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Therapeutic effect of organoselenium dietary supplementation in a sporadic dementia of Alzheimer's type model in rats

Simone Pinton, César A. Brüning, Carla E. Sartori Oliveira, Marina Prigol, Cristina Wayne Nogueira*

Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, SM, RS, CEP 97105-900 Santa Maria, Brazil

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Abstract

It is known that selenium (Se) might play different roles in the progression of Alzheimer's disease (AD), but there is a lack of evidence that proves whether supplementation with Se is beneficial or not for the treatment of AD. Thus, the aim of the current study was to investigate the therapeutic effect of *p,p'*-methoxyl-diphenyl diselenide [$(MeOPhSe)_2$], an organoselenium compound, against streptozotocin (STZ)-induced sporadic dementia of Alzheimer's type (SDAT) in rats. Male Wistar rats received STZ twice daily (1.0 mg/8 μl; 4 μl/ventricle) for 21 days. After 21 days of STZ injection, regular-diet-fed rats were supplemented with 10 ppm of $(MeOPhSe)_2$ during 30 days. At the end of this period, the rats were challenged in the Morris water maze and step-down passive avoidance tasks. The activity of acetylcholinesterase (AChE), deficit in cerebral energy metabolism (measurement of adenosine 5-triphosphate and adenosine 5-diphosphate levels), and oxidative and nitrosative stress were determined in the cortex and hippocampus of rats. The results demonstrated that $(MeOPhSe)_2$ dietary supplementation reverted STZ-induced memory impairment of rats in both cognitive tasks. The findings also indicated that $(MeOPhSe)_2$ dietary supplementation reverted oxidative stress in the STZ group (decreased reactive species and tyrosine nitration levels and enhanced nonprotein thiol levels). Moreover, $(MeOPhSe)_2$ dietary supplementation normalized AChE activity, which was enhanced by STZ injection, but did not revert the deficit in cerebral energy metabolism caused by STZ. The results of the present study indicated the therapeutic effect of the $(MeOPhSe)_2$-supplemented diet in a rat model of SDAT.

Keywords: Alzheimer's disease animal model; Selenium; Nitration; Oxidative stress; Energy metabolism

1. Introduction

Alzheimer's disease (AD) is estimated to affect approximately 39 million people worldwide, being the neurological disorder with a greater prospect for growth in the world [1,2]. There is currently no accurate description of the etiology of AD; moreover, its pathophysiology is complex and involves multiple pathways of neuronal damage [1]. It is characterized by the accumulation of extracellular amyloid beta (Aβ) into aggregated amyloid plaques and the hyperphosphorylation of tau leading to neurofibrillary tangles. This pathologic process is also associated to neuroinflammation and oxidative stress. In this context, there is strong evidence that free radicals play an important role in AD [1,4].

Recently, there has been heightened interest in the role of the trace element selenium (Se) in health and neurologic disorders. Se is an essential trace mineral nutrient with multiple roles in the growth and functioning of living cells of animals. This trace element is known to provide protection from free-radical-induced cell damage [5,6]. There are data showing that Se is involved in most of the molecular pathways that are important in the progression of AD. Although there are controversial data associating the supplementation of Se with cognitive improvement and AD pathophysiology, some authors demonstrated favorable aspects of Se supplementation in AD patients [6–8]. Vural and colleagues [9] have reported that plasma Se levels are lower in AD patients when compared to healthy patients. Furthermore, Se status decreases with age and may contribute to decline in neuropsychological functions among aging people [10]. These data further suggest that alterations in the Se concentration and its related enzymes may play a role in the etiopathogenesis of AD. Se has been associated to the reduction of Aβ production and of Aβ induction of neuronal death in cells culture [11]. In animal models of AD, Se prevented oxidative damage and modulated the cholinergic system [12,13].

A piece of evidence indicates that Se organic compounds have a higher biodisponibility and biological activity than Se inorganic compounds [14]. For this reason, the interest in organoselenium chemistry and biochemistry has increased in the last decades. In fact, some studies have demonstrated the neuroprotective action, among other properties, of these compounds [3,15,16]. In this way, diphenyl diselenide $[(PhSe)_2]$, a simple organoselenium compound, ameliorates memory impairment induced by scopolamine in mice [13] and enhances cognitive performance in rodents without inducing neurotoxicity [17,18].

