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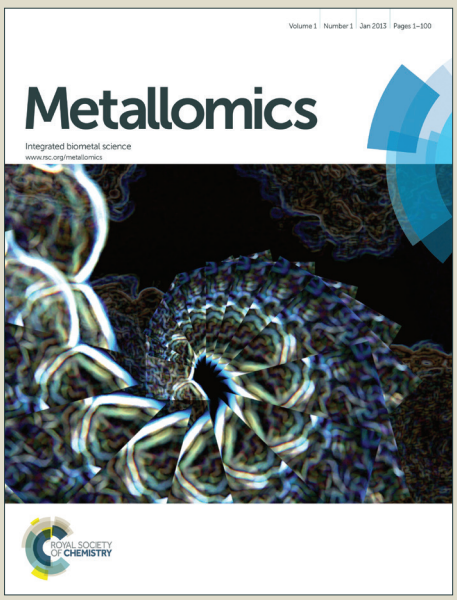

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ARTICLE TYPE

Rat brain oxidative damage in iron and copper overloads

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This study reports on the acute brain toxicity of Fe and Cu in male Sprague-Dawley rats (200 g) that received 0 to 60 mg/kg (ip) $FeCl_2$ or $CuSO_4$. Brain metal contents and time-responses were determined for rat survival, *in situ* brain chemiluminescence and phospholipid and protein oxidation products. Metal doses hyperbolically defined brain metal content. Rat survival was 91% and 60% after Fe and Cu overloads. Brain metal content increased from 35 to 114 µg Fe/g and from 3.6 to 34 µg Cu/g. Brain chemiluminescence (10 cps/cm^2) increased 3 and 2 times after Fe and Cu overloads, with half maximal responses (C_{50}) at 38 µg Fe/g brain and 15 µg Cu/g brain, and with half time responses ($t_{1/2}$) at 12 h for Fe and 20 h for Cu. Phospholipid peroxidation increased 56% and 31% with C_{50} of 40 µg Fe/g and 20 µg Cu/g and with $t_{1/2}$ of 9 h and 14 h. Protein oxidation increased 45% for Fe with C_{50} at 40 µg Fe/g and 18% for Cu with 10 µg Cu/g and $t_{1/2}$ of 12 h for both metals. Fe and Cu brain toxicities are likely mediated by a Haber-Weiss type $HO^{\bullet}$ formation with subsequent oxidative damage.

Keywords: Iron toxicity; copper toxicity; brain oxidative damage; brain chemiluminescence; lipid peroxidation; protein oxidation.

1. Introduction

The mammalian brain is a highly evolved organ with a very active aerobic metabolism. For instance, the human brain while accounting for only 2% of body weight receives 15% of the cardiac output and takes 25% of total glucose consumption and 20% of total body O_2 uplake[1]. In addition, the brain has limited glycogen and fat reserves with a low anaerobic metabolic activity[1]. Thus, dyshomeostasis or situations disrupting the well coupled reactions of mitochondrial energy production have deleterious effects in the brain with eventual lethal consequences.

The mammalian brain, as a fully aerobic organ, requires Fe and Cu for essential mitochondrial energy yielding processes. Fe is in the active centre of cytochromes and Cu in the catalytic centres of cytochrome oxidase and of Cu,Zu–SOD[2]. This is the major cuproenzyme of the brain and constitutes the 25% of total brain Cu[3]. Rat and human brain mitochondria contain about 3 µg Fe/g wet brains, two thirds as Fe-S and His-Fe centres and one third as cytochromes. Mitochondrial Fe accounts for about 8% of total brain Fe; the rest is mainly cytosolic ferritin. Brain mitochondrial Cu, is restricted to cytochrome oxidase and accounts for about 2 µg Cu/g wet brain in rats and humans. The brain content of cytosolic Cu,Zn-S0D is 1 µg Cu/g wet brain in rats and 4 µg Cu/g wet brain in humans[3].

The mammalian brain actively concentrates Fe and Cu[4,5]. The normal Fe content in rats and humans is 35-40 µg Fe/g wet brain[6,7] (or 210 µg Fe/g dry brain[2,8]). Total Cu content in the brain is about 2 µg Cu/g wet brain in rats[8] and 6 µg Cu/g wet brain in humans[6].

