# Silicic Acid and Beer Consumption Reverses the Metal Imbalance and the Prooxidant Status Induced by Aluminum Nitrate in Mouse Brain.

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Abbreviations: AD, Alzheimer disease; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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E-mail address: Aluminum, aluminum nitrate, antioxidants, beer, brain, copper, enzyme expressions, iron, manganese, magnesium, silicic acid, Silicon, TBARS, TNFα , zinc

**Authors declare that non-conflict of interest exists.**

**Running title: Brain metal and beer**

## Abstract

Background: Emerging evidence suggests that by affecting mineral balance, aluminum (Al) may enhance some events associated with neurodegenerative diseases. Aim: To examine the

effect of Al(NO3)3 exposure on brain Al, cooper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), silicon (Si), and zinc (Zn) levels, and the metal-change implication in brain oxidant and inflammatory status. Methods: Four groups of six-week-old male NMRI mice were treated for three months: i) controls, administrated with deionized water; ii) Al, which received Al(NO3)3; iii) Al+silicic acid, which were given Al(NO3)3 plus silicic acid; and iv) Al+beer, which received Al(NO3)3 plus beer. Results: Brain Al and TBARS levels and TNFα and GPx expressions increased, while Cu, Mn, and Zn levels, and catalase and CuZn-SOD expression decreased (at least, $p<0.05$) in Al versus control animals. Al, Si, and TBARS levels and TNFα expression decreased ($p<0.05$) in Al+silicic acid and Al+beer specimens while Cu, Mn, and Zn levels and antioxidant expression increased versus the Al group. Brain Al levels correlated negatively with those of Cu, Fe, Mn, and Zn, and catalase, CuZn-SOD, and GPx enzyme expressions but positively with Si and TBARS levels and TNFα expression. Two components of the principal component analysis (PCA) explained 71.2% of total data variance ($p<0.001$). PCA connected the pro-oxidant markers with brain Al content, while brain Zn and Cu levels were closer to antioxidant enzyme expression. Conclusion: Administration of Al(NO3)3 induced metal imbalance, inflammation, and antioxidant status impairment in the brain. Those effects were blocked to a significant extent by silicic acid and beer administration.

**Keywords:** Aluminum, Beer, Silicic acid, Metals, Antioxidants, Enzyme expressions, TNF, TBARS, Brain.

## Abbreviations

AD, Alzheimer disease; CAT, catalase; GPX, glutathione peroxidase; SOD, superoxide dismutase; PCA**,** Principal component analysis; TBARS, thiobarbituric acid-reactive substances; TNFα, tumor necrotic factor.

## Introduction

Metal dyshomeostasis, especially for endogenous metal ions such as copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) or the exogenous contaminant aluminum (Al), has attracted recent interest on the etiology of a variety of neurodegenerative conditions [1]. Maintaining transition metal homeostasis is known to be important in a wide variety of biological functions, such as anti-oxidant defense mechanisms. However how metals, such as Al, Cu, Fe and Mn, contribute to oxidative stress and protein aggregation leading to neurodegenerative diseases remains unclear [2]. Among several possible mechanisms, the promotion of α-synuclein aggregation and fibril formation, the activation of microglial cells leading to inflammation and impaired production of metalloproteins have been proposed [2]. It has been suggested that Al may be involved in the formation of neurofibrillary tangles in the brain [3]. An unusual aspect of the biochemistry of this non-redox active metal is its pro-oxidant activity, which might be explained by the formation of an Al superoxide semireduced radical ion $AlO_2^{\cdot 2+}$ [3].

Yang et al.,[4] found that Fe, Zn, Mn, and Cu concentrations were significantly decreased in serum after administrating Al to rats, possibly because Al load caused an increase in the transport of Fe, Mn, Cu, and Zn into liver cells. There is evidence that the exposure to Al causes an increase in both oxidative stress and inflammatory events [5]. Furthermore, this neurotoxic metal can modify the levels of other metals in the brain, and this phenomenon has been suggested to be part of its mechanism of action [6].

