Neuroprotective effects of tibolone against oxidative stress induced by ozone exposure

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Introduction. Oxidative stress increases brain lipid peroxidation, memory and motor deficits and progressive neurodegeneration. Tibolone, a treatment for menopausal symptoms, decreases lipid peroxidation levels and improves memory and learning.

Aim. To study the effect of chronic administration of tibolone on lipid peroxidation, memory and motor deficits in ozone induced oxidative stress.

Materials and methods. 100 male Wistar adult rats were randomly divided into 10 experimental groups: control (C) was exposed to an airstream for 60 days; C + tibolone, airstream exposure plus 1 mg/kg of tibolone for 60 days; groups 3-6 were exposed to ozone for 7, 15, 30, and 60 days, and groups 7-10 received 1 mg/kg of tibolone treatment by oral gavage for 7, 15, 30 and 60 days and were then exposed to ozone. We determined the effect of tibolone on memory and motor activity. Hippocampus was processed to determine the content of 4-hydroxynonenal and nitrotyrosine by Western blot. Four animals were perfused and processed for analysis of neuronal death.

Results. In the hippocampus, administration of 1 mg/kg of tibolone for 30 days prevented increased levels of lipid peroxidation and protein oxidation, whereas after 60 days prevented neuronal death in the CA3 region caused by exposure to ozone. Therefore, tibolone prevents cognitive deficits in short- and long-term memory on the passive avoidance task and prevents a decrease in exploratory behavior and an increase in freezing behavior.

Conclusion. Our results indicate a possible neuroprotective role of tibolone as a useful treatment to prevent oxidative stress neurodegeneration.

Key words. 4-hydroxynonenal. Hippocampus. Learning. Lipoperoxidation. Memory. Oxidative stress. Neurodegeneration. Nitrotyrosine. Ozone. Tibolone.

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Introduction

Ozone (O_3) is a primary component of air pollution in large cities and due to the accumulation of photochemical smog [1-3]. Damage caused by O_3 exposure depends on the dose inhaled [1,4]. O_3 causes an increase in the generation of reactive oxygen species (ROS), oxidation or peroxidation of biomolecules with loss of functional groups of proteins and cell damage or death [4-6].

When the O_3 level overwhelms the antioxidant defense systems in the lungs [7,8], ROS can then reach the central nervous system (CNS) through the bloodstream, causing oxidative stress (OS) [9-11], OS in the CNS plays a major role in neuronal death caused by severe vulnerability of the brain to a lost oxidation-reduction balance [12].

In the CNS, OS caused by O_3 increases lipoperoxidation (LPO) [13], with the olfactory bulb, hippocampus, striatum, cerebellum and prefrontal cortex being the most vulnerable areas [14-18]. In the hippocampus also causes neuronal death [1] and can lead to impaired brain plasticity, resulting in learning, memory and motor deficits and ending in progressive neurodegeneration [1]. OS has also been described in the pathophysiology of neurodegenerative diseases, such as Alzheimer and Parkinson [19,20].

It has been reported that the administration of several exogenous compounds, such as taurine [21], vitamin E [22] and estradiol (E_2) [23], have antioxidant effects and may decrease lipid peroxidation induced by O_3 exposure. E_2 also decreases neuronal death, cognitive deficit and impaired social recognition memory caused by exposure to O_3 in adult rats [23,24]. E_2 is likely to exert antioxidant activity through its inherent ability to scavenge ROS and may inhibit LPO [25,26]. However, it is well known that the chronic administration of E_2 alone and in combination with progestins as hormone therapy

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(HT) has side effects as endometrial and breast cancers and cardiovascular events [27,28].

Tibolone (TIB) is a selective tissue estrogenic activity regulator that is widely prescribed to treat menopausal symptoms and to prevent bone loss [29]. TIB is metabolized into three biologically active metabolites, the 3-α-hydroxyestrogenic metabolite, the 3-β-hydroxyestrogenic metabolite, and the Δ^4-TIB, which display progestogenic and androgenic effects [30,31]. TIB metabolites circulate in inactive forms, and their conversion to bioactive forms depends on tissue-specific desulfation [32,33].

