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RESEARCH ARTICLE

OXIDATIVE BALANCE IN BRAIN AFTER EXPOSURE TO ARSENIC IN *EX VIVO* AND *IN VIVO* MODELS.

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Abstract

The hypothesis of this work was that radical generation after exposure to As may contribute to its toxic effects in the brain tissues. Oxidative status of brain was studied, both using *ex vivo* and *in vivo* protocols of exposure to the toxic. The generation rate of lipid radicals (LR[•]), and ascorbyl radical (A[•]) content, were measured by Electron Paramagnetic Resonance (EPR). In the *ex vivo* model control brain homogenates were exposed to As, and a 2-fold increase was detected in the LR[•] generation rate, with no changes in A[•] radical content. A significant decrease of 33% and 30% in the content of glutathione (GSH) was measured after exposure to 3.3 and 4.0 pmol As/mg FW, respectively. In the *in vivo* model, As was ip injected to rats and the rate of generation of LR[•] by homogenates of brain tissue, was increased by 81 and 122%, as compared to control animals after the injection of 3.0 and 5.8 mg As/kg, respectively. Neither GSH, nor α -tocopherol (α -T) nor ascorbate (AH) content was affected in As-treated rats, as compared to the values from control animals. The LR[•]/ α -T content ratio was significantly increased in As-treated animals as compared to control brains. The A[•]/AH⁻ content ratio was not affected by As exposure. The content of total Fe showed non-significant differences between control and rat brains after 24 h of As administration. The data presented here showed new evidence on the generation of specific radical species by As treatment employing EPR methodologies in both, *ex vivo* and *in vivo* models. The data suggested the triggering of different pathways leading to some reactive species generation may occur accordingly to the via As reaches the brain, even when the same concentration of the toxic was achieved by the tissues.

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Introduction:

Arsenic (As) is a metalloid element, and its environmental behavior and metabolism are like the physicochemical characteristics of both metal and non-metal elements (Bonetto et al., 2014). As induces health effects in mammals,

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including cancer, cardiovascular disorders, and metabolic disease in humans (Jomova et al., 2011). Although the toxic and carcinogenic effects on humans exposed to As have been well documented in several tissues, such as liver, blood, kidney and spleen, only a few of them included the brain (Bonetto et al., 2014). As is mainly metabolized in liver by repetitive reduction and oxidative methylation. The arsenic methyltransferase (AS3MT) (+3 oxidation state) (Lin et al., 2002) and an independent minor AS3MT methylation pathway (Drobna et al., 2006) are the suggested main pathways of As metabolism. However, the role of other putative metabolic routes, including free radical reactions, is still under debate. Potential modes of action have been proposed, such as cytotoxicity and regenerative repair, formation of reactive oxygen species (ROS) resulting in oxidative stress and DNA damage, impaired DNA repair, dysregulation of signaling pathways that control the fate of cells (cycle progression, proliferation, differentiation, and apoptosis), and perturbations of gene structure (National Research Council. National Academy of Sciences, 2013).

ROS include superoxide anion (O_2^-), hydroxyl radical ($\cdot OH$), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), and peroxy radicals ($ROO\cdot$). The production of ROS in cellular and extracellular systems should be tightly controlled since they are highly reactive oxidants and mainly regarded as hazardous species. However, recently it has been recognized the importance of radical species in cellular signaling and in the maintenance of homeostatic conditions (Gonzalez et al., 2012). To be able to maintain dangerous reactive species at low steady state concentrations, the presence of many components is required to act as an adequate antioxidant defense system. The mechanisms for the action of these antioxidants involve three levels: (1) deactivation of reactive species by either enzymatic (e.g. catalase, CAT; and superoxide dismutase, SOD) or non-enzymatic compounds, both hydrophilic (e.g. glutathione, GSH and ascorbate, AH^-) and lipophilic (e.g. α -tocopherol, α -T) compounds; (2) prevention of radical formation (Halliwell, 1994), such as by ferritin (Ft) action chelating Fe; and (3) repair of already performed damage, such as by the activity of DNA repairing enzymes (Halliwell, 1994). Zamora et al. (2014) examined the capacity of trivalent arsenic species arsenous acid (iAs(III)), monomethylarsonous acid (MMA(III)), and dimethylarsinous acid (DMA(III)) to generate ROS through a theoretical analysis of their structures, redox properties, and their reactivities to various ROS using a density functional theory approach and by employing electron paramagnetic resonance (EPR) techniques. Spin trapping studies showed a higher propensity for methylated arsenicals to generate radicals than iAs(III) upon treatment with H_2O_2 . However, in the presence of Fe(II, III), all showed radical generation where MMA(III) gave predominantly C-centered adducts, while acidified iAs(III) and DMA(III) gave primarily HO-adducts, and their formation was affected in the presence of SOD suggesting a As(III)-OO/OOH radical intermediate. Although these EPR studies concluded that the As toxicity pathway might involve ROS, there are not EPR reports using biological systems. Recently, oxidative stress ratios (damage/protection indexes), such as ascorbyl radical ($A\cdot$)/ AH^- content, and lipid radical ($LR\cdot$)/ α -T content have been described as useful tools for stress diagnosis (Malanga et al., 2009; González et al., 2013).