Silicon (Si) has recently been recognized to exert health benefits with regard to skeletal and neurological function and status [7]. Very recently our group found that organic Si protected neuroblastoma cells from $H_2O_2$ aggression [8, 9] and improved the antioxidant status of aged rats fed cholesterol-enriched diets [10]. Although action mechanisms are not-well known, it has been proposed that Si among others may decrease Al bioavailability by blocking its uptake through the gastrointestinal tract [11] and by impeding reabsorption [12]. Bioavailable Si, that is, Si in the form of silicic acid or orthosilicic acid, is mainly found in

foods rich in fiber and whole grains [13], with beer being one of the main sources of this element. Si in beer is present chiefly in a monomeric form [14]. A previous paper [15] demonstrated that beer intake affects the kinetics of Al uptake and excretion, possibly due to an interaction between Al and Si in the digestive tract. Therefore, Si in the form of silicic acid may lower Al bioavailability, and hence should be considered an element that may protect against Al toxicity in the brain. In addition, our group has also demonstrated that the ingestion of Si as silicic acid or beer prevented lipid oxidation and pro-inflammatory effects induced by Al exposition [5] reverting the decline of mRNA expression of several endogenous antioxidant enzymes. Thus, keeping in mind all these results present paper hypothesized that Al administration negatively affects brain level of various metals, some of them involved in antioxidant/pro-oxidant reactions; thus, causing oxidative stress and inflammatory deleterious effects. In addition, the simultaneous incorporation of Al and Si, as silicic acid or beer, arrests those negative effects.

The aims of the present study were to (1) evaluate the relationship between levels of Al and other transition metals (i.e., Cu, Fe, Mg, Mn, Si and Zn) in the mouse brain, (2) establish correlations between metal levels obtained and gene expression of certain antioxidant enzymes, and (3) attempt to elucidate other possible mechanisms of Al induced toxicity.

## Material and methods

### *Reagents*

Al nitrate [$Al(NO_3)_3$ .9H2O] (Aldrich, CAS 7784-27-2) and silicic acid, $Si(OH)_4$ (Fluka Chemie, Buchs, Switzerland) were used. Silicic acid was dissolved in 0.9% saline solution and 2% ethanol. Al nitrate was dissolved in 0.9% saline. Both chemicals were orally administered at a volume of 0.5 ml.

### *Animals and treatments*

Six-week-old male NMRI mice (weighing approximately 30 g) were obtained from the Animal Research Center, University of Alcalá, homologated by the Spanish Competent Authority, Register Number ES280050001165 implementing the Spanish Royal Decree 53/2013 and the European Directive 63/2010/EU. The protocol employed in this experiment was approved by the Ethics Committee of the Universidad de Alcalá (Spain). All procedures

were performed in compliance with the Directive 63/2010/EU, for the protection of animals in research.

The animals were randomly divided into four groups (n = 12 per group). A control group (Group 1), receiving only deionized water, was used as the negative control. The three treatment groups were exposed for three months to aluminum nitrate, $Al(NO_3)_3 \cdot 9H_2O$, at 450 μg/ml via drinking water. The solutions were prepared daily and the content of Al was measured in each batch. This methodology for long-term exposure of mice to Al is based on previous studies from our lab [15] and others [16]. Group 2 received only Al nitrate (Al group or positive control); Group 3 (Al+Si group) received Al nitrate and a solution of silicic acid ($Si(OH)_4$) at a concentration of 50 mg/ml; and finally group 4 (Al+beer group) was given the same dose of Al nitrate and an amount of commercial beer (5.5% alcohol by volume) equivalent to moderate to high consumption in humans (1 l/day). A previous study [17], demonstrated that the same doses of alcoholic beer were more effective than non-alcoholic beer. According to [15], the Si concentration in the tested beer was 24.56 ± 2.45μg/g while that of Al 0.40 ± 0.12 μg/g. All animals were weighed weekly. The mice were housed in an animal room under standard conditions of temperature (21 ± 1º C) and humidity (55 ± 10%), with a 12-h light/12-h dark cycle.

