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Copper(II) and iron(III) ions inhibit respiration and increase free radical-mediated phospholipid peroxidation in rat liver mitochondria: Effect of antioxidants

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ABSTRACT

Rat liver mitochondria (1.5–2.1 mg protein·mL^{-1}) supplemented with either 25 and 100 μM Cu^{2+} or 100 and 500 μM Fe^{3+} show inhibition of active respiration (O_2 consumption in state 3) and increased phospholipid peroxidation . Liver mitochondria were supplemented with the antioxidants reduced glutathione, *N*-acetylcysteine or butylated hydroxitoluene, to evaluate their effects on the above-mentioned alterations. Although the mitochondrial dysfunction is clearly associated to phospholipid peroxidation, the different responses to antioxidant supplementation indicate that the metal ions have differences in their mechanisms of toxicity. Mitochondrial phospholipid peroxidation through the formation of hydroxyl radical by a Fenton/Haber-Weiss mechanism seems to precede the respiratory inhibition and to be the main fact in Fe-induced mitochondrial dysfunction. In the case of Cu^{2+}, it seems that the ion oxidizes glutathione, and low molecular weight protein thiol groups in a direct reaction, as part of its intracellular redox cycling. The processes involving phospholipid peroxidation, protein oxidation and mitochondrial respiratory inhibition characterize a redox dyshomeostatic situation that ultimately leads to cell death. However, Cu^{2+} exposure involves an additional, yet unidentified, toxic event as previous reduction of the metal with *N*-acetylcysteine has only a minor effect in preventing the mitochondrial damage.

1. Introduction

A recent report indicates that Cu^{2+} and Fe^{3+} ions added to isolated rat liver mitochondria produce inhibition of the active (adenosine triphosphate (ATP)-producing, state 3) respiration. The process was recognized as associated with phospholipid peroxidation [1]. Both, Cu^{2+} and Fe^{3+} produced decreases in O_2 consumption in state 3 with malate-glutamate or with succinate as substrates (Concentration for half maximal response, C_{50}: 50–60 μM Cu^{2+} and 1.25 mM Fe^{3+}). Concerning mitochondrial phospholipid peroxidation, both metal ions produced a marked increase in the free-radical mediated process, with a 3 to 5-fold increase in thiobarbituric acid reactive species (TBARS) contents and a C_{50} of 40 μM Cu^{2+} and of 1.75 mM Fe^{3+}. Although it seems proved that a Fenton/Haber-Weiss reaction mechanism, with generation of the highly oxidant and toxic hydroxyl radical (HO·) occurs in these metal-ion induced mitochondrial toxicity, different responses to the metal ions suggest differences in the mechanism of intracellular toxicity [1].

The concept of oxidative stress is central to the understanding of Cu^{2+} and Fe^{3+} intracellular toxicity. The classic concept defined by Sies implied an unbalance situation between oxidants and antioxidants [2]. The contemporary update as the “redox hypothesis” [3] amplified the classical definition of oxidative stress to a new concept for oxidative stress, as due to an oxidative process which alters the redox balance of thiol (−SH) and disulfide (−SS−) groups in low molecular weight molecules (as reduced glutathione, GSH) and in whole proteins, where the thiol/disulfide balance keeps the protein conformation that is involved in signal pathways and in the regulation of physiological functions [4].

Antioxidants are normal cell constituents whose function is to decrease the level of oxidative chemical species by different mechanisms. Cellular antioxidants include as the main mechanism to the classic antioxidant enzymes: superoxide dismutases (Cu,Zn-SOD and Mn-SOD) [5], catalase [6], glutathione peroxidase [7] and the thioredoxin system

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[8]. In addition, there are small molecules that are cell constituents, such as GSH, in the mM range, and α-tocopherol and β-carotene, in the μM range, that are able to trap free-radicals and excited species and to reduce the extent of membrane phospholipid peroxidation and protein oxidation [6].