Experimental results have shown that As exposure of some cell lines generated O_2^- and H_2O_2 (Garcia-Chavez et al., 2003; Jing et al., 1999; Wang et al., 1996). It was described that arsenite inhibits glutathione reductase (GR) activity and diminishes the intracellular level of GSH (Thomas et al., 2001). Bharti et al. (2012) reported that rats exposed to As showed an increase in lipid peroxidation in brain with a significant decrease in the GSH level and in the activities of CAT, SOD, glutathione peroxidase (GPx), and GR activities. Rao and Avani (2004) and Chaudhuri et al. (1999) also reported increased neurotoxic oxidative stress in mouse brains after oral administration of arsenic trioxide. Aung et al. (2013) in an *in vitro* study suggested the induction of neural apoptosis since neurite outgrowth was suppressed by sodium arsenite. Deficits in spatial memory and in executive and motor function were shown in animal studies (Tolins et al., 2014). Also, low to moderate concentrations of arsenic, were associated to neurologic deficits, particularly detected in children by IQ tests (Wasserman et al., 2014). The mechanisms appear related to oxidative stress-induced apoptosis and effects on neurotransmitters (National Research Council. National Academy of Sciences, 2013). Moreover, arsenicals can release Fe by the activation of the enzyme heme-oxygenase (HO-1) (Albores et al., 1989). Negishi et al. (2013) reported that the adverse effects of exposure to diphenyl arsenic acid (DPAA) in the drinking water during development could be due to an increase in HO-1 in the cerebellar astrocytes. Previously, Ahmad et al. (2000) suggested the direct reduction of Ft Fe by dimethyl arsenic acid (DMAIII) as the predominant pathway, and that DMAIII and AH^- -mediated Fe-release from Ft (not via O_2^-) also may be a significant route *in vivo*. The hypothesis of this work was that radical generation induced by the exposure to As may contribute to its toxic effects in the brain tissues, even in the absence of previous metabolism of As by the liver. To fulfil this goal, oxidative status of brain was studied, by employing both *ex vivo* and *in vivo* protocols of exposure to the toxic, with direct detection of radical species using EPR.

Material and Methods:

Experimental design:

The School of Pharmacy and Biochemistry, University of Buenos Aires, provided the male Albino Wistar rats (200 ± 20 g) used in this study through the Animal Facility. The animals were housed under standard conditions of light, temperature and humidity with unlimited access to water and food. The rats were injected intraperitoneally (ip) with one dose of sodium arsenite (NaAsO_2) of 1 to 5.8 mg As/kg body weight. Saline solution was employed to sham-ip injected control rats. Brains from control and As-treated animals were excised from euthanized animals in a CO_2 chamber, and rinsed in cold saline solution. Except where noted otherwise, the samples were freeze-clamped immediately following removal and stored under liquid N_2 until used. Whole blood was taken by cardiac puncture. Experimental animal protocols and animal procedures were in agreement with the Guide for the Care and Use of Laboratory Animals (1985), and were carried out according to the principles and directives of the European Communities Council Directives (1986). The procedures also received approval from the Institutional Animal Care and Use Committee-School of Pharmacy and Biochemistry (CICUAL-FFyB, RES N°1037).

As content:

The quantification of As species [As^{V} + As^{III} + acidmonometilarsónico (MMA) + dimethyl arsinic acid (DMA)] content in brain and whole blood was performed by hydride generation-atomic absorption spectrometry (HG-AAS), after dry mineralization. A hydride generator VGA77 and an atomic absorption spectrometer Varian Spectra AA 200 were employed according to Navoni et al. (2010).

