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Beer consumption reduces cerebral oxidation caused by aluminum toxicity by normalizing gene expression of tumor necrotic factor alpha and several antioxidant enzymes

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Abstract

Aluminum (Al)-induced neurotoxicity is well known and different salts of aluminum have been reported to accelerate oxidative damage to biomolecules. The present study has examined whether silicon consumed in the form of silicic acid or beer could potentially inhibit aluminum toxicity in the brain. Male mice were administered with $Al(NO_3)_3$ orally at a dose of 450 mg/kg/day in drinking water for 3 month. Experimental mice were given $Al(NO_3)_3$ along with 50 mg/L of silicic acid or with 0.5 ml/day of beer. Al brain levels in the Al group were four times higher than those of control mice while silicic acid and beer group values were 40% lower than those of the Al group. We have observed that beer prevented accumulation of lipid damage significantly, which resulted from aluminum intake. Decline in the expression of mRNA of endogenous antioxidant enzymes associated with aluminum administration was also inhibited by beer and silicic acid. The tumor necrosis factor alpha (TNFα) RNA expression was normalized in silicic acid and beer groups. Very high and significant correlations were found for the different parameters tested suggesting that moderate consumption of beer, due to its silicon content, effectively protects against the neurotoxic effects of aluminum.

Keywords: Aluminum; Antioxidant enzymes; Beer; Brain; Silicon; TNFα

1. Introduction

Aluminum (Al) is a highly neurotoxic element that may be involved in neuronal degeneration in human and experimental animal brains (Yumoto et al., 2001). This highly reactive element, known to cross-link hyperphosphorylated proteins, may play an active role in the pathogenesis of critical neuropathologic lesions in Alzheimer's disease (AD) and other related disorders (Perl and Moalem, 2006). Walton (2006) hypothesizes that Al could be implicated in the formation of neurofibrillary tangles in the brain and should therefore be considered as a causative factor in AD. Furthermore, Al is found associated with beta-amyloid in the brains of AD patients (Exley, 2005, 2006). In addition, this toxic metal is known to induce neuronal apoptosis *in vivo* as well as *in vitro* (Kawahara, 2005).

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

Abbreviations: AD, Alzheimer disease; CAT, catalase; GPx, glutathione peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TNFα, tumor necrotic factor alpha.

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radical ion (AlO_2^{2+}) (Exley, 2005). Reactive oxygen species (ROS) interact with all biological macromolecules, including lipids, proteins, nucleic acids, and carbohydrates. The resulting stress increases neuronal death, which contributes to the neuropathology associated with several diseases (Baydas et al., 2003). Although the exact mechanism by which the metal may influence disease processes in unknown, there is evidence that the exposure to Al causes an increase in both oxidative stress and inflammatory events. Al initiates and/or propagates an inflammatory response within the aging brain. Since inflammatory events are reported to be up-regulated in the AD brain, this may be one of the mechanisms by which the metals induce neurodegeneration (Campbell, 2006). In transgenic mice AD models, dietary Al markedly increased lipid peroxidation and Aß levels (Pratico et al., 2002). In isolated systems, Al can increase the oxidative stress produced by transition metals such as iron (Bondy and Kirstein, 1996) or Cu (Becaria et al., 2003). As Al is present in trace amounts in the drinking water; the possibility for low-dose chronic exposure should not be discarded (Campbell, 2006). Furthermore, average Al intake is estimated to be 2–5 mg per day in adults (Pérez-Granados and Vaquero, 2002). Some experts have, in fact, recently issued a strong warning that human exposure to Al should be limited (Aikoh et al., 2005).

In contrast, the health benefits of silicon (Si), with regard to skeletal and neurological function and status, have recently been recognized (Chumlea, 2007). Si and silicic acid may decrease aluminum bioavailability by blocking its uptake through the gastrointestinal tract (Parry et al., 1998) and by impeding reabsorption (Reffitt et al., 1999). However, as far as the authors know, there is currently no information available regarding the possible beneficial effects of Si on neural toxicity, the antioxidant system and stress markers of certain neurological disorders. 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 (Pérez-Granados and Vaquero, 2002), with beer being one of the main sources of this element. Si in beer is present chiefly in a monomeric form (Sripanyakorn et al., 2004).