Moreover, preadministration of *p,p'*-methoxyl-diphenyl diselenide $[(MeOPhSe)_2]$, a substituted analogue of $(PhSe)_2$, improves

* Corresponding author. Tel.: +55 55 3220 8140; fax: +55 55 3220 8978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

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memory of mice in the model of sporadic dementia of Alzheimer's type (SDAT) induced by intracerebroventricular (icv) injection of streptozotocin (STZ) [19,20]. Icv injection of STZ to rodents has been reported as an appropriate SDAT model, characterized by an impairment of memory [21]. Icv injection of STZ reduces the glucose utilization and energy-rich phosphate levels [21]. It also alters the cholinergic system, leading to a reduction in choline acetyltransferase (ChAT) activity [12] and an increase in acetylcholinesterase (AChE) activity [22]. There have also been data indicating the involvement of oxidative stress in STZ-induced SDAT model [12,23]. Based on these positive results [19,20], we planned this study to investigate the therapeutic effect of $(MeOPhSe)_2$ against STZ-induced SDAT model. The involvement of AChE activity, deficit in cerebral energy metabolism, and oxidative and nitrosative stress in the therapeutic effect of $(MeOPhSe)_2$ in the cognitive impairment induced by STZ was examined.

2. Materials and methods

2.1. Drugs

$(MeOPhSe)_2$ was synthesized according to the previously published method [24]. Analysis of the ^1H NMR and ^{13}C NMR spectra showed that the compound obtained presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(MeOPhSe)_2$ (99.9%) was determined by gas chromatography–mass spectrometry. 3-Nitrotyrosine (3-NT), tyrosine and STZ were obtained from Sigma Chemical (St. Louis, MO, USA). All other chemicals were obtained in an analytical grade or from standard commercial suppliers.

2.2. Animals

Experiments were conducted using male Wistar rats (350–400 g) about 7 months old. Animals were maintained at 22°C–25°C with free access to water and food under a 12-h:12-h light/dark cycle with lights on at 7:00 a.m. All manipulations were carried out between 8:00 a.m. and 4:00 p.m. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil. All efforts were made to minimize animals' suffering and to reduce the number of animals used in the experiments.

2.3. Experimental design

Fig. 1 illustrates the experimental design of this study. The animals were separated into four groups: (a) sham, (b) STZ, (c) $(MeOPhSe)_2$ and (d) STZ+$(MeOPhSe)_2$. The animals used in the step-down passive avoidance task at days 21 and 51 were the same. Other animals from all groups performed the Morris water maze task. All protocol steps used in this study are described below.

2.4. Surgery

Animals were anesthetized under intraperitoneal Equithesin (1% phenobarbital, 2% magnesium sulfate, 4% chloral hydrate, 42% propylene glycol, 11% ethanol; 3 ml/kg) [artificial cerebrospinal fluid] injection and placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). The scalp was shaved and swabbed with iodine, and an incision was made along the midline of the scalp, exposing the bregma. Burr holes were drilled in the skull, and cannulae were implanted on both sides over the lateral ventricles using the following coordinates: 1.0 mm posterior to bregma, 2.0 mm lateral to sagittal suture (both right and left), 4.0 mm beneath the surface of the brain at day 0 [25]. Rats received injections of STZ (1.0 mg/8 μl; 4 μl/ventricle) at days 1 and 3. The sham group received only the icv injection of vehicle (aCSF — 147 mM NaCl, 2.9 mM KCl, 1.6 mM MgCl, 1.7 mM CaCl and 2.2 mM dextrose) (4 μl/site). Injections were carried out at 1 μl/min using a Hamilton 10-μl syringe with a 26-gauge needle.

2.5. Dietary supplementation

Animals were fed daily with 50 g/animal standard diet chow or standard chow supplemented with 10 ppm of $(MeOPhSe)_2$ during 30 days. The supplementation began 21 days after the icv injection of STZ. The concentration of 10 ppm of $(MeOPhSe)_2$ was chosen based on previous study of toxicity in rats (data not shown). The preparation of supplemented standard chow was based on a previous study published by de Bem et al. [26]. The standard diet was pulverized with ethyl alcohol, whereas the supplemented diet was pulverized with $(MeOPhSe)_2$ [1 mg of $(MeOPhSe)_2$/100 g standard chow] dissolved in ethyl alcohol (1 mg/10 ml). The standard and supplemented diets were stored at room temperature for 3 h to evaporate the alcohol and then kept at 4°C for no more than 1 week.

2.6. Behavioral tests

2.6.1. Step-down passive avoidance task

The step-down passive avoidance task has been used to study nonspatial long-term memory [27]. The apparatus consisted of a single box where the floor was made of a metal grid connected to a shock scrambler. The box had a safe platform. During the training session (acquisition trial), each rat was placed on the platform; the rat often stepped down from the platform to explore the box. When it stepped down and placed its four paws on the grid floor, an electric shock (0.5 mA) was delivered for 2 s. Some seconds later, the rat was removed from the step-down passive avoidance apparatus and returned to its home cage. The retention trial was performed 24 h after training. Each rat was placed again on the platform, and the transfer latency time (i.e., time took to step down from the platform) was measured in the same way as in the acquisition trial, but foot shock was not delivered and the transfer latency time was recorded to a maximum of 600 s. The criterion for learning was taken as an increase in the transfer latency time on retention (second) trial as compared to the acquisition (first) trial. So, short transfer latencies indicate poor retention.

