Coenzyme Q₁₀ – Its role as a prooxidant in the formation of superoxide anion/hydrogen peroxide and the regulation of the metabolome

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Abstract

Coenzyme Q₁₀ plays a central role in cellular bioenergy generation and its regulation. Closed membrane systems generate a proton motive force to create transient localized bio-capacitors; the captured energy is used for the synthesis of mitochondrial ATP but also for many other processes, such as metabolite translocations, nerve conduction and a host of other bioenergy requiring processes. Coenzyme Q₁₀ plays a key role in many of these sub-cellular membrane energy generating systems. Integral to this phenomenon is the prooxidant role of coenzyme Q₁₀ in generating the major superoxide anion/hydrogen peroxide second messenger system. This messenger system, largely but not exclusively, arises from coenzyme Q₁₀ semiquinone function; it contributes to the regulation of sub-cellular redox potential levels; transcription/gene expression control; is essential for modulated protein turnover and activation; mediates hormone and growth factor extracellular signaling. The regulated prooxidant formation of the superoxide anion/H₂O₂ second messenger system is essential for the normal physiological function of the metabolome. The normally functioning metabolome is the expression of a finely tuned dynamic equilibrium comprised of thousands of anabolic and catabolic reactions and all cellular signaling systems must be finely regulated. There is still much to be learnt about the up/down regulation of the H₂O₂ messenger system. The concept that superoxide anion/H₂O₂ cause random macromolecular damage is rebutted. The administration of antioxidants to quench the inferred toxicity of these compounds as a therapy for age associated diseases is unsupported by extant mammalian clinical trials and should be subject to serious re-evaluation. The role of ascorbic acid as a beneficial hydrogen peroxide prodrug is discussed.

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1. Introduction

This paper, in the main, focuses on the formation of superoxide anion and hydrogen peroxide and its role as a second messenger. The essential prooxidant role of coenzyme Q₁₀ and other superoxide anion/H₂O₂ generating systems is discussed. We continue to elaborate the thesis that the concept that superoxide anion/H₂O₂ are toxic products which randomly damage macromolecules and must be quenched by interventionist antioxidant therapy is flawed. On the contrary, there is an essential physiological requirement for superoxide anion/H₂O₂ formation, with H₂O₂ acting as a major second messenger required for normal metabolome function.

There is nothing more critical for all anabolic and catabolic cellular functions than an adequate, constant supply of bioenergy. Coenzyme Q₁₀ was shown by Crane et al. (1957), some 50 years ago to be a key component of the mitochondrial electron transport system. Soon after coenzyme Q₁₀ or its analogues were shown to be ubiquitous essential members of the electron transport and oxidative energy generating systems of bacteria and eukaryotic cells. Mitchell’s (1974) revolutionary work concerning the mechanism of conservation of mitochondrial redox potential energy, overturning a 25 year phosphorylated intermediate...
hypothesis, owed much to his formulation of the Q cycle hypothesis (Mitchell, 1975). Essentially the Q cycle concept centred around mitochondrial proton motive force generated by vectorial separation of protons across impermeable biomembranes, with coenzyme Q10 playing a key role. It has been established that a wide range of biomembrane systems are energized via a redox process which create transient localized bio-capacitors, which are utilized by the metabolome (for review, Linnane et al., 2007b).

At about this time, Boveris, Chance and colleagues reported in a series of papers that the mitochondrial electron transport system through the agency of coenzyme Q10 semiquinone gave rise to high concentrations of superoxide anion, and in turn, hydrogen peroxide (for review Chance et al., 1979). It was calculated that 1–3% of inspired oxygen was converted to superoxide anion and in such amounts would be highly toxic to tissues. These reports appeared to support the free radical theory of aging first proposed by Harman (1956). A voluminous literature has arisen which has concentrated upon establishing the essential need for antioxidant systems to prevent random oxidative damage to cells and among other compounds, orally administered coenzyme Q10 functions as an antioxidant (Ebadi et al., 2001). A major problem with the concept of antioxidant therapy for the treatment of age associated systemic diseases is that there are no human clinical trials which support such a conclusion. By way of example, antioxidant therapy has been promoted for many years for the prevention and treatment of cancers, based on non-physiological in vitro studies (Ames et al., 1993). A wealth of data speaks to the contrary.

In considering/reviewing the encompassing biological function of coenzyme Q10 and prooxidants the subject matter can only be dealt with in a reductionist manner in this limited review. We have attempted to integrate a large body of work and publications emanating from diverse fields and as such include only two over-riding rather complex cartoon summary figures, relying on the text for clarification. Elsewhere in a series of papers the data summarized herein has been elaborated in more detail, we refer the reader particularly to Linnane et al. (2007b) for a more extensive treatment.

