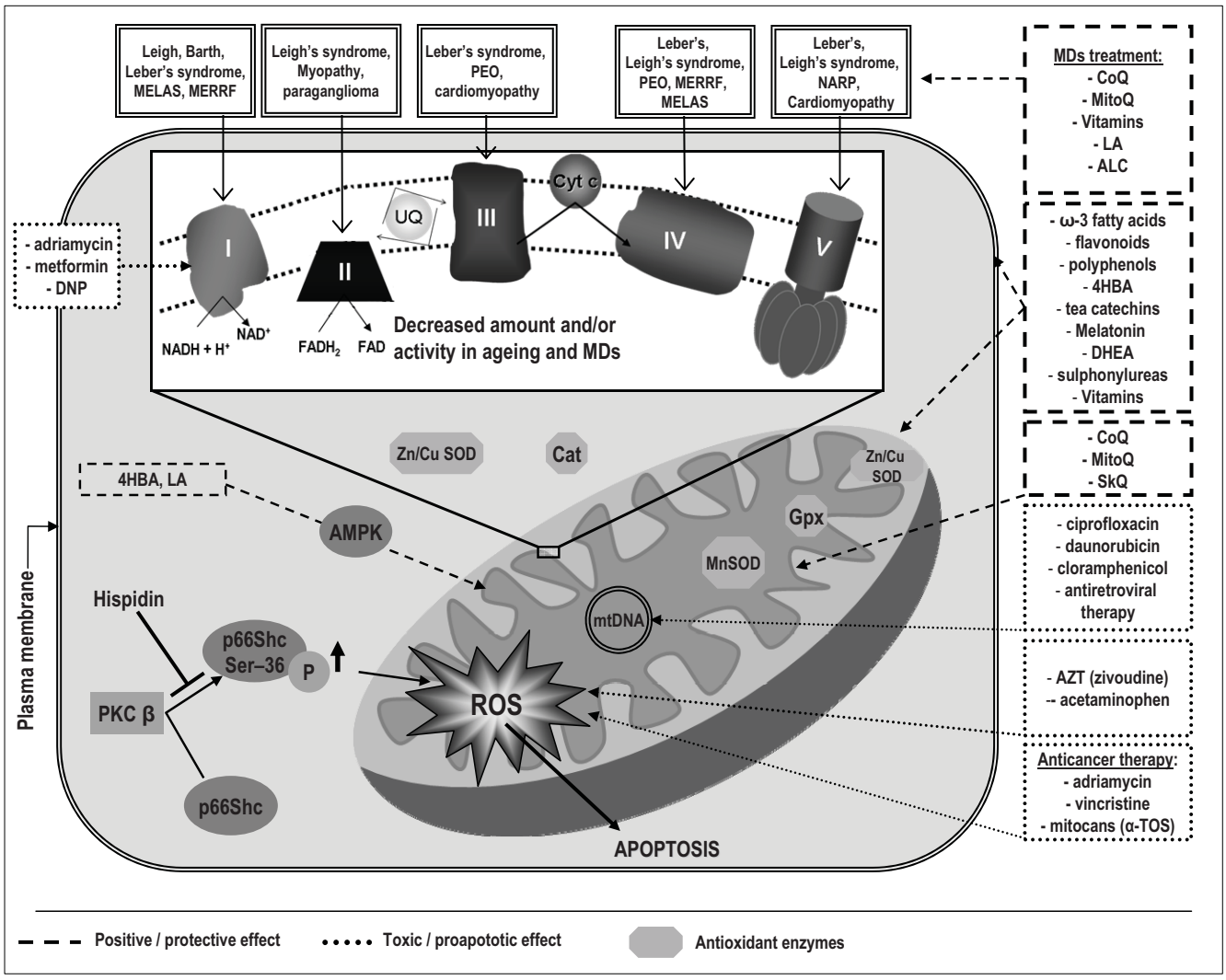

Fig. (3). Positive and negative effects of compounds commonly used in treatment of mitochondrial disorders, anticancer therapy and anti-ageing dietary supplements employed in everyday life. Zn/Cu SOD, SOD1, superoxide dismutase 1; DNP, dinitrophenol; Cat, catalase; MDs, mitochondrial disorders; LA, lipoic acid; 4HBA, 4-hydroxybenzyl alcohol; AMPK, AMP-activated protein kinase; MnSOD, SOD2, superoxide dismutase 2; GPx, glutathione peroxidase; PKC β, protein kinase C β; CoQ10, coenzyme Q10; DHEA, dehydroepiandrosteron; ALC, acetyl-L carnitine; (PEO), Progressive External Ophtalmoplegia; (MELAS), Mitochondrial Encephalomyopathy, Lactic Acidosis, and Strokelike episodes; (MERRF), Myoclonic Epilepsy with Ragged Red Fibers; (NARP), Neuropathy, Ataxia and Retinitis Pigmentosa.

CONCLUSIONS AND PERSPECTIVES

In conclusion, we have demonstrated how mitochondria and various mitochondria-associated pathways respond to a variety of pharmaceutical compounds (Fig. **3**). These factors include registered drugs and other chemicals, and account for diverse consequences which vary depending on the physiological condition. Research provides clear evidence that certain compounds present in drugs or nutraceuticals may cause positive and negative alterations in the mitochondrial metabolism which as a matter of fact sometimes depend on the age of treated subjects. Such knowledge is particularly important because, as was stated in this review, some agents demonstrating beneficial effects in certain age groups may be ineffective in others or even account for additional toxicity. There is no unambiguous tendency that the effectiveness or toxicity prevails in a certain age group, in general it is without doubt that age-dependent metabolism is an essential factor. Although many of the presented agents have only been tested on animals and cell lines a clear conclusion that can be drawn from the research is that a great potential lays in available compounds as long as they are used with an understanding of certain mechanisms underlying age-dependent aptitude. It is therefore particularly important always to take into consideration individual predispositions such as diagnosed disorders or simply the stage of one's life when engaging rejuvenating treatments or prescribing medications or diet supplements. Such precautions should particularly be undertaken when working with young patients. Profound understanding of treatment mechanisms combined with deep comprehension of medical conditions should enable the development of new therapies bringing hope of overcoming diseases incurable at the moment.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Higher Education, Poland, grants N301 092 32/3407, N407 075 137 and by the Polish Mitochondrial Network for MRW, JD, ML and JS. JS was also supported by PhD fellowship from The Foundation for Polish Science (FNP), UE, European Regional Development Fund and Operational Programme 'Innovative economy', PP is supported by the Italian Association for Cancer Research (AIRC), Telethon (GGP09128), local funds from the University of Ferrara, the Italian Ministry of Education, University and Research (COFIN), the Italian Cystic Fibrosis Research Foundation and Italian Ministry of Health. PJO is supported by research grants from the Foundation for Science and Technology, Portugal (PTDC/SAU-OSM/64084/2006 and PTDC/SAU-OSM/ 104731/2008).

We apologize to the authors whose papers could not be cited due to space constraints.

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Received: March 25, 2010 Revised: June 29, 2010 Accepted: June 30, 2010

PMID: 21269267