Previous reports have indicated that the 3-α- and 3-β-hydroxy-TIB are the predominant free metabolites in the brain tissues of ovariectomized (OVX) cynomolgus monkeys [32-34]. High doses of TIB (1 and 10 mg/kg) improved learning observed in T-maze compared to the vehicle. In this same study, in the hippocampus, the choline acetyl transferase content decreased with increasing doses of TIB, whereas the tryptophan hydroxylase content increased with doses of 1 and 10 mg/kg of TIB [35]. TIB causes less vaginal bleeding, breast tenderness, and mammographic density with cardiovascular safety not different from other HTs [27,28].

In premenopausal women, TIB administration has reversed the cognitive damage caused by leuprolide acetate and improved the mood and quality of life in patients receiving uterine leiomyoma treatment [36]. In hypothalamic neurons, TIB administration rapidly attenuated the response of γ-aminobutyric acid receptor B [37]. In the cortex and hippocampus of OVX rats, TIB decreased lipoperoxidation levels, produced greater total antioxidant capacity [38], and improved memory in a inhibitory avoidance task [39]. Another study reported more intense staining for glial fibrillary acidic protein (GFAP) and c-fos in the brain cortex and hippocampus of female rats treated with TIB than in control animals [40]. In hippocampal and cerebellar tissues, the chronic administration of TIB significantly decreased the protein content of hyperphosphorylated tau and increased the levels of dephosphorylated tau, and these correlated with increases in the phosphorylated form of glycogen synthase kinase 3 (GSK3) [41]. All these results indicate that the chronic administration of TIB may exert a neuroprotective effect.

However, the effects of the chronic administration of TIB on memory deficits and motor impairment in an OS rat model induced by O_3 exposure are unknown. Therefore, in this work, we analyzed the effect of administering TIB at different times on memory deficit and motor impairment using the passive avoidance task and the motor activity test. We also analyzed the correlation of TIB treatment with the expression of OS markers and neuronal death in the male rat hippocampus.

The aim of this work was to study the effects of chronic administration of TIB on lipoperoxidation, memory and motor deficits, and neuronal death on a well-established noninvasive OS model using a chronic exposure of animals to low doses of O_3 similar to a day of high pollution in México City.

Materials and methods

Animals

One hundred male Wistar rats weighing 250-300 g were housed in acrylic boxes (five animals per cage) with free access to water and food (Purina, Minnetonka, MN) and kept in a clear air room maintained on an artificial 12 h light/dark cycle (lights on at 08:00 h). Animals were treated in accordance with the guidelines and requirements of the World Medical Association Declaration of Helsinki and those of the Ethical Committee of the Faculty of Medicine at the Universidad Nacional Autónoma de México.

Treatments

The animals were randomly divided into ten experimental groups, with ten animals per group. In the first group (C), ten animals were exposed to an airstream free of O_3 for 60 days; in the second group (C+TIB), ten animals were exposed to an airstream free of O_3 and received 1 mg/kg of TIB (Livial ® Organon, Mexico) for 60 days. Groups 3, 4, 5, and 6 were exposed to O_3 for 7, 15, 30, and 60 days, respectively. Groups 7, 8, 9, and 10 received 1 mg/kg of TIB treatment by oral gavage for 7, 15, 30, and 60 days (O_3+TIB), respectively, and were then exposed to O_3. We selected this dose and time periods because previous studies have reported that TIB exerts effects on cognitive processes during the first two months of treatment with doses of 0.5 and 1 mg/kg [35,39].

O_3 exposure

Animals were put daily for 4 h, exposed to 0.25 ppm of O_3 inside a chamber with a diffuser connected to an ozone generator (5 L/s). The procedure used has been described elsewhere [1,17,21,24].

