Convergent mechanisms for dysregulation of mitochondrial quality control in metabolic disease: implications for mitochondrial therapeutics

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Abstract
Mitochondrial dysfunction is associated with a broad range of pathologies including diabetes, ethanol toxicity, metabolic syndrome and cardiac failure. It is now becoming clear that maintaining mitochondrial quality through a balance between biogenesis, reserve capacity and mitophagy is critical in determining the response to metabolic or xenobiotic stress. In diseases associated with metabolic stress, such as Type II diabetes and non-alcoholic and alcoholic steatosis, the mitochondria are subjected to multiple ‘hits’ such as hypoxia and oxidative and nitrative stress, which can overwhelm the mitochondrial quality control pathways. In addition, the underlying mitochondrial genetics that evolved to accommodate high-energy demand, low-calorie supply environments may now be maladapted to modern lifestyles (low-energy demand, high-calorie environments). The pro-oxidant and pro-inflammatory environment of a sedentary western lifestyle has been associated with modified redox cell signalling pathways such as steatosis, hypoxic signalling, inflammation and fibrosis. These data suggest that loss of mitochondrial quality control is intimately associated with the aberrant activation of redox cell signalling pathways under pathological conditions. In the present short review, we discuss evidence from alcoholic liver disease supporting this concept, the insights obtained from experimental models and the application of bioenergetic-based therapeutics in the context of maintaining mitochondrial quality.

Introduction
Metabolic diseases including metabolic syndrome, diabetes and ALD (alcoholic liver disease) are prevalent causes of illness and death in developed nations. In all of these pathologies, bioenergetics dysfunction is a prominent feature that contributes significantly to the pathophysiology. Recent advances in mitochondrial biology now allow a concerted effort to address the mechanisms involved and the development of bioenergetic-specific therapies. A key feature of metabolic diseases is that a large proportion of the populations affected possess multiple risk factors for developing severe life-threatening symptoms, but only a relatively small proportion progress to more severe pathologies. This observation has raised the possibility that secondary stressors or hits that differentiate the severity of metabolic diseases among individuals. Examples of secondary hits vary and can include those associated with the cardiometabolic syndrome such as obesity, hyperlipidaemia and insulin resistance, and environmental factors such as diet, pathogens and toxicants. In the present review, we use the example of alcohol-induced hepatotoxicity to highlight these concepts.

Chronic alcohol consumption can cause severe liver diseases including steatohepatitis, fibrosis/cirrhosis and hepatocellular carcinoma [1,2]. Classically, it has been thought that the development of ALD depends on the total amount and duration of alcohol consumption [3]. However, this simple hypothesis has been challenged as many heavy drinkers do not develop end-stage liver disease following decades of drinking suggesting that more than one hit contributes to disease progression [4]. In the context of the second hit paradigm, data have emerged showing that genetic, epigenetic, metabolic and environmental factors influence and drive the progression from simple steatosis to cirrhosis and cancer [5,6]. This concept serves as the basis of the multi-hit mechanism of ALD in which secondary stressors or hits contribute to disease pathogenesis. Importantly, alcohol, obesity and cigarette smoke may accelerate fibrosis and have been shown to be key synergistic risk factors for hepatocellular carcinoma [7,8]. Although correliative results from epidemiologic studies are important, the molecular mechanisms responsible for the interactive additive or synergistic effects of alcohol with other risk factors remain poorly understood. Furthermore, how these confounding factors work together to impact mitochondrial function as a key contributing factor to chronic alcohol-induced diseases is unclear.
In the present review, we address the emerging hypothesis that underlying differences in bioenergetics between individual patients is a key mechanism influencing the susceptibility and severity of the disease. At the molecular level, we discuss how the balance between mitochondrial biogenesis, cellular bioenergetics and mitophagy are integrated into a program to regulate mitochondrial quality control and how this may be altered by alcohol intake, diabetes, smoking and in chronic alcohol disease pathogenesis. Furthermore, we discuss the mitochondrial-targeted antioxidant, MitoQ (Mitoquinone) in experimental therapies against metabolic pathologies.