2.6.2. Morris water maze task

Spatial learning and memory were assessed using the Morris water maze task according to the method of Morris [28]. The water maze consisted of a basin (diameter: 180 cm, wall height: 40 cm) made of black plastic and filled with water (22°C±2°C) at a height of 30 cm. The pool was placed in a room with several extra-maze visual cues, such as counters, posters, a dangling wire and a pole. For the acquisition phase, the rats were submitted to four trials [starting in the north (N), south (S), east (E) and west (W)] for 4 consecutive days. The escape platform was hidden 1 cm below water level in the middle of the northwest (NW) quadrant. The rats remained on the platform for at least 40 s after each trial. Whenever the rats failed to reach the escape platform within the 1-min cutoff period, they were retrieved from the pool and placed on it for 40 s. The latencies to reach the platform were calculated as the mean of total time spent in four trials of each day. Twenty-four hours after the acquisition phase, a probe trial was conducted by removing the platform and placing the rat next to and facing the S side. The time spent in each quadrant, the number of crossings over the former platform position, and the times spent in the platform quadrant and in the opposite quadrant were measured for a single 1-min trial.

2.6.3. Open field

Spontaneous locomotor activity was measured in the open field test [29]. The floor of the open field was divided into nine squares. Each animal was placed individually in the center of the arena, and the number of segments crossed (four-paw criterion) and rearings were recorded in a 4-min session.

2.7. Ex vivo assays

Fifty-one days after the first injection of STZ, the animals were killed by decapitation, and the cerebral cortex and hippocampus were dissected. Blood samples were collected for glucose determination to further confirm that 1 mg/site of STZ is a subdiabetogenic dose. The cortex and hippocampus were homogenized in 50 mM Tris–

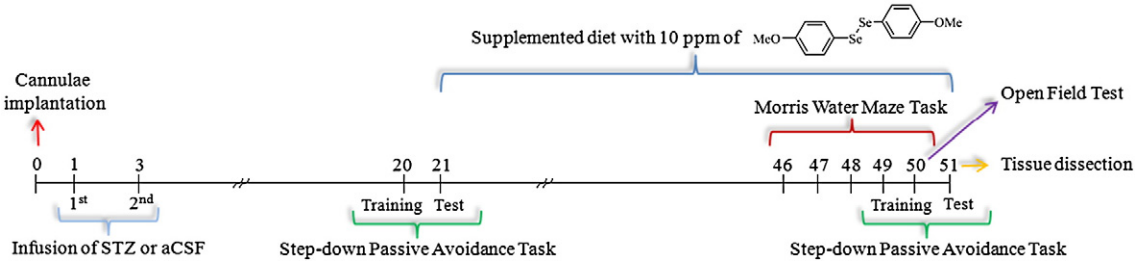

Fig. 1. Experimental procedure.

HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged at 2400*g* for 10 min at 4°C, and a low-speed supernatant fraction (S_1) was used for the following determinations: reactive species (RS) and nonprotein thiol (NPSH) levels.

2.7.1. RS levels

The RS production was determined by diluting S_1 (1:10) in 50 mM Tris–HCl (pH 7.4). S_1 was incubated with 10 μl of 2′,7′-dichlorofluorescein diacetate (DCHF-DA; 1 mM) at room temperature for 30 min. The RS levels were determined by a spectrofluorometric method using the DCHF-DA assay. DCHF-DA is a nonfluorescent compound that easily crosses cell membranes and, in the presence of RS, is rapidly oxidized to its fluorescent derivative dichlorofluorescein (DCF) [30]. The DCF fluorescence intensity emission was recorded at 520 nm (with 480-nm excitation) 30 min after the addition of DCHF-DA to the medium. The RS levels were expressed as arbitrary unit (AU).

2.7.2. NPSH levels

The NPSH levels were determined by the method of Ellman [31]. S_1 was mixed (1:1) with 10% trichloroacetic acid. After the centrifugation, the protein pellet was discarded and free –SH groups were determined in the clear supernatant. An aliquot of supernatant was added in 1 M potassium phosphate buffer (pH 7.4) and 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid). The color reaction was measured at 412 nm. NPSH levels were expressed as μmol NPSH/g tissue.

2.7.3. Tyrosine nitration levels

Determination of 3-NT and tyrosine in rat cortex was performed by high-performance liquid chromatography/ultraviolet (HPLC-UV) detection method based on Erdal and colleagues [32]. Tyrosine nitration was not determined on hippocampus since 3-NT levels were below the method detection limit.

