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Update on Fe-dependent oxidative metabolism *in vivo:* An integrative view

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ABSTRACT: Fe is essential for human life because it constitutes the required cofactor for proteins of diverse biological functions. However, the development of oxidative stress by exposure to excessive Fe, share signaling pathways with other treatments including activation of redox-sensitive factors. This study was focused on the comparison on the effects of Fe in the brain and other organs *in vivo.* The oxidative effects triggered by Fe overload strongly depend not only on the administration protocol, but also on the Fe-compound used, and the studied organ. In both the liver and the brain, Fe content drastically increased after Fe-dextran administration. However, the comparatively low lipid peroxidation in the brain as compared to the liver, suggested that Fe-dependent oxidative stress might involve mechanisms of different nature. In the brain, acute and subchronic administration of Fe-dextran triggered signaling processes that lead to the prevention of injury by the participation of catalase activity as an antioxidant protection. This brief summary opens a huge range of possible points of risk, as well as opportunities, to encounter situations in which the appropriate election of the Fe management protocol could be able of allow oxidative stress to exert beneficial effects.

Fe is essential for life because it constitutes the required cofactor for a multitude of proteins of diverse biological functions. The one-electron interconversion between Fe(II) and Fe(III), which makes Fe the key factor for biological redox processes, is also the process by which Fe becomes toxic through the generation of O_2-derived radicals and other damaging species. The development of oxidative stress by exposure to excessive Fe, shares signaling pathways with other treatments including activation of redox-sensitive factors. However, the nature of the model used for Fe administration seems as a key issue. The analysis presented here, studying the Fe-related changes by the generation of oxidative stress using resonance electron spectroscopy techniques (EPR), antioxidant capacity by HPLC and biochemical tools, was focused on the comparison on Fe effects in the brain and other organs *in vivo*. The possible role of hormesis, i.e., the induction of protective mechanisms by moderate conditions of oxidative stress previous to a challenge, will be discussed.

Fe overload in humans has been observed when dietary Fe is excessive, such as in the severe siderosis in Bantu people who drink acidic beer out of Fe pots, or in excessive Fe treatment of anemias (Puntarulo and Galleano, 2009). Also, Fe overload is found in inherited diseases, such as congenital atransferrinemia (lack of circulating transferrin) or during the medical treatment of thalassemia (a genetic disorder in which the rate of synthesis of one or more of the hemoglobin chains is diminished; Puntarulo, 2005). To characterize these conditions, several models of Fe-administration in rats were used. Experimental Fe overload using dietary supplementation with carbonyl-Fe in the rat is a well-established model, where Fe deposition occurs mainly in periportal hepatocytes, in a distribution as it was observed in idiopathic hemochromatosis (Powell *et al.*, 1980). Moreover, it has been reported that dietary Fe overload

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TABLE 1

Effect of acute Fe-dextran administration on Fe content in several rat organs

Organ	Fe content					
	Control			+ Fe-dextran		
		µg/g FW	%		µg/g FW	%
Liver	[1]257±11 µg/g DW	77±4	100	[1]1837±205 µg/g DW	552±62	100
Kidney	[2]14±3 µmoles/g DW	156±33	203	[2]113±15 µmol/g DW	1260±167	228
Plasma	[3]126±20 µg/dl	1.3±0.2	1.6	[3]1538±158 µg/dl	15±2	2.8
Brain	[4]635±187 pmol/mg FW	35±10	45	[4]5403±1551 pmol/mg FW	301±87	55

[1] Galleano and Puntarulo, 1992; [2]Galleano *et al.,* 1994; [3]Galleano and Puntarulo, 1995;[4]Piloni *et al.,* 2013
Plasma density was considered as 1 g/ml, kidney water content as 80%. Fe content in Fe-dextran treated animals was measured after 6 h of treatment in liver and brain, and after 20 h of treatment in kidney and plasma.
DW indicates dry tissue weight, and FW indicates fresh tissue weight.

led to an increase in lipid oxidation (Dabbagh *et al.,* 1994); however, Bacon *et al.* (1989) concluded that Fe-induced peroxidative damage to hepatic membrane lipids in chronic Fe overload depended on the hepatic Fe concentration. In our model, a carbonyl-Fe (2.5% w/v) diet was used for 6 weeks. The treatment led to a drastic increase (from 69±16 to 1091±178 µg Fe/g fresh tissue weight, (µg/g FW) after 6 weeks of administration in the Fe content in the liver and plasma, respectively; with a slight increase in the content of thiobarbituric acid reactive substances (TBARS, used as an index of lipid peroxidation; Galleano and Puntarulo, 1997). The significant increase in Fe content suggested a pattern of incorporation of Fe in a gradual manner that facilitates the incorporation of Fe in deposit molecules (i.e., ferritin) that would be effective in preventing oxidative damage (Galleano and Puntarulo, 1997).

Treatment of rats with Fe-dextran resembles hemochromatosis secondary to Fe-loading (anemias treated with repeated transfusions) and high Fe oral intake (Powell *et al.,* 1980). It has been suggested that increased intracellular Fe may initiate several hepatotoxic processes (Bacon and Britton, 1989); however, there were no confirmatory studies of the presence of an expanded intracellular Fe pool (Galleano and Puntarulo, 1992). Acute Fe overload employing Fe-dextran (500 mg /kg) was studied in liver (Galleano and Puntarulo, 1992), kidney (Galleano and Puntarulo, 1994), plasma (Galleano and Puntarulo, 1995) and brain (Piloni *et al.,* 2013). Table 1 shows Fe distribution in control and overload conditions, expressed both as the concentration per gram of dry tissue weight (µg/g DW), fresh tissue weight (µg/g FW) and as % of the concentration in fresh liver tissue. Both the DW and FW concentrations increased drastically after Fe overload, but the % distribution was essentially the same as in controls. Thus, the protection afforded by the blood-brain barrier would not avoid the adverse Fe excessive entry. Moreover, the drastic Fe increase seen in brain strongly suggested that brain could be a target organ for Fe-dependent oxidative damage. After acute treatment, an increase in the total Fe content was also measured in the isolated brain regions (cortex, hippocampus, striatum) (Piloni and Puntarulo, 2014), 6 h after Fe-dextran treatment.

