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Review Article

Rat Brain Damage due to Iron and Copper Toxicity

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Abstract

Brain damage is associated to oxidative stress in iron (Fe) and copper (Cu) overloads in rats, in a dose- and time-dependent accumulation of the metals in the organ. The generation of singlet oxygen in brain measured *in vivo* by *in situ* chemiluminescence indicates that Fe and Cu overloads increased phospholipid and protein oxidation, and decreased non enzymetic endogenous antioxidants content in the organ, mainly glutathione (GSH). These results fit with a Fenton/Haber-Weiss type reaction between iron, copper and endogenously produced superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) to yield hydroxyl radical ($OH^{\bullet}$), as well as reactions involving thiol groups of GSH and proteins.

Keywords: Brain; Iron; Copper; Oxidative stress; Transition metals

Abbreviations

ATOX 1: Antioxidant Protein 1; ATP7A: ATPase copper transporting alpha; ATP7B: ATPase copper transporting beta; BBB: Blood–brain barrier; C_{50}: Metal concentration in brain for half maximal effects; Ctr1: Copper transport protein; Cu, Zn-SOD: Cytosolic copper-zinc superoxide dismutase, SOD1; GPx: Glutathione peroxidase; GSH: Glutathione; H_2O_2: Hydrogen peroxide; $OH^{\bullet}$: Hydroxyl radical; $^1O_2$: Singlet oxygen; $O_2^{\bullet-}$: Superoxide anion; $R^{\bullet}$: Alkyl radical; $ROO^{\bullet}$: Lipidhydroperoxyl radical; ROOH: Lipid hydroperoxide; TBARS: Thiobarbituric acid reactive substances; $t_{1/2}$: Time for half maximal effects; Tf: Transferrin; TfR: Transferrin receptor-mediated iron transport.

Introduction

The mammalian brain is a highly evolved organ with very active aerobic metabolism [1]. Iron (Fe) and copper (Cu) are essential elements widely employed due to their redox properties by enzymes in the catalysis of complex reactions involving electron transfer. These biometals are present into the active site of catalase, cytochrome oxidase and superoxide dismutase (Cu, Zn-SOD, and SOD1). While Fe and Cu show a great utility for living organisms due to its ability to undergo redox cycling, this would entail a potential hazard in cases where the cells were not able to control metal ions concentration [2]. For this reason, the free Fe or Cu concentration is kept at extremely low levels in the intracellular milieu by several proteins and small molecules which chelate it and prevent its potentially deleterious reactions.

Iron in brain

The mammalian brain is the organ with the highest Fe content, after liver [3]. The normal Fe content in rats and humans is 35–40 μg of Fe g^{-1} of wet brain [4,5]. The movement of Fe across the blood–brain barrier (BBB) is regulated and there is no passive diffusive flux of the metal from blood to the brain. Trannsferrin (Tf) receptor-mediated (TfR) iron transport at the BBB is responsible for Fe entry into the brain parenchyma, and once within the brain, Fe is transported from the interstitial fluid to neurons by Tf [6,7].

There are differences in Fe content in different human brain areas: in the cortex is 45 $\mu g g^{-1}$; in the hippocampus, 37 $\mu g g^{-1}$; in the caudate nucleus, 27 $\mu g g^{-1}$; in the putamen, 26 $\mu g g^{-1}$ and in the substantia nigra, 70 $\mu g g^{-1}$ [8]. Similarly, rat brain shows differences in metal content in different brain areas. Of the total content of about 35–40 μg of Fe per g of whole wet brain and, 7 μg of Fe per g are present in the brain cortex and 21 μg of Fe per g in the hippocampus [9,10].

Copper in brain

Cu is a redox active metal able to undergo redox cycling and is thereby potentially toxic for cells. The metal transportation and distribution among the different tissues and cells is tightly regulated by a set of transporters which have just recently started to be understood. After ingestion, Cu reaches the intestine where it is taken up by the enterocytes at the lumen surface of the microvilli by copper transport protein (Ctr1), which forms a pore in the membrane allowing the passive influx of Cu(I) to the cell. Once within the cell, Cu(I) binds to antioxidant protein 1 (ATOX1), a Cu chaperone which mediates the intracellular trafficking of the metal. Another chaperone, the ATPase copper transporting alpha (ATP7A), is located at the basolateral membrane of enterocytes, where it mediates the efflux of the metal into the bloodstream. Ceruloplasmin is the main Cu binding plasma protein and its function is not only restricted to systemic copper transport but is also a ferroxidase involved in iron metabolism [11].