Detection of LR^\bullet generation rate by EPR:

LR^\bullet generation rate was detected by a spin trapping technique using N-t-butyl- α -phenyl nitron (PBN) or N-tert-Butyl- α -(4-pyridyl-1-oxide) nitron (POBN) depending of the experimental protocol. In the *in vivo* experiments, a 40 mM PBN stock solution was prepared in dimethyl sulfoxide (DMSO) immediately prior to use. Brain tissue was homogenized in DMSO-PBN (stock solution) in a concentration of 25 mg/ml, incubated for 30 min and immediately transferred to a Pasteur pipette for LR^\bullet detection. In the *ex vivo* experiments, brain tissue was homogenized in 100 mM potassium phosphate buffer (pH 7.4) with 40 mM of POBN in a concentration of 75 mg/ml, incubated at 37 °C in presence of As between 1.3 and 4 pmol As/g FW of tissue, and transferred to a Pasteur pipette for LR^\bullet detection. Instrument settings were as follows: modulation frequency 50 kHz, microwave power 10 mW, microwave frequency 9.75 GHz, centered field 3487 G, time constant 81.92 ms, modulation amplitude 1.20 G and sweep width 100 G, according to Lai et al. (1986). Quantification of the spin adduct was performed using TEMPO introduced into the same sample cell used for spin trapping. EPR spectra for both sample and TEMPO solutions were recorded at the same spectrometer settings and the first derivative EPR spectra were double integrated to obtain the area intensity, then the concentration of spin adduct was calculated according to Kotake et al. (1996).

Reduced GSH content:

The content of GSH in brain exposed to As was performed according to Rodriguez-Ariza et al. (1994) with modifications. Brain homogenates (20% p/v) were prepared in 1 M perchloric acid and 2 mM EDTA soon after isolation of the tissues in the *in vivo* model. For the *ex vivo* model, the homogenate from control brain (20% p/v) in 100 mM phosphate buffer (pH 7.4), was incubated at 37°C for 5 min and the reaction was stopped with the addition of 1 M perchloric acid and 2 mM EDTA. Then reaction mixture was filtered at 10,000g for 5 min at 4°C. The supernatant was recovered and filtered using 0.2 μm filters. The samples were kept at -80°C for quantification by HPLC-EQ assay employing commercially available standard GSH.

Detection of A^\bullet content:

A Bruker EMX plus X band spectrometer was used for A^\bullet measurements. Brain tissue was homogenized in dimethyl sulfoxide (DMSO) in a concentration of 25 mg/ml and the spectra were immediately scanned under the following conditions: 50 kHz field modulation, room temperature, microwave power 10 mW, modulation amplitude 1 G, time constant 655 ms, receiver gain 1×10^5 , microwave frequency 9.81 GHz, and scan rate 0.18 G/s (Piloni et al., 2013). Quantification was performed as previously described, according to Kotake et al. (1996).

AH⁻ content:

The content of AH^- , was measured by reverse-phase HPLC-EQ. The samples were homogenized in metaphosphoric acid (10%, w/v) according to Kutnink et al. (1987).

α -T content:

The content of α -T in brain tissues was quantified by reverse-phase HPLC-EQ with a Perkin Elmer 250 pump and a ESA Cuolochem II electrochemical detector, at an applied oxidation potential of 0.6 V according to Desai (1984). D,L- α -Tocopherol (Sigma) were used as standards.

Total Fe content:

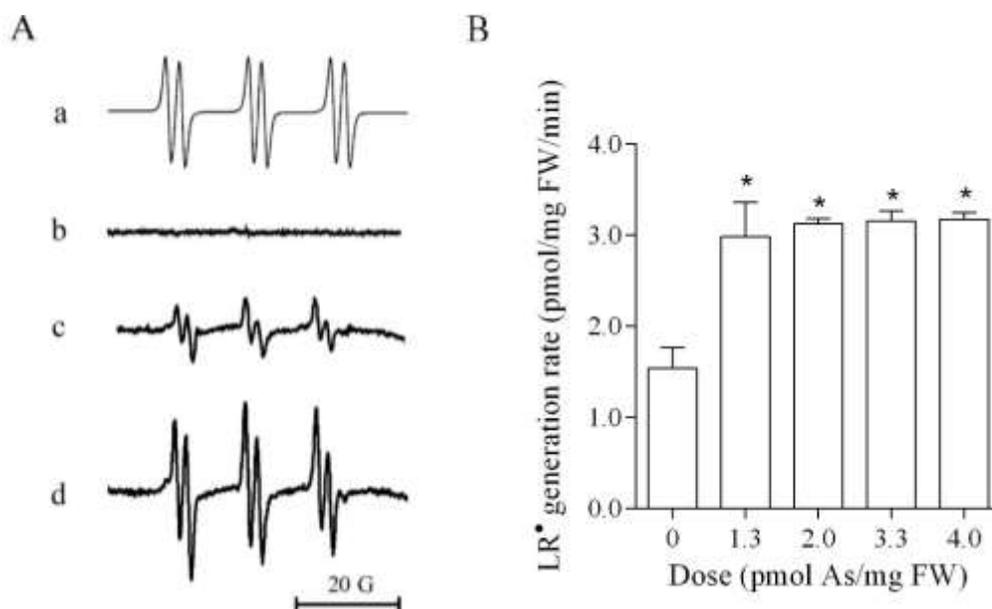
The whole brain was dried in an oven at 60°C until constant weight, were mineralized in HNO₃ according to Laurie et al. (1991). Fe concentration was spectrophotometrically determined after reduction with thioglycolic acid measuring the absorbance at $\lambda = 535$ nm in the presence of bathophenanthroline according to Brumby and Massey (1967).

