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RESEARCH ARTICLE

Biological activities of Zn(II)-*S*-methyl-cysteine complex as antiradical, inhibitor of acid phosphatase enzyme and *in vivo* antidepressant effects

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Abstract

The antidepressant effect of simple Zn(II) salts has been proved in several animal models of depression. In this study, a coordination metal complex of Zn(II) having a sulfur containing ligand is tested as antidepressant for the first time. Forced swimming test method on male Wistar rats shows a decrease in the immobility and an increase in the swimming behavior after treatment with [Zn(S-Met)$_2$] (*S*-Met=*S*-methyl-L-cysteine) being more effective and remarkable than $ZnCl_2$. The thiobarbituric acid and the pyranine consumption (hydroxyl and peroxyl radicals, respectively) methods were applied to evaluate the antioxidant activity of *S*-Met and [Zn(*S*-Met)$_2$] showing evidence of attenuation of hydroxyl but not peroxyl radicals activities. UV-vis studies on the inhibition of acid phosphatase enzyme (AcP) demonstrated that *S*-methyl-L-cysteine did not produce any effect but, in contrast, [Zn(*S*-Met)$_2$] complex behaved as a moderate inhibitor. Finally, bioavailability studies were performed by fluorescence spectroscopy denoting the ability of the albumin to transport the complex.

Keywords

Antidepressant effect, antioxidant activity, phosphatase inhibitory effects, S-methyl-cysteine, Zn(II) complex

History

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Introduction

The relevant physiological and metabolic functions of Zn together with its participation in life processes and their presence as active centers of many enzymes is well-recognized[1,2]. Particularly, Zn is present in the central nervous system, cerebral cortex, pineal gland and hippocampus. Alterations in zinc homeostasis may also be involved in the mechanism of pathophysiology and treatment of depression. Clinical data have revealed lower zinc concentration in serum in patients with depressive disorder that can be restored after an effective antidepressant therapy[3,4]. Furthermore, others evidences that zinc deficiency induces depressive behavior have been confirmed in new animal models for depression[5]. It has been proved that dietary Zn deprivation increases the immobility time in the forced swim and in the tail suspension tests in mice and rats indicating a depressive behavior[5]. Usually, simples salts ($ZnCl_2$ or $ZnSO_4$)[6,7] have been used as zinc supplementation in the body. Only one metal coordination complex named zinc hydroaspartate (ZINCAS®)[8] (dietary supplement) was proved as antidepressant. In this context, our research group became interested in the development and assessment of Zn(II) coordination compounds in order to evaluate their potential antidepressant activity.

Previous studies have shown that depression is related to increased levels of oxidative stress biomarkers suggesting that this process may be a significant factor in the pathogenesis of depression[9]. Zinc also plays an important role in protecting cells from oxidative stress. The deficiency of this essential element increases the osmotic fragility of erythrocyte membranes and the levels of lipid peroxidation in mitochondrial and microsomal membranes, while the presence of zinc prevents lipid peroxidation[5]. In addition, the antioxidant activity of the amino acid *S*-methyl-L-cysteine has been reported[10]. It prevents the oxidation of species from lipid hydroperoxide (LOOH) during formation of the human low-density lipoprotein (LDL)[11]. Besides, this compound shows neuroprotector behavior in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)[12].

Bearing in mind the involvement of: (i) oxidative stress as the major contributor to the etiology of depression[5], (ii) the function of Zn(II) cation against oxidative processes, (iii) the antioxidant effect of the *S*-methyl-L-cysteine and (iv) the fact that Zn(II)-sulfur containing amino acids were effective as antioxidant compounds[13]; the radical scavenging ability of the Zn(II)-*S*-methyl-cysteinato complex was investigated.

On the other side, the design of metal complexes as enzyme inhibitors became of great interest for scientists due to their versatile and pharmacological applications[14]. In the last years, it has been demonstrated that Zn(II) and several metal complexes have been involved in enzyme inhibition processes linked to cancer, hypertension disease process, neurological applications; tumor hypoxia, uric acid production, among others[14]. In particular, in this work, authors are interested in phosphatase acid (AcP) enzymes as a target for inhibition processes in view of the

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application of these compounds on the development of inhibitors for chemotherapeutic treatments like osteoporosis, AIDS encephalopathy, Gaucher disease, hairy cell leukemia, Alzheimer disease and bone metastases, hyperparathyroidism, and prostate cancer[15,16], etc. The enzymatic inhibitory ability of Zn(II) ion and some complexes on AcP have been demonstrated[17] and for this reason, authors decided to prove the behavior of the Zn-*S*-methyl-L-cysteine complex against AcP activities.