In the present study, GSH, *N*-acetylcysteine (NAC) and butylhydroxitoluene (BHT) are used to test their effects on the Cu^{2+} and Fe^{3+} mitochondrial toxicity as recently mentioned [1]. GSH is the hydrophilic main intracellular antioxidant, as coenzyme of glutathione peroxidase and itself a free-radical scavenger, GSH content in liver and brain was severely decreased upon Cu and Fe overloads [9,10]. NAC is a precursor for GSH, widely used as antioxidant in *in vitro* and *in vivo* experimental models, it provides thiol groups, increases GSH synthesis or prevents its deficiency. NAC has proved to be effective in the protection of metal-induced oxidative damage [11,12,13]. BHT is a lipophilic organic compound with antioxidant properties by a free-radical trapping mechanism and that is widely used in food and cosmetics.

As said, to evaluate antioxidant protection and the mechanisms for oxidative damage and impairment of the mitochondrial respiration by Cu^{2+} and Fe^{3+} ions we have analyzed the antioxidant effects of molecules (GSH, NAC and BHT) that are able to inhibit the free-radical mediated chain reaction of phospholipid peroxidation that could lead to mitochondrial respiratory inhibition.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Other reagents were of analytical grade.

2.2. Animals

Sprague-Dawley male rats (200–220 g) were from the Central Animal House, School of Pharmacy and Biochemistry, University of Buenos Aires, and were acclimatized under laboratory conditions for 7 days before starting the experiment. The rats were provided with standard diet and water *ad libitum*, and were maintained under controlled conditions of temperature (23–25 °C) and humidity (50%) with an alternating 12 h light–dark cycle. Rats were anesthetized with 15% (*w/v*) urethane at 1.5 g/kg (ip) and liver was excised and suspended in 250 mM sucrose and 10 mM HEPES buffer solution, pH 7.40 (mitochondria isolation/re-suspension medium) at 0 °C. Animal care was given in compliance with Argentine regulations (ANMAT) and with the Guidelines for Ethical Treatment in Animal Experimentation of the American Physiological Society (Bethesda, MD, USA).

2.3. Mitochondrial isolation

Liver was cut into small pieces and suspended in EDTA-free isolation/re-suspension medium in a ratio of 9 mL per g of tissue. Liver pieces were washed 3 times [14]. The preparation kept at 0 °C was passed through a Potter-Elvehjem homogenizer and centrifuged at 700*g* (Sorvall-Du Pont, Model RC5S) during 10 min at 0–2 °C. Pellet (nuclei, unbroken cells and non-subcellular tissues) was discarded and the supernatant was centrifuged at 8000*g* during 10 min at 0–2 °C. The supernatant was re-centrifuged and the new pellet, containing mitochondria able to perform oxidative phosphorylation, and the pellet was re-suspended in 0.5 mL of the isolation/re-suspension medium [14].

2.4. Mitochondrial oxygen consumption

Oxygen uptake was determined polarographically by high resolution respirometry, using a Clark-type electrode (Hasantech Oxygraph System DW1). For mitochondrial oxygen uptake the following reaction medium was prepared: 120 mM KCl, 5 mM KH_2PO_4 and 20 mM HEPES. Since metal ion addition will follow, EGTA or EDTA were avoided, and this was proved not to affect mitochondria quality, viability or function. This respiratory buffer, adjusted to pH 7.20 with 1 M HCl, was placed in a final volume of 1 mL in the electrode chamber, and that chamber was covered. Substrates were added with a Hamilton syringe. The state 4 respiratory rate was determined either with 2 mM glutamate and 5 mM malate or with 10 mM succinate as substrates. The mitochondrial suspension was added to the reaction medium and rate of respiration was recorded.

For copper and iron exposure, Cu^{2+} and Fe^{3+} were added to the chamber before the mitochondrial suspension, from a 10 mM Cu^{2+} solution ($CuSO_4 \cdot 5H_2O$) or a 100 mM Fe^{3+} solution ($FeCl_3 \cdot 6H_2O$), to obtain the final concentrations of 25 μM of Cu^{2+} and of 100 and 500 μM Fe^{3+}.