Statistical analyses:

Data in the text and tables are expressed as mean values \pm standard error of the mean (S.E.M.). Statistical tests were carried out using Graph Pad Prism 6, Unpaired Student's t-test or one-way ANOVA with Dunnett's post-test, depending on the experimental protocol. The level of statistical significance adopted was $p < 0.05$.

Results:

The *ex vivo* effect of As over brain tissues was studied, by EPR, assessing the generation rate of LR[•] as a measurement of lipid peroxidation. LR[•] combined with the spin trap POBN resulting in adducts that gave a characteristic EPR spectrum with hyperfine coupling constants of $a_N = 15.8$ G and $a_H = 2.6$ G (Fig. 1A), in agreement with computer spectral simulated signals obtained using those parameters. Even though these constants could be assigned to LR[•], spin trapping studies cannot readily distinguish between peroxy, alcohoxyl and alkyl adducts, owing to the similarity of the corresponding coupling constants (Buettner, 1987). Control brain homogenates were exposed to 1.3-4 pmolAs/mg FW, and an approximately 2-fold increase in the LR[•] generation rate was recorded (Fig. 1B).



*significantly different from values in homogenates non-exposed to As. Student's T-test, $p < 0.05$.

Figure 1: LR[•] generation rate in brain homogenates after exposure to As *ex vivo*. **A.** EPR signal for LR[•] in control and rat brain homogenates exposed to As, (a) computer simulated-spectrum employing the following spectral parameters: $g = 2.005$ and $a_H = 1.8$ G, (b) POBN alone, (c) control rat brain, (d) rat brain homogenate exposed to 2.0 pmol As/mg FW in the presence of 40 mM POBN in buffer. **B.** Quantification of LR[•] generation rate in rat brains after exposure to several As concentrations.

Brain homogenates from control rats showed the typical EPR spectrum of A^\bullet , with the characteristic two lines at $g = 2.005$ and $a_{H^\bullet} = 1.8$ G (Fig. 2A). The *ex vivo* exposure to 1.3 to 4.0 pmolAs/mg FW during 5 min did not significantly affect the generation rate of A^\bullet by brain samples (Fig. 2B).

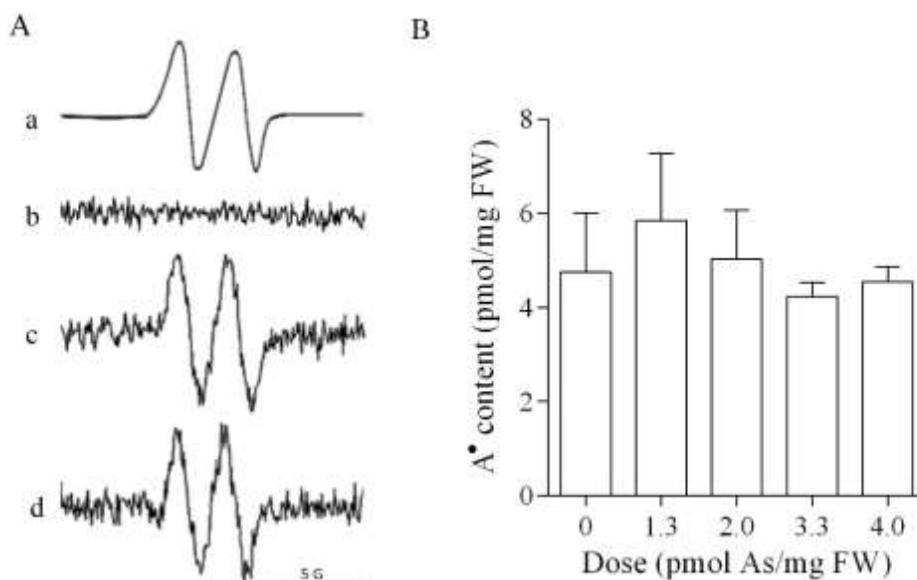
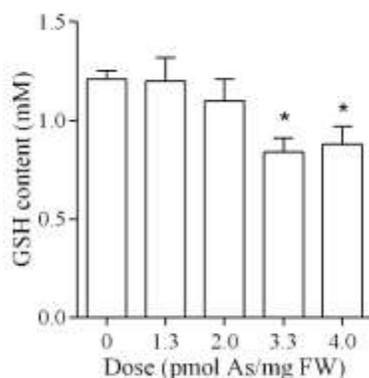