Since the metabolism, distribution and efficacy of many drugs in the body are associated with their affinities towards the plasma binding protein serum albumin, the analysis of compounds with respect to albumin binding ability becomes a relevant item to study. In this investigation, bovine serum albumin (BSA) was selected as an accepted model due to its structural similarity with the human one, the stability of the solutions, its medical relevance, low cost and ready availability[18]. We have demonstrated herein that the poor interaction of *S*-methyl-L-cysteine with BSA could be improved by Zn complexation. A value of the binding constant of $K_b = 7.48 \times 10^4 \pm 0.18\,M^{-1}$ (298 K) and reversible binding of the complex with BSA with non-bonded (van der Waals) interactions have been determined, indicated that the Zn coordination complex could be transported in serum.

Methods and materials

$ZnCl_2$ and *S*-methyl-L-cysteine were obtained from Biopack (Buenos Aires, Argentina) and Sigma-Aldrich, Inc. (St. Louis, MO) respectively, being used as supplied. All the solvents used were from analytical grade.

Preparation of *bis*(*S*-methyl-L-cysteinato)Zn(II) {$(CH_3\text{-}S\text{-}CH_2\text{-}CH\text{-}NH_2CO_2)_2Zn$=[Zn(*S*-Met)$_2$]}

The investigated complex was prepared according to the method described by Shindo et al.[19]. In the mentioned procedure, 0.01 moles of *S*-methyl-L-cysteine were dissolved in a 50 mL solution containing 0.1 mol of NaOH. After that, a solution of $ZnCl_2$ (0.005 moles in 3 mL of distilled water) was added dropwise under continuous stirring. Immediately after, a white precipitate appeared. The suspension was left under stirring for 2 h, and then it was filtered and washed several times with distilled water to ensure complete removal of chloride ions by negative reaction to the formation of AgCl. For this preparation, the formulation indicated refers: $C_8H_{16}N_2S_2O_4Zn$. Calc.: C = 28.79%, H = 4.83%, N = 8.39%; Exp.: C = 28.63%, H = 5.09%, N = 8.30%

The proposed structure of the complex is as follows:

Antidepressant activity

Forced swimming test experiments

Animals. Experiments were carried out on male Wistar rats weighing 200–310 g. The animals were maintained on a 12 h light (08:00–20:00 h)–12 h dark cycle, with free access to food and water, except during testing. Abundant evidence indicates their free access to food and water except during testing, to possible interference with experimental data[20].

Rats were housed in groups of four, in individual polyethylene cages (55 × 38 × 30 cm). Their weights were recorded before and at the end of each experiment. Animals were used only in one experiment. All studies described were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health, USA and AVMA Guidelines for the Euthanasia of Animals, 2013 Ed.

The experiments were performed after approval of the protocol by the Ethics Committee for the care and use of Laboratory Animals of the Universidad Nacional de La Rioja, Argentina.

Drugs and treatment. $ZnCl_2$ was administered in a doses of 30 mg/kg of the animal and also [Zn(*S*-Met)$_2$] (153 mg/kg) and *S*-methyl-L-cysteine (41 mg/kg) in the equivalent quantities because it was proved that $ZnCl_2$ produced the most robust effect in the forced swimming test (FST) under similar experimental conditions[21]. In order to estimate some degree of dose-response behavior, a second dose of $ZnCl_2$ (10 mg/kg) lacking antidepressant effect[21] was chosen and a dose of [Zn(S-Met)$_2$] (51 mg/kg) containing the equivalent of Zn(II) content and *S*-methyl-L-cysteine (30 mg/kg) quantities were used to make comparisons with the active one.

All of the control rats received injections of saline solution (0.9% NaCl). Rats were treated with the saline solution (rats control) or $ZnCl_2$, *S*-methyl-L-cysteine, [Zn(S-Met)$_2$] once a day and all solutions were administered by oral route in a constant volume of 10 ml/kg body weight.

The rats were randomly divided into four groups and conducted the following treatments: Group 1: saline (control group), Group 2: *S*-methyl-L-cysteine and Group 3: $ZnCl_2$ and Group 4: [Zn(S-Met)$_2$].

FST. The forced swimming test is a well-accepted model to test the antidepressant action of drugs and to identify by rat treatments the antidepressant efficacy in humans[22]. Stress is a well-known risk factor in the development of depression. The forced swimming test employs forced swimming stimuli as stressor to generate a behavior characterized by increased immobility time.

Swimming sessions were conducted by placing rats in individual Plexiglas cylinders (46 cm tall × 20 cm in diameter), filled with water (23–25 °C) up to 30 cm from bottom. All swimming sessions were carried out between 12:00 and 18:00 h.

