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Labile iron pool and ferritin content in developing rat brain γ-irradiated *in utero*

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ABSTRACT

This study was aimed to assess the content of total Fe, Ferritin (Ft) and labile Fe pool (LIP) in developing rat brain exposed *in utero* to 1 Gy of γ-irradiation. A significant increase (2.3-fold) in the total Fe content of the fetal rat brain irradiated *in utero* was observed from 1 to 4 h post-irradiation, as compared to the content in non-irradiated brain. Ft was analyzed by immunoblotting. The Ft protein was composed by 20 kDa subunits. According to the analysis of the band density in the Western blot, the Ft content decreased by 77 ± 15% 2 h after γ-irradiation, as compared to the values in non-irradiated samples. The effect of γ-irradiation on the LIP was studied by both electron paramagnetic resonance (EPR) and by a fluorescence technique employing calcein (CA). A reduction on the LIP was detected at 2 h post-irradiation, independently of the methodology employed for the assay. Since NO content increased in the same time frame of LIP decreasing, a protective role for NO is suggested in fetal rat brain exposed to γ-irradiation. The data presented in this work are the first experimental evidence suggesting that, as part of the network of the cellular response to limit irradiation-dependent injury, a complex interaction between Fe and NO could be triggered.

1. Introduction

Developing central nervous system (CNS) is especially sensitive to radiation damage (Hays et al., 1993). The more immature antioxidant defenses and the higher abundance of labile Fe found in the developing CNS, together with the high proportion of dividing neuroblasts, might be some of the reasons for the high radiosensitivity of developing brain (Guelman et al., 2004). Mammalian brain exposure to ionizing radiation might happen as a consequence of military or industrial accidents, or after antineoplasic therapeutic treatments (Guelman et al., 2005). A broad spectrum of congenital abnormalities, growth retardations, developmental delays and functional defects are associated with irradiation of the mammalian fetus (Gisone et al., 2003). Epidemiological studies on individuals exposed *in utero* to the atomic bomb explosions at Hiroshima and Nagasaki suggest that a few hundred mGy of ionizing radiation can have deleterious effects on the developing brain in human embryos (Kimmler, 1998). Developmental radiation-induced abnormalities of the brain cortex of fetuses are expressed in different ways, depending on the dose and on the gestational day of exposure (Dimberg et al., 1992). It is well known that ionizing radiation induces tissue damage on developing CNS through different simultaneous mechanisms, including apoptosis and reactive species-dependent effects, as it was reported by Guelman et al. (2004) employing cultures of cerebellar granule cells.

Fe is an essential element for the growth and well-being of almost all living organisms. Fe is involved in many biological functions by varying the ligands to which it is coordinated. Moreover, Fe has access to a wide range of redox potentials and can participate in many electron transfer reactions, spanning the standard redox potential range (Galatro and Puntarulo, 2007). However, Fe can be associated with toxic effects if its cellular concentration exceeds certain values (Lu and Koppenol, 2005). The proteins responsible for keeping Fe homeostasis in the brain are similar to those found in other organs, as extracellular transferrins and intracellular ferritins (Fts) (Connor et al., 2001). Both proteins retain Fe in the form of Fe^{3+}, which unless mobilized, will not be able to efficiently catalyze the production of free radicals. Fe is stored mainly intracellularly, where its potentially damaging effects are greatest. Thus, Ft play a key role in preventing Fe toxicity because of its ability to sequester several thousand of Fe atoms in their central cavity in a soluble, non-toxic bioavailable form (Galatro and Puntarulo, 2007). It was reported that brain Ft expression is affected by development, being high at birth, decreasing for the first 2 weeks after birth, and increasing afterwards until adult levels are reached (Connor et al., 2001).

The labile iron pool (LIP) is defined as a low-molecular-weight pool of weakly chelated Fe that rapidly passes through the cell. It likely consists of both forms of ionic Fe (Fe^{2+} + Fe^{3+}) associated with low affinity ligands for Fe ions (Kakhlon and Cabantchik, 2002). It has been proposed that Fe is complexed by several

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chelators, such as citrate and other organic ions, phosphate, carbohydrates, etc. (Petrat et al., 2002). The LIP can catalyze the conversion of normal by-products of cell respiration, like superoxide anion and hydrogen peroxide, into highly damaging hydroxyl radical through the Fenton reaction, or by the Fe^{2+} catalyzed Haber-Weiss reaction, or into equally aggressive ferryl ions or oxygen-bridged Fe^{2+}/Fe^{3+} complexes. Moreover, Fe^{3+} can be reduced either by superoxide anion or by ascorbate leading to further radical production (Galatro and Puntarulo, 2007) that could be responsible for lipid peroxidation, DNA strand breaks and degradation of other biomolecules (Harrison and Arosio, 1996).