Mitochondrial quality control and dysfunction in ALD and metabolic disease

Mitochondria are important intracellular organelles that provide cellular energy and control reactive species and retrograde signalling to the nucleus for gene expression [9–12]. Within the cell, the quality of the mitochondrial population varies due to protein expression, post-translational modifications, assembly of respiratory chain proteins, membrane potential, and specific and non-specific modifications by reactive species to lipids, proteins, DNA and RNA. Dysfunctional mitochondria may arise owing to accumulation of mitochondrial DNA mutations and mis-folded and oxidatively modified proteins [13–15]. In ALD, it has been well established that mitochondria are targeted in cellular injury and that cytochrome P450 (CYP2E1) and iNOS (inducible nitric oxide synthase) expression are up-regulated [16–18]. This results in aldehyde and peroxynitrite formation, which further induces mitochondrial reactive oxygen and nitrogen species formation, respiratory chain dysfunction, and mtDNA (mitochondria DNA) damage [19–21] (Figure 1). These events are all thought to contribute to liver steatosis. This naturally raises the question of whether bioenergetic dysfunction is an epiphenomenon or intimately related to the pathology of the disease.

How do cells maintain normal mitochondrial bioenergetics? Typically, this is carried out by a mitochondrial quality control system to remove damaged proteins through mitochondrial-specific repair pathways [22,23]. If this process is overwhelmed, then the entire organelle is targeted for disposal and removed by the autophagic machinery in a process known as autophagy of the mitochondria, or mitophagy [23] (Figure 1). Mitophagy is a complex programme that the cell uses to recognize damaged mitochondria by mitochondrial network morphologies (e.g. fission) and bioenergetic parameters (e.g. decreased mitochondrial membrane potential). As intracellular protein and organelle degradation can be a dangerous undertaking, autophagy is highly regulated by the participation of more than 30 proteins that are important for synthesis and maturation of double membrane vesicles encircling cellular content, including damaged mitochondria and formation of autophagosomes [23]. The autophagosomes then fuse to lysosomes in order for the contents of the autophagosomes including mitochondria to be degraded. This may seem to be a drastic measure, but low-quality mitochondria can damage remaining healthy mitochondria through the release of ROS (reactive oxygen species) and Ca²⁺ from the organelle. Concomitantly, the cell also stimulates mitochondrial biogenesis to maintain the mitochondrial population (Figure 1). This is a complex process that is controlled by communication between the nucleus and the organelle to activate transcription factors that control fusion and fission processes to divide and segregate damaged mitochondria to increase the population of healthy mitochondria [24]. For example, damaged mitochondria undergo fission to segregate the dysfunctional components, and fusion to supplement the deficiencies of damaged mitochondria with segments of the respiratory chain [24–26]. Thus maintaining mitochondrial dynamics has emerged as a critical element in the contribution of mitochondria to cell division and physiological maintenance. Preservation of mitochondrial quality in alcohol toxicity could represent a threshold for cell survival since damaged mitochondria that are not removed by mitophagy are more susceptible to uncoupling and contribute to cell death [22,27–29].

In alcohol-dependent hepatotoxicity, primary (alcohol) and secondary stressors conspire to promote an inflammatory response in the liver. Specifically, previous studies implicate mechanistic links between alcohol (primary hit) and cigarette smoke, insulin resistance and hyperlipidaemia (secondary hits), which include oxidative and/or nitrative damage, disrupted redox signalling and hypoxic stress [6,17,30–32]. Notably, mitochondria are primary targets for alcohol and secondary stressors, which can also heighten liver inflammatory processes. Studies show that chronic alcohol consumption alone increases mitochondrial ROS production, causes mtDNA damage, impairs oxidative phosphorylation and alters mitochondrial dynamics [33,34]. Cigarette smoke and hyperlipidaemia also disrupt mitochondrial function [35]. Thus chronic alcohol consumption, when combined with these secondary stressors such as cigarette smoke or hyperlipidaemia, amplifies liver injury with increased mtDNA damage, mitochondrial proteome alterations and enhanced hypoxia via mitochondrial damage [36]. Nitric oxide is central to these mechanisms and is exacerbated by the fact that the responsive of the respiratory chain to nitric oxide is increased by chronic alcohol consumption [16,17]. Indeed, hypoxia and disrupted nitric oxide signalling increase the vulnerability of mitochondria in the alcohol consumer to additional damage when exposed to metabolic and/or environmental stressors. These conditions will lead to irreversible modification and inactivation of mitochondrial proteins, which will negatively impact mitochondrial bioenergetics. The inability to generate ATP needed for metabolism, detoxification and repair renders hepatocytes vulnerable to secondary stressors leading to hepatocyte cell death; a factor critical in ALD progression and severity.

