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Nrf2-ARE signaling pathway associated to antioxidant response in rat brain after acute iron and copper overloads

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Abstract

The Nrf2-ARE (nuclear factor erythroid 2 (NFE2)-related factor 2- antioxidant responsive element) signaling pathway is involved in detoxification and elimination of reactive oxidants through antioxidant and conjugation reactions and by enhancing cellular antioxidant content. The molecule that seems to be the signal for the genomic transcription is a soluble phospholipid hydroperoxide (ROOH). Rats (220 ± 8 g) were given single doses of 30 mg/kg iron (Fe) or 5 mg/kg copper (Cu). The activities and expressions of brain cytosolic superoxide dismutase (SOD1), catalase and levels of Nrf2 in brain were determined in the period of 0-24 h after metal ion loads. SOD1, catalase and Nrf2 increased their expressions in response to oxidative stress: SOD1, 2.5 and 1.8 times; catalase 2 and 2.5 times after 24 h ($p<0.01$). Nrf2 expression increased 1.5 times after 6 h ($p<0.01$) in brain of rats with Fe(II) or Cu(II) overload compared to control rats. Nrf2-ARE signaling pathway regulates brain antioxidant enzymes and ROOH seems to be the signaling molecule for the adaptive response after Fe and Cu loads.

Introduction

The transcription factor Nrf2 (nuclear factor erythroid 2 (NFE2)-related factor 2) binds to ARE *(*antioxidant responsive element) in the Nrf2-ARE signaling pathway. Nrf2-ARE is *i*nvolved in detoxification and elimination of reactive oxidants through antioxidant and conjugation reactions and by enhancing cellular antioxidant content (Nguyen *et al.,* 2009).

The bioelements iron (Fe) and copper (Cu) exhibit the phenomenon of hormesis: they are needed at low concentrations for health in mammals but they become

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toxic at higher than normal concentrations. Both metals are known by its ability to catalyze the formation of hydroxyl radical (OH•) through the Fenton/Haber-Weiss reaction. Fe(II) and Cu(II) overloads produce oxidative damage in rat brain, among other tissues (Musacco Sebio *et al.*, 2014).

The classical concept of oxidative stress (Sies, 1991) evolved with the recognition of the roles of cellular thiols (–SH) and of antioxidant enzymes in the antioxidant defenses. The current interpretation of cellular oxidative stress is that the condition implies an unbalance between oxidants and antioxidants in favor of the oxidants that leads to a disruption of redox signaling and cell control (Sies, 2015).

Fe(II) and Cu(II) induced oxidative damage to rat brain is responsible for an adaptive response of the antioxidant enzymes that is regulated at a transcriptional level: the activation of Nrf2-ARE signaling pathway, one of the physiological mechanisms in cellular defense against oxidative stress that controls the expression of genes whose protein products, is involved in detoxification and elimination of reactive oxidants and by enhancing cellular antioxidant content.

The aim of the present study was to determine the characteristics of the adaptive response in rat brain after Fe(II) and Cu(II) overloads. The activities and expressions of brain cytosolic superoxide dismutase (SOD1), and catalase were determined as well as the levels of the regulatory transcription factor Nrf2 in the cytosol and nucleus.

Materials and Methods

Experimental model

Sprague-Dawley male rats (220 ± 8 g, three months old) were given intraperitoneally (ip) single doses of 30 mg/kg Fe(II) or 5 mg/kg Cu(II), to determine the time course of metal ion effects (Musacco Sebio *et al.*, 2014; Semprine *et al.*, 2014). Control rats received equal volumes of 0.9 % NaCl. Rats received a standard diet and water and were maintained under controlled conditions of temperature (23-25 ºC) and humidity (50%) in 12 h light/dark cycles. Rats were anesthetized with 15 % (w/v) urethane at 1.5 g/kg (ip). Animal care was given in compliance with the Guidelines for Ethical Treatment in Animal Experimentation of School of Pharmacy and Biochemistry (CICUAL), University of Buenos Aires (Argentina).

In vivo and in situ brain chemiluminescence

Brain photoemission was determined following a previously used protocol (Boveris *et al.*, 1980). Brain chemiluminescence was measured with a Johnson Foundation photon counter (University of Pennsylvania, Philadelphia, PA, USA) and photoemission is expressed as counts per second per cm^2 of exposed brain surface (cps/cm^2).