At the end of the treatment period, animals were euthanized after anesthesia with isofluorane by heart puncture and total and terminal bleeding, and brain tissue samples were taken for subsequent analysis. The right hemibrain was used to determine mineral content, while the left hemibrain was washed with saline solution, minced and homogenized (10%, w/v) separately in ice-cold 1.15% KCl–0.01M sodium, potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000*g* for 20 min at 4 $^{o}$C, and the resultant supernatant was used for biochemical analysis.

*Analytical methods*

The mineral levels in the brain were measured after ashing of the organic matter according to the method proposed by Granero et al., 2004 [18]. Each sample (2.5 ml) was digested with 2 ml of 65% nitric acid (Suprapur, Merck, Darmstad, Germany) in Teflon bombs for 8 h at room temperature, and subsequently heated at 100 º C for 12 h. After cooling, solutions were filtered and made up to 25 ml with deionized water.

Si content of the brain was measured by means of inductively coupled plasma atomic emission spectrometry ICP-OES (Perkin Elmer Optima model 3200 RL), using Si emission lines of 254.611 nm and 212.412 nm. Brain levels of Al were determined by means of inductively coupled plasma mass spectrometry (Perkin Elmer Elan model 6000, ICP-MS), using the only Al isotope, Al27. The emission lines and isotopes used are free of spectral interferences in these matrix types. Multi-element analysis of Mn in digested samples was performed with ICP-MS spectrometry, while the other elements (Cu, Mg, Fe, Zn) were analyzed with ICP-OES spectrometry. Elements with the highest isotopic abundance, free from isobaric and polyatomic interferences, were selected as analytical mass to perform determinations by ICP-MS spectrometry. When ICP-OES spectrometry was employed, possible interferences and selection of analytical lines were checked selecting three of the most sensitive spectral lines.

Validation of the methods, based on the ICP-OES and ICP-MS techniques, was performed according to EURACHEM guidelines, 1998 [19] with regard to accuracy, precision, sensitivity, and linearity, using the experimental setting that provided the optimal conditions. According to these guidelines, intra-assay and inter-assay imprecision, measured as variation coefficients, should be below 5 and 10% respectively. Sensitivity of the determination of each chemical element was expressed by the slope of the linear regression equation. Linearity was assessed by the correlation coefficients of calibration curves and was considered acceptable when $r \geq 0.9995$. Detection limits were calculated on the basis of the 3s criterion for ten replicate measurements of blank solutions subjected to the same treatment as the samples.

The accuracy of the instrumental methods was validated by replicating all samples and by taking measurements of reference material (lobster hepatopancreas, NRC Canada TORT 2) every 10 samples. Quantification was based on the most abundant isotope of each element free of analytical interferences. The mean recovery rates were between 90% and 95%.

*Biochemical assays*

Using an Uvikon 930 spectrophotometer, lipid peroxidation was measured by following the formation of malonaldehyde (MDA), according to the presence of thiobarbituric acid reactive substances (TBARS) in the brain homogenates [20]. Concentrations were calculated using a standard curve obtained with MDA. TBARS values were expressed as μmols of MDA per mg

protein. Protein was determined by the Bradford method [21], with bovine serum albumin as the standard.