A previous paper of our research group (González et al., 2007) demonstrated that beer intake affects the kinetics of aluminum uptake and excretion, possibly due to an interaction between Al and Si in the digestive tract. Therefore, it can be hypothesized that Si in the form of silicic acid may lower Al bioavailability, and hence should be considered an element that may afford protection against Al toxicity in the brain.

Taking into account the potential relationship between Al exposure, oxidative stress, inflammation, and certain neurological disorders, the aims of the present study were to evaluate (1) the oxidative and inflammatory alterations induced by Al intoxication in the murine brain, (2) the changes in gene expression of some antioxidant enzymes and inflammatory factors after dietary supplementation of Al-intoxicated mice with Si in the form of silicic acid or as a drinking beer compound, and (3) to assess the possible relationships between brain tissue inflammation and antioxidant defense markers.

2. Materials and methods

2.1. Reagents

Aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$] (Aldrich, CAS 7784-27-2) and silicic acid, SiO_2 or $Si(OH)_4$ (Fluka Chemie, Buchs, Switzerland) were used. All reagents were of the highest quality available and obtained from commercial sources. Silicic acid was dissolved in 0.9% saline solution and 2% ethanol, while Al nitrate was dissolved in 0.9% saline. Both chemicals were orally administered at a volume of 0.5 ml.

2.2. Animals and treatments

Six week old male NMRI mice weighing approximately 30 g were obtained from Animal Research Center, University of Alcalá, homologated by the Spanish Ministerio de Agricultura, Reference 28005-22A, Real Decreto 233-88.

The mice were housed in an animal room under standard conditions of temperature (21 ± 1 °C) and humidity (55 ± 10%), with a 12-h light/12-h dark cycle. All experiments were performed in compliance with Directive 86/609/EEC of November 24, 1986, for the protection of scientific research animals.

The animals were divided into four groups ($n = 12$). A control group consisting of mice that received only deionized water. The mice of the other three groups received aluminum nitrate, $Al(NO_3)_3 \cdot 9H_2O$, at the level of 450 μg/ml dissolved in deionized water, in their drinking water, for three months. This long-term exposure of mouse to Al is based on previous studies from our research group (González et al., 2007) and in those of bibliography (Pandya et al., 2004). One group consisted of intoxicated mice that received only aluminum nitrate (Al group); other group (Al + silicic acid group) received aluminum nitrate and a solution of silicic acid [SiO_2 or $Si(OH)_4$] at a concentration of 50 mg/ml; and finally the last group (Al + beer group) was also given the same dose of aluminum 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 (Peña et al., 2006) demonstrated that same doses of alcoholic beer were more effective than non-alcoholic beer. According to González et al. (2007) the Si concentration in the tested beer was 24.56 ± 2.45 μg/g while that of Al 0.40 ± 0.12 μg/g. All animals were weighed weekly.

At the end of the treatment period animals were euthanized by cardiac puncture under anesthesia with halothane, and brain tissue samples were taken for subsequent analysis. The whole right hemibrain was used to determine mineral content, while the whole left hemibrain was washed with saline solution, minced and homogenized (10%, w/v) separately in ice-cold 1.15% KCl–0.01 M 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 °C, and the resultant supernatant was used for biochemical analysis.

2.3. Analytical methods

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, Al^{27}. The emission lines and isotopes used are free of spectral interferences in these matrix types. Brain Al and Si contents were measured after wet ashing of the organic matter according to the method proposed by Granero et al. (2004). In short, 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 to 100 °C and held at that temperature for 12 h. After cooling, solutions were filtered and made up to 25 ml with deionized water. Accu-

racy of the instrumental methods was validated by replicating all samples as well as 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. Mean recovery rates were between 90% and 95%.

2.4. Biochemical assays

Using a Uvikon 930 spectrophotometer (Mihara and Uchiyama, 1978), , 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. 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 (Bradford, 1976), with bovine serum albumin as the standard.

2.5. RT-PCR real time analysis

Total RNA was extracted from frozen brain samples following the guanidinium thiocyanate/phenol reagent method (Chomeczynki and Sacchi, 1987). 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 °C for 30 min. β-actin cDNA was used as an internal control.