2. Superoxide anion and hydrogen peroxide formation

2.1. The role of coenzyme Q10 and sub-cellular signaling

Coenzyme Q10 acting through formation of its semiquinone is a major source of cellular and mitochondrial superoxide anion and consequently H2O2 formation. It also has a major role in mitochondrial energy generation actively participating in the establishment of the mitochondrial membrane’s proton motive force ($\Delta p = \Delta \psi + \Delta pH$). Coenzyme Q10 occurs in most, if not all, cellular membranes and, it is again therein an important source of superoxide anion/H2O2, for example, the Golgi apparatus and lysosomal system contributing to the proton motive force established for these closed membrane systems.

We have earlier reported that coenzyme Q10 functions in the process of gene regulation (Linnane et al., 2002a,b). This conclusion arose from administering coenzyme Q10, in a placebo controlled trial, to patients for a period of 4 weeks prior to undergoing hip replacement surgery. Subsequent to surgery, vastus lateralis muscle specimens were analyzed using histochemical analyses, microarray gene display, differential gene display and proteome analysis technologies to establish the profound effect coenzyme Q10 had on muscle fibre type composition, gene expression and the protein expression profile of human skeletal muscle. Coenzyme Q10 is anchored in cellular membranes as a member of oxidoreductase systems from which superoxide anion and H2O2 will arise. To explain the far reaching effect of coenzyme Q10 administration we concluded that H2O2 acted as a second messenger moving through the cell to deliver the coenzyme Q10 redox message from a number of sub-cellular membrane locations. Thus the fluctuating redox poise of coenzyme Q10 within its various membrane oxidoreductase systems generates a fluctuating amount of superoxide anion and in turn second messenger H2O2 which acts to signal the necessary modulation of the sub-cellular microenvironment activities required for the normal function of the metabolome. The overarching role played by coenzyme Q10 in the holistic regulation of cellular function (Figs. 1a and b) acting largely as a key source of H2O2 has been discussed in more detail elsewhere and later herein (Linnane et al., 2002b; Linnane and Eastwood, 2006; Linnane et al., 2007a).

**Fig. 1.** Coenzyme Q$_{10}$ oxido-reductase bioenergy system(s). (a) The cartoon summarizes the role of coenzyme Q$_{10}$ oxido-reductase systems in energy generation and uncoupling and hydrogen peroxide second messenger generation regulating metabolic flux and redox poise subcellular microenvironments: the fluctuating oxidation/reduction status of coenzyme Q$_{10}$ within the subcellular membranes results in fluctuating pH values and consequently the energy status of the various subcellular compartments creates a range of bioenergy microenvironments. Metabolic flux modulation: the fluctuating energy status of the various subcellular compartments will result in the fluxing of localized metabolic activity. (b) Prooxidant coenzyme Q$_{10}$ regulatory signaling system(s). The cartoon summarizes the roles of coenzyme Q$_{10}$ and H$_2$O$_2$ in the regulation of transcription and gene expression. For chloroplast details refer Pfannschmidt et al. (1999) and Linnane and Eastwood (2004, 2006). Redox poise refers to fluctuating oxidation reduction states of coenzyme Q$_{10}$ and other redox systems which constitutes a dynamic metabolome signaling system, largely modulated by H$_2$O$_2$ turnover.

**2.2. Mitochondria**

Central to a consideration of the roles of superoxide anion and hydrogen peroxide formation as related to their putative adverse effects and role as a second messenger system are the amounts formed under physiological conditions. Early reports suggested that 1–3% of inspired oxygen was converted to superoxide anion (Chance et al., 1979). These papers set the stage for future interpretations of the putative toxicity of metabolically generated oxygen radicals; indeed 1–3% of inspired oxygen converted to superoxide anion and H$_2$O$_2$ would be potentially physiologically catastrophic. A voluminous literature extending from the 1970’s to the present day, has grown describing the toxic effects of excessively high concentrations of H$_2$O$_2$ on enzyme systems and cultured cells. However hydrogen peroxide is not a toxic compound, except at non-physiological unrealistically high concentrations; the common experimental use of millimolar concentrations has lead to extrapolations and misinterpretations of the physiological role of superoxide anion and H$_2$O$_2$. It has erroneously become conventional knowledge in the field of antioxidant studies that excessively high levels of superoxide anion and H$_2$O$_2$ are produced in vivo as a by-product of energy metabolism and as such they are highly toxic to cells and that it is essential that they be quenched by antioxidants as rapidly as possible.

More recently the original, and much of the subsequent published data, emanating from many laboratories has since been shown to be interpretively misleading, in that normal (uninhibited) respiring mitochondria produce only very low, trace levels of superoxide anion and H$_2$O$_2$ (Nohl et al., 2001, 2005) confirmed and further elaborated by St-Pierre et al. (2002). St-Pierre et al. (2002) reported that the studies of Boveris, Chance and colleagues over estimated the amount of superoxide anion and H$_2$O$_2$ formed by mitochondria by about two orders of magnitude; the early estimate being about 10 nM H$_2$O$_2$/mg mitochondrial protein/min versus actual 0.1 nM H$_2$O$_2$/mg mitochondrial protein/min.