The movements of Fe and Cu across the blood brain barrier (BBB) are regulated and there is no passive diffusive flux of the metals from blood to the brain[9]. Iron TfR – mediated transport at the BBB are responsible for Fe entry to the brain parenchyma, and once within the brain, Fe is transported form interstitial fluid to neurons by transferrin. Copper is internalized in the brain by the BBB Cu transporter (CTr1) and by the Cu importing ATPase (ATP7A)[10].

There are differences in metal contents in different brain areas. The point is relevant for brain areas that are particularly sensitive to some pathology, as substantia nigra and striatum body in parkinsonism. The following numbers are given refered to g of human wet brain. Fe content in cortex is 45 µg/g; in hippocampus, 37 µg/g; in caudate nucleus, 27 µg/g; in putamen, 26 µg/g and in substantia nigra, 70 µg/g[11]. A quantitative MR imaging of human brain reported 23 µg Fe/g in caudate nucleus and 38 µg Fe/g in the putamen[12]. Concerning Cu, the contents are: 2.5 µg/g in the cortex, 2.1 µg/g in hippocampus and 6 µg/g in substantia nigra[8].

Similarly, rat brain shows differences in metal content in different brain areas. From a total contents of about 35-40 μg Fe/g whole wet brain and 3.5 μg Cu/g whole wet brain, there are 7 μg Fe/g and 1.5 μg Cu/g in brain cortex; and 21 μg Fe/g and 1.1 μg Cu/g in hippocampus[8,13].

Brain Fe and Cu deficiencies or excesses describe metal dyshomeostasis as a threshold to pathologic situations. Both metals duly illustrate the concept of hormesis: they are required at low levels for physiological function but at higher levels they show toxic effects[14].

The transition metals Fe and Cu are biochemically redox active: the ferrous form (Fe^{2+}) and the cuprous form (Cu^{+}) are able to antioxidize whereas the ferric (Fe^{3+}) and the cupric (Cu^{2+}) forms are able to catalyze the homolysis of H_2O_2 and of ROOH with formation of the powerful oxidant $HO^{\cdot}$[14,15].

This study reports on the acute brain toxicity of Fe and Cu by determining rat survival, brain metal contents, *in situ* brain chemiluminescence and phospholipid and protein oxidation products to describe the sensitive and free-radical mediated processes after metal overloads.

2. Experimental methods

2.1. Experimental animal model

Sprague-Dawley male rats (200 g) received increasing doses of ferrous chloride ($FeCl_2$, 0-60 mg/kg, ip, n = 24) or cupric sulfate ($CuSO_4$, 0-30 mg/kg, ip, n = 24) while control rats received the same volume of 0.9 % NaCl and were sacrificed after 16 h of metal administration, when a maximal and stable metal level in the brain was reached. In order to determine the time course of the effects after metal injections, rats received in a separate experiment a single dose of 30 mg $FeCl_2$ (n=24) or 10 mg $CuSO_4$ (n=18) and were sacrificed after 0 to 48 h.[14] Rats were anesthetized with 15 % (w/v) urethane at 1.5 g/kg (ip).

In situ brain chemiluminescence was determined 15 min after anesthesia, with stable photoemission readings, usually after 5 min. After recording brain chemiluminescence, animals were sacrificed and brains were excised for the determinations of oxidative damage. 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).

2.2. Animal survival

Rat survival was followed during 48 h after acute treatments with Fe and Cu and with control rats receiving the same volume of 0.9 % NaCl.

2.3. *In situ* brain chemiluminescence

The whole procedure followed a previously used protocol[14-16]. The head skin of the anesthetized rats was removed, the parietal bones were cut out with a curved scissor, and the exposed brain was washed with 0.9 % NaCl to remove blood from the cranial cavity. Brain was exposed and the animal was covered with aluminum foil, in which an about 2.0 cm^2 window allowed brain cortex exposure. Brain chemiluminescence was measured with a Johnson Foundation photon counter (Johnson Research Foundation, University of Pennsylvania, Philadelphia, PA, USA) and photoemission was expressed as counts per second (cps/cm^2) of exposed brain surface.

