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Quinone analogs prevent enzymes targeted in Friedreich ataxia from iron-induced injury *in vitro*

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1. Introduction

Friedreich ataxia (FRDA) is an autosomal recessive degenerative disease (1/30,000 live births) characterized by progressive limb and gait ataxia with lack of tendon reflexes in the legs, pyramidal syndrome of the inferior limbs and hypertrophic cardiomyopathy [8,11]. The disease gene has been mapped to chromosome 9q13 and encodes an ubiquitous 210 aminoacid protein, frataxin, targeted to the mitochondria [1–3,5,12,16,20]. FRDA is primarily caused by a GAA repeat expansion in the first intron of the frataxin gene, which accounts for 98% of mutant alleles [2]. Interestingly, in yeast strains carrying a deleted frataxin gene counterpart both an intra-mitochondrial iron accumulation and a respiratory chain deficiency were reported [1].

We have recently reported a deficient activity of the iron-sulphur (Fe-S) cluster containing proteins (ISP) in endomyocardial biopsies of FRDA patients, namely complexes I, II and III of the mitochondrial respiratory chain and aconitases, which cytosolic activity regulates cell iron homeostasis [17]. Considering the high sensitivity of ISP to oxygen free radicals on the one hand [7,13,19] and the reported iron deposit in heart tissues of FRDA patients on the other [18], we have hypothesized that (i) mitochondrial iron accumulation in FRDA is the consequence of a deregulation of a mitochondrial iron import system, triggered by the decreased amount of frataxin, normally acting as a regulator of the mitochondrial iron homeostasis, and that (ii) mitochondrial iron overload in FRDA would cause a secondary alteration of mitochondrial functions, through the iron-catalyzed Fenton chemistry. Accordingly, we have found that ISP lose their catalytic activity in both FRDA patients and in yeast strains carrying a deleted frataxin gene counterpart [17].

2. Methods

Yet, the tissue-specific expression of the disease remains unexplained and no animal model is presently available in FRDA. In order to elucidate the mechanism of the damages caused to Fe-S proteins by iron

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overload and to devise new strategies for future therapeutic trials, we developed an *in vitro* system using human heart homogenates prepared under low iron-chelating conditions, both to mimic the action of iron on mitochondrial ISP and to test the potentially protective effect of various substances. Homogenate from frozen human heart was obtained surgically and prepared at ice-melting temperature in a 0.1 M Tris-HCl buffer (pH 7.3) by five strokes of hand-driven ground glass potter. After filtering through a 90 μm nylon net, homogenate was aliquoted and frozen at -80°C for enzyme studies. Under these conditions, the endogenous antioxidant defenses of heart tissue were neutralized by simple dilution and free access of iron to its specific targets was achieved by freeze-thaw disrupting mitochondrial membranes.

2.1. Iron-induced damages to human heart enzymes in vitro

The activities of the soluble (mitochondrial *plus* cytosolic) aconitases and membrane-bound respiratory chain complex II were monitored in the presence of either reduced or oxidized iron under various iron chelating conditions (Table 1). Complex II activity was measured on freeze-thaw heart homogenate as the dichlorophenolindophenol quinone reductase as previously described [17]. Aconitase activity in heart homogenate, corresponding to the combined mitochondrial and cytosolic enzyme activities, was measured by following the aconitate accumulation at 240 nm in the presence of citrate. Lipoperoxidation, a marker of non-specific peroxidative damages caused to membrane lipid components, was simultaneously monitored using *cis*-parinaric acid as a fluorescent probe [6].

It was firstly shown that reduced iron tested as ferrous chloride resulted in a rapid lipoperoxidation and a significant loss of complex II activity (more than 75%) after a 1 min incubation of heart homogenates with 5 nM Fe^{2+} (Table 1). While reduced iron caused rapid damages to membrane lipids and membrane-bound complex II activity, no damage could be observed when an oxidized iron solution, 5 nM Fe^{3+}, was substituted for ferrous ions. The redox status of cellular iron has long been known to play a crucial role in the triggering of the oxidation cascade [10]. For this reason, reducing agents (ascorbate, glutathione) can be harmful to patients with iron overload, as these drugs are likely to reduce free iron *in vivo* [4]. In keeping with this, it is worth remembering that giving ascorbate to patients with iron overload has been reportedly harmful, because of increased peroxidation, triggered by ascorbate/iron salt mixture [14].