The St-Pierre paper is a notable contribution in its dissection of the topology of mitochondrial superoxide anion production; most significantly these authors reported that isolated rat skeletal muscle mitochondria respiring on complex I/III substrates release superoxide anion into the matrix while complex II/III substrates release superoxide anion into the medium. This vectorial synthesis of superoxide anion, extrapolated to cells would indicate that the resultant H$_2$O$_2$ formation enables it to act as a mitochondrial second messenger signaling to both nuclear and mitochondrial genomes. This signal would reflect the extant metabolic state of the mitochondrial organelle and its temporal requirement for appropriate nuclear and mitochondrial gene expression and metabolome modulation. Another notable feature of this paper is that heart muscle and skeletal muscle mitochondria oxidizing palmitoyl carnitine, vectorially release H$_2$O$_2$ differently. Isolated heart mitochondria oxidizing fatty acids (the heart’s main energy substrate) release superoxide anion into the mitochondrial matrix while muscle mitochondria release it into the medium. These observations perhaps reflect that different tissue metabolisms have a requirement for different metabolic regulatory signaling messages.

Studies of knock out mice and superoxide dismutases provide some further insight on the role played by the superoxide anion/H$_2$O$_2$ couple. Construction of homozygous transgenic mice, null (−/−), for Mn SOD resulted in neonates being severely affected. Such animals as were born (no data were presented on prenatal deaths) died within a few days of birth as a result of severe cardiopathy, neurological and other pathological changes (Melov et al., 1998; Wallace, 1999). However, the interesting finding with this mouse model was that apparently only nuclear encoded proteins imported into the mitochondria from the cytosol were oxidatively damaged, as exemplified particularly by the Fe–S centre enzyme aconitase and nuclear
encoded complex II proteins of the electron transport chain. There was little effect on complex I, III and IV activities which require mtDNA encoded proteins for activity and it may be concluded therefore that mtDNA was not significantly oxidatively damaged in Mn SOD (−/−) animals. In any event, our interpretation of these SOD (−/−) animal results, is to suggest that such a major disruption to the H2O2 messenger signaling system would be expected to have a catastrophic outcome. Mitochondrially generated H2O2 is required for normal cell function hence Mn SOD null transgenic mice have no real survival value. We have elsewhere discussed the metabolic consequences of transgenic mice manipulations of cytosolic Cu/Zn SOD (Linnane and Eastwood, 2004; Linnane et al., 2007b). We concluded that experimental manipulations of Cu/Zn SOD leading to excessive depletion, or over production, of cytosolic essential second messenger H2O2 is detrimental to metabolome regulation and to cell function.

2.3. Lysosomal system/Golgi apparatus/secretory granules system

The early endosome arises from the plasma membrane and a proton translocating system functions to lower the internal pH of this membranous inclusion from about pH 7.4 to 6.2. This pH lowering process continues through to the late endosome (pH 5.3) and finally the lysosome (pH 5.0). Gille and Nohl (2000) have described a lysosomal redox chain. NADH was identified as a substrate for the system with a cytochrome b559, a flavoprotein and coenzyme Q10 as components. Proton translocation into the strongly acidic lumen of the lysosomes through the agency of coenzyme Q10 was reported. The formation of superoxide anion was also described.

The early Golgi apparatus as it arises from the E.R. has an internal pH of about 6.7 progressing through to 5.4 at the secretory granule interface; it has been shown to contain a coenzyme Q10 oxido-reductase (Barr et al., 1984; Crane et al., 1994a) which will contribute to the acidification process and again superoxide anion H2O2 will be formed to function in a signaling mode.