The sequences of the primers were as follows:

CAT sense: 5′-GTGAGAACATTGCCAACCAC-3′;
CAT antisense: 5′-CTCGGGAAATGTCATCAAAAG-3′;
SOD sense: 5′-GCCGTGTGCGTGCTGAA-3′;
SOD antisense: 5′-TTTCCACCTTTGCCCAAGTCA-3′;
GPx sense: 5′-AGTCCACCGTATATGCCTTC-3′;
GPx antisense: 5′-TCTGAGGGGATTTTTCTGGA-3′;
TNFα sense: 5′-TGGCCCAGACCCTCACACTC-3′;
TNFα antisense: 5′-CTCCTGGTATGAAATGGCAAATC-3′;
β-Actin sense: 5′-TACAACCTCCTTGCAGCTCC-3′;
β-Actin antisense: 5′-GGATCTTCATGAGGTAGTCAGTC-3′.

The number of PCR cycles was adjusted to avoid saturation of the amplification system: 94° for 30 s, 55° for 45 s and 72° for 30 s (30 cycles) for CAT; 95° for 30 s, 55° for 1 min and 72° for 30 s (30 cycles) for SOD; 94° for 30 s, 56° for 45 s and 72° for 1 min (30 cycles) for GPx, 94 °C for 1 min, at 59 °C for 1 min and 72 °C for 1 min (35 cycles) for TNFα and 94° for 30 s, 58° for 45 s and 72° for 30 s (24 cycles) for β-actin with a final elongation at 72° for 10 min. Amplification products were visualized on 1.8% agarose gels containing ethidium bromide (1 μg/ml): CAT product, 395 bp; SOD product, 383 bp; GPx product, 697 bp; TNFα product, 281 bp; β-actin product 630 bp. A 100 bp DNA ladder was used as marker. The products were quantified by laser densitometry.

2.6. Statistical analysis

All analysis were performed in triplicate. Data were expressed as means ± SD and median (minimum–maximum). The Kruskal–Wallis non-parametric test followed by multiple non-parametric comparison test (Hollander and Wolfe, 1973) were used. Spearman correlations were performed to study the relationship between aluminum and TBARS and the different expressions of TNFα and antioxidant enzymes. *P* values of <0.05 were considered statistically significant. Statistical analyses were performed using the SPSS statistical software package (version 13.0) and the SAS (version 9.1).

3. Results

3.1. Mortality rate and Growth

There were no deaths during the period of Al exposure in any of the groups studied. Similar growth rates, without any significant differences between groups ($p > 0.05$) were found for all four test groups (data no shown).

3.2. Brain tissue Al and Si levels

Table 1 presents brain weights and Al and Si brain tissue concentrations in the different groups. Brain weight did not significantly differ ($p < 0.05$) between mouse groups. Silicon intake significantly lowered Al levels in the brain compared with the Al-exposed mouse. However, the exposure to beer did not yield the same decreasing Al-effect as providing by silicic acid.

3.3. Effects of beer and silicic acid on TBARS levels in aluminum-intoxicated mice

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 + silicic acid and Al + beer groups were significantly lower ($p < 0.001$) than those of the Al group (Table 1).

3.4. Effects of beer and silicic acid on RNA levels of antioxidant enzymes in aluminum treated mice

The relative mRNA levels of the different intoxicated groups, expressed in arbitrary unities of CAT, MnSOD, CuZnSOD and GPx in brain homogenate of basal animals, are included in Table 1. Representative gene expression profiles of the control, Al + beer, Al + silicic, and Al groups assayed by RT-PCR analysis are presented in Fig. 1. Results show that expression of CAT, MnSOD and CuZnSOD enzymes was significantly lower ($p < 0.001$) in the Al group than in the control animals. Enzyme expressions were normalized in the brains of the Al + beer and Al + silicic acid group animals. Administration of Al alone increased control GPx mRNA levels more than 10-fold ($p < 0.001$), while simultaneous administration of Al and beer or Al and silicic acid significantly decreased ($p < 0.001$ or $p < 0.05$, respectively) the expression of the same antioxidant enzyme with respect to the Al group.