Briefly, cortex samples were homogenized in 50 mM Tris–HCl, pH 7.4 (1/10, w/v), and an aliquot was hydrolyzed in HCl (12 N; 1:1 v/v) at 60°C for 24 h. Digested samples were filtered through a membrane (0.45-μm pore size; Millipore) before injection onto the HPLC instrument. Samples were analyzed on a Shimadzu HPLC apparatus. The analytical column was a 5-μm particle and 100-Å pore size Phenomenex ODS-2 C_{18} reverse-phase column (4.6×250 mm, Allcrom, Brazil). The mobile phase was 50 mM sodium acetate, 50 mM sodium citrate and 8% (v/v) methanol, pH 3.1 (corrected with 12 N HCl). The HPLC analysis was performed under isocratic conditions at a flow rate of 1 ml/min and with the UV detector set at 274 nm. 3-NT levels were expressed as 3-NT (μM)/total tyrosine (μM).

2.7.4. Adenosine 5-triphosphate (ATP) and adenosine 5-diphosphate (ADP)

The energy-rich phosphate (ATP, ADP) in rat cortex and hippocampus was determined by the HPLC-UV detection modified method of Özogul and colleagues [33]. Briefly, the cortex and hippocampus samples were homogenized in 0.6 mM perchloric acid (1/5, w/v) and centrifuged at 2400*g* at 4°C for 10 min. The supernatant fraction was neutralized to pH 6–6.5 with 1 M potassium hydroxide. The neutralized fractions were kept on ice for 30 min to ensure the total precipitation of potassium crystals. After that, they were filtered through a membrane (0.45-μm pore size; Millipore) before injection onto the HPLC instrument. Samples were analyzed on the same apparatus described for 3-NT and tyrosine determinations. The mobile phase was 0.04 M potassium dihydrogen orthophosphate and 0.06 M dipotassium hydrogen orthophosphate dissolved in purified distilled water and adjusted to pH 7 with 0.1 M potassium hydroxide. The HPLC analysis was performed under isocratic conditions at a flow rate of 1 ml/min and with the UV detector set at 257 nm. The results were expressed as ATP concentration/ADP concentration ratio.

2.7.5. AChE activity

Samples of cortex and hippocampus were homogenized in 0.25-M sucrose buffer (1/10, w/v) and centrifuged at 2400*g* at 4°C for 15 min. The activity of AChE was carried out according to the method of Ellman and colleagues [34] using acetylthiocholine as substrate. The activity of AChE was spectrophotometrically measured at 412 nm. The activity of AChE was expressed as nmol/min/mg protein. Protein concentration was measured according to the method of Bradford [35].

2.7.6. Plasma glucose levels

Plasma glucose levels were determined by enzymatic colorimetric method using a commercial kit (Labtest Diagnostica, MG, Brazil). Glucose levels were expressed as mg/dl.

2.8. Statistical analysis

The behavioral data were analyzed by the nonparametric test (Kruskal–Wallis analysis of variance) followed by the Dunn's multiple comparison test when necessary or *t* test analysis (GraphPad software, San Diego, CA, USA; for the step-down passive avoidance at 21 days). The behavioral data are given as the median±interquartile range. Data from *ex vivo* assays were calculated by means of two-way analysis of variance followed by the Duncan's test when necessary. Experimental results of *ex vivo* assays are given as the mean±S.E.M. Probability values less than .05 ($P<.05$) were considered to be statistically significant.

3. Results

$(MeOPhSe)_2$ improved memory decline induced by STZ in the rat Morris water maze and step-down passive avoidance tasks. At the 21st day after the STZ infusion, STZ induced an impairment in memory of rats ($P=.0334$) in the step-down passive avoidance task (Fig. 2A), which is in agreement with the literature data.

Thirty days of $(MeOPhSe)_2$ dietary supplementation were effective in improving memory of rats since they took more time to descend from the platform in the step-down passive avoidance task [$H(3)=13.93$; $P<.005$] (Fig. 2B, see the retention phase). Animals of the sham group supplemented with $(MeOPhSe)_2$ diet had a memory of the first shock preserved [$H(3)=12.86$; $P<.005$] (Fig. 2B, in the acquisition phase). These findings support the hypothesis that $(MeOPhSe)_2$ diet recovered and enhanced the memory of rats.

On the Morris water maze task, the analysis of the spatial learning behavior on the memory acquisition phase revealed a significant effect of training days. These results showed that rats of all groups learned to find the platform during the 4 days of training. There was no significant difference in the latency to reach platform in all days of the acquisition phase among groups, except at the fourth day [$H(3)=9.497$, $P<.05$] (Fig. 3). The results showed that STZ induced an impairment on spatial memory of rats because rats from the STZ group spent less time in the platform quadrant [$H(3)=9.034$; $P<.05$] (Fig. 4C) and more time in the opposite platform quadrant when compared to the sham group [$H(3)=14.98$; $P<.005$] (Fig. 4D). $(MeOPhSe)_2$-supplemented diet not only reverted this behavior but also decreased the latency to reach platform in the probe [$H(3)=9.497$, $P<.05$] (Fig. 4A). There was no significant difference in the