Surface brain chemiluminescence, by measuring the photons emitted by the tissue, determines: 1) directly, the steady state concentration of $^1O_2$, the electronically excited state of normal atmospheric oxygen, 2) indirectly, the rate of free-radical chain reactions occurring in the tissue.[16] The spectral analysis of *in situ* organ chemiluminescence shows a red emission in the 620 to 700 nm region that indicates that $^1O_2$ is the main photon source.[17,18] Control rats showed an *in situ* brain chemiluminescence of 10 ± 1 cps/cm^2 in physiological conditions. Considering 10 % of efficiency of the photomultiplier and a 0.5 factor for the 180° geometry of the surface, brain emission is estimated in 200 photons/s cm^2. As the active tissue depth from the detected photoemission is 0.1 mm, brain photoemission is estimated as 2 x 10^4 photons/s.mL, or 2 x 10^7 photons/s. L, or 3.3 x 10^{-17} einsteins/s (1 einstein = 6.02 x 10^{23} photons).[16] The photoemission is due to the dimol emission of $^1O_2$, with a rate constant of the diffusion controlled process of $k = 1 \times 10^{10}\ M^{-1}\ s^{-1}$. The calculated physiological steady state concentration of $^1O_2$ in the brain[16] is 5.8 x 10^{-14} M[11], according to the following equation: $d(hv)/dt = k\,[^1O_2]\,[^1O_2]$.

2.4. Brain homogenate

After determination of brain chemiluminescence, the whole brain was rapidly excised, weighed and homogenized in a medium consisting of 120 mM KCl, 30 mM phosphate buffer, pH 7.4, at a ratio of 1 g brain/9 mL of buffer at 0 °C. The homogenates were centrifuged at 600 g for 10 min to discard nuclei and cell debris. The supernatant, a suspension of organelles and plasma membranes in diluted cytosol, was used as tissue homogenate. The homogenates were frozen and thawed to disrupt mitochondrial membranes and to release mitochondrial contents.[19,20]

2.5. Phospholipid peroxidation

This assay was performed by the thiobarbituric acid-reactive substances (TBARS), as described by Fraga *et al.*[21] The homogenate was added to 20 % w/v thrichloroacetic acid, 4 % w/v buthylhydroxytoluene in ethanol and 0.7 % w/v thiobarbituric acid. The deproteinized supernatant was heated at 100 °C for 20 min, the absorption of the pink solution was determined at 535 nm ($\varepsilon = 156\ mM^{-1}\ cm^{-1}$) and expressed as nmol TBARS/ g brain.

2.6. Protein oxidation

Protein oxidation was assessed spectrophotometrically at 340 nm, by determining protein carbonyl groups in brain homogenates after conjugation with 2,4 dinitrophenylhydrazine.[22]

2.7. Oxidative damage index

The index 1/3 [(TBARS-metal/TBARS-control) + (carbonyls-metal/carbonyls-control) + (chemiluminescence-metal/ chemiluminescence-control)] was calculated for each metal content and time point. The three members of the equation contribute equally to the index that is 1.0 in the absence of oxidative damage.

2.8. Brain metal content

Brain metal contents were measured in an atomic absorption spectrometer (Buck model 200 A, East Norwalk, Connecticut, USA). Samples were incinerated for 4 h in a graphite muffle at 500 °C. Calibration was made by using standard solutions of 0.1 to 3 mg/L of Fe and Cu. Results are expressed in µg metal/g wet brain.

2.9. Protein determination

Protein contents were measured by the Folin reagent using bovine serum albumin as standard.

2.10. Chemicals

Chemicals were purchased from Sigma-Aldrich Chemical Co (St Louis, Mo).

2.11. Data analysis

Data were analyzed statistically using the Tukey-Kramer multiple comparison test. Statistical significance was indicated by p values ($p < 0.05$ and $p < 0.01$). Samples were duplicates and the results indicate mean values ± standard error of the mean (SEM).

3. Results

3.1. Animal survival

Rat survival at 48 h after metal overload was 91 % after a dose of 60 mg Fe/kg and 60% after a dose of 30 mg Cu/kg. The calculated Cu lethal dose to produce 50 % animal death (LD_{50}) was 60 mg/kg (Fig. 1A).

Rat mortality linearly correlated with brain metal content ($r = 0.97$ for Fe and $r = 0.92$ for Cu), and with the time after treatment ($r = 0.82$ for Fe and $r = 0.94$ for Cu) ($p < 0.01$).