In the protocol, two sessions were conducted: an initial 15 min pretest on Day 1 followed by a 5 min test on Day 15. Drugs treatment began on Day 1 after the pretest session and it were administered from Day 1 to 14[23]. At the end of both swimming sessions, rats were removed from the cylinders, dried with towels, placed in heated cages for 15 min, allowed to rest and recover, and then returned to their home cages. The cylinders were emptied and cleaned between rat treatments. Each animal was assigned randomly to the treatment, and was only employed for one pretest/test session.

Behavioral scoring. For behavioral sampling, rats were rated at 5 s intervals throughout the duration of the forced swimming session. At each 5 s interval, the predominant behavior was assigned to one of three categories: (i) immobility: floating in the water without struggling, and making only those movements necessary to keep the head above the water; (ii) swimming: making active swimming motions, more than necessary to merely keep the head above water (i.e. moving around in the cylinder); and (iii) climbing: making active movements with forepaws in and out of the water, usually directed against the walls. Scores for each behavior were expressed as total behavioral counts per 5-min session[23].

Open field test. Independent groups of animals were submitted to the open field test in order to investigate if the treatments used induced any significant motor effect, which would interfere in the FST results.

Until Day 14, these studies were conducted exactly as the forced swimming test studies: all rats underwent the first day of the forced swimming test but instead of re-testing in the forced swimming test on Day 15, animals were subjected to an open field session. All animals were placed gently in the center of the open field arena, owed to explore freely and its locomotion was

measured by the number of squares entered with all four paws (counts), during a period of 5 min. The apparatus for the open field test consisted of a black, square open field (60 cm by 60 cm) with the floor divided in squares (15 × 15 cm) by means of white lines. Testing was performed between 1400 and 1700 h, illuminated with a 75 W electric bulb, hung 75 cm above it, in a quiet room. During all the experiments the laboratory room was dark. After each animal was removed, the open field was carefully cleaned with a damp cloth. The behavior was scored by an observer who was unaware of the experimental procedures previously performed on the animals and the results were expressed as mean ± standard error of mean (SEM).

Acid phosphatase inhibition test. Acid phosphatase inhibition test was performed according to Blum and Schwedt procedures[24] using acid phosphatase (AcP from potato, product number P-3752, Sigma Chemical Co. (St. Louis, MO). Acetate buffer was prepared by dissolving a volume of 5.72 mL of concentrated acetic acid in distilled water (final volume of 250 mL) adjusting the pH value to 5.60 with 0.5 M NaOH. The stock solution of the enzyme was prepared by mixing 12.5 mg of the 0.25 U/mL acid phosphatase powder in 2.0 ml acetate buffer. For the measurements, 100 µL of the stock solution was diluted with 1.9 mL acetate buffer. For the substrate solution 0.170 g of *p*-NPP were dissolved in 2.5 mL distilled water.

Test procedure: the compounds solutions were prepared by diluting the stock solutions prepared in DMSO with acetate buffer. A volume of 0.50 mL of each compound solution was mixed with 0.10 mL of the enzyme solution and 1.00 mL of buffer. The mixture was kept at 25 °C for 20 min (incubation time). After starting the reaction by adding 0.10 mL of the substrate solution, the tube was kept at 25 °C for 20 min. The reaction was stopped with the addition of 0.50 mL of a 0.5 M sodium hydroxide solution. The final concentration of DMSO resulted in 1.14%. The enzymatic activity was finally calculated by measuring the absorbance of 4-nitrophenolate at 405 nm against a blank prepared without the enzyme. Three independent replicates of each point were measured.

In both experiments, the 100% of the enzyme activity is assigned to a basal measurement containing all the reaction media including the same volume of DMSO in all the tests. It is worthy to mention that the presence of that very low quantity of DMSO did not affect the enzyme activity.

Antioxidant properties

Scavenging of the hydroxyl radical

The ascorbate-iron-H_2O_2 system was used for hydroxyl radical's generation[25]. In a few words, the reaction mixture contains 3.75 mM 2-deoxyribose, 2.0 mM H_2O_2, 100 µM $FeCl_3$ and 100 µM EDTA without or with the tested compounds (0–50 µM) in 20 mM KH_2PO_4–KOH buffer, pH 7.4. The reaction was triggered by the addition of 100 µM ascorbate and the mixture was incubated at 37 °C for 30 min. Solutions of $FeCl_3$, ascorbate, and H_2O_2 were made up in deaerated water immediately before use. The thiobarbituric acid method was applied to evaluate the extent of deoxyribose degradation by hydroxyl radical.