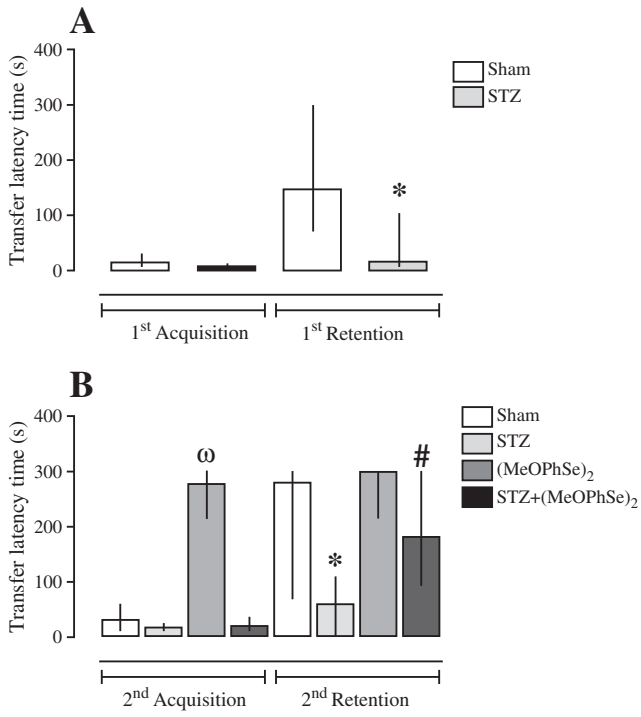

Fig. 2. Effects of $(MeOPhSe)_2$ on STZ-induced memory deficit in passive avoidance test: (A) transfer latency time (s) to fall from the platform in the acquisition and retention phase at 20–21 days after STZ infusion; (B) transfer latency time(s) to fall from the platform in the acquisition and retention phases after $(MeOPhSe)_2$ diet supplementation (50–51 days after STZ infusion). Data are median and interquartile range for $n=12$–13 animals per group (task at 21 day) and $n=6$–7 in each group (task at 51 day). $^{\omega}P<.05$ as compared to the sham group in the acquisition phase; $^{*}P<.05$ as compared to the sham group; $^{\#}P<.05$ as compared to the STZ group in the retention phase.

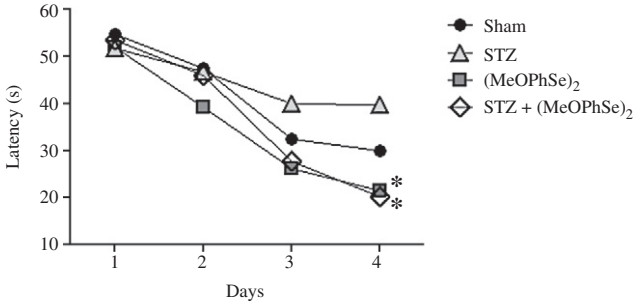

Fig. 3. Effects of $(MeOPhSe)_2$ on STZ-induced memory deficit in the Morris water maze test: latency (ies) to reach the platform in the acquisition phase. Data are median and interquartile range for $n=8$ in each group. *$P<.05$ as compared to the STZ group.

number of crossings in the platform local among groups [$H(3)=5.779$; $P>.05$] (Fig. 4B).

STZ- and/or $(MeOPhSe)_2$-supplemented diet caused alteration neither in the spontaneous locomotion [$H(3)=2.104$; $P>.05$] nor in exploratory activities [$H(3)=1.241$; $P>.05$] in the open field test (data not shown).

The dose of STZ did not induce diabetes. The results confirmed that 1 mg/site of STZ is a subdiabetogenic dose since there was no significant difference in plasma glucose levels among groups [STZ×$(MeOPhSe)_2$ interaction ($F_{1,36}=0.322$; $P>.05$)] (data not shown).

$(MeOPhSe)_2$ supplementation alleviated oxidative and nitrosative stress induced by STZ in rats. As shown in Table 1, STZ induced an increase of RS levels in cortex (by ~110%), and $(MeOPhSe)_2$ was effective against this increase [STZ×$(MeOPhSe)_2$ interaction ($F_{1,25}=4.341$; $P<.05$)]. There was no significant difference in RS levels in hippocampus of rats from all groups [STZ×$(MeOPhSe)_2$ interaction ($F_{1,25}=0.000$; $P>.05$)]. STZ induced a decrease of NPSH levels in hippocampus (by ~15%), and the $(MeOPhSe)_2$-supplemented diet reverted this reduction [main effect of STZ ($F_{1,25}=7.036$; $P<.05$) and $(MeOPhSe)_2$ ($F_{1,25}=8.999$; $P<.01$)]. In the cortex, the results revealed an effect of $(MeOPhSe)_2$-supplemented diet on NPSH levels [main effect of $(MeOPhSe)_2$ ($F_{1,25}=7.031$; $P<.05$)]. The icv injection of STZ increased tyrosine nitration in cerebral cortex of rats, and the $(MeOPhSe)_2$-supplemented diet was effective against the increase [STZ×$(MeOPhSe)_2$ interaction ($F_{1,8}=7.125$; $P<.05$)] (Table 1).