Antioxidants (4 mM GSH, 4 mM NAC or 50 μM BHT) were added to the respiratory medium before mitochondria, that were added to a final content of 1.5–2.1 mg protein mL^{-1}. The concentration of NAC and GSH was chosen based on the intracellular concentration of GSH whereas that of BHT, which is not normally found in the cell and may be toxic at high concentrations, was chosen as a function of the concentration used for antioxidant activity in scientific literature. This antioxidant compound has a mechanism of action different from the first, since it is distributed and acts specifically at the level of the membranes where it acts interrupting the lipid peroxidation reactions and is efficient at very low concentrations. We used the same concentration of NAC as of GSH to be able to analyze the effect of the −SH group in GSH protection.

Active respiration (O_2 consumption in state 3) was established by addition of 1 mM ADP. The slope of the trace corresponding to about 1–2 min of respiration, was used to calculate the rate of mitochondrial O_2 consumption and expressed in ng-at O min^{-1} mg $protein^{-1}$. Respiratory control (RC), the accepted index to measure mitochondrial quality by the coupling between respiration and phosphorylation, was calculated as the ratio between the rate of O_2 consumption in states 3 and 4. Typical RC values range from 3 to 10, varying with the substrate and the quality of the preparation [15].

2.5. Mitochondrial phospholipid peroxidation

Mitochondrial phospholipid peroxidation was assessed by the TBARS assay, as described by Fraga et al. [16]. The sample (rat liver mitochondria, at about 5 mg protein mL^{-1}) was incubated for 10 min at room temperature with Fe^{3+} or Cu^{2+} in the reaction medium for O_2 consumption (about 1/5 dilution) with the respiratory substrate (2 mM glutamate and 5 mM malate) and in absence or presence of the antioxidant compounds (GSH, NAC and BHT). After this exposure, mitochondria were washed with phosphate buffer solution by centrifugation at 8000 g (10 min, 2–4 °C). After that, samples were added with 4% w/v BHT in ethanol and 20% w/v trichloro-acetic acid were added to the samples and centrifuged in a table top centrifuge at low speed for 10 min. 0.7% w/v thiobarbituric acid was added to the deproteinized supernatant which was heated at 100 °C for 20 min, and the absorption of the pink solution was determined at 535 nm (ε = 156 $mM^{-1} \cdot cm^{-1}$) and expressed as nmol TBARS mg of $protein^{-1}$.

2.6. Total thiol groups

Mitochondria were suspended in phosphate buffered saline solution, pH 7.3 containing 1% Triton-X-100. After 5 min incubation at 0 °C to obtain a solubilized preparation, the preparation was centrifuged at 13600*g*, and the supernatant containing the thiol groups was kept. Quantification was assessed by the reaction of the −SH groups with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to form 5-thio-2-nitrobenzoate (TNB) which was measured spectrophotometrically at 412 nm

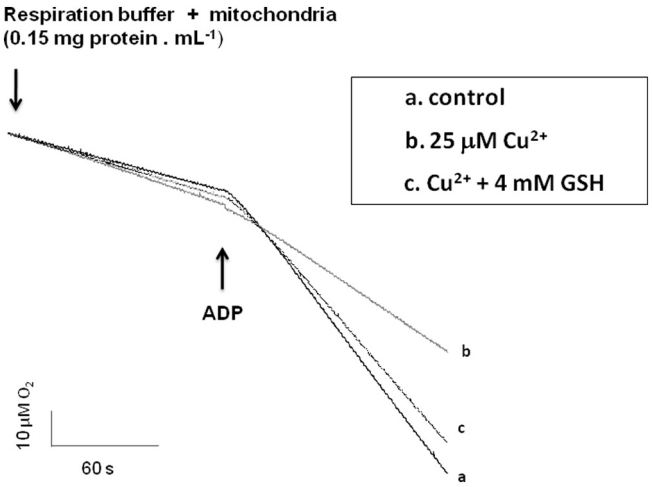

Fig. 1. Representative trace of oxygen consumption upon 25 µM Cu overload for 2 min in rat liver mitochondria respiring in state 4 and with 4 mM GSH supplementation. Here, we show the last 2 min before adding ADP to switch to state 3.