2.4. Plasma membrane

Embodied within the plasma membrane are a number of complex signaling systems for the regulation of the cellular metabolome. There are two plasma membrane oxido-reductase systems (CNOX and Nox) which give rise to the superoxide anion/H2O2 system. The plasma membrane systems generation of H2O2 is incorporated into Fig. 2. NADH oxido-reductase complex (later denoted CNOX, constitutive NADH oxidase) was first recognized as a coenzyme Q10 flavoprotein–cytochrome b5 system localized on the outer face of the plasma membrane by Crane and colleagues some years ago. The activity of this system is quite low unless cells are exposed to extracellular growth promoting effectors, e.g., platelet derived growth factor (PDGF) and epidermal growth factor (EGF). There are few details extant on the mechanism of activation of the CNOX system by the extracellular effectors. Crane et al. (1994b) proposed that the increased induced CNOX activity resulted in the formation of H2O2 which acted as a second messenger for the regulation of cell growth. The significance of the finding of a functional prooxidant activity of plasma membrane coenzyme Q10 as reported by Crane and associates has been largely overlooked with a focus centred on the putative antioxidant role of coenzyme Q10. Perhaps the antioxidant/prooxidant role of coenzyme Q10 might be reconciled, for example, by consideration of the role of ascorbate acting as a H2O2 generating prodrug (Chen et al., 2005; Linnane et al., 2007b). In such a consideration the NADH CNOX–CoQ10 system functions to reduce extracellular ascorbate radical thus enabling ascorbate to be recycled, to continue in turn, to produce the H2O2 second messenger as part of the overall regulation of the metabolome (Crane and Low, 2001). Erythrocyte membrane studies add some weight to such a suggestion. Low et al. (1995) have reported the activation by H2O2 of erythrocyte glycolytic enzymes aldolase, phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase. These enzymes when bound to the cytoplasmic domain of intrinsic Band 3 protein of the erythrocyte membrane are inactive. Hydrogen peroxide (or ferricyanide resulting in H2O2 formation) treatment of the cells leads to activation of a tyrosine kinase which phosphorylates tyrosine residues of the intracellular domain of Band 3 resulting in the release of the three enzymes and their co-ordinate activation. The authors conclude that a plasma membrane electron transport system mediates the prooxidant formation of H2O2. Subsequently May and Qu (1999) reported that intracellular ascorbate was a key component in the generation of H2O2 by erythrocytes.

The CNOX system can be readily envisaged as concerned with cellular energy generation. NADH plasma membrane oxidation with oxygen as terminal acceptor, together with the mitochondrial bioenergy system and cytosolic glycolysis interactively contribute to cellular energy maintenance (Lawen et al., 1994). NADH is a major substrate required for mitochondrial energy generation while NAD+ is required to maintain glycolytic ATP production. Lowered mitochondrial respiratory activity due to the aging process or its elimination in respiratory deficient r0 cells is compensated by the upregulation of the plasma membrane NADH oxidizing systems to maintain NAD+ levels and glycolytic activity (Larm et al., 1994; Kopsidas et al., 2000). This system can also provide other cellular needs for NAD+, for example, as required by the sirtuin family of gene regulators. Caloric restriction (CR) increases average rodent life expectancy by as much as 50%; human epidemiological studies suggest that dietary CR can also increase average human life expectancy (Willcox et al., 2006). CR will result in the downregulation of overall metabolic pathways due to a less demanding metabolic substrate load. This proposition finds support from
the reported upregulation of the sirtuin family of NAD⁺-dependent histone deacetylases, which function to silence gene expression. Crane and Low (2005) have recently invoked the plasma membrane oxidase systems as sirtuin activity regulators by dint of being major sources of cytosolic NAD⁺. Again superoxide anion/H₂O₂ formation will play a key role in the regulation of such systems and presumably in this instance, gene silencing.

The second plasma membrane NADPH oxidase system (Nox) does not contain coenzyme Q₁₀ and may be subdivided into essentially two classes. One class is neutrophil/macrophage located, and produces high concentrations of superoxide anion/H₂O₂ (mM) functioning to kill sequestered bacteria. The second class, occurs probably in all cells and produces superoxide anion/H₂O₂ in the non-toxic nanomolar signaling range (for review, Linnane et al., 2007b). Most significantly the non-macrophage NADPH Nox activities are upregulated, analogously to CNOX, by a range of extracellular effectors (growth factors, cytokines and hormones) to produce H₂O₂ in a regulated manner presumably tailored to respond to the needs of the metabolomes characterizing the various tissues (for review, Werner, 2004). As a part of the activation process, the G protein Rac I, is recruited to become part of these NADPH oxidase complexes functioning to activate them to produce superoxide anion/H₂O₂ required for the downstream activation of Src protein phosphokinases which precipitate a regulatory protein phosphorylation cascade. The components involved and nature of the cascade, varies from one tissue to another and will include, differentially, among others Raf, MEK, MAPK, MAPKK in turn regulating transcription factors Myc, Fos and Jun. On the other hand, as part of the overall regulatory activation/deactivation processes, protein phosphatases under cysteine/cystine redox cycling function to terminate and regulate the signals; also contributing will be catalase and glutathione peroxidase to the cellular equilibrium of the H₂O₂ signaling system.

3. H₂O₂ plasma membrane signaling specificity

The fact that the same extracellular effectors, e.g., PDGF, can stimulate both the CNOX and Nox systems to produce second messenger H₂O₂ raises the major unresolved problem of localized specificity. Does PDGF exposure lead to simultaneous H₂O₂ production by both systems, or is there some steric properties inherent in the plasma membrane which confers some selective effector regulatory specificity, and/or are there specific carriers (e.g., proteins) which move the H₂O₂ through the cell to elicit specific sub-cellular responses. There remains much to be learnt about H₂O₂ regulation and its second messenger role.