Since reactive oxygen species (ROS) are strongly compromised in radiation-induced effects, and Fe is both a catalytic agent of ROS generation and of cellular damage, this study was aimed to assess the response to γ-irradiation of the total Fe, Ft and LIP content in developing rat brain exposed *in utero*. The data obtained here suggested that the induced damage in the developing rat brain could be auto-limited by affecting the cellular availability of catalytically active Fe. This finding could be of interest to develop therapeutical approaches to endure natural control mechanisms of defense when the endogenous systems of protection are overwhelmed.

2. Materials and methods

2.1. Breeding and irradiation

Female Wistar rats, from the School of Pharmacy and Biochemistry, University of Buenos Aires, were mated overnight and the following morning sperm-positive vaginal smear was checked to assess day 0 of pregnancy. After 17 days of gestation, the rats were placed in plastic restraining cages and exposed to 1 Gy gamma irradiation from ^{60}Co source at a rate of 0.7 Gy/min. Control rats were sham-irradiated under similar conditions. Fetal brains were removed under general anesthesia (sodium pentobarbital 120 mg/kg i.p. to the pregnant rat) 1, 2 and 4 h after γ-irradiation. Blood samples from the pregnant rat were taken by cardiac puncture under general anesthesia and immediately centrifuged at 600 × *g* for 10 min to obtain the plasma. The cortical plate was dissected out freehand on an ice-cold glass Petri dish. Samples were immediately frozen and stored under liquid N_2 until analysis.

2.2. Labile iron pool (LIP)

The LIP was determined by both EPR at 77K, according to Woodmansee and Imlay (2002), and a fluorescence technique employing the Fe sensor calcein (CA) according to Darbari et al. (2003) with modifications by Robello et al. (2007). For EPR measurements at 77K fetal rat brains were transferred to a Dewar finger. The measurements were performed using the following instrument settings: modulation frequency, 50 kHz; microwave power, 20 mW; microwave frequency, 9.42 GHz; center field, 1600 G; time constant, 81.92 ms; modulation amplitude, 4.759 G; and sweep width, 800 G. For the fluorescence measurements fetal rat brains or maternal plasma were used. Fetal rat brains were homogenized in 40 mM potassium phosphate buffer, 120 mM KCl, pH 7.4 (500 mg fetal brain/ml). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was passed through filters with 30 kDa nominal molecular weight limit. The filtered solution was then reduced with thioglycolic acid to a final concentration of 4% (v/v). The maternal plasma was filtered (30 kDa nominal molecular weight limit) previously to the reduction with thioglycolic acid (final concentration 4%, v/v). Fe content in the reduced solutions was measured in the presence of 1 μM CA solution in 40 mM potassium phosphate buffer, 120 mM KCl, pH 7.4. The fluorescence (λ_{exc} = 485 nm, λ_{em} = 535 nm) was monitored until stabilization of the signal, and then deferoxamine mesylate (DF) was added to a final concentration of 800 μM. The fluorescence was monitored until a new stabilization of the signal. Then Fe^{2+} + Fe^{3+} concentration was assessed according to Robello et al. (2007).

2.3. Total Fe content

Fetal rat brains were dried until constant weight in an oven at 70 °C. Then, the samples were mineralized in $HNO_3/HClO_4$ (1:1) according to Laurie et al. (1991). Fe content was determined spectrophotometrically after reduction with thioglycolic acid, measuring the Fe^{2+}-batophenanthroline complex absorbance at 535 nm (Brumby and Massey, 1967).