Inhibition of peroxyl radical

Peroxyl radicals were generated by the thermal decomposition of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH). AAPH was chosen due to its ability to generate free radicals at a steady rate for extended periods of time (half-life of 175 h). The consumption of pyranine was followed spectrophotometrically by the decrease in absorbance at 454 nm with a thermostated cell at 37 °C. The reaction solutions contained AAPH (50 mM), pyranine (50 µM) and several concentrations of the tested compounds (0–50 µM). The delay of pyranine consumption (lag phase) was calculated as the time before the consumption of pyranine started (notable reductions in absorbance)[25].

Binding affinity to albumin by fluorescence spectroscopy

BSA was dissolved in Tris–HCl (0.1 M, pH 7.4) buffer to attain a final concentration of 4% w/v (~0.6 mM). *S*-methyl-L-cysteine and [Zn(S-Met)$_2$] solutions were added dropwise to the 2% w/v BSA solution (~0.3 mM) to ensure the formation of a homogeneous solution and to obtain the desired concentration of 0.05–0.10 mM. Adequate solubility was reached under these experimental conditions in which the compounds did not show any fluorescence intensity that could interfere with the measurements. For each sample and concentration, three independent replicates were performed at 25 and 37 °C. These solutions were used for fluorescence measurements, which were carried out on a Perkin-Elmer LS-50B luminescence spectrometer (Beaconsfield, England) equipped with a pulsed xenon lamp (half peak height <10 µs, 60 Hz), an R928 photomultiplier tube and a computer working with FL Winlab software. Both excitation and emission slits were set at 5 nm throughout this study. BSA 2% w/v was titrated by successive additions of *S*-methyl-L-cysteine and [Zn(*S*-Met)$_2$] solutions from 0.05 to 0.10 mM and the fluorescence intensity was measured (excitation at 280 nm and emission at 348 nm) at 25 and 37 °C. All the fluorescence quenching data were analyzed according to previous studies performed in the laboratory applying a traditional mathematical procedure[18,25] to obtain the apparent binding constant (K_b), the binding site value (n) and the thermodynamic parameters.

Results

The experiments for the antidepressant activity were performed using Zn(II) (as $ZnCl_2$), and *S*-methyl-L-cysteine and the [Zn(S-Met)$_2$] complex, for the sake of comparison.

As it was expected, the higher $ZnCl_2$ (30 mg/kg) dose reduced immobility (−33%) and increased swimming behavior indicating an antidepressant effect (Figure 1A) while a lower dose of $ZnCl_2$ (10 mg/kg) had no significant effect on FST in agreement to the previous reported data[21]. Administration of *S*-methyl-L-cysteine (both doses 30 and 41 mg/kg) had no considerable effect on FST. Treatment with a lower [Zn(S-Met)$_2$] complex dose (51 mg/kg) which proportionately contains a sub-effective dose of Zn(II) (10 mg/kg) did not decreased immobility neither improved the swimming behavior (Figure 1A).

Remarkably, the administration of the higher [Zn(S-Met)$_2$] complex dose containing equivalent Zn(II) content (effective dose) produce a reduction of a 56% of immobility respect to the control group and it was 20% more effective than $ZnCl_2$.

The effects of different treatments on spontaneous locomotor activity in rats are shown in Figure 1(B). None of these treatments affected activity levels, confirming the specificity of the FST results.

The effect on the AcP activity was tested at pH = 5.60 varying the concentrations of the complex and the ligand. The result of the action of the [Zn(*S*-Met)$_2$] complex on AcP activity is shown in Figure 2. As it could be observed, the complex behaves as inhibitor in a 10–500 µM range being the IC_{50} value of 250 µM. The ligand, *S*-methyl-L-cysteine, does not produce any effect on AcP activity when tested in the same concentration range (not shown).

On view of the proven reduction of the antioxidant stress of the cysteine-containing agents and taking into account the existing data of the effectiveness of the compound on diminishing the