3.1. Proton motive force and cellular bioenergy

Membrane associated electrochemical energy can be calculated from measured membrane potential (∆ψ) and the
pH gradient across the membrane (ΔpH), whereby the sum of the values is equal to the proton motive force (Δp) generated across the membrane. This relationship is expressed by the following equation as:

\[ \Delta p = \Delta \psi + \Delta \text{pH} \]

Cells are in the main comprised of a number of closed sub-cellular membrane systems of individual fluctuating proton motive forces. The immediately localized specific metabolic activity of the sub-cellular organelles will be under the control/ regulation, of these energy fluxes. This information is developed more fully elsewhere (refer Linnane and Eastwood, 2004, 2006; Linnane et al., 2007b).

Membrane electrochemical energy (proton motive force) will vary, say, in millisecond time and serve to modulate sub-cellular organelle microenvironments and hence sub-cellular metabolic fluxes (Fig. 1a). The bioenergy generated by the proton motive force is used metabolically in the cellular regulation of ion, macromolecular and other substrate movements across membranes and in the case of mitochondria also for ATP synthesis. However coenzyme Q₁₀ also functions as a source of superoxide anion of mitochondria also for ATP synthesis. However coenzyme Q₁₀ also functions as a source of superoxide anion. However coenzyme Q₁₀ also functions as a source of superoxide anion formation, induced by a range of cell effectors (e.g., cytokines, hormones) and regulated by Ras 1 (G protein), a transduction phosphorylation acts to phosphorylate the cell from random proteolytic action. The role of the cathepsins was supposedly to randomly hydrolyze proteins as part of the process of autophagy and endocytosis. Later the ubiquitin proteasome system was discovered and it is now recognised that cellular proteolysis is a regulated multi-system phenomenon which includes the lysosomal cathepsins.

The 26S proteasome enzyme complex is involved in the selection of proteins for degradation by the 26S unit. Ubiquitination of a protein is a three step process (E1, thioester bond formation; E2, transfer to protein sulphhydryl group) with the third step catalysed by ATP-dependent ubiquitin protein ligases (E3’s). It is necessary to appreciate that there are hundreds of E3’s present in cells, specific to one or more proteins to be ligated to ubiquitin and thus tagged for 26S proteasome destruction. Further, polyubiquitination (4-50 ubiquitin residues) is required before final acceptance for hydrolysis by the 26S proteasome. The ubiquitin-ATP-dependent system which tags proteins for degradation by the proteasome is the major effector of protein turnover. Recently is has been recognised that ubiquitination is a key signal for targeting membrane intrinsic and extrinsic proteins for endosomal sorting and delivery to the proteolytic interior of the lysosome (for review Urbe, 2005).

Consider a commonly held view, that proteins are oxidized in an uncontrolled random process by superoxide anion/H₂O₂ and NO and peroxynitrite not considered herein, refer Linnane et al., 2007b and that such damage, unequivocally commits the damaged proteins to proteasome hydrolysis. This concept is no longer tenable, it is at best a gross over-simplification; we have discussed the processes more extensively elsewhere (Linnane et al., 2007b), a summary follows.

The oxidation of protein amino acid residues since their discovery some decades ago has been almost universally reported as leading to protein inactivation and requiring mandatory proteolysis to prevent their deleterious cellular accumulation. However it is clear that oxidatively modified proteins do not simply arise as the result of random oxidative damage (hydroxylations of various amino acid residues, sulphotation of methionines, nitrations of sulphhydryl groups and so on). There are an increasing number of situations where free radical protein modifications can be shown to be part of normal cellular regulatory signaling activity.

The important nuclear transcription factor NFκB activity is regulated by superoxide anion formation. NFκB is maintained in an inactive form bound to the inhibitor IκBα. Following plasma membrane superoxide anion and H₂O₂ formation, induced by a range of cell effectors (e.g., cytokines, hormones), and regulated by Ras 1 (G protein), a transduction phosphorylation acts to phosphorylate IκBα and dissociate the complex leading to selective IκBα ubiquitination and proteasome destruction, in turn releas-
It may be envisaged that the superoxide anion/H\textsubscript{2}O\textsubscript{2} system is a universal messenger regulator of transcription, acting under the direction of all manner of cell effectors. The Jak/Stat system discovered by Darnell (for early review Darnell, 1998), is a major multifactorial transcription control system. The plasma membrane Nox oxidases produce superoxide anion/H\textsubscript{2}O\textsubscript{2} messengers which activate the Jak–Stat system (Schieffer et al., 2000; Grote et al., 2005) again in response to a very similar range of extracellular factors (hormones, cytokines, growth factors) to that which activates the CNOX system (Figs. 1b and 2).