Since alcohol consumption is a severe metabolic stress caused by the production of ROS/RNS (reactive nitrogen species), and reactive aldehydes leading to protein and
It is well known that during ALD cytochrome P450 (CYP2E1) and iNOS are up-regulated and this leads to aldehyde (R-CHO) and peroxynitrite (ONOO\(^{-}\)) generation. These highly oxidative/nitrative compounds further induce mitochondrial ROS/RNS generation, electron transport chain (ETC) dysfunction and mtDNA damage. Collectively, these events are thought to contribute to liver steatosis. To maintain mitochondrial population and quality, the cell stimulates mitochondrial biogenesis and mitophagy pathways. Mitochondrial biogenesis is a complex process that is controlled by communication between the nucleus and the organelle to activate transcription factors that control fusion and fission processes. dynamin-1-like protein (DRP1) and optic atrophy 1 (OPA1) are fission proteins located on the mitochondrial outer and inner membranes respectively, which divide and segregate damaged mitochondria to increase the population of healthy mitochondria. In contrast, mitophagy is initiated by the formation of cytoplasmic double membrane structures that recognize damaged mitochondria. Furthermore, expansion of the autophagosomal structures is dependent on the conversion of LC3-I into LC3-II, and insertion of LC3-II into the autophagosomal membranes. Completion of autophagic flux requires fusion of autophagosomes with lysosomes, where damaged mitochondria are degraded and cleared from the cell. MitoQ, a mitochondrial-targeted antioxidant, has been shown to prevent ALD-mediated oxidative and nitrative stress and steatosis, supporting the pivotal role of mitochondrial oxidants in ALD pathogenesis.
As oxidative stress is implicated as a key player in ALD, NAFLD (non-alcoholic fatty liver disease) is a serious medical problem as the percentage of obese individuals is growing in many countries [41,42]. Moreover, the aetiology of NAFLD is also linked to multiple risk factors including obesity, visceral adiposity, inflammation, Type 2 diabetes, insulin resistance and hyperlipidaemia. Mitochondrial dysfunction and proteomic changes are a feature of NAFLD, suggesting that overlapping mechanisms between obesity-dependent and ALD, including enhanced hypoxia and increased mitochondrial sensitivity to nitric oxide [43,44]. These results are important as they reveal novel targets in mitochondria that should be amenable for future NAFLD therapeutic studies.

Mitochondrial dynamics and therapeutics

As oxidative stress is implicated as a key player in ALD, numerous studies have been carried out using antioxidant therapies. Unfortunately, the results from these studies show minimal effects of antioxidants to prevent and/or reverse alcohol hepatotoxicity in experimental animal models and clinical trials with alcoholic patients. Importantly, few studies have assessed whether antioxidants prevent alcohol-dependent mitochondrial dysfunction and oxidative damage. We have shown previously that SAM (S-adenosylmethionine) and betaine; key methionine metabolism intermediates, prevent alcohol-dependent steatosis, with preservation of the mitochondrial thiol proteome and inhibition of oxidative and nitrative stress [37,45,46]. These results are important as they support the concept that SAM serves a key role in maintaining mitochondrial quality.

In a previous study, a new category of antioxidant-based therapeutics have been developed that target mitochondria. The strategy employed attaches the antioxidant to a lipophilic cationic moiety TPP+ (triphenylphosphonium), which results in a several hundred-fold accumulation of the antioxidant into the mitochondrial matrix compartment [47]. To date, the most successful mitochondrial-targeted antioxidant is MitoQ [48–51]. Studies show that the protective effects of MitoQ stem from changes in mitochondrial ROS production, which affect redox and hypoxia-sensitive signalling pathways. For example, MitoQ prevented alcohol-dependent oxidative damage, hypoxia and steatosis [16,20]. MitoQ has also been shown to decrease liver injury in hepatitis C patients [52]. Whether the mechanisms of action of MitoQ in hepatoprotection involve regulation of mitochondrial biogenesis, dynamics and mitophagy are currently being investigated. In other cell models, MitoQ has been shown to decrease mitochondrial fragmentation and increase autophagy [53].