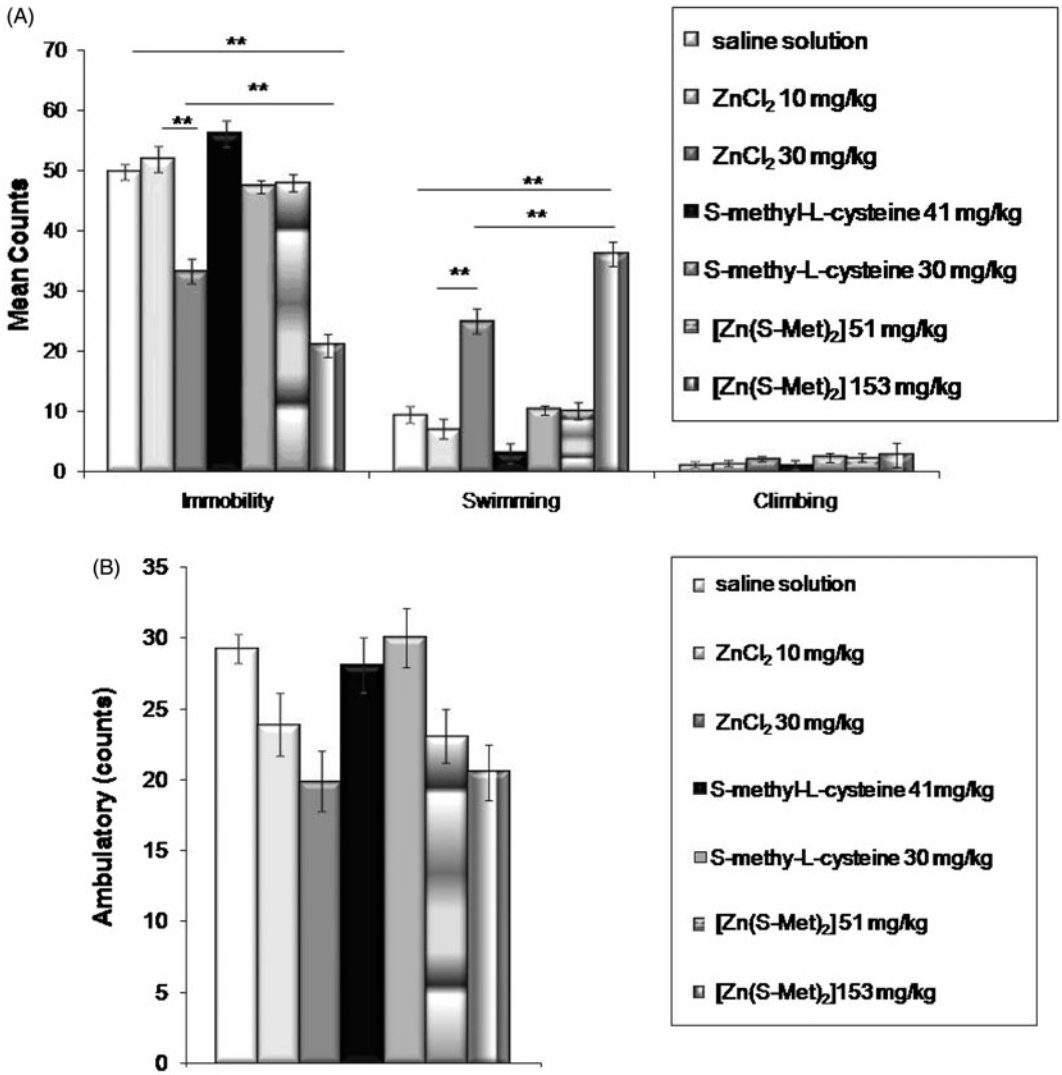

Figure 1. Behavioral effects of the rats produced in the forced swimming (FST) (A) and open field tests (OFT) (B) by the administration of saline solution, *S*-methyl-L-cysteine, $ZnCl_2$ and [Zn(*S*-Met)$_2$]. Values represent mean (±SEM) counts of immobility, swimming and climbing behaviors when sampled every 5 s during the 5-min test period. $*p < 0.05$, $**p < 0.01$, $n = 12$ rats per group. Data were analyzed by one-way analysis of variance followed by Tukey's test for multiple comparisons.

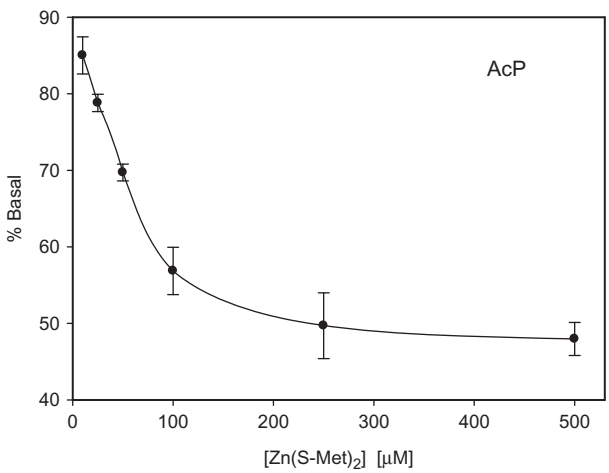

Figure 2. Effect of [Zn(*S*-Met)$_2$] on AcP activity. Initial rate was determined by incubation of the enzyme at 37 °C for 20 min in the absence or presence of variable concentrations of the inhibitors. The values are expressed as the mean ± SEM of at least three independent experiments.