2.4. Ft identification by Western blotting

For Western blot analysis total protein extract of fetal rat brain was performed according to Siles et al. (2002) with modifications. Briefly, fetal rat brains (100 mg/ml) were homogenized at 4 °C in protein extraction buffer (30 mM Tris-HCl, 0.5 mM DTT, 1 mM EDTA, 1% SDS, pH 7.4, and protease inhibitors: 0.5 μg/ml leucopeptin, 0.7 μg/ml pepstatin A, 40 μg/ml phenylmethylsulfonyl fluoride, 0.5 μg/ml aprotinin and 0.6 mM $MgSO_4$). Homogenates were centrifuged at 16,266 × *g* for 20 min at 4 °C. The supernatant was ultracentrifuged at 100,000 × *g* for 1 h at 4 °C. The pellets were suspended in 10 mM potassium phosphate buffer, 0.15 M NaCl, pH 7.0. The protein concentration was determined according to Bradford (1976). The samples were separated by electrophoresis in 15% SDS-polyacrylamide gel on denaturating conditions. Gels were either stained with coomassie blue, or layered onto a nitrocellulose membrane 0.2 μm (BioRad) to transfer the proteins by electroblotting. An anti-horse spleen Ft primary antibody developed in rabbit and a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, USA) were used. Chemiluminescence was followed using an Immun-StartTM HRP chemiluminescent kit (BioRad). In order to analyze a one-dimensional electrophoretic gel the Scion Image program for Windows was used. This software was employed to mark the lanes of scanned image of the gel, and then to generate a lane profile of it. Drawing a base line in the lane profile, a defined closed area was defined for each band of the gel. Finally the area of the β-actin and Ft bands were calculated, and the Ft area/β-actin area ratio for each treatment was used as an index of expression in the data analysis. The Ft area/β-actin area ratio of the control was used as a reference (relative area = 100%) within each performed gel.

2.5. In vitro exposure of isolated rat brains to a NO donor

Samples of non-irradiated brains from fetuses at the 17th gestational day (GD) were homogenized in 40 mM potassium phosphate buffer, 120 mM KCl, pH 7.4 (300 mg fetal brain/ml). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was passed through filters with 30 kDa nominal molecular weight limit. The filtered solution was then treated with 10 μM S-nitrosoglutathione (GSNO) for 2, 15 and 30 min at 37 °C. Afterwards, the samples were reduced with thioglycolic acid at a final concentration of 4% (v/v), and LIP was determined as previously described.

2.6. Statistical analyses

Data in the text and tables are expressed as mean ± S.E. of four independent experiments, with two replicates in each experiment.

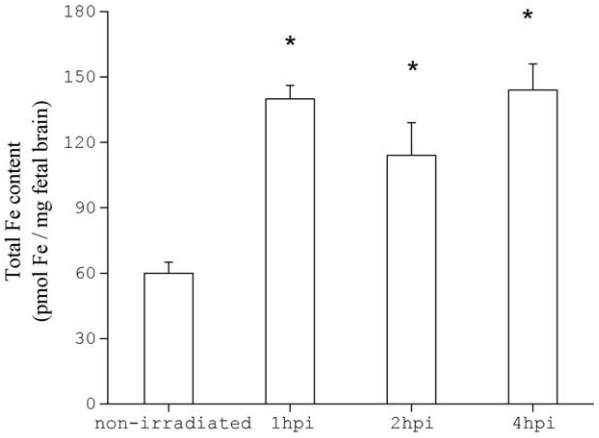

Fig. 1. Effect of γ-irradiation in the total Fe content in developing rat brain. *Significantly different from non-irradiated brain samples. ANOVA, $P \leq 0.05$.

For each experiment, the fetuses from an entire litter were pooled and two aliquots of the pool were analyzed. Statistical tests were carried out using ANOVA followed by a Bonferroni post-test. All statistical analysis was performed using the computer program GraphPad InStat® Version 3.01,32 bit for Win 95/NT.

3. Results

Lipid peroxidation is a measurement of oxidative damage. Previous work employing this model indicates that 4 h after the exposure to γ-irradiation, a significant increase in the TBARS content was measured in the rat brain (Gisone et al., 2003). Since Fe is a recognized catalyst for lipid peroxidation, the role of this metal upon the early stages after γ-irradiation was studied. A significant increase (2.3-fold) in the total Fe content of the fetal rat brain irradiated *in utero* was observed from 1 to 4 h post-irradiation as compared to the content in non-irradiated fetal rat brain (Fig. 1). Ft has been described as the main storage protein for Fe, thus the effect of γ-irradiation on Ft content of the developing rat brain irradiated *in utero* was studied by Western blot. Total fetal rat brain protein was analyzed by immunoblotting. By comparison with commercially available Ft, a band corresponding to a protein of 20 kDa was assigned to fetal brain Ft (Fig. 2A). According to the analysis of the band density in the Western blot, the Ft content decreased by 77 ± 15% 2 h after γ-irradiation, as compared to values observed in non-irradiated brains (Fig. 2B). Non-significant alterations have been detected either after 1 or 4 h post-irradiation as compared to values in non-irradiated samples. However, the assay performed here did not allow to identify the contribution of each type of subunit of the cytosolic Ft that assemble together to form a 24-subunit protein, termed heavy chain (FHC, with ferroxidase activity) and light chain (FLC, that contributes to the nucleation of the Fe core). The second band obtained could be interpreted as a degraded form of the Ft, not seen in the commercially available Ft.