Even though lipid peroxidation is thought a key factor for the deleterious effects of Fe in biological systems (Lu and Koppenol, 2005), TBARS content was not equally affected by acute Fe-dextran treatment in all of the studied organs. TBARS generation rate in liver of Fe-dextran treated rats was increased by 175% (Galleano and Puntarulo, 1992) and by 27% in kidney (Galleano *et al.,*1994), and TBARS content was unchanged in brain (Piloni *et al.,* 2013) and increased by 285% in plasma (Galleano and Puntarulo, 1995). These results suggested that the different antioxidant system profiles may be responsible for controlling lipid deterioration in the different organs.

The massive change in brain Fe content led to an increase in the oxidative stress index in the hydrophilic medium, the ascorbyl radical content ($A^{\bullet}$)/ascorbate content (AH^{-}) ratio, by 55%, and the activity of the enzyme catalase (CAT) enhanced in the areas studied at 6 h post-treatment (Piloni and Puntarulo, 2014). Also, the content of glutathione (GSH) decreased after 6 h and 8 h post-injection in cortex, hippocampus and was unchanged in striatum (Reiteri *et al.,* 2014). These results suggested that the antioxidant response is not uniform in all brain areas, because certain areas seem less likely to express oxidative damage than others do. Piloni

et al. (2013) reported that increased Fe in the acute overload condition was related to an increase in the binding capacity of factor NF-KB to DNA, and to a significant increase in the activity of the antioxidant enzyme CAT.

For a subchronic treatment, the rats were injected intraperitoneally (ip) with Fe-dextran (50 mg/kg every 48 h, 6 injections). This treatment changed Fe content from 3.3±0.3 to 75±7 μg/g FW in the liver (Tapia *et al.,* 1998) and from 47±1 to 360±14 μg/g FW in the brain (Piloni *et al.,* under consideration). The total Fe content in the whole brain and in the cortex, hippocampus and striatum was increased at 2 h post-administration of the 6th dose. The generation rate of RL$^{\bullet}$ and the A$^{\bullet}$/AH^{-} ratio, 2 h post-treatment, increased by 97% and 3.9-fold, respectively. CAT activity increased in the cortex at 2, 4, 6, and 8 h post-treatment (Reiteri *et al.,* 2014). The data suggested that after both Fe-dextran protocols (acute and subchronic), the brain protection mechanisms that were triggered were based on CAT activity.

Due to this close association between oxidative stress and Fe, the effect of the establishment of an oxidative condition and the alteration of Fe oxidative metabolism at the cellular level was considered. Robello *et al.* (2012) reported that fetal brain cells in culture (obtained from rats γ-irradiated *in utero*, a well-known trigger for oxidative stress), the content of total Fe and the level of lipid peroxidation were increased 4 h post-treatment. However, the direct irradiation of cells *in vitro* did not generate any changes in the fetal brain total Fe content, even though the A$^{\bullet}$/AH^{-} ratio was significantly increased (Robello *et al.,* 2012). Thus, these authors postulated that *in vivo* irradiation affected the oxidative metabolism of Fe in the fetal brain mainly by Fe leak from maternal membranes damaged by radiation-dependent processes. The excess Fe coming from the injured membranes could be routed to fetal brain tissues through maternal blood circulation (Robello and Puntarulo, 2014), which indicates the need to be cautious when postulating direct mechanistic links between different oxidative conditions and Fe alterations.

Even more, in some cases, the displacement of Fe from membranes by *in vivo* treatment with toxic compounds (such as As-compounds), was postulated as a contributing factor to lipid peroxidation of brain membranes, probably by removing Fe from ferritin (Ahmad *et al.,* 2000). Bonetto *et al.* (2014) and Piloni *et al.* (2014) reported an increase in the As content in blood and brain of rats, 8 h after i.p. administration of 5.8 mg As/kg (as sodium arsenite). Neither the A$^{\bullet}$/AH^{-} ratio nor the generation rate of the hydroxyl radical ($^{\bullet}$OH) were significantly different between treated and control brains. However, the rate of generation of the RL$^{\bullet}$ was significantly increased in brain from animals receiving As (Bonetto *et al.,* 2013).

From an integrative view of the experimental evidence, the following conclusions would be suggested: (1) the oxidative effects triggered by Fe overload strongly depend not only on the way of administration, but also on the Fe-compound used (carbonyl-Fe, dextran-Fe) and the studied organ (liver, kidney, plasma or brain); (2) in the liver and the brain, Fe content drastically increased after Fe-dextran administration; however, the comparatively low lipid peroxidation in the brain as compared to the liver suggested that Fe-dependent oxidative stress could involve different mechanisms in those tissues; and (3) in the brain, both acute and subchronic administration of Fe-dextran, may be triggering signaling processes leading to prevention of injury, with the participation of CAT activity. This hormetic effect should be considered for the development of possible medical-therapeutic uses.

This view opens a range of possible risks and opportunities, because the appropriate selection of a Fe management protocol may allow oxidative stress to exert beneficial effects that could be applied to the treatment of critical conditions such as neurodegenerative diseases.

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