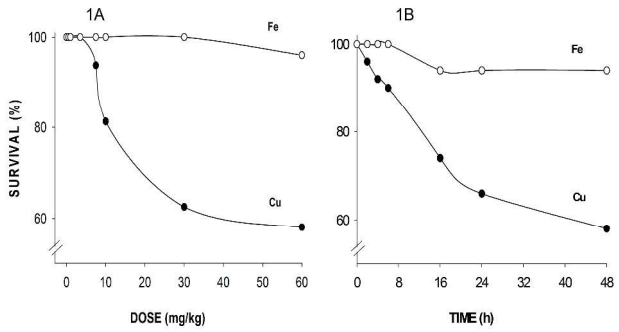

Fig.1. Rat survival after acute Fe and Cu overloads.

3.2. Fe and Cu brain accumulation after metal overload

The brain content of Fe and Cu increased up to 3 and 8 times, respectively, after acute metal overloads, from 35 ± 5 to 114 ± 3 µg Fe/g brain and from 3.6 ± 0.8 to 34 ± 2 µg Cu/g brain (Fig. 2A). The Fe and Cu contents for normal brains are agree with previous reports.[6,8] The maximal metal contents in the brain were reached at doses of 30 mg Fe/kg and 10 mg Cu/kg. The C_{50} of metal accumulation are summarized in Table 1.

Table 1. Brain metal content (C_{50}) and time ($t_{1/2}$) that produces 50% of the maximal oxidative response.

Oxidative stress/damage	C_{50}Fe (µg/g brain)	$t_{1/2}$ Fe (h)	C_{50} Cu (µg/g brain)	$t_{1/2}$ Cu (h)
Metal brain content	80	10	20	12
Brain chemiluminescence	38	12	15	20
Phospholipid oxidation	40	9	20	14
Protein oxidation	40	12	10	12

Time course of metal accumulation was followed after administration of 30 mg/kg of Fe or 10 mg/kg of Cu, and the determinations were made in the period of 0 to 48 h. The time for half maximal effect ($t_{1/2}$) in metal accumulation are shown in Fig. 2B and Table 1.

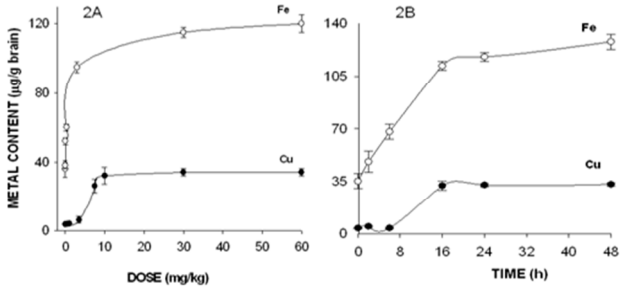

Fig.2. Dose and time dependence of brain metal content in acute Fe and Cu overloads. 2A, Fe (○) and Cu (●) contents and dose

dependence; 2B, Fe (○) and Cu (●) accumulation during time of acute exposure.

3.3. Organ chemiluminescence and oxidative stress indicators

The increase in the brain content of both metals produced significant increases in *in situ* brain chemiluminescence, which indicates an increased rate of free-radical-mediated reactions in the *in vivo* situation[14,16,17,23] (Fig. 3A). The time course (Fig. 3A) and the dose-effect (Fig. 3B) dependences of brain chemiluminescence after Fe and Cu intoxication showed that photoemission increased up to 3 times after Fe overload and about twice after Cu overload. Brain chemiluminescence correlated (r = 0.91 and 0.97) with Fe and Cu brain content ($p < 0.01$). The time for half maximal effect ($t_{1/2}$) and C_{50} for brain chemiluminescence are listed in Table 1.