LOOH in human LDL, the antioxidant effect of the *S*-methyl-L-cysteine and the complex were tested on hydroxyl and peroxyl radicals. None of them showed any anti peroxyl activity at least in the tested concentration range (0–50 μM). Related to hydroxyl attenuation, *S*-methyl-L-cysteine and [Zn(S-Met)$_2$] behaved as a good hydroxyl scavenger (Figure 3).

The interaction and binding of the compounds with the albumin were studied by fluorescence spectroscopy. In this experiment a quenching effect is produced on the fluorescence intensity of the typical BSA signal being indicative of the degree of the interaction. This quenching effect could be associated to a variety of molecular interactions. When a binding to a macromolecule is produced, the equilibrium between free and bound molecules could be evaluated by the following equation:

$$\log[(F^{o} - F)/F] = \log K_b + n \log[Q]$$

where, F^{o} and F are the measured fluorescence intensity without and with the presence of the quencher, K_b is the apparent binding constant, and n is the number of the binding sites per BSA. For *S*-methyl-L-cysteine the measured interaction was not strong enough and this behavior did not allowed us to perform calculations (data not shown). For the Zn(II)-complex, the parameters K_b and n were

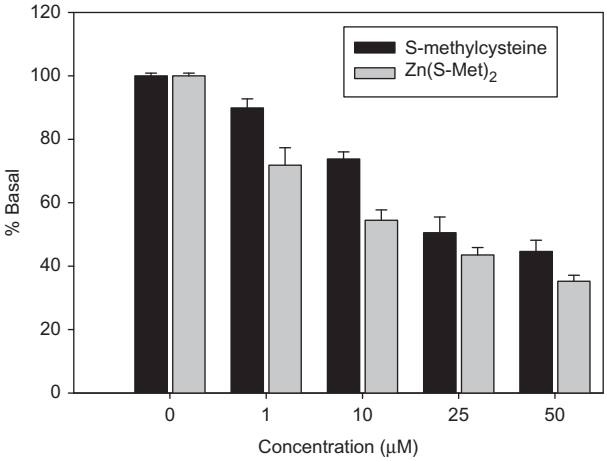

Figure 3. Effect of [Zn(*S*-Met)$_2$] on the extent of deoxyribose degradation by hydroxyl radical, measured with the thiobarbituric acid method. The values are expressed as the mean ± SEM of at least three independent experiments.

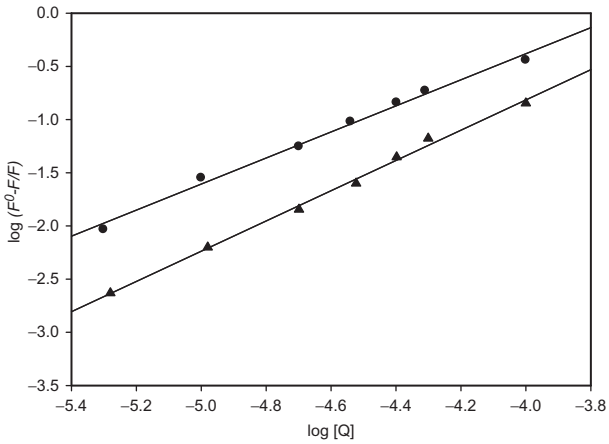

Figure 4. Plots of log[$(F^o-F)/F$] versus log[Q] for the [Zn(*S*-Met)$_2$]-BSA system at pH 7.40: 298 K (filled triangle) and 310 K (filled circle).

calculated by the ordinate and slope of double logarithm regression curve (Figure 4, Table 1).

To obtain information about the type of interaction, the thermodynamic parameters were calculated.

The standard enthalpy change (ΔH^0) is calculated using Van't Hoff equation:

$$\ln(K_{b2}/K_{b1}) = -\Delta H^0/R(1/T_2 - 1/T_1) \quad (1)$$

where, T_1 and T_2 are the temperatures at which K_{b1} and K_{b2} were determined. The standard free energy change (ΔG^0) and the standard free entropy change (ΔS^0) were evaluated according to the well-known thermodynamic relationships:

$$\Delta G^0 = -RT\ln K_b \quad (2)$$

$$\Delta S^0 = (\Delta H^0 - \Delta G^0)/T \quad (3)$$

The results are shown in Table 1.

Discussion

Depression is a highly prevalent and disabling condition associated with significant morbidity and mortality. Despite the availability of many antidepressant drugs approved for the treatment of major depressive disorders, nearly 50% of patients are treatment-resistant[26]. In addition, Lin et al.[27] have reported a high rate of premature treatment discontinuation mostly due to drug side effects. Other therapeutic concerns are also faced in the treatment of bipolar disorders and perinatal and childhood depression, especially due to the queries regarding drug efficacy and safety.