Air exposure

The same chamber was used when treating the con-

trol group where flow of ozone-free purified air was used [1,17,21,24].

Passive Avoidance Task (PAT)

The PAT described in reference [13] was employed. Training was performed in a conditioning chamber with two compartments of the same size (30 × 30 × 30 cm), one considered the safe compartment and the other the punishment compartment. They were separated by a guillotine-style door. In the safe compartment the floor consisted of an aluminum grill made of bars 0.5 cm in diameter separated by a distance of 1.5 cm. A stainless-steel layer covered the lateral walls and the floor of the punishment compartment. There was a gap of 1 cm in the middle of the floor. The floor was connected to a constant-current unit (Grass, Model PSIU6), which in turn was connected to a Grass stimulator (Model S48) that delivered 50 square pulses per second at an intensity of either 2 to 4 mA, with a duration of 5 ms per pulse for 5 s. Passive avoidance training of 10 rats per group with a 3 mA footshock was performed two hours following the last air or O_3 exposure. Either 10 min (short-term memory) or 24 h (long-term memory) after this training procedure, a retention test was performed.

Motor Activity Test (MAT)

The MAT described in reference [13] was employed. Immediately after air or ozone exposure had finished, animals were returned to their home cages. Two hours later, motor activity of each animal of each group was quantified. The home cage was positioned on a locomotion sensitive plate, while the animal remained inside it. Motor activity was measured using an electronic activity monitor (EAM; Stoelting Co., Wood Dale, IL, USA). Ten minutes after having placed the cage on the plate, so as for the animal to adapt to the new location of the cage, motor activity was measured for 5 min. Quantification of the motor activity (exploratory and freezing) was carried out in all 10 animals of each group. The motor activity of the rats in the control groups was compared to that of the animals in the other experimental groups.