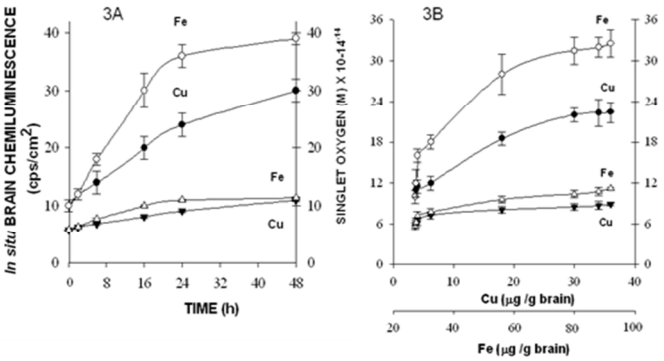

Fig.3. 3A. Time course of *in situ* rat brain chemiluminescence determined after Fe (○) and Cu (●) overloads (left ordinate), and calculated singlet oxygen steady state level (right ordinate) after Fe (Δ) and Cu (▼) overloads; 3B, *In situ* rat brain chemiluminescence after Fe (○) and Cu (●) overloads (left ordinate), and singlet oxygen steady state level (right ordinate), after Fe (Δ) and Cu (▼) overloads.

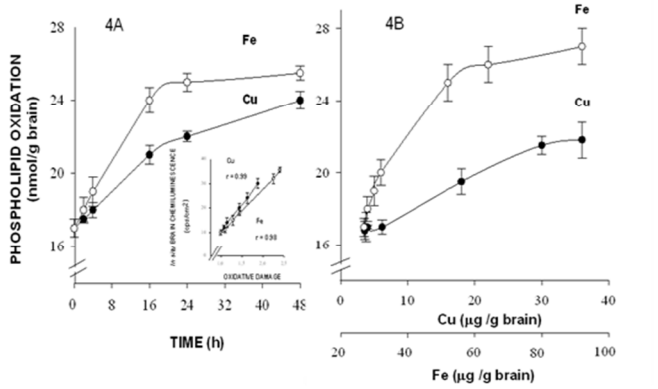

Fig.4. Time (4A) and content dependence (4B) of phospholipid peroxidation, measured as TBARS in brain homogenates after Fe (○) and Cu (●) overloads. Insert: Brain chemiluminescence as a function of oxidative damage index for Fe (○) and Cu (●) overloads ($p < 0.05$).

The content of phospholipid peroxidation products, measured as TBARS, increased in brain homogenates after Fe and Cu overloads. In Fe-treated rats, lipid peroxidation products increased 56 % and for Cu overload, the increase was 31 % (Fig. 4A and 4B). The content of lipid peroxidation products correlated with brain metal content (r = 0.97 for Fe and r = 0.98 for Cu) ($p < 0.01$). The time for half maximal effect ($t_{1/2}$) and C_{50} for the content of phospholipid oxidation products are given in Table 1.

Protein oxidation products, measured as protein carbonyl groups in brain homogenates, markedly increased after Fe (45 %) and Cu (18 %) accumulation in the brain (Figs. 5A and 5B). The $t_{1/2}$ and C_{50} in metal accumulation are listed in Table 1. Protein oxidation products correlated with brain metal content (r = 0.92 for Fe and r = 0.96 for Cu); ($p < 0.01$ for both).

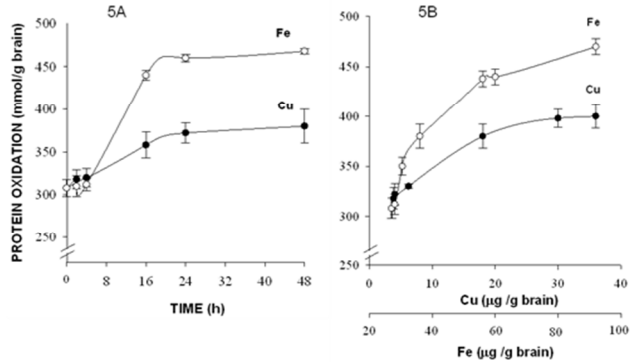

Fig.5. Time (5A) and content (5B) dependence with protein oxidation, measured as protein carbonyl groups in brain homogenates, after acute Fe (○) and Cu (●) overloads.

The oxidative damage index combines three indicators, brain TBARS and protein carbonyls in homogenates and *in situ* brain chemiluminescence, and gives a quantification of the free-radical-mediated damage in cells and tissues. A positive correlation between oxidative damage and time after metal overload showed the significant correlations of r = 0.79 and 0.96 for Fe and Cu (Fig. 6A), and a subjacent correlation between oxidative damage and brain metal content of 0.92 for Fe and 0.85 for Cu (Fig. 6B). The three indicators of oxidative damage show strong correlations between them (r = 0.98 to 0.99, $p < 0.05$ to 0.01).