*RT-PCR real time analysis*

Total RNA was extracted from frozen brain samples following the guanidinium thiocyanate/phenol reagent method [22]. Reverse transcription and amplification using the Titan system involved the preparation of a master-mix 1 and 2 on ice. Mix 1 was comprised of dNTPs, primers, dithiothreitol (DTT), extracted RNA (1 μg) and sterile pre-chilled deionized water. Mix 2 consisted of RT-PCR buffer, enzyme mix (AMV reverse transcriptase, Taq DNA polymerase) and sterile pre-chilled deionized water. All reagents were thawed, vortexed briefly and centrifuged before setting up the reactions. Twenty-five microliters each of master mix 1 and 2 was added to a 0.2 ml PCR tubes kept on ice. This was vortexed and centrifuged briefly to collect the sample at the bottom of the tube, and RT was carried out at 50 oC for 30 min. β-actin cDNA was used as an internal control.

The sequences of the primers were as follows:

SOD sense: 50-GCCGTGTGCGTGCTGAA-30;

SOD antisense: 50-TTTCCACCTTTGCCCAAGTCA-30;

b-Actin sense: 50-TACAACCTCCTTGCAGCTCC-30;

b-Actin antisense: 50-GGATCTTCATGAGGTAGTCAGTC-30.

The number of PCR cycles was adjusted to avoid saturation of the amplification system: 95º for 30 s, 5º for 1 min and 72º for 30 s (30 cycles) for SOD; 58º for 45 s and 72º for 30 s (24 cycles) for b-actin with a final elongation at 72º for 10 min. Amplification products were visualized on 1.8% agarose gels containing ethidium bromide (1 μg/ml): SOD product, 383 bp; b-actin product 630 bp. A 100 bp DNA ladder was used as marker. The products were quantified by laser densitometry.

*Principal component analysis*

PCA test was applied to understand possible associations between some of the mineral determined and the pro- and antioxidants parameters analyzed. Ten different variables were tested, five, related to mineral content (Zn, Cu, Mn, Si, and Al), and five about antioxidant

markers (CuZnSOD, Catalase, GPx, TNF-alpha and TBARs). PCA create new variables (principal components (PCs)) containing the initial variables which correlate among them.

*Statistical analysis*

All analyses were performed in triplicate. Data were expressed as means ± SD. The Kruskal–Wallis non-parametric test followed by multiple non-parametric comparison test [23] were used. Spearman correlations were performed to study the relationship between minerals and TBARS, TNFα and the different expressions antioxidant enzymes. P values of <0.05 were considered statistically significant. Statistical analyses were performed using the SPSS statistical software package (version 15.0) and the SAS (version 19.0).

**Results**

*Brain tissue Al and trace metals levels*

**Table 1** presents the concentrations of Al, Cu, Fe, Mg, Mn and Zn in mouse brain from the different treatment groups. Al exposition significantly increased brain Al and Si contents but significantly decreased ($p < 0.05$) Cu, Mn and Zn levels. In presence of Al exposition, beer or silicic acid, significantly lowered Al and Si levels and normalized those of Cu, Mn and Zn in brain.

*Brain TBARS levels and* TNFα and *antioxidant enzymes expression*

Brain tissue levels of TBARS, measured as the lipid peroxidation end product malondialdehyde (MDA), were significantly higher ($p < 0.001$) in the Al group than in control mice. Brain levels of TBARS in the Al+Si and Al+beer groups were significantly lower ($p < 0.001$) than those of the Al one (**Fig. 1**).

The relative TNFα expression of the Al group significantly increased *vs.* control ($p < 0.001$). This expression was significantly reduced in the Al+Si and Al+beer groups *vs*. their Al counterparts and non-differing ($p > 0.05$) from that of control animals. (**Fig. 1**).