Figure 2: A^\bullet content in brain homogenates after exposure to As *ex vivo*. **A.** EPR signal for A^\bullet in control rat brain homogenates exposed to As during 5 min, (a) computer simulated-spectrum employing the following spectral parameters: $g = 2.005$ and $a_{H^\bullet} = 1.8$ G, (b) DMSO alone, (c) control rat brain homogenate, (d) rat brain homogenate exposed to 3.3 pmol As/mg FW. **B.** Quantification of A^\bullet generation rate in rat brains after exposure to several As concentrations.

A dose-dependent significant decrease of 33% and 30% in the content of the antioxidant GSH was measured after exposure to 3.3 and 4.0 pmolAs/mg FW, respectively (Fig. 3).

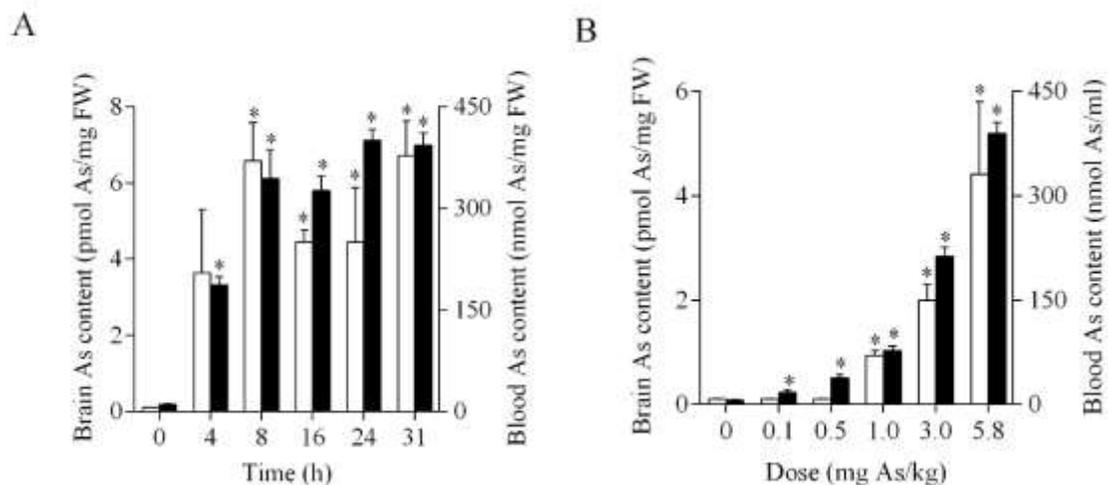


*significantly different from values in homogenates non-exposed to As. Student's T-test, $p < 0.05$

Figure 3: GSH quantification in brain homogenates after exposure to different doses of As *ex vivo*.

Since As is mainly metabolized in liver by repetitive reduction and oxidative methylation (Bonetto et al., 2014), for the *in vivo* studies the As content, both in whole blood and brain, was measured after the ip injection of a single dose of As. In all the As concentration range studied no morphological damage was observed in liver tissue by employing histology with hematoxylin and eosin staining techniques (data not shown). The data in Fig 4A showed that As

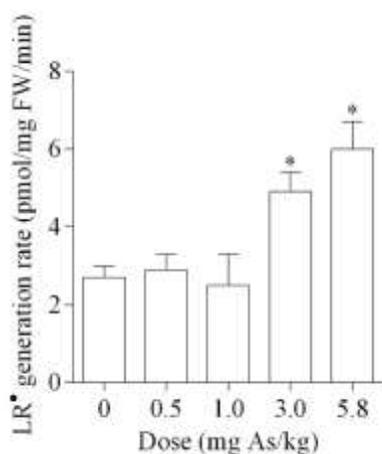
content reached its maximum value, in both compartments, after 24 h of the administration of the single dose of As. In both tissues, the As content depended on the administrated dose of As (Fig. 4B).