($\varepsilon = 13.6\ mM^{-1}\ cm^{-1}$) [17]. The content of thiol groups after addition of Cu^{2+} or Fe^{3+}, in the range 0–100 µM was measured in the sample. To assess the sole ability of the metals to interact with thiols ruling out possible interferences from the sample, this titration was run replacing the sample by a standard solution of GSH, in the same concentration of the sample (15 µM −SH).

2.7. Protein content

Protein contents were measured using the Folin reagent with bovine serum albumin as standard [18].

2.8. Data analysis

Data in text, figures and tables are expressed as mean ± standard error of the mean (SEM) from at least 3 independent experiments. The software GraphPad InStat3 was used and One-way ANOVA test and Dunnett's test as a *post hoc* test were performed. Significance is indicated by p value, where $p < 0.05$ was considered significant, $p < 0.01$ was considered very significant and $p < 0.001$ was considered extremely significant.

3. Results

3.1. Effect of GSH, NAC and BHT on the O_2 consumption of rat liver mitochondria exposed to Cu^{2+} and Fe^{3+} overloads

Fig. 1 shows a representative trace of the O_2 consumption of isolated rat liver mitochondria in control conditions, and after supplementation of the respiration medium with 25 µM Cu^{2+}, or with Cu^{2+} and 4 mM GSH.

Table 1 shows the effects of 25 µM Cu^{2+} and of 100 µM and 500 µM Fe^{3+} in the respiratory rates of rat liver mitochondria. The condition for the highest H_2O_2 production (the main oxygen reactive species) in state 4 was established as 10 min, so the respiration rates were measured with mitochondria respiring in state 4 for 10 min. 100 µM Fe^{3+} corresponds to the minimum concentration needed of this metal to observe phospholipid oxidation while a decrease in the mitochondrial functions is only seen from 100 µM Fe^{3+} in the mentioned condition of high $O_2\cdot^-/H_2O_2$ production [1].

A significant decrease of 47% in the O_2 consumption in active respiration (state 3), was observed with 25 µM Cu^{2+} (first column). A small partial uncoupling effect, as an increased respiration in state 4, was also observed.

The three antioxidants had an effective improving effect in the effect of Cu^{2+} on the state 3 rate of O_2 consumption, which was recovered by 92% for GSH, by 79% for NAC and by 69% for BHT.

Table 1 also shows the effects of 100 µM and 500 µM Fe^{3+} that were assayed in state 3 mitochondria. The lower Fe^{3+} concentration was almost without effect, and 500 µM Fe^{3+} were required for a respiratory inhibition of 61%. BHT was the only antioxidant that exhibited an effect, with a recovery of 93% of the active respiration rate.

The small uncoupling effect, the increase in state 4 respiration, was not prevented by any of the three antioxidant assayed.

Table 1
Respiration rates of rat liver mitochondria exposed to Cu^{2+} and Fe^{3+} in absence and presence of the antioxidants GSH (4 mM), NAC (4 mM) and BHT (50 µM). Malate-glutamate was used as substrate. Metal ions and antioxidants were added to state 4 mitochondria 2 min before respiration measurements.

	Oxygen consumption (ng-at O·min^{-1}·mg protein^{-1})			
	Without antioxidant s	With antioxidant		
		GSH	NAC	BHT
Control				
State 4	6.7 ± 0.3	8.0 ± 0.3	7.8 ± 0.9	7.6 ± 0.6
State 3	36.0 ± 0.3	38.4 ± 2.1	34.3 ± 2.0	38.0 ± 1.8
RC[a]	5.4 ± 0.2	4.8 ± 0.3	4.4 ± 0.3	5.0 ± 0.3
Cu^{2+} (25 µM)				
State 4	10.4 ± 0.4**	9.3 ± 0.4	11.3 ± 0.2	10.3 ± 0.4
State 3	19.2 ± 0.5**	33.3 ± 0.9###	28.3 ± 1.2###	24.7 ± 1###
RC	1.9 ± 0.1**	3.6 ± 0.2###	2.5 ± 0.2##	2.4 ± 0.1#
Fe^{3+} (100 µM)				
State 4	9.1 ± 0.5**	9.1 ± 0.2	12.8 ± 0.2#	8.8 ± 0.06#
State 3	37.4 ± 0.4	39.4 ± 0.7	36.5 ± 1.0	32.0 ± 0.7
RC	4.1 ± 0.3**	4.7 ± 0.1	3.4 ± 0.2###	6.7 ± 0.1 ###
Fe^{3+} (500 µM)				
State 4	6.9 ± 0.3	7.9 ± 0.3	7.1 ± 0.2	6.4 ± 0.2
State 3	22.1 ± 1.2**	29.2 ± 1.1	23.2 + 0.5##	33.4 ± 0.4###
RC	3.2 ± 0.3**	2.8 ± 0.3	1.9 ± 0.2#	5.3 ± 0.1##