Advances in the neurobiology of depression have suggested a number of novel targets for antidepressant treatment. Based on an improved understanding of the neurobiology of depression, several novel pharmacologic and non-pharmacologic interventions are being developed. New pharmacologic developments include NMDA antagonists, like zinc[28].

In this study, we used the forced swimming test (FST), a well-accepted model to test the antidepressant action of agents and an important tool to study neurobiological mechanisms involved in the antidepressant responses. The FST employs forced swimming stimuli as stressor to generate a behavior characterized by increased immobility time. The appearance of this behavioral failure has been associated to changes in neurotransmitters and cell signaling pathways in the brain[29].

Some studies have reported antidepressant effects of zinc after acute administration of $ZnSO_4$ at a dose of 30 mg/kg[30] and subchronic (three times: 24, 5 and 1 h before the test)[7] in the forced swimming test in mice and rats, respectively. Moreover, $ZnCl_2$ induces an antidepressant effect after acute (30 mg/kg intraperitoneal) administration using the same experiment[31]. Moreover, recent studies have demonstrated that zinc (as hydroaspartate salt) in single, subchronic or chronic administration reduced the immobility time in different animal models of depression (as olfactory bulbectomy, chronic mild, stress, chronic unpredictable stress and forced swimming test)[32,33].

The results showed, to our knowledge for the first time, that $ZnCl_2$ (30 mg/kg) after chronic administration is effective in reducing the immobility time in the FST, consistent with an antidepressant effect in this predictive test[34]. Moreover, the decrease in immobility or the increase in the swimming behavior induced in the FST by this treatment can be considered as specific ones since they are not attributable to changes in the locomotor activity.

Related to *S*-methyl-L-cysteine, there was no data in the literature involving this amino acid in any antidepressant studies. Furthermore, a combination of two compounds could possible show different effects with respect to those of the individual parts. When the difference is observed as an enhanced effect, usually it referred as synergistic effect. Our results revealed that the ligand was capable to produce a synergistic effect on Zn(II) (at the higher measured dose) after forming a *bis*-chelate metal coordination complex because of the marked difference in the antidepressant activity in comparison to that obtained with *S*-methyl-L-cysteine and $ZnCl_2$. From Figure 1, it can be seen that the coordination complex produced a decrease of 56% in the immobility time with respect to the basal and the ligand and resulted 20% more effective than Zn(II).

We can determine herein that the immobility time of rats or the antidepressant activity upon [Zn(*S*-Met)$_2$] complex increased in a dose-response manner and that rats treated with the higher [Zn(*S*-Met)$_2$] complex dose decreased immobility and increased swimming behavior in a magnitude that exceeded $ZnCl_2$ individual effects.

To our knowledge, this is the first report showing the antidepressant effect of [Zn(*S*-Met)$_2$] complex. However, the mechanisms underlying the additive and the synergistic effects observed could not been established through this experiments. Nevertheless, reports from basic and clinical studies demonstrate that increased cell proliferation and increased neuronal number may be a mechanism by which antidepressant treatment overcomes the stress-induced atrophy and loss of hippocampal

Table 1. Apparent binding constants K_b, ''n'' binding site and relative thermodynamic parameters of the system of [Zn(*S*-Met)$_2$]-BSA.

pH	T (K)	K_b (M^{-1}) ± SD	n ± SD	R^a	ΔH^0 (kJ/mol)	ΔG^0 (kJ/mol)	ΔS^0 (J/mol.K)
7.4	298	7.48×10^4 ±0.18	1.42 ± 0.04	0.993	−52.42	−27.79	−82.65
	310	3.30×10^4 ±0.20	1.21 ± 0.05	0.996			

[a]R, correlation coefficient for the K_b values.
SD, standard deviation.

neurons and may contribute to the therapeutic actions of chronic antidepressant treatment[35,36]. They are characterized by a delayed onset of action without noticeable clinical effects that have not seen before 3–4 weeks of treatment suggesting adaptive mechanisms induced by chronic use[37].

Overall, the data could have important consequences in the medical practice and in the development of new antidepressant treatment strategies.

The inhibition behavior against AcP was also determined. As it was mentioned, high AcP in sera are associated with several diseases being this enzyme a target for the development of new drugs. It is well known that some metal ions including Zn(II) were able to inhibit acid phosphatases. This inhibition behavior depends of the reaction media pH and the inhibitor concentration[38].