*significantly different from values in animals non-exposed to As. One way ANOVA with Dunnett's post-test, $p < 0.05$.

Figure 4: As content in rat blood and brain. **A.** Brain (white bars) and blood (black bars) content as a function of time after the administration of a single dose of 5.8 mg As/kg body weight. **B.** Brain (white bars) and blood (black bars) content as a function of the doses of As administrated to the animals. Measurements were performed after 24 h of the ip injection of As.

Moreover, as it was seen after exposure to As in the *ex vivo* experiments, the rate of generation of LR^{\bullet} by homogenates of brain tissue excised 24 h after the As administration to the animals, was increased as compared to values observed in brain from control rats. Data in Figure 5 showed that the LR^{\bullet} generation rate in the brain was increased by 81 and 122%, after the administration of 3.0 and 5.8 mg As/kg, respectively.



*significantly different from values in animals non-exposed to As. Student's t-test, $p < 0.05$.

Figure 5: LR^{\bullet} generation rate in brain homogenates after exposure to As *in vivo*. Quantification of LR^{\bullet} generation rate in rat brains excised after 24 h of exposure to several As doses. Brain homogenates were incubated in the presence of 40 mM PBN-DMSO for 30 min.

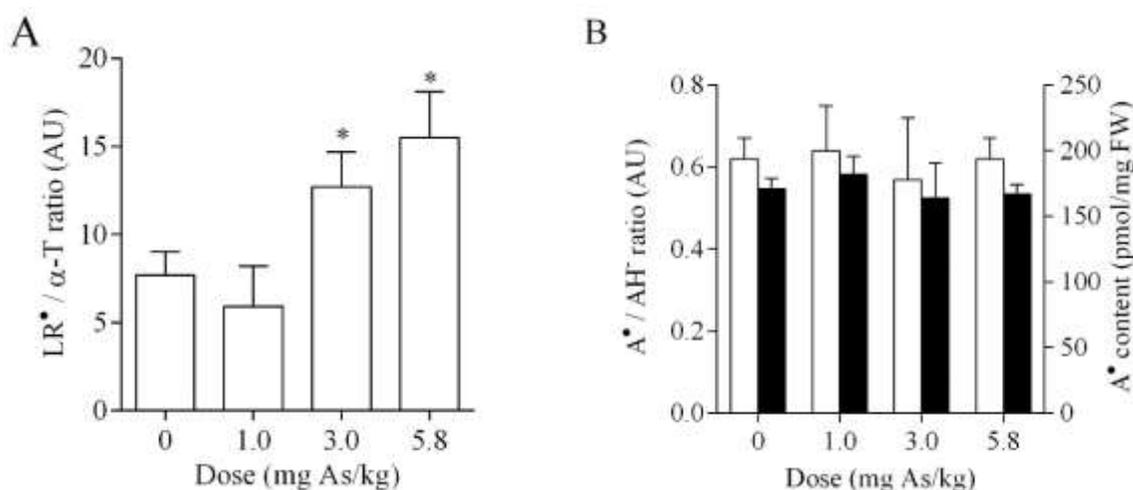
However, neither the content of GSH, nor α -T nor AH^- in brain tissues from animals receiving 5.8 mg As/kg was significantly affected, as compared to the values from control animals (Table 1).

Table 1

		α -T content (μ mol/mg FW)	AH^- content (μ mol/mg FW)	GSH content (mM)
Control		11 \pm 1	171 \pm 8	1.9 \pm 0.1
As (mg/kg)	1.0	13 \pm 1	182 \pm 14	non-determined
	3.0	12 \pm 1	164 \pm 27	non-determined
	5.8	12 \pm 1	167 \pm 7	2.0 \pm 0.1

Measurements were performed after 24 h of the ip injection of As.

The LR^\bullet/α -T content ratio, considered as an indicator of the balance between free radical damage and antioxidant protection in the hydrophobic medium (Malanga et al., 2009), was significantly different between samples obtained from control and As-treated animals in a dose-dependent way (Fig. 6A). However, the A^\bullet content in brain samples from control rats was not significantly affected 24 h after As administration in any tested dose (Fig. 6B). The A^\bullet/AH^- content ratio, understood as an index of oxidative stress in the hydrophilic medium (González et al., 2013), was not significantly different between the obtained data in control and As-treated animals (Fig. 6B).