EPR detection of LIP is understood as a reliable method for Fe detection, and the fluorescence method employing CA is seen as a very sensitive approach (Tarpey et al., 2004). LIP was measured by both methods to confirm the accuracy of the data. The typical EPR signal of Fe^{3+} is shown in Fig. 3. A significant decrease in the signal (80.5%) was observed in rat brain 2 h after γ-irradiation as compared to values in non-irradiated brains. The effect of γ-irradiation on the LIP in the fetal rat brain was studied by the fluorescence technique employing CA. As it is shown in Fig. 4, LIP in the fetal brains was significantly decreased at 2 h post-irradiation (76%), without significant changes at 1 and 4 h post-irradiation, as compared to values in non-irradiated fetal rat brain. Even though the assessment of LIP by EPR reflects Fe^{3+} content and the CA-dependent measurements represent the content of Fe^{2+} + Fe^{3+} in the fetal brains, the data shown here indicate that LIP was significantly decreased at 2 h post-irradiation, independently of the methodology employed. Thus, the performed analysis confirmed that the total amount of Fe available for catalysis decreased 2 h after exposure of the fetal brain to irradiation. Moreover, the LIP content in the fetal brain recovered to non-irradiated values at 24 h post-irradiation (data not shown).

In the model used here where the effect of irradiation is studied in the fetus during prenatal life, the analysis of Fe available from the maternal plasma is an important issue. Moreover, according to Umegaki et al. (2001) the increased Fe content could lead to an enhancement of the redox active Fe, thus an increase in LIP in maternal plasma could be responsible of an increased Fe trafficking from the placenta. Data in Fig. 5 show that total body γ-irradiation of pregnant rats induced an increase in the LIP, assessed by CA-dependent technique, in the maternal plasma. The LIP kinetically increased after 1 h post-irradiation, as compared to values in non-irradiated brains. Preliminary work indicates that Fe in the LIP in maternal plasma recovered to non-irradiated values at 24 h post-irradiation.

Previous reports from Gisone et al. (2003) showed that ionizing radiation induced an early increase in nitric oxide (NO) steady-state concentration in the developing rat brain irradiated *in utero* with 1 Gy of γ-radiation on the 17 GD. The measured increase on NO steady-state concentration could favor the formation of nitrosyl–Fe complexes, or similar adducts, that would be able to take out Fe from the pool of Fe complexes assessed by the CA-fluorescent-dependent method. To test this hypothesis, the effect of an NO donor on the LIP

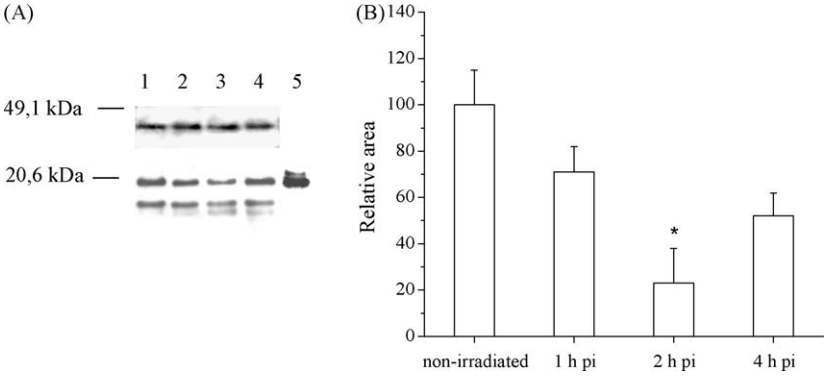

Fig. 2. (A) Immunoblotting of total protein extracts of rat brain. Lane 1: non-irradiated fetal brains; lane 2: irradiated fetal brains after 1 h; lane 3: after 2 h, and lane 4: after 4 h of exposure to γ-irradiation; and in lane 5: horse spleen Ft, as standard. (B) Analysis of the Ft expression by Western blotting shown in panel A. *Significantly different from non-irradiated brain samples. ANOVA, $P \leq 0.05$.