[a] RC: respiratory control.
** $p < 0.01$ compared to control group.
# $p < 0.05$ compared to metal ion treated and antioxidant-untreated group.
$p < 0.01$ compared to metal ion treated and antioxidant-untreated group.
$p < 0.001$ compared to metal ion treated and antioxidant-untreated group.

Table 2
Phospholipid peroxidation in rat liver in mitochondria added with Cu^{2+} or Fe^{3+} in the absence or presence of the antioxidants GSH (4 mM), NAC (4 mM) and BHT (50 μM) and incubated for 10 min. Metal and antioxidants were incubated with state 4 mitochondria for 10 min before the TBARS assay. ($^{**}p < 0.01$ and $^{***}p < 0.001$ compared to control (antioxidant-untreated); $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to metal-treated and antioxidant-untreated groups.)

	Lipid peroxidation (nmol TBARS·mg protein^{-1} × 10)			
	Control (without antioxidant)	GSH	NAC	BHT
Mitochondria	0.8 ± 0.1	0.8 ± 0.1	0.56 ± 0.05	0.58 ± 0.03
+ 25 μM Cu^{2+}	1.8 ± 0.2**	0.9 ± 0.1$^{\#\#}$	1.0 ± 0.1$^{\#}$	1.5 ± 0.3
+ 100 μM Fe^{3+}	2.95 ± 0.06***	2.30 ± 0.02$^{\#\#}$	3.2 ± 0.2	0.70 ± 0.06$^{\#\#}$

3.2. Effect of GSH, NAC and BHT on the phospholipid peroxidation of rat liver mitochondria supplemented with Cu^{2+} and Fe^{3+}

The process of phospholipid peroxidation in rat liver mitochondria was determined as TBARS content in Cu^{2+} and Fe^{3+} added mitochondria in absence and presence of the antioxidants GSH (4 mM), NAC (4 mM) and BHT (50 μM) (Table 2).

In the absence of antioxidant compounds, rat liver mitochondria exposed to 25 μM Cu^{2+} or to 100 μM Fe^{3+} showed an increased phospholipid peroxidation that amounted to about 2.3 and 3.7 times, respectively, as compared with the organelles not exposed to the metal ions. GSH was slightly effective in decreasing iron-induced mitochondrial phospholipid peroxidation. The chain-breaker antioxidant BHT was fully effective in preventing phospholipid peroxidation. Concerning Cu^{2+}, complete protection against metal-induced phospholipid oxidation was achieved by the GSH and NAC treatments (Table 2).

3.3. Effect of Cu^{2+} and Fe^{3+} on the content of thiol groups in rat liver mitochondria

The ability of Cu^{2+} and of Fe^{3+} to react with the sulfhydryl groups (−SH) of the mitochondrial matrix was assessed in samples of the mitochondrial matrix obtained from disrupted and solubilized mitochondria. Cu^{2+} was able to decrease the content of -SH in a 1:1 stoichiometric ratio. The Cu^{2+} concentration necessary for depleting the 50% of total mitochondrial −SH groups content (C_{50}) was 10 μM Cu^{2+} (Fig. 2A). Cu^{2+} consumed stoichiometrically the −SH groups up to a minimum of 2 μM, while no significant differences were observed after Fe^{3+} overloads (Fig. 2A).

To assess whether other soluble compounds were reacting with the metal ions and interfering with thiol group oxidation in the mitochondrial sample, the experiment was repeated using a GSH solution at the same final concentration of −SH than in the mitochondrial sample (Fig. 2B). The same response after metal ion exposure was observed with a C_{50} of 10 μM Cu^{2+}.