3.5. Effect of beer and silicic acid on TNFα expression in aluminum-intoxicated mice

Table 1 and Fig. 1 show that TNFα expression in aluminum-intoxicated mice increased more than 3-fold

Table 1
Effect of supplementing silicon in the diet in the form of silicic acid or beer on brain weight, Al, Si, and TBARS, and in the expression of CAT, CuZnSOD, MnSOD, GPx and TNFα mRNA, assayed by RT-PCR analysis, in brain homogenates of mice intoxicated with $Al(NO_3)_3$

	Basal	Aluminum	Al + beer	Al + silicic acid	*p*
Brain weight (g)	0.23 ± 0.04	0.22 ± 0.03	0.22 ± 0.03	0.22 ± 0.04	0.257
	0.24 (0.14–0.27)	0.22 (0.17–0.30)	0.23 (0.15–0.24)	0.21 (0.16–0.29)	
Brain Al (μg/g)	0.98 ± 0.36^{a}	4.0 ± 0.33^{b}	2.5 ± 1.1^{ab}	1.3 ± 1.1^{a}	<0.001
	0.90 (0.37–1.9)	3.8 (2.3–7.4)	2.7 (0.14–4.0)	1.4 (0.5–2.2)	
Brain Si (μg/g)	13.6 ± 11.1^{a}	52.9 ± 9.9^{b}	47.2 ± 7.8^{b}	45.3 ± 11.2^{b}	<0.001
	14.9 (4.3–21.0)	41.9 (14.2–110.4)	37.8 (28.4–46.5)	43.4 (30.7–61.4)	
Brain Al/Si ratio	0.08 ± 0.05	0.10 ± 0.07	0.06 ± 0.04	0.07 ± 0.03	0.370
	0.08 (0.03–0.19)	0.08 (0.03–0.28)	0.05 (0.01–0.12)	0.07 (0.03–0.12)	
TBARS (μmol/mg protein)	0.13 ± 0.02^{a}	0.28 ± 0.08^{b}	0.12 ± 0.01^{a}	0.14 ± 0.01^{a}	<0.001
	0.13 (0.10–0.16)	0.28 (0.17–0.42)	0.12 (0.09–0.13)	0.13 (0.11–0.16)	
Catalase expression (AU)	62.2 ± 4.2^{b}	34.0 ± 4.0^{a}	60.8 ± 6.2^{b}	62.0 ± 5.4^{b}	<0.001
	62.5 (56.8–67. 9)	35.4 (27.4–39.4)	59.9 (51.6–71.3)	62.2 (51.3–68.3)	
CuZnSOD expression (AU)	591.3 ± 58.3^{b}	390.7 ± 31.4^{a}	547.7 ± 47.9^{b}	600.0 ± 36.3^{b}	<0.001
	591.6 (499.0–681.5)	390.2 (314.7–431.8)	542.0 (489.3–601.9)	600.2 (527.3–678.3)	
MnSOD expression (AU)	$94{,}690 \pm 11009.4^{b}$	$61{,}071 \pm 10{,}334^{a}$	$95{,}011 \pm 19{,}032^{b}$	$97{,}165 \pm 25{,}609^{b}$	<0.001
	93,561 (77,359–112,523)	63,686 (41,235–76,457)	87,350 (71,457–132,046)	83,879 (68,457–142,358)	
CuZnSOD/ MnSOD ratio	161.6 ± 24.9	157.8 ± 34.6	173.8 ± 32.2	162.2 ± 42.2	0.676
	161.1 (122.6–214.0)	157.4 (113.1–243.0)	168.9 (131.3–220.7)	142.1 (116.1–217.6)	
GPx expression (AU)	4.6 ± 0.6^{a}	57.2 ± 6.5^{c}	14.8 ± 3.5^{b}	18.7 ± 4.0^{b}	<0.001
	4.8 (3.8–5.4)	56.8 (49.0–67.3)	14.3 (10.1–21.8)	18.0 (13.8–26.9)	
TNFα expression (AU)	20.4 ± 4.8^{a}	66.5 ± 9.1^{b}	28.7 ± 4.7^{a}	27.2 ± 6.1^{a}	>0.001
	19.2 (14.7–29.2)	69.1 (44.2–78.3)	4.8 (3.8–5.4)	28.6 (19.6–35.1)	

AU Arbitrary units. Values [mean ± SD; median (minimum–maximum)] bearing different letters (a–c) were significantly different (Kruskal–Wallis test followed by multiples comparison non-parametric test). All significant differences at the level of $p < 0.01$ except for Al group *vs.* Al + silicic acid group for Gpx expression ($p < 0.05$).

($p < 0.001$) with respect to the control animals. This expression was normalized in the Al + silicic acid and Al + beer groups.