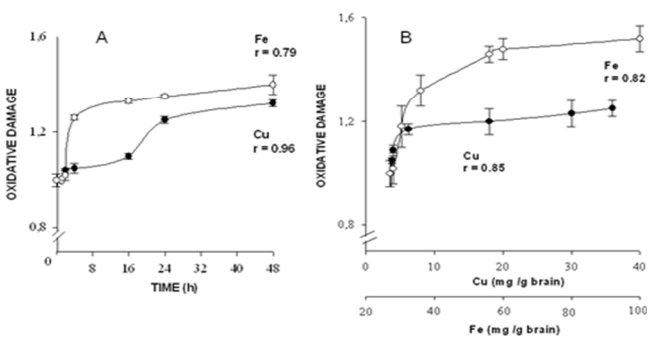

Fig.6. Oxidative damage index as a function of time (A) and of brain Fe (○)and Cu (●) content (B) after metal overloads.

The times ($t_{½}$) and brain metal contents (C_{50}) that produced half maximal oxidative effects in the determined indicators are compared in Table 1. The direct indicators of oxidative damage (brain chemiluminescence, TBARS and protein carbonyls) show C_{50} in the ranges of 38-40 μg Fe/g brain and of 10-20 μg Cu/g brain. The ranges identify a main and common chemical process. Moreover, the kinetic description given by the $t_{½}$ points out to establish the approximate sequence of their occurrence[20] (Fig.7).

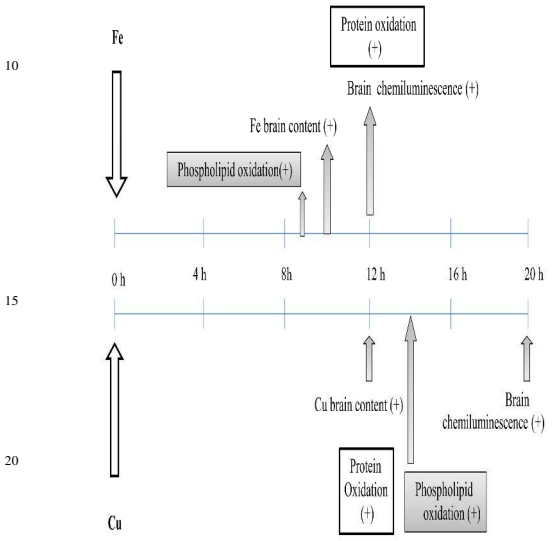

Fig.7. Scheme of the time course of the oxidative damage processes in rat brain after Fe (up) and Cu (down) overloads. (+) indicate increased values in metal overloads.

Concerning to the mechanism of the oxidative stress and damage that follows to brain Fe and Cu overloads, the indicators of the oxidative damage processes, brain chemiluminescence and phospholipid and protein oxidation, show a similar C_{50} for Fe overload (Table 1), indicating that free radical generation, brain chemiluminescence and phospholipid and protein oxidation share a common biochemical mechanism, consistent with a Fenton-Haber-Weiss mechanism with similar dependence on the rate limiting factors.

In Cu overload, brain Cu contents were hyperbolically related to metal doses. The time courses show identical values for brain Cu content and protein oxidation with a similar $t_{½}$ for phospholipid oxidation and a slight delay for the maximal brain chemiluminescence.

4. Discussion

Brain blood barrier regulates Fe and Cu ion levels. However, intraperitoneal metal overloads lead to a increase in brain metal contents (Fig.2). Metal transport through the BBB results in significant increments in brain metal levels, which indeed lead to neurological dysfunction and neuronal death.

Brain accumulation of Fe and Cu, in order to keep effective reserves of these two vital transition metals, is an efficient process, considering the linear part of the accumulation/dose ratio, that is 3.4% and 3.7% for Fe and Cu salts (Fig.2A). In liver, a recent report with the same experimental model of Fe and Cu overloads showed 7 % for Fe and 20 % for Cu accumulation/dose ratios.[14] It is understood that brain oxidative damage of Fe and Cu occurs when the metal overloads overwhelm brain storage proteins, which mainly consist of ferritin[24] and mitochondrial ferritin[25] for Fe, and metallothionein[26] for Cu storage. Ceruloplasmin[27] and DMT1[10] are involved in Cu transport.