The normal Cu content in humans is 100–120 mg of Cu, approximately distributed 50% in bones and skeletal muscle, 15% in skin, 15% in bone marrow, 8-15% in liver an 8% in brain. About of 95% of Cu is bond to ceruloplasmin and 5% to albumin and other molecules [12].

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Table 1: Rat Brain Oxidative Damage after Fe and Cu overlods[1].

	Fe			Cu		
Property	Effect %)[2]	$t_{1/2}$ (h)	C_{50}(µg Fe/g)[3]	Change (%)[2]	$t_{1/2}$ (h)	C_{50}(µg Cu/g)[3]
Metal content	(+) 225	10	75	(+) 850	12	20
In situ brain chemiluminescence	(+) 200	12	40	(+) 100	15	20
Lipid peroxidation	(+) 56	9	40	(+) 35	15	20
Protein oxidation	(+) 45	12	40	(+) 18	12	15
Hydrophilic antioxidant	(-) 50	10	33	(-) 70	12	10
Lipophilic antioxidant	(-) 75	15	38	(-) 60	10	15
GSH content	(-) 89	8	30	(-) 45	12	10
GSH/GSSG ratio	(-) 75	4	30	(-) 75	2	4
SOD1 activity	(+) 200	10	50	(+) 90	12	25
Catalase activity	(+) 150	8	20	(+) 90	10	40
GPx activity	(-) 73	8	30	(-) 27	15	20

[1]Adapted from refs. [26-27]: [2]in % of increased (+) or decreased (-) property compared with control rats; [3]determined by atomic absorption.

Brain damage from iron toxicity

A sudden delivery of Fe (Fe^{2+} or Fe^{3+}) to the brain can take place after hemorrhagic stroke, which is the bleeding in the brain or within the subarachnoid space as a consequence of a ruptured cerebral aneurysm or head injury. Here, an acute delivery of Fe to the brain occurs when the blood spilled into the brain is metabolized by heme-oxygenases yielding biliverdin, carbon monoxide and Fe. This sudden iron overload in brain areas overwhelms cellular ferritin storage, generating oxidative stress and further contributing to the damage of the affected tissue [13].

Currently, researchers have reported that deregulated Fe and Cu in the brain cortex of patients with Alzheimer's disease, is associated with amyloid plaques deposition and protein aggregation in affected tissue [14-16], and in Parkinson's disease [17,18]. Because of this, is interesting to study the Fe and Cu effect in brain by characterizing the oxidative stress and damage that it produces by free radical production through Fenton/Haber-Weiss reaction mechanism.

Brain damage from copper toxicity

Wilson's disease is a autosomal recessive disorder, with an incidence of 1:30.000 to 1:100.000, characterized by the excessive accumulation of Cu in liver that leads to cirrhosis and chronic hepatitis, which ultimately end in liver failure, and in brain, neurological defects (parkinsonian features, seizures) and psychiatric symptoms (personality changes, depression, and psychosis). The genetic alteration underlying the pathology involves a defective pump, the ATPase copper transporting beta (ATP7B), required for excretion of Cu from the hepatocytes to the bile. The impaired or absent function of ATP7B leads to an increased intracellular concentration of Cu within the hepatocytes, and toxic biochemical oxidation process that end in cell death [13].

Toxic mechanism of iron and copper overload in rat brain

The toxicity of Fe and Cu are mainly due to their ability to change oxidation states when taking part in redox reactions such as Fenton [19] and Haber-Weiss [20] involved in the $HO^{\bullet}$ generation [21,22]. An examination of the intracellular steady-state concentrations of the reactive oxygen species: $O_2^{\bullet-}$ (10^{-11}-10^{-10} M), H_2O_2 (10^{-7}-10^{-6} M), $HO^{\bullet}$ (10^{-17}M), alkyl radical ($R^{\bullet}$, 10^{-12}M), lipid hydroperoxyl radical ($ROO^{\bullet}$, 10^{-10}M), lipid hydroperoxide (ROOH, 10^{-7}-10^{-6} M), singlet oxygen ($^1O_2$, 10^{-14}M) clearly indicates that H_2O_2 and ROOH are quantitatively the predominant species by factor of 10-10^6 in physiological conditions [23]. However, in a non-physiological situation the steady state concentration of specific oxygen reactive species may rise above others and led to the oxidative stress condition.