3.6. Brain inflammation and oxidation relationships

Table 2 shows the non-parametric correlations between Al and the different expressions of TNFα and antioxidant enzymes. Al correlated negatively and significantly with CAT, MnSOD, CuZnSOD, GPx and TNFα (all, $p < 0.001$) and with TBARs ($p = 0.008$). High and significant correlations were found between TBARS levels and the expressions of all antioxidant enzymes and TNFα (all, $p \leqslant 0.001$).

4. Discussion

The present study is the first to show that inclusion of Si in the diet, in the form of beer or silicic acid, reduces the harmful effects of increased cerebral peroxidation by lowering Al levels in the brain. This finding is relevant because increased levels of oxidative stress and products of lipid peroxidation in cerebral tissue are the major contributing factors in the development of neurodegenerative diseases (Vargas et al., 2004).

Treatment did not affect final body and brain weights. The weights recorded fell within the range of weights for these mice (IFFA CREDO) specified by Charles River Laboratories, Inc. Nonetheless, results show that the mouse brain is a target organ for Al, which accumulates to a significant degree in that organ as a result of chronic exposure. A number of studies have shown that animals exposed to Al have increased Al concentrations in whole brain tissue (Gómez et al., 2005; Srivastava and Jain, 2002). As expected, the lowest concentrations of Al in the brain were recorded for animals in the basal group, whose levels were within the normal range for mammalian brain tissue of between 1.1 and 1.9 Al/g (Cacabelos, 1990). Al values of animals in the beer and silicic acid groups were about 30% lower than those of the Al group. These findings were consistent with the faecal and urinary Al levels found in a previous paper (González et al., 2007) where the higher rate of excretion in the treated animals could be a cause of the lower accumulation of Al in the brain. Therefore, silicic acid could not only able to reduce the gastrointestinal absorption of Al but also to release and excrete Al from the body.

Si concentrations in the brains of Al group animals 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 (Srivastava and Jain, 2002), allowing more Si to enter the brain tissue. Si is considered by many to be an essential element. However, one prevailing theory is that Si may protect, in virtue of an evolutionary mechanism, against aluminum toxicity (Exley, 2006). A number of biological sites have been identified in which Si and Al are co-deposited or co-localized. One of the areas investigated in most detail is the senile pla-

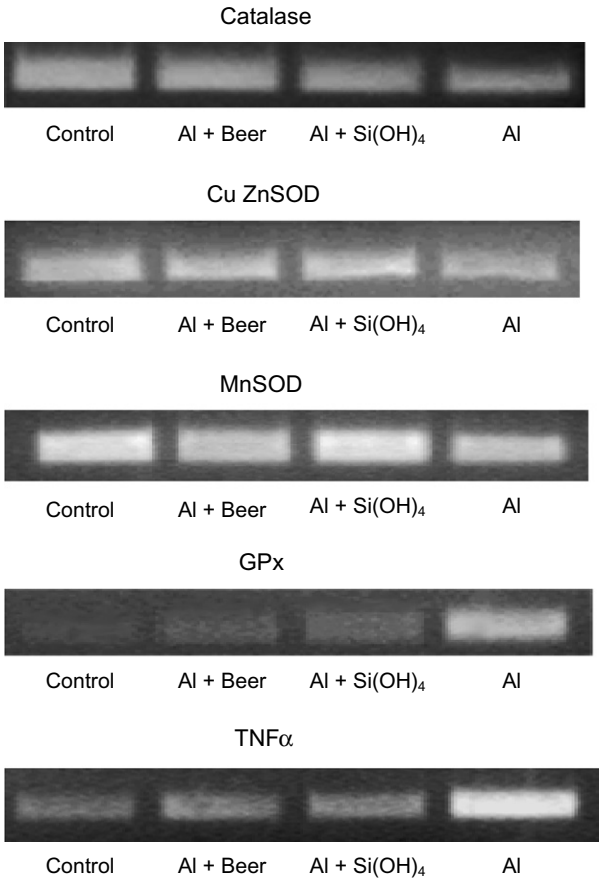

Fig. 1. Effect of supplementing silicon in the diet in the form of silicic acid or beer on CAT, MnSOD, CuZnSOD, GPx and TNFα mRNA levels, assayed by RT-PCR analysis, in brain homogenates of mice intoxicated with $Al(NO_3)_3$. Panels show a representative gene expression profile of these antioxidants assayed by RT-PCR analysis.