The expression of MnSOD, CuZn-SOD and GPX enzymes was significantly lower ($p<0.001$) and GPX higher ($p < 0.001$) in the Al group *vs.* control mice. Enzyme expressions ere normalized in the brains of the Al+Si and Al+beer, as their values did not differ from those of the control group ($p>0.05$) (**Fig. 1).**

*Metal levels, TBARS and gene expression relationships*

**Table 2** shows the non-parametric correlations between mineral and TBARS levels and the TNFα and antioxidant enzymes expression. Al correlated negatively and significantly with Cu ($p < 0.01$), Mn and Fe (both $p < 0.05$) and Zn ($p < 0.001$) but positively with Si ($p < 0.001$). Cu was positively correlated with Fe ($p < 0.01$), Mg ($p < 0.001$), Mn ($p < 0.01$), while Fe positive and significantly correlated with Mn ($p < 0.001$) and Zn ($p < 0.01$). Zn showed positive correlation ($p < 0.001$) with Mg levels. TBARS levels and TNFα expression were positively correlated with Al (both $p < 0.001$) and Si ($p < 0.05$ and $p < 0.01$, respectively). Cu ($p < 0.01$) and Zn ($p < 0.05$) negatively correlated with TBARS and TNFα (both $p < 0.001$).

CAT gene expression showed significant positive correlations with Cu ($p < 0.001$), Fe ($p < 0.05$), Mg ($p < 0.05$), Mn ($p < 0.05$) and Zn ($p < 0.001$), while CuZn-SOD gene expression with Cu ($p < 0.01$) and Zn ($p < 0.001$). Both CAT and CuZn-SOD expressions negatively correlated with Al ($p < 0.001$) and Si ($p < 0.001$) whereas GPx expression negatively with Cu ($p < 0.01$), Fe ($p < 0.01$), Mn ($p < 0.05$) and Zn ($p < 0.01$) but positively with Al ($p < 0.001$) and Si ($p < 0.001$).

*PCA results*

PCA was conducted to ascertain possible relationships between mineral and TBARS contents and TNFα and antioxidant enzymes expressions. Ten different variables were evaluated (Zn, Cu, Mn, Si and Al, CuZnSOD, Catalase, GPx, TNFα and TBARs). PCA created two components which explained the 71.2% of the total variance in the data set (**Fig. 2a**). First component (PC-1) accounted for 59.48% of the variance. It strongly and positively correlated with GPx, TNF-alpha, Al, and TBARs. Their values indicated in **Table 3** were close to 1. Si was found relatively close to them with a 0.532 of PC-1. On the other hand, there were CAT and CuZn-SOD, with values close to -1, located on the left side of the plot (**Fig. 2a**), which correlated negatively with the previous ones. Second component (PC-2) explained 11.73% of the total variance. It positively correlated with Zn and Cu, both appearing negatively correlated also with PC-1 (**Fig. 2a**). Mn was in the middle of the two components, with a positive value of PC-2 but negative of PC-1.

The PCA connected the pro-oxidant markers with Al content, and to a lesser extent with Si. On the contrary, Zn and Cu were closer to antioxidants enzymes. The PCA scatter plot (**Fig. 2b**) indicates the position of the studied groups (control, Al, Al+Si, Al+beer) in a graph compiled with two components (PC-1 and PC-2). It showed how the Al group was located on the positive part of PC-1 and close to 0 of PC-2. Control group appeared

oppositely in the negative part of both PC-1 and PC-2. Finally Al + Si and Al + beer were both located in the negative part of PC-1 as control group, but with more positive values of PC-2 than that group.

## Discussion

Present results clearly show that Al administration induced changes in mineral contents and the in the inflammatory and oxidant/antioxidant status of brain. These negative effects were significantly reversed by the conjoint administration of Al with silicic acid or beer.

There has been growing interest in the role of metal ions (especially Zn, Cu and Fe) in neurobiological processes. They participate in many essential activities, and their deficiencies can be lethal. Loss of Fe, Cu and Zn can lead to neurological disease, but conversely their accumulation or abnormal interactions with proteins, lipids or nucleic acids can also contribute to neurological disease [24]. Chronic exposure to Al induced reduction of Cu, Mn and Zn levels in the mouse brain. Yang et al.,[4] found that Fe, Zn, Mn, and Cu concentrations decreased in rat serum after Al administration, possibly because Al load caused liver uptake increase of those minerals. Thus, it can be speculated that the Cu, Mg and Zn decrease observed in brain following the Al administration could be a consequence of the lower plasma content of these metals. Others authors have suggested that also Al and some metals (i.g. Fe) compete for a common mechanism of gastrointestinal absorption [25, 26]; explaining, at least in part, the lower brain levels observed. In contrast, the level of Si increased in Al treated animals. Si has been suggested to interact with Al through the formation of non-toxic aluminosilicates that decrease free Al concentrations [11]. Formation of aluminosilicate prevents Al intestinal absorption, and thus prevents chronic aluminum accumulation that may cause or enhance neurodegeneration in the brain [27].