The current knowledge in metallo-biochemistry sustains two main hypotheses for the molecular mechanism of the transition metal toxicity in mammalian cells and organs. The first concept is that the reduced forms of Fe and Cu ions catalyze the homolytic scission of the O-O bond in H_2O_2 and ROOH with formation of $HO^{\bullet}$ and $RO^{\bullet}$. These two free radicals are able to initiate toxic rates of phospholipids peroxidation and protein oxidation.[14,28] The second concept is that Fe^{3+} and Cu^{2+} ions react with intracellular reduced glutathione, deplete the cells of this antioxidant and disrupt the thiol redox signalling of physiological regulation.[29] Both hypotheses are not exclusive and both processes are likely to take place simultaneously. Moreover, the two processes are bound to show synergic effects leading to the same oxidative damage in phospholipids and proteins, which are clearly supported by the experimental evidence.

The first hypothesis is based on the cyclic redox properties of the couples $Fe^{2+}/Fe^{3+}$and Cu^{1+}/Cu^{2+}. The oxidized forms are complexed and stored in intracellular proteins, ferritin for Fe^{3+} and methalothionein for Cu^{2+}, from where they are released after reduction by cytosolic O_2^{-}. The reduced forms, Fe^{2+} and Cu^{1+}, react with H_2O_2 or ROOH and catalyze the homolytic scission of the O-O bond in a Fenton-like reaction, which is considered the rate-limiting step for the free-radical-mediated processes of phospholipid peroxidation and protein oxidation. [28] The cytosolic steady-state concentrations of Fe^{2+} and Cu^{1+} that would be able to produce a 2-3 times enhanced rate of $HO^{\bullet}$ formation are not known. However, the reaction of $HO^{\bullet}$ formation is a second order reaction with an estimated steady state concentrations of 1.0 and 0.1 μM for the ions Fe^{2+} and Cu^{1+}, and 1.0 μM for H_2O_2. Nevertheless, there is evidence of a 2-3 times enhanced brain chemiluminescence, a process occurring *in vivo* under physiological conditions, that is consistent with a similarly increased rate of free-radical mediated reactions. The sequence (1) increase in $HO^{\bullet}$ formation, (2) increase in phospholipid peroxidation and brain chemiluminescence, and (3) cytotoxicity, is supported by experimental evidence from *in vitro* phospholipid mixtures supplemented with H_2O_2 and Fe or Cu,[15] and from rat liver after Fe and Cu overloads.[14] Considering the O-O bond homolysis, there are two points of view. The classical one is that the free ions Fe^{2+} and Cu^{1+}catalyze the reaction. The second is that Fe^{2+} and Cu^{1+} bind to a specific peptide or protein sites and

react with H_2O_2 generating $HO^{\bullet}$ that immediately oxidizes neighbouring amino acids with cross-linking, fragmentation and denaturation.[30] This mechanism seems to apply to β-amyloid in Alzheimer disease.[31]

The second hypothesis implies depletion, from partial to markedly significant, of intracellular glutathione (2-3 mM in brain cytosol) and other essential thiol groups in enzymes, regulatory factors and proteins. The high affinity of the cysteine –SH group for the transition metals Fe and Cu provides support for the hypothesis.[31] However, it is not clear if the levels of Fe^{3+} and Cu^{2+} in the sub-μM range are enough to deplete cells of reduced glutathione (GSH) which is in the mM range. The heavy metals Pb[32] and Hg[33], have a divalent oxidation state that does not undergo redox cycling. However, they have been reported to produce oxidative stress and damage. This fact gives support to the involvement of thiol groups in the mechanism of metal toxicity.

The two hypotheses imply, respectively, an increase in the oxidant $HO^{\bullet}$ and a decrease in the antioxidant GSH, two equivalent actions that establish the oxidative stress situation, according to the classic definition by Sies.[29] Subsequent elaborations of the oxidative stress situation and definition, as the one that considers the increases in the steady-state concentrations of the reactive oxygen species ($O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$, $ROO^{\bullet}$, and $^1O_2$)[23] and the one that considers the disruption of the homeostasis of cell thiol groups[34] are in agreement with the increase in the rate of formation and level of $HO^{\bullet}$ radical and the decrease in GSH and other thiol levels. It is then clear that the Fe and Cu overloads lead to an oxidative stress and damage situation with increased rates of the free-radical mediated processes of brain chemiluminescence, phospholipid peroxidation and protein oxidation.