*significantly different from values in animals non-exposed to As. Student's t-test, $p < 0.05$

Figure 6: Oxidative balance in the lipophilic and hydrophilic environment in rat brain after exposure to As. **A.** LR^\bullet/α -T content as a function of the doses of As administrated 24 h before the measurement. **B.** A^\bullet/AH^- ratio (white bars) and ascorbyl radical content (black bars) as a function of the doses of As administrated 24 h before the measurement.

Since arsenicals can release Fe, the content of total Fe was measured. No significant differences between brain homogenates from control and treated animals for 24 h with 5.8 mg As/kg 24 h (1.4 ± 0.2 and 1.5 ± 0.2 μ mol/mg FW, respectively) were seen.

Discussion:

Even though the main forms responsible for As toxicity, as compared to the pentavalent forms, are the highly toxic intermediate arsenicals monomethylarsonous acid (MMAIII) and DMAIII (Catalayud et al., 2013), As and ROS metabolisms may be closely associated. Several metabolic pathways were proposed for As-dependent oxidative

damage to tissues. Yamanaka et al. (1989, 2009) postulated the formation of an oxidative stress promotor, a dimethylated As peroxide from DMA, the generation of dimethylarsenic $[(\text{CH}_3)_2\text{As}^\bullet]$ and dimethylarsenic peroxy radicals $[(\text{CH}_3)_2\text{AsOO}^\bullet]$. However, up to now no experimental evidence was presented about the absolute requirement of the liver contribution to the formation of radical species by As exposure. Brain cells may be at particular risk for oxidative stress. The brain derives its energy almost exclusively from oxidative metabolism through the mitochondrial respiratory chain, and is relatively deficient in protective mechanisms compared to other tissues. It contains reduced quantities of CAT, GPx, GSH and α -T than the liver or the kidney (Rodriguez et al., 2003), but Kharroubi et al. (2017) studying microglial BV-2 cells treated with sodium arsenate showed neurotoxic effects of high concentrations of As^{V} by increased levels of mitochondrial complexes I, II, and IV, followed by increased O_2^\bullet generation. These authors suggested that As^{V} triggered mitochondrial dysfunction, which may lead to defective oxidative phosphorylation subsequently causing mitochondrial oxidative damage, which in turn, induces an apoptotic mode of cell death with an alteration of plasma membrane integrity. This proposal is consistent with the results presented here. The rate of generation of LR^\bullet , showed a compatible effect *in vivo*, as compared to the response seen in the *ex vivo* study. Moreover, the LR^\bullet/α -T content ratio was significantly increased in the *in vivo* model, in agreement with the development of oxidative damage to membranes by the exposure to the toxic. Thus, it seems that liver processing of As is not a key factor for radical generation in brain at the lipophilic medium. The production of A^\bullet , a resonance stabilized tricarbonyl species, is easily observable by EPR in aqueous solutions at room temperature since it is a quite stable radical with a biologic half-life of 30-60 min (Ahola et al., 2004). Homogenates of rat brain exposed *ex vivo* to As showed no changes in the content of A^\bullet , and neither did the homogenates from *in vivo* animals exposed to As, as compared to control brain homogenates. Since the content of A^\bullet was not affected by the single doses of As in all the range of concentrations tested, the data from the *ex vivo* and *in vivo* studies suggested that the oxidative balance in the hydrophilic cellular environment was not affected by As exposure. Thus, in agreement with the response observed in the lipophilic medium, the nature of the protocol of administration of As does not seem to be directly involved as a factor for modifying the As-dependent radical generation. Moreover, A^\bullet signal intensity is a function of pH, temperature, catalytic metal concentration, oxygen concentration and AH^\bullet concentration. Only when these variables can be controlled, the intensity of the EPR signal of the A^\bullet in steady-state concentration serves as a marker for the oxidative stress in a system (Buettner and Jurkiewicz, 1993). In intact biological systems it is very difficult to control all these variables, especially the total AH^\bullet content, which can be modified by the pro-oxidant conditions in a given cellular preparation. In such cases, the A^\bullet content is not applicable as oxidative stress indicator by itself, and the ratio $\text{A}^\bullet/\text{AH}^\bullet$ content should be used. The *in vivo* experiments reported here using EPR spectroscopy, in conjunction with HPLC detection of the AH^\bullet content, a powerful tool for detection of the initial stages of oxidative stress in the cellular hydrophilic compartment, agreed with the *ex vivo* experiments. The data reported here are consistent with the hypothesis that the liver is not absolutely required for causing these changes in the oxidative metabolism, either because the transformations to the active forms of As can be performed by the brain or that oxidative stress could be triggered directly by the inorganic As. Further studies are needed to clarify the mechanism(s), however these observations are the first indications in this regard. The tested doses of As employed in the *ex vivo* study were consistent with the As content achieved in brain 24 h after the administration of a single doses of As (5.8 mg/mg FW brain). Moreover, achieving comparable content of As in brain by *ex vivo* and *in vivo* experiments allowed to exclude the effect of the As content when comparing the assays protocols used in the studying of the effect of As in the cellular redox balance. On the other hand, GSH metabolism seems to play a role in As toxicity. The conjugation of iAs^{III} with GSH has been described by Hayakawa et al. (2005) and Pastore et al. (2003). Since As interacts with thiol groups, it can be directly toxic by blocking essential sulfhydryl groups of protein and enzymes, such as succinic and pyruvate dehydrogenases (Aposhian, 1989), or by binding to free sulfhydryl groups of membrane proteins inducing a marked decrease of free sulfhydryl groups and altering the intracellular signaling mechanisms (Zhang et al., 2000). DMAG^{III} was described to be reduced to a dimethylarsine by a GR and NADPH, and this product may react with molecular O_2 to form dimethylarsine radical. Monomethylarsonic diglutathione (MMDG^{III}) was present in the reaction mixture (Hayakawa et al., 2005). Accordingly, the exposure to As in the *ex vivo* model studied here shown a significant decrease in the content of GSH in rat brain. Rezaei et al. (2017) showed that acute, but not chronic contact with As generates significant changes in the normal glucose tolerance pattern that may be due fundamentally to over production of ROS and oxidative stress, and is preventable by using N-acetylcysteine, a thiol containing antioxidant. The data presented in this work is showing that not only the pattern of administration of As, but also the employment of an *ex vivo* or an *in vivo* acute contact could be a key factor to generate a differential oxidative response. Thus, the data presented here, by comparing the effects of comparable doses of As administrated either *ex vivo* or *in vivo*, suggested that even though oxidative stress is detected at the membrane level, the nature of the