Brain homogenates

Brain was homogenized at a ratio of 1 g brain/9 mL was obtain following a previously used protocol (Gonzalez Flecha *et al.*, 1991). The supernatant, a suspension of subcellular fractions in diluted cytosol, was used as brain homogenate.

Antioxidant enzymes expression

The time course of the genomic response that involves the expression of SOD1, catalase and Nrf2 in rat brain was evaluated in the period of 0-24 h after Fe(II) and Cu(II) loads.

Expression of SOD1, catalase and Nrf2 are expressed per g of wet brain and were determined by western blot.

Brain metal content

Brain metal content was measured in an atomic absorption spectrometer (Buck model 200 A, East Norwalk, CN).

Statistical analysis

Results indicate mean values ± standard error of the mean (SEM). Statistical analysis was done by one-way ANOVA with Tukey-Kramer multiple comparison test using Graph Pad InStat software; p-values lower than 0.05 were considered statistically significant.

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Results and Discussion

The adaptive response of rat brain to acute Fe(II) or Cu(II) intoxications is adequately described by the response times of the expression of cytosolic SOD1 and of homogenate catalase.

Adaptive response regulates brain antioxidant enzymes in acute Fe(II) and Cu(II) overloads. Figure 1 (A and B) shows the densitometry data of SOD1 and catalase expression in the 0-24 h period after Fe(II) and Cu(II) overloads, in full agreement with the previous report showing increments of 50% on antioxidant response activities with increasing Fe(II) and Cu(II) overload in rat brain (Semprine *et al.*, 2014).

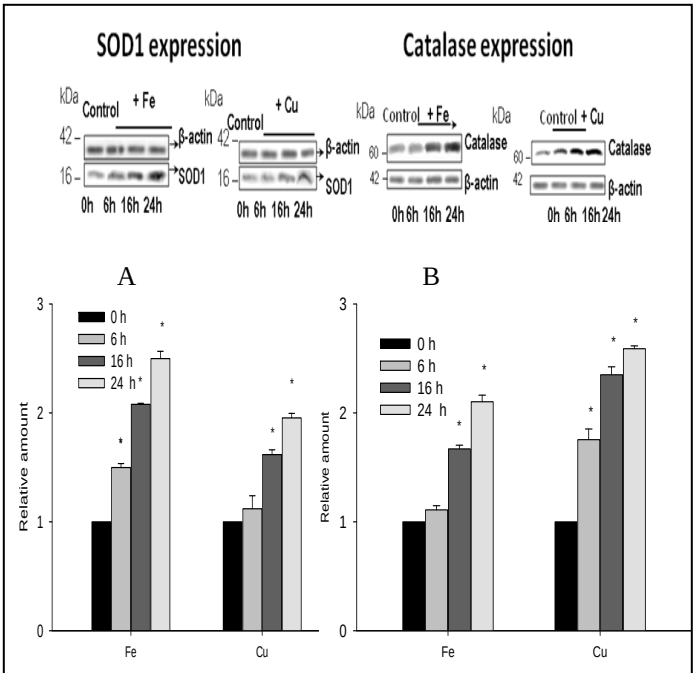

Figure 1. *Western blots and quantitative densitometry of SOD1 (A), catalase (B) in rat brain homogenates, 0-24 h after Fe(II) and Cu(II) loads.*

After 24 h of Fe(II) and Cu(II) load, enzyme expression of SOD1 was increased 2.5 times and 1.8 times respectively; and catalase, 2 and 2.5 times ($p<0.01$) (Fig.1).

Regarding the response times, SOD1 protein expressions increased 50% after 6 h and 16 h of metal ion overloads, whereas catalase expression increased 50% after 16 h of Fe treatment and 30 h after Cu overload (Figures 1 and 2). This antioxidant response increment correlates with *in vivo* brain chemiluminescence (CL) (Figure 2).

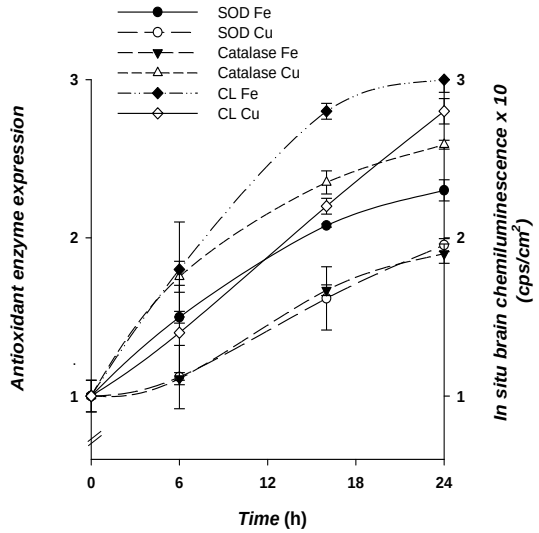

Figure 2. *Quantitative densitometric relative data of superoxide dismutase (SOD1), and catalase* expressions compared with *in situ* brain chemiluminescence (CL) in rat brain 0-24 h after Fe and Cu overloads.