4. Discussion

Recently we have described the deleterious effects induced by Cu^{2+} and Fe^{3+} added to respiring rat liver mitochondria, consisting of a respiratory inhibition and a promotion of the phospholipid peroxidation process [1]. We interpreted that Cu^{2+} and Fe^{3+} toxicities are associated with the mitochondrial production of H_2O_2, which according to the Fenton/Haber-Weiss reaction mechanism, leads to the formation of $HO^{\bullet}$ radicals. In this study, we have assayed the protection provided by the antioxidants GSH, NAC and BHT by adding the antioxidant compounds to the respiration medium.

Regarding Fe^{3+}, the metal-ion induced oxidative damage seems due mainly to the phospholipid peroxidation process, considering the effect of BHT, that was totally effective in preventing the peroxidative process and almost similarly, the respiratory inhibition. BHT is a radical trap of hydrophobic nature that would be exerting its action incorporated in the mitochondrial membrane, where phospholipid peroxidation takes place. The fact that 100 μM Fe^{3+} were able to induce a marked phospholipid peroxidation but were almost ineffective in inhibiting active respiration, an effect that was evident at 500 μM Fe^{3+} could be interpreted as an indication that phospholipid peroxidation precedes the respiratory inhibition. This concept is illustrated in Fig. 3.

Regarding Cu^{2+}, a different antioxidant response profile was observed with antioxidant supplementation. GSH and NAC were effective in preventing the respiratory inhibition. There is evidence that Cu^{2+} toxicity involves the interaction of the metal ion with -SH groups and formation of Cu^{2+} complexes.

Intracellular Cu is physiologically handled by chaperones in the form of Cu^{1+}, which coordinate the metal delivery to specific targets within the cell [19]. However, the sudden increase of free metal ions in an overload context gives rise to a whole new set of potentially reactive targets. On one side, Cu^{1+} is likely to react with H_2O_2 in a Fenton like reaction giving rise to OH· and consequent phospholipid peroxidation (Table 2). The concomitant oxidation of Cu^{1+} to Cu^{2+} gives rise to a new oxidant which effectively oxidizes −SH groups. The Cu^{2+} reaction with thiol groups, not observed as a similar Fe^{3+} reaction, was evident

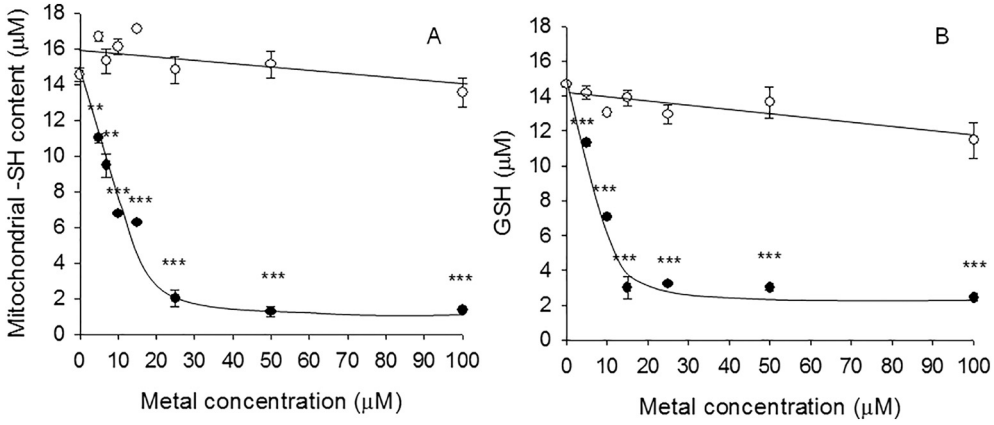

Fig. 2. A) Thiol group (− SH) content from mitochondria that were disrupted and solubilized by Triton-X-100. The extract was exposed to increasing Cu (●) and Fe (○) concentrations. B) Thiol group content of a standard GSH solution exposed to increasing Cu (●) and Fe (○) concentration ($^{**}p < 0.01$; $^{***}p < 0.001$).