$(MeOPhSe)_2$ inhibited AChE activity but did not change the energy metabolism. No significant difference was found in ATP/ADP ratio in the cerebral cortex of rats [STZ×$(MeOPhSe)_2$ interaction ($F_{1,28}=0.000$; $P>.05$)] (Table 2). In the hippocampus, the $(MeOPhSe)_2$-supplemented diet was not effective in restoring the decrease in ATP/ADP ratio caused by STZ [main effect of STZ ($F_{1,28}=7.517$; $P<.05$)]. The $(MeOPhSe)_2$-supplemented diet inhibited AChE activity in both structures: cortex [main effect of STZ ($F_{1,27}=14.028$; $P<.05$) and $(MeOPhSe)_2$ ($F_{1,27}=6.348$; $P<.05$)] and hippocampus of rats [STZ×$(MeOPhSe)_2$ interaction ($F_{1,27}=4.356$; $P<.05$)] (Table 2).

4. Discussion

In the present study, we demonstrated the therapeutic effect of $(MeOPhSe)_2$ dietary supplementation on memory and learning of rats in a model of SDAT induced by STZ. The results indicate that the $(MeOPhSe)_2$-supplemented diet rescued spatial learning and memory and nonspatial long-term memory in STZ-treated rats. $(MeOPhSe)_2$ restored AChE activity and had antioxidant and antinitrosative effects in rats. The therapeutic action of $(MeOPhSe)_2$, the improvement of cognitive function, could be tentatively explained by its antioxidant property. The therapeutic effect of $(MeOPhSe)_2$ dietary supplementation seems not to be related to the energetic metabolism because $(MeOPhSe)_2$ did not alter the levels of

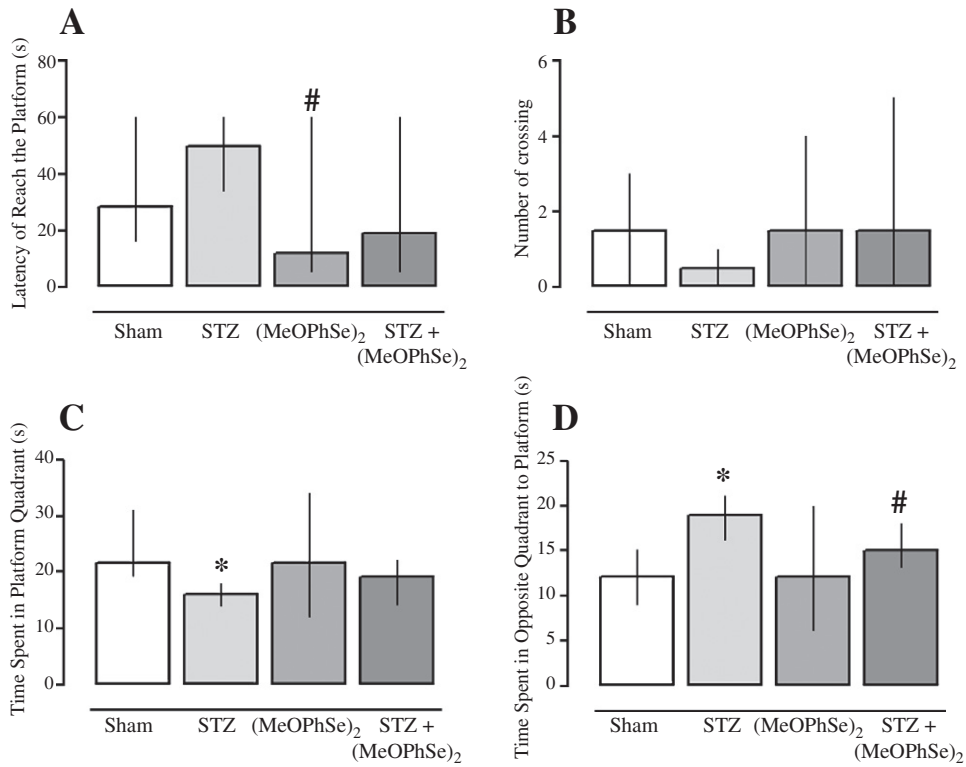

Fig. 4. Effects of $(MeOPhSe)_2$ on STZ-induced memory deficit in the Morris water maze test: (A) latency (ies) to reach the platform in the probe test; (B) number of crossing over the former platform position; (C) time (s) spent in the platform quadrant; (D) time (s) spent in the opposite quadrant to platform. Data are median and interquartile range for $n=8$ in each group. *$P<.05$ as compared to the sham group; #$P<.05$ as compared to the STZ group.