The free radicals $HO^{\bullet}$ and $RO^{\bullet}$ are certainly able to start chains of free-radical mediated processes. Non-enzymatic phospholipids peroxidation is a free-radical-driven chain reaction in which one radical $HO^{\bullet}$ or $RO^{\bullet}$ induces the oxidation of a large number of phospholipids containing unsaturated fatty acids. The process is considered here as central, due to the use of intact brain chemiluminescence as one indicator of organ oxidative stress and damage. Light emission is one of the secondary processes associated to phospholipids peroxidation. The molecular mechanism of chemiluminescence is that two $ROO^{\bullet}$, produced in the peroxidation of unsaturated fatty acids, collide and generate singlet oxygen ($^1O_2$), according to the Russell mechanism. In turn, two $^1O_2$ collide and by dimol emission release photons of 640 and 710 nm.[16] The consequence of a massive process of phospholipid peroxidation is lethal for cells. Secondary and highly reactive intermediates are produced ($^1O_2$ is one of them) that lead to fragmented fatty acids in the membrane structures, to lipid-lipid and to protein-lipid cross-linking, and to impairment in membrane function and enzyme activity. Some aldehydes that are products of the phospholipid peroxidation process, such as malonaldehyde and 4-hydoxynonenal, react with protein lysine amino, cysteine sulfhydryl and histidine imidazole groups.[35,36]

A sudden delivery of iron to the brain can occur after hemorrhagic stroke, which is the bleeding into the brain or within the subarachnoid space as a consequence of a ruptured cerebral aneurysm or head injury. After this injury, an acute delivery of iron to the brain occurs. The blood spilled into the brain is metabolized by heme-oxygenases yielding biliverdin, carbon monoxide and iron. Brain areas affected by the haemorrhage will face a sudden iron overload, which would overwhelm cellular ferritin storages, generating oxidative stress and further contributing to damage the affected tissue. However, while chelating therapy does lower brain iron content, some authors have found it ineffective in improving the outcome of patients.[37]

Due to the essential role of Fe and Cu on mitochondrial oxidative metabolism, alterations in organ or blood Fe and Cu contents are a topic of interest in a series of pathologies as: cardiovascular diseases and atherosclerosis[38], rheumatoid arthritis[39] and haemochromatosis.[40] Cu dyshomeostasis has been implicated in diabetes [41] and in Wilson [42] and Menkes diseases.[43]

One subject of major interest related to the scope of this report is the role of the transition metals, Fe and Cu, in neurodegenerative diseases, especially in Alzheimer´s disease. There was a current hypothesis that dysregulated Fe in the cortex of Alzheimer´s patients, through an interaction with β-amyloid, leads to neuronal oxidative stress and to cell damage.[44] However, the experimental data do not support such concept, according to the recent metaanalysis by Schrag et al.[45] Moreover, it has been reported that several brain regions from patients with Alzheimer's disease have decreased Cu contents[46] stated that Cu supplementation may be protective against Alzheimer disease[47].

5. Conclusions

Brain oxidative damage is produced by increased Fe and Cu contents which are hyperbolically related to metal doses. Increased brain Fe and Cu contents produced similar increases of *in vivo* brain chemiluminescence and of phospholipids and protein oxidation products in the homogenates. The observed effects are produced at similar Fe and Cu C_{50} for the metal-promoted processes and indicate a common biochemical mechanism. The $t_{1/2}$ point out to a free-radical mediated processes of phospholipids oxidation in the case of both metals with an early protein oxidation in the case of Cu.

Abbreviations

$^1O_2$: singlet oxygen
BBB: blood-brain barrier
C_{50}: metal brain content that produces 50% of the maximal oxidative response
cps: counts per second
Cu,Zn-SOD: cu,Zn- Superoxide dismutase
LD_{50}: lethal dose 50, dose that reduces survival to 50 %
SEM: standard error of the mean
$t_{1/2}$: time that produces 50% of the maximal oxidative response

TBARS: thiobarbituric acid-reactive substances

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The authors declare that they have no conflict of interest.

Notes and references

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Graphical Abstract