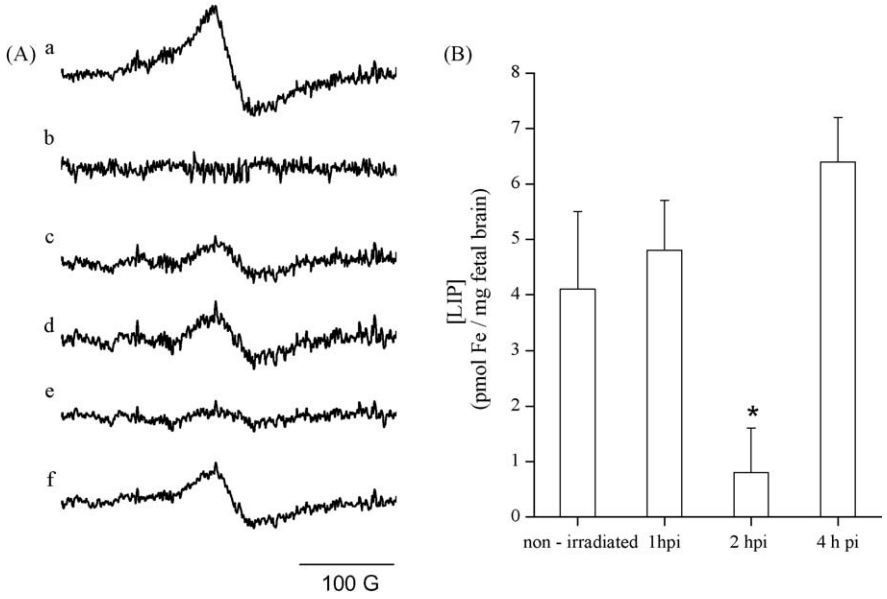

Fig. 3. EPR detection of LIP (Fe^{3+}) in developing rat brain. (A) EPR signal from a, 30 μM DF-Fe^{3+} complex (10:1), as standard; b, DF by itself; c, non-irradiated fetal brain; d, fetal brain 1 h post-irradiation; e, fetal brain 2 h post-irradiation; and f, fetal brain 4 h post-irradiation. (B) Quantification of the LIP from the EPR spectra. *Significantly different from non-irradiated brain samples. ANOVA, $P \leq 0.05$.

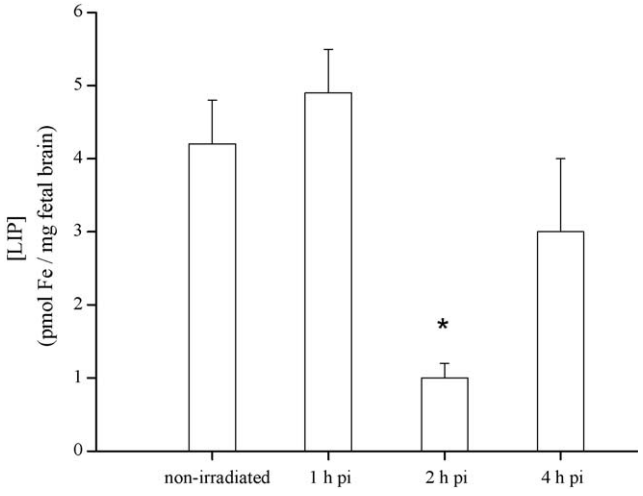

Fig. 4. Effect of γ-irradiation on the LIP in developing rat brain detected by a fluorescent technique. *Significantly different from non-irradiated brain samples. ANOVA, $P \leq 0.05$.