As it was stated, the ligand has not inhibition effect on AcP on the contrary that occurs after metal complexation. It is therefore evident that the complexation with Zn(II) produces a synergic action improving the inhibitory ability having specific action on AcP. As authors have recently reported[39], there are not so much examples related to metal(II) coordination compounds inhibiting AcP and this was the reason to begin to study this effect for copper(II) and zinc(II) complexes (this work). It can be shown that the [Zn(*S*-Met)$_2$] behaves as a better inhibitor than the Cu(II)-methimazole complexes especially in comparison with the ternary [Cu(MeimzH)$_2$(phen)(H$_2$O)$_2$]Cl$_2$ (Cu-Met-phen, MeimzH = methimazole, phen = phenanthroline) which has an IC_{50} of 300 μM. Although the measured IC_{50} value of the [Zn(*S*-Met)$_2$] complex cannot be considered too low, it corresponds to the first data obtained for the inhibitory effect of AcP by a Zn(II)-complex.

Antioxidant activity was also evaluated. The better effect found for the complex in comparison with the ligand is given at 10 μM in which the ligand showed a decrease of 26.21% from the basal and the complex had a stronger effect lowering the initial concentration of the radicals in 45.43%. Even though in the whole concentration range the complex is more effective than the ligand causing a 65% of reduction at 50 μM, at 10 μM concentration the higher difference in percentage between the effectiveness shown by both compounds (19.22%) has been determined. Again, there is an improvement of the antioxidant activity of the *S*-methyl-L-cysteine by means of the coordination with Zn(II).

The Interaction with the BSA was studied with the aim to obtain information about the bioavailability of the complex.

It is well known that albumin is the most abundant plasma protein and that its physiological role comprises the control of osmotic blood pressure, transport, metabolism and distribution of several endogenous or exogenous substances having the ability to improve the dissolution in the biological medium including the most hydrophobic ones. For this reason, the interaction and transport of the drugs by this protein is a crucial point to determine its bioavailability[18,25].

The calculated binding constant (Table 1) is in the 103–106 M^{-1} range compatible with a reversible binding to BSA which agreed with the common affinities of drugs for serum albumin[18,25]. The number of binding site is about 1.0, which indicated that one binding site is formed between the Zn(II)-complex and BSA.

There are basically four types of noncovalent interactions: hydrogen bonds, van der Waals forces, electrostatic and hydrophobic interactions that play an important role in ligand binding to proteins[25,40].

Based in several studies done by Ross et al.[27] it is revealed that the sign of the thermodynamic parameters discloses the type of interaction that is taking place during the complex formation between the studied compound and the albumin. For the [Zn(*S*-Met)$_2$], negative enthalpy and entropy changes are suggested to arise from non-bonded (van der Waals) interactions and hydrogen-bond formation in low dielectric media and protonation accompanying association. The experimental conditions of the experiments lead us to suggest that metal complexation takes place mainly *via* van der Waals (stacking) interactions.

Conclusions

The compounds, *S*-methyl-L-cysteine and [Zn(*S*-Met)$_2$] have been studied on the base of potential new pharmacological activities. Focus the interest on the antidepressant activity and taking into account the recent demonstrated antidepressant effect for free Zn(II), it was established that spontaneous locomotor activity was not affected to a relevant degree confirming the specificity of the FST results. It was also proved that *S*-methyl-L-cysteine had no significant effect on the FST while the higher dose of [Zn(*S*-Met)$_2$] (153 mg/kg) reduced the immobility time and increased their swimming behavior after 14 days of treatment being these effects more effective and remarkable than those observed when administering $ZnCl_2$ in the equivalent quantities. These results allow us to confirm the generation of a synergic effect as a result of the coordination of the Zn(II) with *S*-methyl-L-cysteine. The scientific interest in new pharmacological compounds proficient in inhibit phosphatases activities has formerly been mentioned. In this work it could be demonstrated that the *S*-methyl-L-cysteine did not produce any effect on AcP activity in contrast to the action of [Zn(*S*-Met)$_2$] complex that behaved as a moderate inhibitor agent (IC_{50} = 250 μM) being the first example of a Zn(II) complex for which the inhibition effect on AcP has been determined. The interest to determine the antioxidant activity of *S*-methyl-L-cysteine was based on previous results that indicated the decrease in oxidative stress under the presence of cysteine-containing agents. For this reason, anti-peroxyl activity and hydroxyl attenuation were tested showing that both *S*-methyl-L-cysteine and [Zn(*S*-Met)$_2$] acted as good hydroxyl radical suppressors being the complex more effective in the whole concentration range but more remarkably at 10 μM. Data about bioavailability was obtained by fluorescence spectroscopy which showed that the complex but not the ligand was able to be transported by BSA and the measured K_b value indicated a reversible binding to BSA. These findings predict the potency

of the new compound as a promising metal based drug with antidepressant, antioxidant and enzyme inhibitor ability.

Declaration of interest

The authors have no declaration of interest for this study.

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