que cores in the cerebral cortex of patients suffering from senile dementia/Alzheimer's type (SDAT). High-resolution solid-state nuclear magnetic resonance measurements on the central regions of these plaques have shown that Si and Al are present as an aluminosilicate species and it has been suggested that Si ameliorates Al toxicity (Birchall and Chappell, 1989). This suggestion has resulted in much debate over the use of dietary Si supplements as a preventative measure for this illness, although such plaque structures have also been observed in mentally normal elderly patients (Perry and Keeling-Tucker, 2000).

The dietary silicic acid supplement was efficient in lowering Al brain depots of Al + silicic acid group mice to the same values observed in the basal group. Similarly, although not significantly, the administration of beer tended to decrease the Al in the brain. These results suggest that beer intake did not produce the same increase in Si brain levels as the dietary silicon supplement. Nonetheless, based on these results, administering Si would appear to be effective in preventing Al from accumulating in the brains of mice, as reported earlier by Granero et al. (2004). The effectiveness of Si could be attributed to its interaction with Al through the formation of non-toxic aluminosilicates that decrease Al concentrations.

According to Gillette Guyonnet et al. (2007), silica is probably the natural antidote of Al and could play a beneficial role by decreasing Al bioavailability. These authors suggest the possible use of silicates as a therapeutic agent for AD, since both model tangles and precipitated β-pleated sheets of βA4 can be reversed to soluble forms by silicates. Likewise, the same authors (Gillette Guyonnet et al., 2005) found that silica in drinking water may reduce the risk of developing AD.

Our results indicate a pattern of Al-induced oxidative stress similar to that found in the others studies, suggesting Al-induced oxidative stress involving free radical generation in the brain (e.g. TBARS accumulation in hippocampus) (Pratico et al., 2002; Gómez et al., 2005; Srivastava and Jain, 2002; Flora et al., 2003; Moumen et al., 2001). Inhibition of TBARS by administration of silicic acid or beer strongly suggests the neuroprotective properties of Si. However, it is possible that other components, in addition to Si, were responsible for the decrease in TBARS levels in the Al + beer group. Alcohol, hop, some polyphenols and folic acid are some of those potential antioxidants. Moderate alcohol consumption has been proven to decrease lipid peroxidation by increasing the total

Table 2
Spearman's correlations between Aluminum and TBARS and the different expressions of TNFα and antioxidant enzymes

	TBARS	Catalase	CuZnSOD	MnSOD	GPx	TNFα
Aluminum	0.381 ($p = 0.008$)	−0.549 ($p < 0.001$)	−0.504 ($p < 0.001$)	−0.530 ($p < 0.001$)	0.713 ($p < 0.001$)	0.646 ($p < 0.001$)
TBARS		−0.566 ($p < 0.001$)	−0.476 ($p = 0.001$)	−0.491 ($p < 0.001$)	0.599 ($p < 0.001$)	0.492 ($p < 0.001$)
Catalase			0.623 ($p < 0.001$)	0.584 ($p < 0.001$)	−0.576 ($p < 0.001$)	−0.571 ($p < 0.001$)
CuZnSOD				0.584 ($p < 0.001$)	−0.576 ($p < 0.001$)	−0.559 ($p < 0.001$)
MnSOD					−0.572 ($p < 0.001$)	−0.578 ($p < 0.001$)
GPx						0.757 ($p < 0.001$)

Data are the correlation coefficient. In parenthesis the bilateral signification.

antioxidant capacity of plasma (Bassus et al., 2004). Furthermore, hop has been found to decrease production of the TBARS and carbonyl groups in the elderly (Valls-Belles et al., 2007). Likewise, hop has also been found to be a relevant source of resveratrol (Callemien et al., 2005); thus explaining, as least in part, its potential antioxidant properties. On the other hand, folic acid is responsible, through cystathionine-β synthase, for producing cysteine. In turn, cysteine is a precursor of glutathione that exerts antioxidant properties. Moreover, hyperhomocysteinemia increased generation of free radicals (Thambyrajah and Townend, 2000) and several studies in humans have reported an inverse association between homocysteine and cognitive impairment or dementia (Ravaglia et al., 2003; Whitmer et al., 2003).