One of the most sensitive amino acids to oxidation is methionine, being converted to methionine sulphoxide (MetO), it is commonly cited as an example of random oxidative damage to proteins. Calmodulin (CM) function and its regulation by superoxide anion/H\textsubscript{2}O\textsubscript{2} oxidation of specific methionine residues, is now well documented (Yin et al., 1999). The oxidation of only two specific methionine residues (Nos. 144 and 145) of calmodulin (there are seven) are involved in the process of downregulating, plasma membrane Ca\textsuperscript{2+}ATPase. The oxidation of other calmodulin methionines does not downregulate calmodulin–plasma membrane–Ca\textsuperscript{2+}ATPase activation (Yin et al., 2000). It has also been reported from the same laboratory (Sun et al., 1999) that methionine sulphoxide reductase, can act reductively, to restore the ability of oxidized calmodulin to regulate plasma membrane–Ca\textsuperscript{2+}ATPase. These results indicate that superoxide anion/H\textsubscript{2}O\textsubscript{2} is functioning as part of the controlled regulation of the CM–PM–Ca\textsuperscript{2+}ATPase complex. Other examples including hemoglobin regulation and free radical reactions are presented elsewhere (Linnane et al., 2007b).

One of the complexities of H\textsubscript{2}O\textsubscript{2} function is that it is theoretically, freely diffusible throughout the cell; but functionally that appears to be unlikely as the possibility would exist for the metabolome to simultaneously receive a multiplicity of messages which may be contradictory, self cancelling or over stimulatory. The H\textsubscript{2}O\textsubscript{2} presumably must be locale specific or perhaps transported by some unknown regulatory system and also regulatory integrated with, at least, catalase and the glutathione system. The claimed toxic (pathophysiological) effects of superoxide anion/H\textsubscript{2}O\textsubscript{2}, may be the out workings of metabolic imbalance, such imbalance arising from dysfunctional hormone, growth factor, cytokine signaling among others. Clearly a plethora of disease states can arise from primary imbalances in cell signaling and does not conditionally arise as a consequence of induced macromolecular damage by superoxide anion/H\textsubscript{2}O\textsubscript{2}. Fig. 2 is a cartoon overview of the superoxide anion/H\textsubscript{2}O\textsubscript{2} couple acting in their capacity as overall second messengers. It summarises their cellular production sites and their roles in mitogen activated phosphokinase activations, protein turnover, sub-cellular metabolic redox modulation, mitochondrial and nuclear gene regulation and extracellular effector activations of the superoxide anion/H\textsubscript{2}O\textsubscript{2} second messenger system.

6. Mitochondrial DNA

Age related decline in bioenergy capacity below a crucial threshold will obviously contribute to cellular malfunction. The development of mitochondrial tissue bioenergy mosaics with age, exemplified by null, low and normal cytochrome oxidase cell content has been stringently correlated at the single cell level, by our laboratory, with mtDNA deletions and the individual cell content of full-length functional mtDNA (Linnane et al., 1989; Nagley et al., 1993; Kovalenko et al., 1998; Kopsidas et al., 2000, 2002; Linnane et al., 2002a). The mtDNA deletion changes reported by us, mainly arise by replication error, due to the asymmetrical nature of the heavy and light strand synthesis and the very large number of base pair repeats which mismatch during replication and result in mtDNA deletions. Cells whose mitochondrial oxidative phosphorylation function approaches zero due to severe mtDNA changes are lost from the tissue by apoptosis. The phenomenon of increasing age is fundamentally, cell loss from post-mitotic tissue. The cellular amount of mtDNA is under nuclear genome control so that if cells contain some full-length unmodified mtDNA it can be amplified back to normal levels, the various mtDNA deletions are eliminated and the cells rescued (Kopsidas et al., 2000). Cell loss occurs when little or no full-length mtDNA remains and therefore they become non-rescuable.

What role does oxidative damage play in inducing irreparable mtDNA dysfunction? Numerous early reports suggested that oxidative damage was a major contributor to increasing nuclear and more particularly mtDNA dysfunction. From such reports an error catastrophe hypothesis was proposed, whereby a vicious cycle of increasing oxidative damage to the mtDNA, lead to increased further oxidative damage and escalating mitochondrial bioenergy dysfunction. There is no extant evidence in support of such a view (Mansouri et al., 2006; Trifunovic et al., 2004, 2005). Urinary 8-oxo-deoxyguanosine was used as the exemplifier of age associated nuclear and mtDNA damage. However one of the major laboratories supporting the error catastrophe hypothesis subsequently reported that urinary measurements of 8-oxo-deoxyguanosine, as an estimation of increasing mtDNA (and nuclear DNA) damage, was unreliable and fraught with technical error and various confounding factors, leading to gross over estimation of oxidized mtDNA damage and uncertainty as to its interpretation (Helbock et al., 1998). The proposed dysfunctional formation of superoxide anion as a major cause of mtDNA damage and disease has yet to be demonstrated. On the contrary, recently Trifunovic et al. (2004, 2005) have reported that transgenic mice expressing an error prone mtDNA polymerase, accumulate substantial somatic
mtDNA mutations which are strongly correlated with a range of premature aging phenotypes and reduced life span. They did not observe any increase in so called oxidatively damaged products. These authors in agreement with our earlier human mtDNA studies conclude that oxidative phosphorylation dysfunction per se, arising from the replicatingly damaged mtDNA (and not oxidatively damaged mtDNA), is the primary inducer of the observed premature aging in the mtDNA mutator mice, and most particularly in a range of diseases normally associated with the aging process and observed in the mice.