The conjoint administration of Al and silicic acid or beer, partially blocked the negative effects of Al in the metal dysbalance observed. Si and silicic acid may decrease Al bioavailability by blocking gastrointestinal tract uptake [11] and reabsorption [12]; thus, explaining the lower amounts of Al in Al+Si and Al+beer brain mice and the normalization of metals affected by Al intoxication. Brain Si concentrations in Al+Si and Al+beer mice were higher than those of the animals in the basal group probably due to a number of different causes. There is evidence that Al is able to produce free radicals that cause lipid peroxidation,

thereby damaging neuronal membranes and increasing blood–brain barrier permeability [28], allowing more Si to enter the brain tissue. The negative correlations between Al with Cu, Fe, Mn, and Zn but positive with Si strengths the potential mechanisms already discussed.

Changes observed in TBARS, TNFα and enzymes expression in Al group suggests a toxic and pro-oxidant effect due to the metal dysbalance induced by Al intoxication. Cu is also required for mitochondrial respiration, neurotransmitter biosynthesis and as a cofactor for antioxidant enzymes [29]. Some other findings suggest that AD could be the result of diminished availability of Cu in neurons. Cu acts at different levels: it participates in AβPP processing into non-amyloidogenic derivatives although its deficiency reduces Aβ degradation [30]. Cu deficiency may also influence the activity of Cu-binding proteins in AD. For example, activity of the Cu-dependent proteins, Cu/Zn superoxide dismutase (SOD) [31] and cytochrome C oxidase [32] has been reported to be reduced in AD patients when compared to controls. This suggests that altered Cu homeostasis exists in AD and that such alteration can lead redox disequilibrium by altering the functioning of important enzymes like Cu,Zn-SOD and ceruloplasmin [33]. Therefore, the decrease in Cu levels caused by Al could explain, in part, the decreased expression of Cu,Zn-SOD observed in rats treated with Al [4]. Results obtained by Bayer et al.[34] and Phinney et al.[35] show that both dietary and genetic manipulations, that increase brain Cu levels, improved amyloid pathology in two strains of AβPP transgenic mice. Silicon administration, as organic silicon or beer, restores brain levels of Cu and regulates expression of Cu,Zn-SOD. The hypothesis that silicon may be beneficial by altering the absorption and utilization of other mineral elements involved in bone metabolism, immune or inflammatory response, or cognitive function cannot be discounted [36]. Silicon, especially in supra nutritional amounts, has been reported to facilitate the absorption, retention, and/or utilization of copper [37] and magnesium [38].These data indicate that some of the metabolic effects attributed to silicon may be manifested through a silicon-facilitated increase in copper utilization [39]. The high positive correlation obtained between brain Cu levels and gene expression of Cu,Zn-SOD, as well as the negative correlation of Al levels with this enzyme support previous comments.

Zn nutritional deficiency is common in advanced age, and a recent report indicated that Zn deficiency in AβPP transgenic mice increased the volume of amyloid plaques [39]. Zinc acts as a neuromodulator at excitatory synapses and has a considerable role in the stress response and in the functionality of zinc-dependent enzymes, contributing to maintaining brain compensatory capacity [40]. The data obtained by Cuajungco and Fagét, [41] suggest a

protective role for Zn(2+) in AD, where plaques form as the result of a more robust Zn(2+) antioxidant response to the underlying oxidative attack. In particular, the mechanisms that modulate the free zinc pool are pivotal for safeguarding brain health and performance. Alterations in zinc metabolism and homeostasis have been reported in Parkinson's and Alzheimer's disease as well as in transient forebrain ischemia, seizures and traumatic brain injury, but little is known regarding aged brain [42].