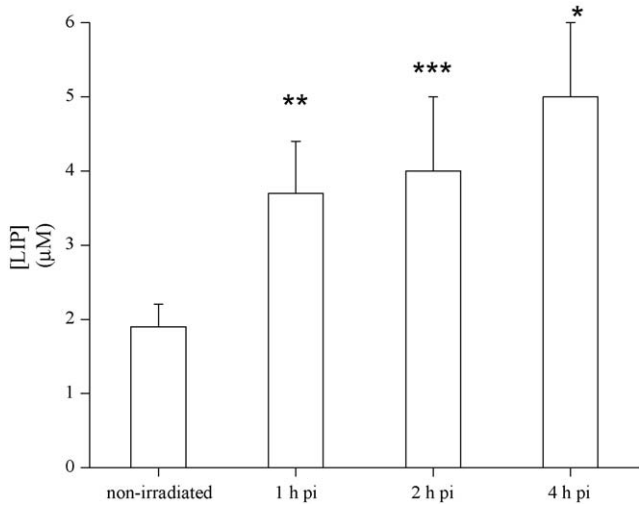

Fig. 5. Effect of γ-irradiation on the LIP in maternal plasma detected by a fluorescent technique. *Significantly different from non-irradiated plasma samples. ANOVA, $P \leq 0.05$, $^{**}P = 0.086$, $^{***}P = 0.094$.

of fetal rat brain not exposed to irradiation was tested. The brain homogenates from non-irradiated rats were exposed *in vitro* to the NO donor S-nitrosoglutathione (GSNO) (10 μM) during 2, 15 and 30 min. The LIP in the samples was assessed afterwards employing the CA-dependent technique. The LIP was decreased by 84, 89 and 96%, respectively after 2, 15 and 30 min of exposure to the NO donor, as compared to non-treated brain homogenates (Fig. 6), suggesting that the LIP content assessed by the CA-dependent technique, did not include the Fe bound to NO.

4. Discussion

A role for reactive species and Fe in tissue, among other factors, was suggested by Guelman et al. (2004) since they have shown that the metal chelator DF partially blocked γ-irradiation injury in cells in culture. The alterations in Fe oxidative metabolism in the brain of the fetus could be due to either direct or indirect irradiation effects. To consider the distribution of Fe as a direct effect of irradiation is a very difficult hypothesis to be maintained since the disruption of subcellular structures after exposure would lead to a substantial increase in the LIP content, and the results shown in this work are not consistent with this postulation. Another possibility is that indirect effects, resulting from changes on maternal tissues, could be responsible for the observed Fe alterations after irradiation in fetal brain. In this regard, the scheme shown in Fig. 7 describes a possible mechanism of response to the exposure to γ-irradiation. Up to now, little is known about the developmental changes in Fe content in the fetus during the gestational period, and even less about how this scenario is affected by the exposure to γ-irradiation. The significant increase in the total Fe content of the fetal rat brain irradiated *in utero* observed from 1 to 4 h post-irradiation, as compared to the content in non-irradiated fetal rat brain, is consistent with previous reports showing that total body irradiation of rats with X-rays significantly increased Fe content in liver and bone marrow at a few hours

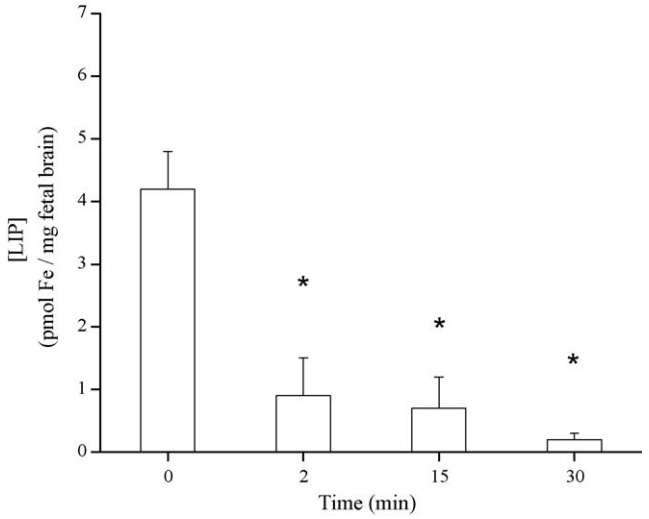

Fig. 6. Effect of *in vitro* GSNO exposure on LIP in fetal brain homogenates. LIP was assessed employing the CA-dependent technique in non-irradiated brain homogenates exposed to 10 μM GSNO for 2, 15 and 30 min at 37 °C. *Significantly different from non-treated brain samples. ANOVA, $P \leq 0.05$.