7. Redox regulation of cellular metabolism and differentiation

The early work of Smith et al. (2000) on rat glial oligodendrocytes progenitor cell differentiation teaches that cellular redox poise regulates the process. Oligodendrocyte/astrocyte progenitor cells can be grown under conditions to establish a more oxidizing redox cytoplasmic environment, such conditions favour cell differentiation to oligodendrocyte or astrocyte formation. By contrast, a more reducing redox cytoplasmic environment favoured the maintenance of the progenitor cells. A particularly important aspect of these studies is that a range of naturally occurring physiological regulators function to modulate the redox state of cells, thus thyroid hormone and bone morphogenic protein 4 function to induce a more oxidizing cytoplasmic redox poise environment while basic fibroblast growth factor and PDGF exposure induce a more reducing cytoplasmic state. Different admixtures of hormones and growth factors favoring oxidation or reduction were used to manipulate cytoplasmic redox poise and cultures were thereby induced to predominantly self replicate or differentiate. Sundaresan et al. (1995) have reported that the response of rat vascular smooth muscle cells to PDGF, which includes a cascade of tyrosine phosphorylation, mitogen activated protein kinase stimulation and DNA synthesis was inhibited when PDGF induced H$_2$O$_2$ formation was blocked. There are numerous other examples of redox poise (H$_2$O$_2$) regulation playing a major role in cell differentiation (refer Linnane et al., 2007b).

8. The chimera of antioxidant (theory and) therapy?

The theme running through this short review is that a random antioxidant scavenging of superoxide anion (H$_2$O$_2$) would catastrophically derange their second messenger function which is essential for the regulation of major metabolome activities. The chimera of antioxidant therapy we have briefly considered elsewhere (Linnane and Eastwood, 2004). There is no compelling evidence from human clinical studies, conducted with sharp end points, to support the claims that the ingestion of small molecule antioxidants such as vitamins C, E, β-carotene and others prevent/ameliorate the development of age associated human diseases presumptively arising from random oxidative damage to cellular systems (Linnane and Eastwood, 2004; Linnane et al., 2007b).

9. Ascorbic acid, a hydrogen peroxide prodrug

Vitamin C has long been promoted as an outstanding antioxidant and of benefit in the prevention/amelioration of age associated diseases proposedly arising from oxygen radical damage but it has yet to demonstrated that it has any role as a meaningful therapeutic antioxidant. However ascorbate has been promoted by some complementary medicine practitioners for the treatment of cancers but the data in support of an efficacious outcome is equivocal. The rationale for this therapy, has been the antioxidant function of ascorbate to prevent oxidative damage which functions to induce cancer by random oxidation attack on cellular macromolecules. In a series of recent studies by Levine and associates they have revisited the use of high doses of ascorbate as a cancer therapy; their findings are largely summarized in Chen et al. (2005). In one of their earlier studies they reported that increasingly large oral doses of ascorbic acid do not lead to increasing levels of plasma vitamin C; the system is saturable and plasma levels plateau at less than 0.3 mM even on oral doses of 10 g/day. However I.V. doses of 10 g/day result in plasma levels of about 6 mM; doses up to 30–40 g/day have been used. This paper also reports the important discovery that ascorbic acid at a concentration of about 5 mM functions in vitro to selectively kill a variety of cancer cell lines. They demonstrate that under appropriate conditions, specifically, ascorbate at a concentration of about 5 mM in the presence of serum, acts as a prodrug promoting the formation of high concentrations of H$_2$O$_2$, which are lethal to a range of human and mouse cancer cell lines. These same high levels of generated H$_2$O$_2$ had apparently no effect on the growth of normal human cell lines; the authors had no suggestion as to why H$_2$O$_2$ was selective in its action on cancer cell lines. The equivocal earlier findings by others of a beneficial effect of I.V. ascorbate on cancers may possibly be explained by the observation that not all cancer cell lines are killed by ascorbate (Chen et al., 2005) and that there has been a wide variation in reported concentrations of ascorbate administered I.V. However the report of Chen et al. (2005) may be a particularly significant paper; they convincingly argue the case for blinded human clinical trials to be conducted on the possible efficacy of I.V. ascorbate on a range of human cancers.