metabolic pathways triggered in the brain differs according to the exposure protocol in terms of GSH consumption. As is a worldwide environmental contaminant, which compromises immunity and causes various associated disorders. Soria et al. (2017) to further investigate its immune toxicity exposed rats to inorganic As^{III} in drinking water for 2 months, and then evaluated subsequent changes of redox enzyme responses in spleen parenchyma cells (splenocytes). Not only As-exposed splenocytes showed γ -glutamyltranspeptidase inhibition, peroxidase induction, and variable responses of nitric oxide synthase, but also established the impact of As bioaccumulation on metallic spatial homeostasis (including Fe content) in the spleen. These authors suggested that As disrupts biometal-dependent immune pathways and redox homeostasis. The data presented here, however, showed that Fe total content was not modified by As administration after 24 h of the treatment. Nevertheless, the potential effect of the As on Fe cellular metabolism in this model could not be discarded. Besides the fact that arsenicals can release Fe by the activation of the enzyme HO-1 (Albores et al., 1989), as previously indicated, Fe is able to chelate As (Asghar and Zahra, 2017). This feature could be of significant relevance in terms of the establishment of oxidative-dependent damage to membranes since the exposure to the toxic could lead to modification of the cellular Fe distribution. Since the catalytic activity of Fe in the ROS metabolism strongly depends on the form in which the metal is present in the cell, the presence of a compound that could affect this scenario may act as a key factor to lead to oxidative-dependent damage by increasing the content of the labile Fe pool (LIP).

Conclusion:

Taken as a whole, the information presented here showed new evidence on the generation of specific radical species by As treatment, both *ex vivo* and *in vivo*, and pointed out to the possibility that the triggering of some oxidative pathways (such as oxidation of GSH) occur accordingly to the via As reaches the brain, even achieving the same concentration of the toxic in the tissues. However, other pathways may be similarly affected under both conditions of treatment, since the oxidative balance in the lipophilic and hydrophilic medium followed the same pattern. On the other hand, further studies analyzing the effect of As exposure on the distribution of Fe in the brain cells, could be an important aspect to achieve a more specific knowledge on As-dependent ROS generation. Also, the effect of As exposure on nitrosative pathways may be explored to complete the knowledge of the oxidative scenario in the presence of As.

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