(Umegaki et al., 2001). Also, an increased plasmatic Fe concentration was reported after total body irradiation in bone marrow transplantation in humans (Dürken et al., 2000). The increase reported here in total Fe in the maternal plasma after exposure to irradiation could be understood as a consequence of the oxidative damage to proteins that released Fe bound either as prostetic groups or forming adducts in the native condition. The total Fe in the brain cells accounts for the LIP and the Fe bound to Ft, heme and non-heme proteins and dinitrosyl, dinitrosyl–diglutathionyl, dinitrosyl–glutathionyl and other nitrosyl complexes, as indicated in Fig. 7. The increase reported here, in the total Fe content observed in the fetal rat brain, could be due to an increased Fe trafficking from the placenta. Thus, according to this hypothesis both, transferrin-bound Fe and non-transferrin bound Fe increased in the extracellular milieu and could lead to an increase in the total Fe content in the fetal brain, and by triggering a signaling network that disturbed the cellular equilibria and decreased the content of Ft and the LIP, would increase the content of Fe forming complexes to NO that could be easily exported from the cell (Fig. 7).

Even though Ft is considered as an antioxidant by its ability of sequestering Fe, Miller et al. (1995) reported that oxidative injury by exposure to ethanol *in utero* resulted in a decrease in the brain Ft. Accordingly, the data presented here showing a significant decrease in Ft content in the brain after 2 h of exposure to γ-irradiation suggest that alteration in the Ft content does not seem to be the cellular mechanism triggered for preventing the damage dependent on the Fe excess in the tissue.

The steady-state concentration of the LIP could be understood as indicated by Eq. (1), where each term refers to the concentration of Fe bound to a physiological available Fe chelator in the cytosol.

$$\left(\frac{d[Fe]}{dt}\right) = \left(\frac{d[Fe]}{dt}\right)_{\text{citrate}} + \left(\frac{d[Fe]}{dt}\right)_{\text{ATP}} + \left(\frac{d[Fe]}{dt}\right)_{\text{ADP}} + \left(\frac{d[Fe]}{dt}\right)_{\text{oxalate}} + \left(\frac{d[Fe]}{dt}\right)_{\text{NO}} + \left(\frac{d[Fe]}{dt}\right)_{\text{other physiological chelators}} \quad (1)$$

NO could be bound to Fe generating dinitrosyl–Fe, dinitrosyl–diglutathionyl–Fe or dinitrosyl–glutathionyl–Fe complexes among others, as indicated by the following equation:

$$\left(\frac{d[Fe]}{dt}\right)_{\text{NO}} = \left(\frac{d[Fe]}{dt}\right)_{\text{dinitrosyl complex}} + \left(\frac{d[Fe]}{dt}\right)_{\text{dinitrosyl–diglutathionyl complex}} + \left(\frac{d[Fe]}{dt}\right)_{\text{dinitrosyl–glutathionyl complex}} + \left(\frac{d[Fe]}{dt}\right)_{\text{other nitrosyl complexes}} \quad (2)$$

The observed decrease in Ft content and the fact that total Fe content in the brain increased at the same time, could lead to the conclusion that catalytically active Fe would be increased. However, the measurements of LIP in fetal rat brain by two independent techniques showed a significant decrease in the LIP, as fast as 2 h after radiation exposure. These data suggested that irradiation of the developing rat brain triggered a network of signals that affected Fe distribution with the objective of limiting cellular injury. Among the possible candidates to exert this action, NO is a multi-faced molecule with dichotomous regulatory roles. The effects of NO are modulated by interactions, that can be dose-dependent and cell-type specific (Kim et al., 2001; Choi et al.,