The oxidative process of the spontaneous chemiluminescence of *in situ* brain, where the photoemission is 3 times and 2 times increased after Fe(II) and Cu(II) load, indicates that phospholipids oxidation process occurs after metal accumulation in the brain. The molecular mechanism by which Fe(II) and Cu(II) produce toxicological effects in rat brain involves the H_2O_2 homolysis and formation of the HO• radical from a Fenton/Haber-Weiss process. The HO• radical reacts immediately with cellular phospholipids and proteins, initiates the O_2-dependent and free-radical mediated process of lipid peroxidation, and generates oxidative damage. As a consequence, free radicals and molecules in the excited state are generated by the Russell reaction, and photoemission of these species is the chemical basis of organ chemiluminescence in Fe(II) and Cu(II) overload.

The whole phenomenon is understood as an adaptive response to the oxidative stress and damage following overdose of Fe(II) or Cu(II) ions with lipid peroxidation mediated by free radicals. The molecule that seems most likely to be the signal for the genomic transcription is a soluble phospholipid hydroperoxide (ROOH), where R indicates a 4-6 carbon chain that immediately increases its steady state concentration along with the increased rates of lipid peroxidation (Figure 2). This molecule should be a normal metabolite that immediately increases its steady state

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concentration along with the increased rates of lipid peroxidation.

Considering the intracellular steady state levels of both species (ROOH = 1-3 × 10^{-6} M; and $^1O_2$ = 0.3-1 × 10^{-13} M) it is unlikely that singlet oxygen ($^1O_2$) could afford a biological signal with its low level. Accordingly, we postulate that ROOH is the likely signaling molecule for the adaptive response involving cytosolic SOD1 and catalase after Fe(II) and Cu(II) loads.

Nrf2 expression in rat brain increased 1.5 times after 6 h (25 and 36% after 16 and 24 h of acute Fe(II) overload and 20% after 16-24 h after Cu(II) overload), compared to control rats ($p<0.01$) (Figure 3). The genomic response seems triggered by Nrf2, and includes SOD1 and catalase, as well as other antioxidant enzymes.

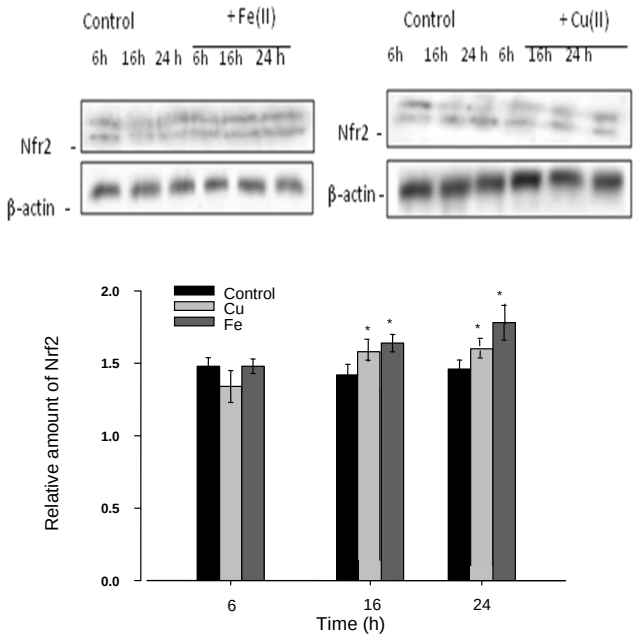

Figure 3: *Western Blots of Nrf2 and quantitative densitometry of Nrf2 expression in rat brain in the period 0-24 h after Fe(II) and Cu(II) overloads, for rats given a single dose of Fe (30 mg/kg) or Cu (5 mg/kg). *p<0.01 compared to control.*

Conclusions

The observed results indicate that the physiologic mechanisms in cellular defense against oxidative or electrophilic stress in acute Fe(II) and Cu(II) overloads is the activation of the Nrf2-ARE signaling pathway.

Acknowledgements

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