CAT, SOD and GPx enzymes are crucial in the cellular defense against ROS and free radicals. The oxidative status of the cell is a primary factor in the regulation of gene expression and enzymatic activity (Rodriguez et al., 2004). As previously commented, Al is a neurotoxic agent and its accumulation may lead to numerous neurological disorders. Our results in the Al group, shows that this toxic element impaired cerebral expression of enzymes engaged in antioxidant defense. Sharma et al. (2007) found induction of oxidative stress in brain tissue and serum after aluminum chloride exposure (172.5 mg/kg/d orally for 10 weeks) and reported that glutathione reductase, reduced glutathione, GPx, CAT and SOD levels decreased significantly while concentrations of TBARS and Glutathione-*S*-transferase increased in brain tissue and serum (Moumen et al., 2001).

SOD and CAT expression in the silicic acid and beer groups returned to basal, while GPx levels in these animals decrease, displaying values that were in between those of basal and the Al groups; thus, suggesting that silicic acid and beer improve the antioxidant defense of the brain.

SOD is considered important in protecting against oxidative stress, but as its increase accelerates H_2O_2 formation and could enhance oxidant toxicity in aerobes (Scott et al., 1987), it has to be coupled to CAT and other enzyme systems. Consequently, our results regarding the enzyme correlations showed that the gene expression of MnSOD, CuZnSOD and CAT are well correlated. Moreover, the negative correlations observed in the brains of our study animals between the expression of these enzymes and that of GPx support the thesis that ingestion of Si has beneficial effects.

Although oxidative and inflammatory events are apparently independent phenomena (Becaria et al., 2006), they are closely related. The relationship between inflammatory response and free radical generation is complex, but current evidence suggests that both oxidative stress and neuroinflammation are early events in the pathogenesis of AD. Thus, a rise in brain TNFα levels precede development of AD in patients with mild cognitive impairment (Tarkowski et al., 2003). The increased expression of TNFα found in the brains of mice exposed to Al concurs with data from the brains of mice treated with Al lactate (Campbell, 2006). Becaria et al. (2006) have shown that Al in drinking water dose-dependently increased TNFα levels selectively in mouse brains. These authors also found that the elevation in TNFα appears to be unique to brain tissue since concentrations in both serum and spleen decreased after exposure to aluminum lactate. This provides evidence that the inflammatory response within the central nervous system consequent to chronic treatment with low levels of Al differs quantitatively from that of the systemic immune response. Furthermore, the Al-induced neuroinflammatory response cannot be attributed to penetration of plasma TNFα into the brain, as indicated by Becaria et al. (2006). Furthermore, in primary neural cells, Al exposure induces expression of inflammatory genes (Lukiw et al., 2005).

Present data suggest that the decrease for TNFα expression in the silicic acid and beer groups with respect to Al group is related to a detoxification mechanism, probably in the form of a successful chelation therapy for metal poisoning through mobilization of the metal and its excretion from the body. This would reduce the body burden of the metal and prevent its toxic effects. As commented before, Si may play a specific role in the detoxification of Al, possibly by forming non-toxic aluminosilicates (Birchall and Chappell, 1989), which are sequestered into lysosomal granules (Desouky et al., 2003).

Winkler et al. (2006) found that beer components may not only act as chemical antioxidants, detoxifying ROS by reducing effects mediated by IFN-γ (pro-inflammatory cytokine interferon-γ), but may also reduce the formation of ROS. At least some of the beneficial health effects of beer may relate to its ability to interfere with pro-inflammatory cytokine cascades. Moreover, hop, probably as a polyphenol source, exerts an anti-inflammatory effect (Valls-Belles et al., 2007; Yamamoto et al., 2000). The beer given to the mice contained alcohol in a ration considered normal or non-toxic for humans (ca. 30 g/day). Nonetheless, taking into account the deleterious effect of high alcohol consumption (Ramstedt, 2007), future studies must be undertaken to investigate whether non-alcoholic beer has a similar anti-inflammatory effect.

In short, the present results clearly show that ingestion of Si as silicic acid or beer blocked prooxidant and proinflammatory actions of dietary Al, decreasing TBARS levels and the expressions of GPx and TNFα and increasing those of SOD and CAT. In order to understand the protective mechanisms involved, further studies with different kinds of beer and doses of Si must be carried out.

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