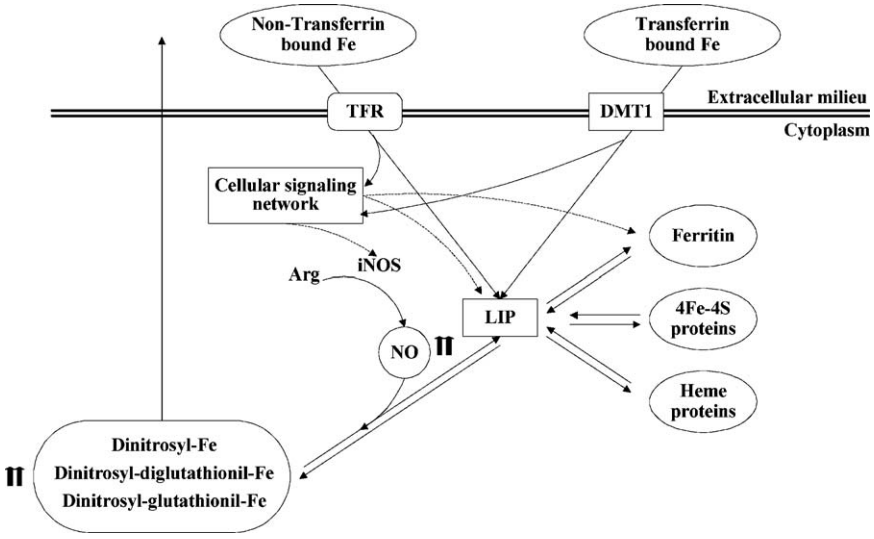

Fig. 7. General scheme describing the presented hypothesis of the intracellular Fe interactions and the effect of γ-irradiation in fetal rat brain. TFR stands for transferrin receptor, DMT1 for divalent metal transporter 1. Dashed lines indicate the possible equilibria affected by the increased Fe trafficking from the maternal plasma.

2002). The rate of NO production and the interaction with biological molecules such as metal ions, thiols, proteins tyrosine, and reactive oxygen species seems to determine the effect of NO (Choi et al., 2002). Previous data from our laboratory (Gisone et al., 2003) using the model employed here, suggested that NO could play a protective role in the developing rat brain, since an early increase in NOS activity was detected 1 h after irradiation with a further increase in NO steady-state concentration 2 h after irradiation. In this work, the observed significant decrease in the LIP by γ-irradiation could strike as a paradox. However, it could be interpreted assuming that Fe was chelated by NO generated as a response to γ-irradiation, leading to an increase in the concentration of dinitrosyl–Fe, dinitrosyl–diglutathionyl–Fe or dinitrosyl–glutathionyl–Fe complexes, among others. These complexes would be unable to induce oxidative stress, as it was suggested by Sergent et al. (1997), in hepatocytes. Also, Lu and Koppenol (2005) demonstrated, employing a chemical system, that NO can inhibit the Fenton reaction by reacting with Fe^{2+} to form a nitrosylferrate^{2+} complex. These authors suggested that in complex biological systems an excess of NO would bind to Fe^{2+} and slow the Fenton reaction. This hypothesis may explain the beneficial effects of NO since the nitrosyl–Fe complexes are stable and, as shown here, they were not detected as components of the catalytically active Fe pool identified by CA.

Within the complex network of cell signals elicited by ionizing radiation, the pathway controlling cell survival has a key role. Cells of the CNS, like other cells, have natural resistance mechanisms to nitro-oxidative stress exerted by the free radicals. Even though the low levels of NO and possible O_2^- generated by normal metabolism can theoretically cause cellular damage, resistance mechanisms have evolved to deal with this condition (Bishop and Anderson, 2005). Bishop and Anderson (2005) found that when motor neurons are pretreated with a subtoxic flux of NO (25 nM/s) they gain resistance to a normal cytotoxic flux (250 nM/s to 1 μM/s) of NO. The role of NO production could be understood (a) as a triggering factor for damaging cellular compounds, (b) as a cellular attempt to minimize cellular injury, or (c) as a combination of the above. For NO both actions could be possible since it has a dual nature (induction of adaptive resistance and toxicity). The final effect will depend on its concentration, rate of release, the cellular environment into which it is released, and the alteration of the cellular LIP. On this scenario, it could be postulated that the radiation-dependent damage, evidenced as cellular deterioration that lead to severe pathologies, could be related to the lack of success of maintaining over time the control of the activity of the potentially damaging species generated by γ-irradiation. Further studies are required in order to fully address NO role. Nevertheless, the data presented in this work are the first experimental evidence showing that, as a part of the network of the cellular responses to limit irradiation-dependent injury, a complex interaction between Fe and NO could be triggered. The effect reported here seems as an endogenous mechanism which would contribute to prevent irreversible damage to fetal brain after exposure to irradiation, and opens the possibility of designing therapeutical strategies to reinforce cellular resistance to damage. Alternatively, it could be speculated that the excess Fe incorporated to the fetal brain after exposure to γ-irradiation on the 17 GD, could be rapidly metabolized by this active developing tissue, and used either in the synthesis of heme-proteins or incorporated as non-heme Fe to structural proteins. This hypothesis could explain the fast recovery to control values in the LIP content in the fetal brain, avoiding both lack of required Fe for the growing developing tissue and the dangerous increase in the content of catalytically active Fe. Even though these aspects exceed the scope of this study, further experiments are required to fully address the kinetic features of the recovery and to identify the fate of Fe under these experimental conditions.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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