Table 1
Effect of $(MeOPhSe)_2$ diet supplementation on RS, NPSH and 3-NT levels in cerebral cortex and hippocampus of rats that received the icv injection of STZ

	RS[a]		NPSH[b]		3-NT[c]
	Cortex	Hippocampus	Cortex	Hippocampus	Cortex
Sham	104.3±23.7	160.2±21.0	5.59±0.12	5.79±0.16	0.207±0.02
STZ	220.14±29.8*	187.2±43.3	5.21±0.26	5.02±0.11*	0.291±0.01*
$(MeOPhSe)_2$	120.14±40.8	158.1±19.6	5.98±0.20#	6.37±0.22#	0.153±0.01*
STZ+$(MeOPhSe)_2$	118.2±24.7#	185.0±36.1	5.74±0.09	5.87±0.33#	0.140±0.03*,#

Data are reported as the mean(s)±S.E.M. for n=7–8 animals per group in RS and NPSH determinations and for n=3 animals per group in 3-NT determination.
[a] Data were expressed as AU.
[b] Data were expressed as μmol NPSH/g tissue.
[c] Data were expressed as 3-NT (μM)/total tyrosine (μM).
* P<.05 as compared to the sham group.
# P<.05 as compared to the STZ group.

ATP and ADP. Moreover, the findings presented here also indicate that the $(MeOPhSe)_2$-supplemented diet improved memory of nontreated rats.

At 21 days after injection of STZ, rats were challenged in the step-down passive avoidance to further confirm the memory impairment. After that, rats were submitted to the $(MeOPhSe)_2$-supplemented diet for 30 days and were retested in step-down passive avoidance. Surprisingly, even after 30 days, in the second acquisition phase, rats supplemented with the $(MeOPhSe)_2$ diet practically did not step down on the grid floor. These findings support the hypothesis that the $(MeOPhSe)_2$-supplemented diet induced cognitive enhancement in rats. In this context, organoselenium compounds have been reported as memory enhancers [17,18].

Moreover, in this study, the therapeutic effect of $(MeOPhSe)_2$ dietary supplementation on STZ-induced SDAT in rats was investigated in Morris water maze and step-down passive avoidance tasks. Although these are different paradigms — Morris water maze evaluates spatial learning and memory, while step-down passive avoidance assesses nonspatial long-term memory — the therapeutic effect of the $(MeOPhSe)_2$-supplemented diet was demonstrated. In fact, the $(MeOPhSe)_2$-supplemented diet was effective in improving spatial learning and memory and nonspatial long-term memory in STZ-treated rats without altering the spontaneous locomotor activity of these animals.

Consistent with previously published data [22,23,36], in the present study, the icv injection of STZ in rats caused learning and memory impairment. The learning and memory impairment was demonstrated by a clear trend to increase the latency to find a platform, a reduction in the time spent by the animals in the quadrant where the platform was formerly located, an augmentation in the time spent in the opposite quadrant in Morris water maze and a decrease in the latency time to step down and place the four paws on the grid floor in step-down passive avoidance. The $(MeOPhSe)_2$-supplemented diet ameliorated the performance of rats in the Morris water maze and passive avoidance tasks. These findings are in agreement with those previously reported, which demonstrated that inorganic Se [12] and organic Se [13,17,18] are effective in preventing, improving or ameliorating memory of rodents.

Table 2
Effect of $(MeOPhSe)_2$ diet supplementation on AChE activity and ATP turnover levels in the cerebral cortex and hippocampus of rats that received the icv injection of STZ

	AChE[a]		[ATP]/[ADP] ratio	
	Cortex	Hippocampus	Cortex	Hippocampus
Sham	7.35±0.94	9.23±0.62	0.779±0.023	0.913±0.039
STZ	11.34±1.14*	11.90±1.06*	0.695±0.047	0.771±0.042*
$(MeOPhSe)_2$	6.46±0.45	9.90±0.89	0.787±0.057	0.969±0.082
STZ+$(MeOPhSe)_2$	8.30±0.73#	9.03±0.71#	0.731±0.066	0.786±0.010*

Data are reported as the means±S.E.M. for n=7–8 animals per group.
[a] Data were expressed as nmol/min/mg protein.
* P<.05 as compared to the sham group.
# P<.05 as compared to the STZ group.

AD is associated with progressive death of neurons, particularly in the cortex and hippocampus [1]. This neurodegenerative process is coupled to oxidative stress, mitochondrial dysfunction, impaired energy metabolism and activation of prodeath signaling pathways [1,37]. In fact, there is strong evidence that free radicals play an important role in AD [38]. In this context, the icv injection of STZ has been reported as an appropriate animal model to mimic the human SDAT characterized by the presence of oxidative stress [36].

A reduction in the level of GSH may impair H_2O_2 clearance and promote the formation of OH, the most toxic moiety to the brain, leading to more oxidant load and consequently oxidative damage [39]. In this way, the results found in the current study demonstrated that the icv injection of STZ in rats caused an increase in RS levels in the cortex and a depletion of NPSH levels in the hippocampus, which were kept at normal levels after dietary supplementation with $(MeOPhSe)_2$. These results suggest that the antioxidant property could be involved in the $(MeOPhSe)_2$ neuroprotective effect on SDAT induced by STZ in rats. Accordingly, the neuroprotective effect of the antioxidant $(MeOPhSe)_2$ was reported [19].

