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Time course and mechanism of brain oxidative stress and damage for redox active and inactive transition metals overload

Nidia FERRAROTTI[1], Rosario MUSACCO-SEBIO[2], Christian SAPORITO-MAGRIÑÁ[2], Juan Manuel ACOSTA[2], Marisa REPETTO[2]*

[1] Clinical Biochemistry Department, Faculty of Pharmacy and Biochemistry, Institute of Biochemistry and Molecular Medicine (IBIMOL), University of Buenos Aires -CONICET, Buenos Aires, Argentina.
[2] General and Inorganic Chemistry, Faculty of Pharmacy and Biochemistry, Institute of Biochemistry and Molecular Medicine (IBIMOL), University of Buenos Aires -CONICET, Buenos Aires, Argentina.

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ABSTRACT: The objective of this work was to study the *in vivo* time course of biochemical processes of oxidative damage in the brain of Sprague-Dawley rats that received an acute overload of the redox active metals iron (Fe) and copper (Cu), and the redox inactive cobalt (Co) and nickel (Ni). Oxidative stress indicators (phospholipid and protein oxidation), glutathione (GSH), antioxidant enzymes and NADPH oxidase activities, and the plasma inflammatory cytokine (IL-6) were measured. The results showed that in brain oxidative mechanisms for both sets of metal are different, however in both cases are irreversible. The mechanism for Fe and Cu oxidative damage is mediated by the generation of the free radical hydroxyl (Fenton reaction and homolytic cleavage of hydroperoxides). Two events of antioxidant protection prior to oxidation of phospholipids and proteins by Fe and Cu are considered. The first process is the use of GSH and the second is the increased activity of the Cu, Zn-SOD and catalase enzymes. The oxidative mechanism for metal redox inactive is the consumption of GSH, NADPH oxidase activation and inflammatory response mediated by IL-6. Co increased protein oxidation as a result of the inflammatory process. Ni produced increments of phospholipid oxidation and SOD activity.

Twenty three elements of the Periodic Table are present in living organisms, since they have essential biological functions and are considered as biometals (Repetto and Boveris, 2012).

Biometals in living organisms generate a phenomenon of hormesis, i.e., a biphasic response in which certain chemical agents at low doses cause desirable effects, but at high doses cause adverse effects. It is also defined as the phenomenon of the living response to the dose of a substance characterized by a beneficial effect at low dose and a toxic effect at high doses; small doses are essential to health, but in large doses have adverse effects (Boveris *et al.* 2012; Repetto and Boveris, 2012).

Reduction-oxidation (redox) reactions are one of the fundamental mechanisms of cell metabolism. Some transition metals ions are effective as intermediates in processes due to the redox reversibility in the oxidation state; this allows them to transfer or accept electrons to or from the substrate or cofactor involved (Halliwell and Gutteridge, 1984). Iron (Fe) and copper (Cu) are the most important for their abundance and functionality.

The toxic effects of transition metals are associated with different mechanisms of action. Fe, Cu, cobalt (Co) and nickel (Ni), like other transition metals, generate cellular toxicity when they exceed the minimum harmless concentrations (Kohen and Nyska, 2002). This condition creates a state of cellular oxidative stress by various mechanisms that, if sustained over time, lead to irreversible oxidative damage (Valko *et al.* 2005).

In a previous research, Fe, Cu, Co and Ni were classified into two groups in terms of their participation in the autoxidation of phospholipids and the Fenton reaction. Fe and Cu were considered as redox active metals, and Co and Ni, as redox inactive metals (Repetto *et al.* 2010).

*Address correspondence to: Marisa Repetto, mrepetto@ffyb.uba.ar
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The objective of this work was to study the in vivo time course of biochemical processes of oxidative damage in rat brain mediated by redox active and inactive transition metals.

Sprague-Dawley rats (250 g, n=24) received a single intraperitoneal injection of $FeCl_3$ (30 mg/kg), $CuSO_4$ (10mg/kg), $CoCl_2$ (10mg/kg), or $NiSO_4$ (7.5mg/kg) or 0.9%w/v NaCl (control, C). Subsequent studies were conducted 2 to 48 h after injection, when the following oxidative stress parameters were determined: phospholipid oxidation measured as thiobarbituric acid reactive substances (TBARS) and protein oxidation, measured as carbonyl groups, antioxidant protection (GSH content, GSG/GSSG ratio) as described elsewhere (Musacco-Sebio *et al.,* 2014, Semprine *et al.*, 2014) and NADPH oxidase activity (Wei *et al.,* 2006). IL-6 content in plasma was determined by ELISA (commercial kit). The animals were treated according to Argentine regulations (ANMAT) and the Guidelines for Ethical Treatment in Animal Experimentation of the American Physiological Society (Bethesda, MD, USA).

Acute toxicity (brain oxidative damage) was generated by Fe and Cu treatments with a similar timing, suggesting they were acting through a common mechanism. Four hours after treatment, Fe and Cu decreased 90% the GSH content and 68% the GSH/GSSG ratio ($p<0.05$) as compared with the respective control values (2.2±0.3 mM and 21±1). Eight hours after Fe treatment, Cu,Zn-SOD activity was increased 4-fold and catalase activity was increased 3-fold over controls ($p<0.05$), and Cu,16 h after treatment, increased both enzyme activities by 90% over their respective controls (339±36 USOD/g and 14±4 pmol/g). In the same experiments, Fe and Cu treatments increased protein oxidation by 45% and 18% ($p<0.05$), respectively, over their control value (308±10 nmol/g). Phospholipid oxidation (TBARS) was increased by Fe treatment by 56% ($p<0.05$) and by Cu treatment by 31% ($p<0.05$) over the control value (17±1 nmol TBARS/g), both 16 h after treatment (Fig. 1A) (Musacco-Sebio *et al.,* 2014).