For the purposes of this review we emphasize that there is no convincing evidence for ascorbic acid acting beneficially in mammals as an antioxidant. On the contrary in large doses it may act as a non-toxic prodrug for the production and delivery of H$_2$O$_2$ to tissues, beneficially for the selective treatment of some cancers. Albeit ascorbic acid and coenzyme Q$_{10}$ (and some other antioxidants)
A large literature, comprehensively reviewed in the monographs by Ebadi (2001) and references in the text. Sports medicine. Performance enhancement (not a pathology)

Vascular disease, diabetes mellitus

Chronic fatigue syndrome

Muscle weakness

Congestive heart failure—prevention/support therapy

Cognitive function decline, others

Mitochondrial neuromuscular diseases

and others

Reported anecdotal therapeutic benefits with coenzyme Q10 on systemic diseases and others (Table 1). For most practitioners these claims have been greeted with scepticism and coenzyme Q10 has not been widely embraced as being therapeutically useful.

One exception has been the use of coenzyme Q10 for the treatment of mitochondrial disease (MERRF, LHON, MELAS etc.), where clearly mitochondrial energy function is impaired. However, there are the true believers, including the present authors, who consider that the largely anecdotal results most likely have a kernel of truth in them, if only because the reports have come from a diverse range of reputable laboratories. Apart from its obvious role in mitochondrial energy generation to explain the reported clinical benefits, an encompassing physiological antioxidant role has been proposed for coenzyme Q10. There are extant many reports that superoxide/H2O2 are toxic products which must be scavenged, otherwise overtime, their formation will result in the development of a plethora of age related diseases. The role of coenzyme Q10 (quinol) as an antioxidant has been repeatedly demonstrated in non-physiologically constructed in vitro scenarios, with the observations extrapolated to explain the purported in vivo clinical benefits of coenzyme Q10. However, there are no substantive clinical findings supporting such a conclusion, just as with the cases of β-carotene, vitamin E, vitamin C and others (Linnane et al., 2007b). In any case this concept appears to be too limited in scope over focusing on supposed excessive oxidative damage, an hypothesis with which we do not agree.

A more encompassing concept would be that the claimed overarching therapeutic benefits of coenzyme Q10 administration (Table 1) revolve around bioenergy generation and H2O2 formation. In this scenario coenzyme Q10 is concerned with the generation of proton motive force (energy) involving a number of sub-cellular organelles and also as a major regulated source of H2O2. It can be readily envisaged that inappropriate up/down-physiological levels of H2O2 in specific tissues could lead to a range of systemic disease states which are commonly age associated. A corollary of this concept would be that administrating coenzyme Q10 to patients acts therapeutically to restore the H2O2 regulated messenger system to its normal physiological level at which it functions to appropriately regulate the metabolome and cell growth/differentiation and resolve the disease problem.

We comment further herein, only on the role of coenzyme Q10 in relation to the immune system. Bliznakov and others in a series of publications dating from the 1970’s reported on the effect of coenzyme Q10 on the immune system responses. Among other phenomena, they reported that coenzyme Q10 increased phagocytic activity, humoral antibody response titers, T4 lymphocyte numbers and decreased immunosuppressive effects of chemotherapeutic drugs (Bliznakov and Hunt, 1986 for overview).

There has not been a satisfactory encompassing explanation as to how coenzyme Q10 might stimulate immune system activity. We suggest, that this broad based cellular response phenomena, may find an explanation in the coenzyme Q10/H2O2 relationship. In this concept H2O2 would function by promoting cellular/clonal cell expansion acting in its role as a second messenger for growth; in response to hormones and cytokine effectors responsible for regulation of cell activity and growth.

By way of a concluding comment, it is obvious that no biological system is a perfect machine and that if there is a small inappropriate leakage of free radicals occurring over time, then they may contribute as a factor in the causation of the age associated diseases. Any regulated second messenger system which becomes dysfunctional as a result of loss of up/down regulation will give rise to physiological dysfunction; recall the loss of upward or downward control of any endocrine messenger system and the complex pathologies which result.

References


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Table 1

Reported anecdotal therapeutic benefits with coenzyme Q10 on systemic diseases and others

Mitochondrial neuromuscular diseases

Neurodegenerative diseases (Parkinson’s disease, dementias, others)

Cognitive function decline, others

Congestive heart failure—prevention/support therapy

Muscle weakness

Chronic fatigue syndrome

Cancer—breast treatment

Chemotherapy amelioration, support for AZT/AIDS treatment

Immuno—regulation

Vascular disease, diabetes mellitus

Sports medicine. Performance enhancement (not a pathology)

Consult Ebadi et al. (2001) and references in the text.

A large literature, comprehensively reviewed in the monographs by Ebadi et al. (2001).
mouse liver driven by NADH and ATP. J. Biol. Chem. 259, 14064–14067.