Moreover, it has been reported that organoselenium moieties are good antioxidants, increasing NPSH levels and promoting RS detoxification [16,19]. Recently, the property of mimicking the activity of glutathione peroxidase, glutathione-*S*-transferase and dehydroascorbate reductase was related to the antioxidant action of $(PhSe)_2$. The data provided in this study further support the idea that $(PhSe)_2$ is not a radical scavenger [40]. In addition, Freitas and colleagues [41] demonstrated that $(MeOPhSe)_2$ was a substrate for mammalian thioredoxin reductase (TxR), which may explain, at least in part, its antioxidant properties. Based on these data, the antioxidant effect of $(PhSe)_2$ and its substituted analogues, like $(MeOPhSe)_2$, has been attributed to the property of mimicking the activity of antioxidant enzymes and by acting as a substrate for TxR.

The rapid interaction between oxygen (O_2) and nitric oxide (NO) produces peroxynitrite ($ONOO^-$). Peroxynitrite is a potent nitration mediator and strong oxidant implicated in AD pathogenesis [42]. It modifies tyrosine residues in protein and thus generates a stable compound, namely, 3-NT. The concentration of 3-NT is markedly elevated in the brains of AD patients and is positively correlated with decreased cognitive functions in these patients [42]. In support of this latter assertion, Horiguchi and colleagues [43] demonstrated that the tau protein is nitrated and co-localized with neurofibrillary tangles in AD brains. Moreover, Zhang and colleagues [44,45] reported that $ONOO^-$ and NO cause modification, accumulation and hyperphosphorylation of tau protein in rat brain. Thus, the inhibition of inducible nitric oxide synthase (iNOS) activity or nitrosative species scavenger could be an alternative to prevent the neurodegeneration in AD.

The results demonstrated here indicate that $(MeOPhSe)_2$ protected against protein nitration induced by icv injection of STZ in cortex of rats. These results support the hypothesis that the neuroprotector effect of $(MeOPhSe)_2$ is attributed to its antinitrosative property. In this way, previous data have demonstrated that the neuroprotective action of organoselenium compounds is related to the decrease in cerebral nitrate/nitrite levels [46] and inhibition of iNOS activity [47] in rodents. Thus, if $(MeOPhSe)_2$ is effective in decreasing 3-NT levels induced by STZ in cerebral cortex of rats and if $ONOO^-$ and NO could simultaneously induce tau nitration and hyperphosphorylation, it is plausible to assume that $(MeOPhSe)_2$ minimizes tau hyperphosphorylation induced by STZ [48].

During the early stages of AD, a reduced number of mitochondria in neurons, decreased brain glucose metabolism, and reduced activities of both tricarboxylic acid cycle enzymes and cytochrome *c* oxidase have been reported [37]. Similarly to AD, the icv injection of STZ induces a desensitization of neuronal insulin receptor and a reduction in activities of glycolytic enzymes [49]. It causes a deficit on cerebral energy metabolism, leading to a cognitive dysfunction by inhibiting the synthesis of ATP and acetyl CoA, which results into cholinergic deficiency, supported by reduced ChAT activity and enhanced AChE activity [12,22]. Moreover, the brains of rats which received icv injection of STZ exhibited an increased expression of genes encoding AChE, tau and amyloid precursor protein [48,50].

The results of the present study confirmed an inhibition in ATP synthesis, demonstrating that STZ induced a deficit in energy metabolism in the hippocampus of rats since the ATP/ADP ratio, an indicator of ATP turnover, was reduced. The $(MeOPhSe)_2$-supplemented diet was not effective in restoring ATP levels. Thus, these results rule out a possible interaction of $(MeOPhSe)_2$ with energy metabolism. By contrast, $(MeOPhSe)_2$ was effective in normalizing AChE activity of the cerebral cortex in rats exposed to STZ. This result strengthens the relationship between the $(MeOPhSe)_2$ effect on memory and cholinergic modulation [20]. Thus, the results on AChE activity associated to $(MeOPhSe)_2$ antioxidant properties could explain the positive results on the Morris water maze and step-down passive avoidance tasks.

In summary, the most relevant additional findings of the present study are that therapeutic $(MeOPhSe)_2$ dietary supplementation (a) reverted STZ-induced memory impairment of SDAT in rats; (b) reverted oxidative stress; (c) normalized AChE activity, which was increased by STZ injection; and (d) did not alter the deficit in cerebral energy metabolism induced by STZ. Thus, the use of $(MeOPhSe)_2$-supplemented diet should be encouraged for the treatment of SDAT due to its therapeutic values.

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