The mechanisms by which Co and Ni generate *in vivo* irreversible oxidative damage of the brain involve sequential binding to proteins and their oxidation (Repetto *et al.,* 2012). Two hours after Co treatment protein oxidation was increased 22% over control values (308±10 nmol/g), and 8 h after Ni treatment it was increased by 52% ($p<0.05$). GSH content was decreased 50%, 4 h after Co injection, and was decreased 75%, 2 h after Ni injection ($p<0.05$) as compared with their controls (2.2±0.3 mM). Eight hours after Co treatment, NADPH oxidase activity was increased 2.5-fold. In the same set of experiments, NADPH oxidase activity was increased 82%, 24 h after Ni treatment, over control values (220±40nmol/min g) ($p<0.05$). The existence of a common mechanism mediated by the inflammatory response was evidenced by the following: Co and Ni were able to increase 100% the interleukin-6 (IL-6) concentration in plasma ($p<0.05$) over control values (13±2 pg/mL), both 8 h after Co treatment and 24h after Ni treatment. So, in the case of Ni, the oxidation of lipids and proteins seems to contribute to an inflammatory response (Fig. 1B).

Redox active metals Fe and Cu are involved in the production of hydroxyl radicals ($HO^{\bullet}$) from hydrogen peroxide (H_2O_2) (Fenton reaction) and the reactions involving the decomposition and cleavage of hydroperoxide (ROOH) and production of alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals (Lloyd, 1997). This mechanism allows the propagation

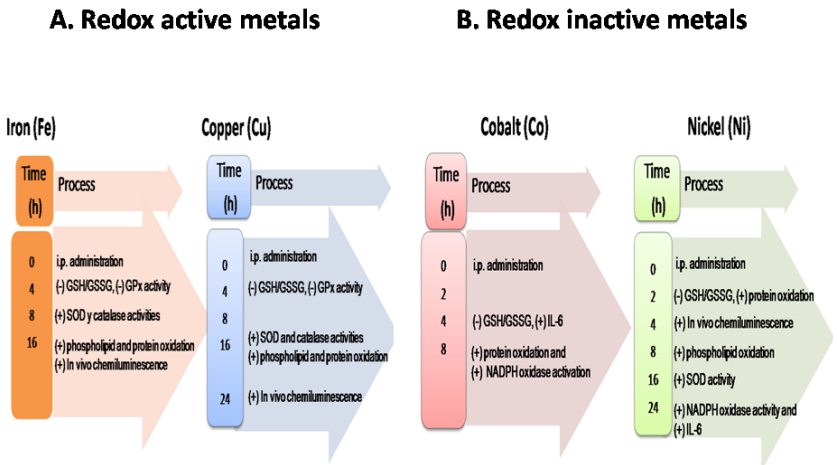

FIGURE 1. Time course of brain oxidative stress and damage for redox active (A) and inactive (B) metals overload. Increases (+) or decreases (-) on the parameters of oxidative stress and antioxidants were evaluated for each metal and compared with control. One way ANOVA and post test Dunnett were used. Each data represents the time that there are significant changes ($p<0.05$) in brain GSH/GSSG, *in vivo* chemiluminescence, phospholipid and protein oxidation, activity of antioxidant enzymes and NADPH oxidase, and IL-6 level in plasma.

of chain reactions of free radicals and damage occurs as a consequence of the interaction of these reactive species with tissue biomolecules. The toxicity of Fe and Cu lies mainly in the reversibility of the oxidation states of the ions that participate in redox reactions such as the Fenton and Haber-Weiss reactions (Fenton, 1894; Haber and Weiss, 1934) involved in the generation of reactive oxygen species (Gutteridge, 1984; Crichton, 2002), thus favoring lipid and protein oxidation.

The toxic effect of redox inactive metals is given by the reaction of these metals with thiol groups (SH) present in molecules with antioxidant function, mainly GSH, and antioxidant enzymes (Repetto *et al.* 2010; Repetto *et al.* 2012). Co and Ni may stimulate the generation of free radicals through indirect mechanisms, by displacement of metals redox active of their enzyme catalytic sites or from storage proteins,.

It should also be considered that Co and Ni may generate proinflammatory responses by increasing cytokines such as IL-6 and IL-8 (Devitt *et al.* 2010) and by activating the nuclear transcription factor NF-kB (Goebeler *et al.* 1993). Data presented herein also support this possibility.

There is now a growing interest in bioinorganic chemistry in both neurochemistry and neurophysiology. The dyshomeostasis of transition metals is a common phenomenon, however, is not currently considered as a risk factor for neurodegenerative disorders, perhaps because the pathophysiological mechanisms are to be explored. The regulation of activity and expression of the antioxidant enzymes, associated to the brain GSH homeostasis, may be considered as a target for designing strategies of protection from oxidative damage due to the toxicity of redox active and inactive metals.

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