Present results suggest that Al poisoning causes a significant decrease in the brain levels of zinc; the effect being reversed when Si was administered. Therefore, the Si could somehow mitigate the damage induced by Al, restoring Zn homeostasis in the brain. These results are correlated with increased expression of Cu/Zn SOD observed after administration of the beer or silicon aluminum intoxicated rats [5]. As for Cu, it found significant positive correlations between brain Zn levels and gene expression of Cu,Zn-SOD.

Fe is required to support the brain's high respiratory rate as well as for myelination, gene expression and neurotransmitters synthesis [43]. According to Yang et al. [4] Al easily occupies the binding sites of Fe, decreasing the interactions between $Fe^{2+}$ and iron transporters. This increase in free $Fe^{2+}$ level and thus, the production of a large amount of active oxygen free radicals, could result in cell membrane lipid peroxidation, accelerated cell apoptosis and necrosis, and mitochondrial function damage that clearly link with the impairment of the antioxidant and inflammation status observed in Al mice. Contini et al. [44] observed that the malregulation of intestinal Fe absorption in Al exposure inducing decrease of serum Fe concentration could be the result of the increased lipid peroxidation (TBARS) observed in this tissue. Mucosal TBARS were increased by Al exposure (+26%). In the present study, TBARS increased by Al exposure 2.15 fold (+46%) vs. control.

As the brain Al levels are diminished after Al+Si or Al+beer administration, it can be suggested that the interaction Al-$Fe^{2+}$ and thus, the active oxygen free radicals, may be reduced, explaining the improvement in the inflammation and antioxidant status observed. In fact, the concentration of brain Fe was significantly and positively correlated with gene expression of catalase and negatively with the expression of GPx and TNFα.

Although direct interaction between Mn and Aβ has not been reported yet, recent reports have highlighted the impact of loss of MnSOD or $SOD_2$ on AD pathology in APP transgenic AD models [45]. MnSOD is found exclusively in mitochondria and heterozygotic genetic ablation of MnSOD leads to increased oxidative stress [46]. This raises the hypothesis

that Mn deficiency might increase the risk for AD and other brain degenerative diseases. Our results showed a significant reduction in Mn levels in the brains of mice intoxicated with Al. Also the MnSOD was highly decresed, suggesting that brain mitochondria metabolism was highly affected in Al group. The administration of Si as silicic acid or beer achieved to reestablish both levels Mn and values MnSOD in brain, suggesting that silicic acid and beer improved the antioxidant defense of the brain through ameliorating Mn-SOD expression. Very recently our group has observed that Si protected neuroblastoma cells from $H_2O_2$ aggression [8, 9]. Moreover, Si increased liver Si content and improved the liver antioxidant status in cholesterol fed-aged rats [10].

As commented Al intoxication, directly or by inducing metal dysbalance, provoked oxidative stress and dysregulation in the antioxidant enzymes expression. Such alterations were blocked and reversed by the conjoint administration of Al+Si or Al+beer. This outcome was easily observed in the PCA scatter plot which located Al+Si and Al+beer groups close to control mice, suggesting a reversion of Al intoxication effects.

## Conclusion

Chronic Al leads to metal dysbalance in brain inducing brain inflammatory and pro-oxidant status impairment that in turn could contribute to neurological disease progression. Present study is the first showing that Si inclusion in the diet, in the form of silicic acid or beer, helps to recover the depleted levels of Cu, Fe, Mn, and Zn in the brain negatively affected by Al administration, allowing to improve oxidation and inflammatory markers and the antioxidant status in brain.

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