The classical concept of oxidative stress was the idea of an unbalance between the production of oxidants and the antioxidant defenses in cells and tissues [24]. The increase in oxidants, usually free radicals and related species, and the shift in the –SH/-SS- redox couple occur simultaneously and have synergistic effects. The process, if sustained, leads to molecular and cellular damage, eventually to neuronal death [25].

The toxicological effects of Fe and Cu overloads were studied in rat brain by the kinetic and holistic analysis, considering the time and metal concentration in brain for half maximal effects ($t_{1/2}$ and C_{50}).

Oxidative damage in brain: Toxicity and oxidative processes in iron and copper overloads

Sprague Dawley male rats (200-210 g) received once a day: (a) for Fe $t_{1/2}$ determination, 6 mg Fe element; (b) for Fe C_{50} determination, 1-12 mg of Fe element; (c) for Cu $t_{1/2}$ determination, 2 mg Cu element; and (d) for Cu C_{50} determination, 0.6-6.0 mg of Cu element. The indicators of oxidative damage in brain and toxic effects of Fe and Cu are summarized in Table 1.

After intraperitoneal injection, Fe and Cu were accumulated in the brain in a dose- and time-dependent manner, reaching the maximal metal content in the organ (110±3 µg Fe/g and 32±2 µg Cu/g) at the time of 16 h after the acute metal overload [26] (Table 1). The intracellular accumulation of the metal might involve an enhanced rate of homolytic cleavage of H_2O_2 by Fe^{2+} and Cu^{1+} which would yield $OH^{\bullet}$, thus initiating the phospholipid and protein oxidation which show a good correlation with the accumulation of the metal in the brain.

In situ brain chemiluminescence is used as a sensitive indicator of oxidative damage to phospholipid membranes. From a kinetic

approach, brain *in vivo* chemiluminescence was observed earlier for Fe than Cu, and simultaneously with decreasing concentration of hydrophilic and lipophilic antioxidants and glutathione peroxidase (GPx) activity (Table 1). Increased in *in vivo* chemiluminescence indicates overproduction of singlet oxygen ($^1O_2$), as a consequence of lipid peroxidation, oxidation of phospholipids (measured as thiobarbituric acid reactive substances, TBARS) and protein (measured as carbonyl groups). The phospholipid oxidation is earlier in Fe than Cu toxicity and protein oxidation follow similar kinetics with both metal overloads, pointing out a common mechanism of oxidative process to proteins (Table 1).

Antioxidants are species which function is to decrease the level of oxidative chemical species. The non enzymatic (hydrophilic and lipophilic) antioxidant consumptions exhibit, from a kinetic approach, similar $t_{1/2}$, coincident with the biochemical markers of oxidative damage and changes for enzymatic activities [27], showing that Fe and Cu toxicities could be responding to a common mechanism of adaptive response in brain (Table 1).

GPx activity was decreased in rat brain with acute Fe and Cu overload [27] in a dose- and time- dependent manner, with different $t_{1/2}$, indicating that the oxidative processes may be due to the toxic products of decomposition of ROOH, that are not detoxified by Gpx, mainly in the case of Cu overload (Table 1).

GSH intracellular content in brain is 2 mM [28]. The endogenous antioxidant GSH shows a sharp decrease in its brain concentration as the Fe and Cu concentration in the organ increases, with a maximal decrease of 89-45% in the GSH content with 80 μg Fe/g brain and 30 μg Cu/g brain, respectively, after 16 h after the acute overload [27].

Conclusion

Fe and Cu accumulate in brain in a dose- and time-dependent manner. However, there is a lag phase of around 16h before the Cu concentration in the organ shows a significant increase, likely due to the presence of the blood brain barrier, which prevents the metal from freely diffusing into the interstitial space of the brain and allows its entrance only through specific transporters. The increased *in situ* chemiluminescence indicates an enhanced steady-state of $^1O_2$ along with an increased production of products of phospholipid peroxidation and protein carbonyl groups as a consequence of oxidative modifications of certain protein amino acids. The GSH concentration decays quickly after Fe and Cu overload, indicating an oxidative stress situation which shows a good correlation with the concentration of Fe and Cu in the organ.

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