Table 1

Effects of chelators and antioxidant compounds on the iron-induced damages to human heart homogenate

Condition	Iron-induced damages to:		
	Lipids	Complex II	Aconitase
5 nM Fe^{2+}	−72	−75	−3
5 nM Fe^{3+}	0	−2	0
5 nM Fe^{2+} + 250 μM desferrioxamine	0	*nm*	−94
5 nM Fe^{2+} + 10 mM succinate + 60 μM idebenone	−1	−5	*nm*
5 nM Fe^{2+} + 10 mM succinate + 8 μM CoQ_{10}	−1	−5	*nm*
5 nM Fe^{2+} + 250 μM desferrioxamine + 6 U SOD + 6 U catalase	*nm*	*nm*	−6

Values are percent of the rate measured in the absence of iron.

2.2. *Quinone analogs protect membranes from iron-induced peroxidation*

Among the numerous drugs known to protect mitochondrial membranes against iron-induced injury, oxidized coenzyme Q_{10} and its short chain analog idebenone were selected for further investigations. While the quinone ring accounts for the common redox properties of these molecules, the length and the composition of the side chain modulate their lipophilicity and their diffusion in the human body [3,9, 15]. Interestingly, low concentrations of idebenone, reduced *in situ* in the presence of succinate by the respiratory chain, efficiently protected both membrane lipids and membrane-anchored complex II against iron injury (Table 1). A similar protection was provided by succinate-reduced CoQ_{10}.

2.3. *Iron hits different targets in the presence of a chelator*

While ferrous ions alone failed to cause any damage to aconitase, the addition of a water soluble chelator such as desferrioxamine led to a significant quinone-resistant loss of activity (Table 1). By contrast, water soluble chelators protected membrane lipid components and membrane-bound Fe-S respiratory chain enzyme proteins against iron-induced injury (Table 1). One possible explanation is that iron chelators shifted iron from the negatively-charged lipid phase to the aqueous phase, and changed the nature of the targeted enzymes rather than they reduced iron toxicity. Involvement of Fenton chemistry in the damages caused by iron *plus* desferrioxamine to aconitase activity was supported by the protective effects of superoxide dismutase and catalase (Table 1).

2.4. *Soluble aconitase is also protected from iron-induced injury by quinones*

Considering that iron-induced mitochondrial enzyme injury depends on the redox status of iron and that quinone prevented iron-induced injury to membrane components, we hypothesized that quinones, reduced *in situ* by the respiratory chain, could secondarily protect soluble aconitase by causing iron-reoxidation. Heart homogenate was therefore first incubated in the presence of CoQ_{10} and 5 nM Fe^{2+} *plus* or *minus* 10 mM succinate for 3 min. The homogenate was subsequently supplemented with 200 μM desferrioxamine and assayed for aconitase activity after an additional 3 min incubation. When the initial incubation medium was devoid of succinate, a 75% decrease of aconitase activity was observed. By contrast, a 3 min incubation with succinate significantly protected aconitase activity (70%). This establishes that, beside their protective effect on mitochondrial membrane lipids and enzymes, quinones can also indirectly protect the soluble enzymes against iron-induced injury in our *in vitro* system FRDA.

3. Conclusion

The present study shows that, as previously established in numerous biological models, reduced iron triggered a rapid lipoperoxidation and a significant loss of complex II activity in human heart homogenate *in vitro* as well. In association with water-soluble iron chelators, reduced iron caused a marked loss of water soluble aconitase activity. Reduced (but not oxidized) quinones efficiently protected lipid membrane components as well as soluble and membrane ISP from iron-induced damages *in vitro*. One possible explanation for this is that positively charged iron is retained by negatively charged membrane phospholipids, thereby causing a membrane accumulation of oxygen free radicals which cannot be prevented by water-soluble superoxide dismutase (SOD) and catalase. By contrast, due to their lipophilicity and redox

properties, quinones reduced *in situ* by the respiratory chain protected mitochondrial membranes against iron-induced injury.

On the other hand, accumulated iron could be shifted from mitochondrial membranes by water-soluble chelators, triggering the production of oxygen free radicals in the aqueous phase where SOD and catalase (but not quinones) have now a protective effect. In FRDA, numerous natural compounds present in the mitochondria could act as natural iron-chelators and might play a role in the partitioning of iron overload between mitochondrial membranes and matrix *in vivo*. For this reason, both membrane ISP and soluble matrix aconitase are expected to be targeted by oxygen free radicals in FRDA [17].

Assuming that the *in vitro* system of iron-induced injury reported here mimics the damages caused to heart (and possibly spinocerebellar tract) in FRDA, we suggest that antioxidant drugs likely to reduce iron and water soluble chelators are potentially harmful and should be avoided in FRDA. Conversely, quinone analogs should protect heart and brain of FRDA patients from iron-induced injury *in vivo*, as these drugs do not increase the reduction status of iron-loaded mitochondria, and, when reduced by respiratory substrates, protect both membrane lipids and targeted mitochondrial enzymes from iron injury *in vitro*.

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