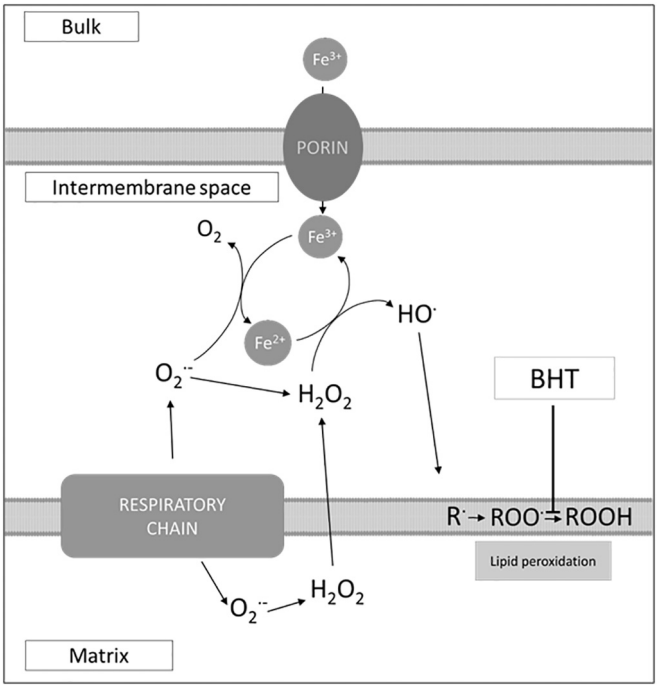

Fig. 3. Proposed mechanism of iron toxicity to isolated rat liver mitochondria.

in the metal ion titration of –SH groups (Fig. 2), where it occurs with a 1:1 stoichiometry at the low Cu^{2+} concentrations. Comparison of the titrations of mitochondrial –SH contents and a standard GSH provided evidence of the Cu^{2+}-GSH reaction. The high intracellular concentration of GSH would of course prevent the random cross-linking among proteins due to Cu^{2+}.

However, if oxidative-stress results in an impaired GSH content, unstrained disulfide formation would become an important target of toxicity. In cells, GSH acts as antioxidant by reacting with hydroperoxides (H_2O_2 or ROOH), directly or indirectly through glutathione peroxidase. GSH also reacts with electrophiles and xenobiotics in conjugation reactions. The oxidized form GSSG activates nuclear transcription factors (NF-κβ) and is reduced by the thioredoxin system, regulating the intracellular redox homeostasis [4]. The intracellular levels of GSH are an important factor in the cell viability in Fe, Cu and Cd ions toxicity [24].

Reasonably, supplementation with GSH or NAC previously to the addition of the metal aids in conserving the respiratory function. Both molecules possess a −SH group with $E^{\circ}_{GSSG/GSH} = -240$ mV which readily reduces Cu^{2+}, thus preventing the direct oxidation of protein –SH groups. However, GSH resulted more effective, likely due to its molecular structure and participation in enzymatic reactions. It is clear the greater ability of GSH to restore the respiratory function upon Cu^{2+} overload (Table 1).

The explanation of the observed results could be afforded by the formation of Cu(I)-$(GSH)_2$ complexes, as reported [20–22] and indicated in Eqs. (1) and (2) [23].

$$2Cu^{2+} + 2GSH \rightarrow 2Cu^{+} + GSSG + 2H^{+} \quad (1)$$

$$Cu^{+} + 2GSH \rightarrow Cu(I) - [GSH]_2 \quad (2)$$

Complexes of the type Cu(I)-$(GSH)_2$ ($K_f > 10^{35}$) have been proposed to work as Cu(I)-carrier in proteins as well as to serve as a mechanism to protect cells from undergoing the damage due to free Cu^{2+} ions. Moreover, Cu(II)-disulfide complexes were found to have antioxidant activity by superoxide dismutase- and catalase-like activities [23].