Mitochondrial genetics and the implications for personalized medicine

The mitochondrial genome codes for key proteins in oxidative phosphorylation and it is now clear that polymorphisms in this genome can influence disease susceptibility or progression [54,55]. In terms of liver disease, recent studies have implicated certain mtDNA polymorphisms with an increased risk for the development of hepatocellular carcinoma associated with either hepatitis virus infection or alcohol abuse [56,57]. Similar associations have also been found linking mtDNA background and epigenetic modifications of the mtDNA with NAFLD. Although these representative studies specifically investigated liver disease, relationships between metabolic diseases and mtDNA genetic background have frequently been reported [55–59].

The rationale connecting disease susceptibility to mtDNA genetic background is based upon the hypothesis that certain mtDNA missense mutations were bioenergetically advantageous during human prehistory [58]. These changes in mitochondrial function were inter-related with caloric availability (diet) and environmental conditions (latitude) in humans, which gave them a reproductive and survival advantage in prehistoric times. For example, it has been hypothesized that mtDNA haplotypes associated with sub-Saharan latitudes have mutations that made the mitochondrion more economical, enabling them to utilize fewer electrons for the generation of a transmembrane potential and thus, ATP synthesis. This would make them well adapted for a low-calorie diet at warm latitudes (i.e. no need to generate heat). In contrast, mtDNAs associated with northern latitudes have altered mitochondrial economies in that more electrons would be used for ATP generation owing to decreased efficiency, translating into generation of greater thermal energy (heat), a metabolic advantage at colder latitudes. Although these mutations decreased mitochondrial efficiency for ATP generation, they were accommodated by a change in diet (increased caloric intake associated with animal
fats). To date, these changes (mutations) in the mtDNA can be maladapted for contemporary diets (excess fat) and a sedentary lifestyle common in western society. Under conditions of high-caloric intake and physical inactivity, individuals with mtDNA backgrounds originating from prehistoric clines characterized by low-caloric availability and tropical latitudes have mitochondria that generate increased mitochondrial oxidants relative to those with geographic origins typified by animal fats and northern latitudes.

An additional factor impacting mitochondrial function in addition to mtDNA background is overall organelle integrity, or the level of epigenetic modification and damage present. It is well established that age and the environment can influence mitochondrial integrity. Numerous studies have shown that environmental oxidants cause significant mtDNA damage and that developmental exposure can significantly impact downstream organelle function and condition [59,60]. Because these factors can significantly influence mitochondrial function and response to exogenous stimuli, it is plausible that future therapeutic strategies for disease treatment and/or prevention will use information about mitochondrial genetic background, function and integrity to determine the appropriate choice of pharmacological or treatment regimen. Interventional strategies could be personalized based on mtDNA background, mtDNA damage, function and oxidative stress measures from a surrogate tissue. For example, mtDNA genetic background may be linked to changes in mitochondrial function and cellular oxidant production in the presence and absence of a particular therapeutic (e.g. SAM or MitoQ) in a surrogate tissue, as a predictive measure for overall efficacy. Previous studies have revealed that mitochondrial priming may provide a means for increasing the efficiency of cytotoxins for the treatment of certain cancers [61].

Summary

As many chronic alcohol consumers are now more likely to be obese, studies are needed to investigate the impact of cardiometabolic risk factors such as Type 2 diabetes and hyperlipidaemia to increase ALD. Moreover, because exposure to environmental pollutants such as second-hand cigarette smoke remains widespread, and concomitant exposure to alcohol and cigarette smoke frequently occurs, investigations of these confounding factors on liver disease are also essential. It is rapidly becoming clear that the central therapeutic strategy in the context of bioenergetics is to maintain mitochondrial quality (Figure 1). The aspect that has received the most attention is the suppression of the dysregulation of mitochondrial hydrogen peroxide generation thus correcting dysfunctional cell signalling and oxidative damage to proteins, lipids and DNA. This is the mechanism of action through which MitoQ appears to be working. However, if the mitophagy pathway is overwhelmed or inhibited and the removal of damaged mitochondria fails then the impact of compounds such as MitoQ will be limited. Similarly, if biogenesis cannot supply new functional mitochondria to replace those removed by mitophagy, the remaining mitochondrial population will be stressed and turnover increased therefore amplifying the initial insult. This framework provides both a model to understand the interaction of multiple hits on the development of metabolic syndromes and the rationale for combination therapies to improve mitochondrial quality in disease.

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