Nonetheless, while the protection bestowed by GSH and NAC may partially be explained due to the reducing equivalents incorporated in the system, the lack of complete protection brought about by NAC despite its concentration being several folds higher than that of Cu suggests an additional target of toxicity mediated by Cu^{1+}, which is likely independent of the −SH oxidation reaction. Cu reactivity extends beyond redox chemistry, being able to form complexes with several ligands including the amide groups in the proteins backbone. Therefore, it is likely that the direct interaction of free Cu^{1+} with peptide bonds [25] further contribute to enzyme inactivation resulting, in the case of isolated mitochondria, in an impaired respiration.

The toxic mechanism of copper in isolated mitochondria and the protective role of GSH is summarized in Fig. 4.

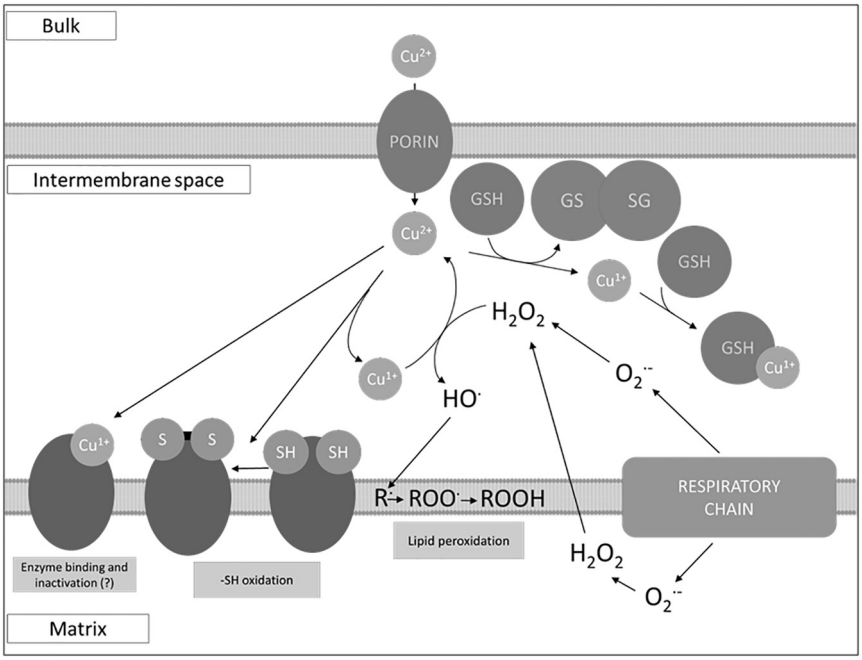

Fig. 4. Proposed mechanism of copper toxicity to isolated rat liver mitochondria. The protective role of glutathione.

5. Conclusion

Overloads of the metal ions Cu^{2+} and Fe^{3+} added to liver mitochondria produce a respiratory inhibition by an enhanced free-radical mediated process of phospholipid peroxidation and by the formation of mitochondrial oxidants. For the case of Fe^{3+} cytotoxicity, the formation of $HO^{\bullet}$ by a Fenton/Haber-Weiss reaction seems to afford the responsible molecular mechanism. For the case of Cu^{2+} cytotoxicity, a different response to antioxidant supplementation suggests that this metal-ion is first reduced and then reacts with GSH to form the complex Cu(I)-$(GSH)_2$. Both metal ions, Fe^{3+} and Cu^{2+}, are able to deeply alter the normal function of cellular processes through phospholipid peroxidation, protein oxidation. However, Cu^{2+} ability to directly react with macromolecules results in an increased toxicity of this metal.

Abbreviations

ANOVA	analysis of variance
ADP	adenosine triphosphate
ATP	adenosine triphosphate
BHT	butylated hydroxytoluene
C50	concentration of half maximal response
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycol-aminoethyl-tetraacetic acid
GSH	reduced glutathione
HEPES	4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid
H_2O_2	hydrogen peroxide
ip	intraperitoneal
NAC	*N*-acetylcysteine
HO·	hydroxyl radical
ROOH	lipid hydroperoxides
ROS	reactive oxygen species
rpm	revolutions per minute
SEM	standard error of the mean
TBARS	thiobarbituric acid-reactive substances

Conflicts of interest

The authors declare that they have no conflict of interest.

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