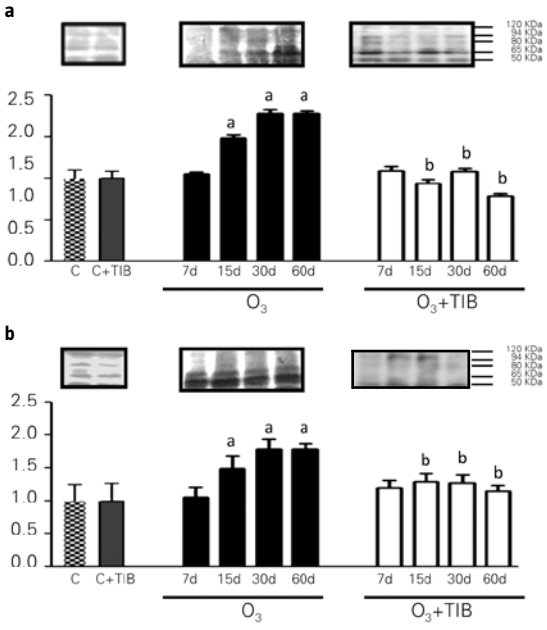
Western blot analysis

After analysis of the different behaviors, six animals from each group were randomly selected and killed by decapitation, and their hippocampus were dissected and processed for Western blotting. The Western blot analysis described in reference [41] was employed. Tissue samples were homogenized in lysis buffer (150 nM NaCl, 20 mM Tris-HCl, 10% glycerol, 5 mM EDTA, and 1% NP-40; Roche, Mannheim, Germany) supplemented with protease inhibitors (50 µg/ml of phenyl methyl sulfonyl fluoride, 10 µg/mL aprotinin, 25 µg/mL leupeptin, all from Sigma). Proteins were obtained by centrifugation for 15 min at 15,000 rpm at 4 °C, and the supernatants were quantified using a modified Bradford assay (BioRad, Munich, Germany). Proteins (30 mg) were resolved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% SDS-PAGE) with a Mini-Protean system (BioRad) and electrophoretically transferred to nitrocellulose membranes (GE Healthcare, formerly Amersham Bioscience, Piscataway, NJ, USA). To verify that the amount of protein was the same in each lane, the membrane was stained with Ponceu S solution (0.1% w/v in 5% acetic acid, Sigma) and washed with 0.05% Tween-20 Tris-buffered saline until the stain disappeared. The membranes were blocked with 5% non-fat dry milk diluted in 0.05% Tween-20 Tris-buffered saline and incubated overnight with the primary antibodies. Pre-stained markers (BioRad) were included for size determination. The following antibodies were used: rabbit polyclonal antibody against nitrotyrosine (NT) (Abcam, diluted 1:1,000); mouse monoclonal antibody against 4-hydroxynonenal (4-HNE) (R&D, diluted 1:1,000). After incubation with the primary antibody, the membranes were washed and incubated with horseradish peroxidase-coupled secondary antibodies (Santa Cruz, diluted 1:10,000). The intensity of the protein bands was quantified by densitometry using a HP Scanner for capturing data and the Kodak 1D Image Analysis Software for densitometric analysis. Five immunoreactive bands were quantified (50, 65, 80, 94 and 120 kDa) in both oxidative stress markers. The sum of the optical density of the 5 bands was used to perform the corresponding graph. To minimize intra-assay variations, samples from all animal groups in each experiment were processed concurrently and under the same conditions.

Histological assessment

For histological assessment of hippocampal damage, the other four animals were perfused with 4% paraformaldehyde in PBS at the end of the last O_3 exposure. Brain slices containing the dorsal hippocampus were processed for paraffin embedding and coronal sections (10 µm) were stained with the Nissl technique. The number of remaining pyrami-

Figure 1. Examples of Western blots and effects of chronic exposure to O_3 and O_3+TIB on NT and 4-NHE content in the hippocampus of rats. Animals were randomly divided into ten experimental groups, (C) animals exposed to an airstream for 60 days; C+TIB animals exposed to an airstream and received 1 mg/kg of TIB for 60 days. O_3 groups were exposed to O_3 for 7, 15, 30, and 60 days, respectively. O_3+TIB groups received 1 mg/kg of TIB treatment for 7, 15, 30 and 60 days respectively, and were then exposed to O_3. Bands immunodetected and densitometric analysis of NT (a) and 4-HNE antibody (b) are showed. Results are expressed as mean ± standard error. $n = 10$. [a] $p < 0.05$ compared to C and C+TIB; [b] $p < 0.05$ 15d, 30d and 60d O_3+TIB compared to 15d, 30d and 60d O_3 respectively.



dal neurons showing normal morphology, distinct cytoplasmic and nuclear outlines, and visible nucleolus, per 385 µm length in the middle part of the CA3 pyramidal cell layer, was counted bilaterally in 2 sections per animal under a light microscope (magnification, 400×). Cell counts from the right and left hippocampus on each of the two sections were averaged to provide a single value for each animal.

Statistical analysis

Behavioral data were analyzed using nonparametric tests: Kruskal-Wallis analysis of variance among all groups and the Mann-Whitney test to compare the control and experimental groups**.** Western blot data and histological assessment were analyzed using ANOVA followed by a post hoc analysis with Tukey's test. Prism 2.01 software (Graph Pad, CA) was used for calculating probability values. Values of $p < 0.05$ were considered statistically significant.

Results

Figure 1 shows Western blot data for OS markers of protein oxidation as NT (Fig. 1a) and lipoperoxidation, such as 4-HNE (Fig. 1b) from animals exposed to O_3 and exposed to O_3 plus treatments of 1 mg/kg of TIB. We observed that the OS produced by chronic exposure to O_3 increased the levels of products of the decomposition of peroxides derived from polyunsaturated fatty acids and the levels of ester lipoperoxidation and protein oxidation. We further observed that these increases were statistically significant upon at least 15 days of exposure. However, treatment with TIB maintained the levels of these OS markers at values similar to those of controls over the same time period (Figs. 1a and 1b).

Figure 2 shows the results of the PAT at 10 min and 24 h after the last exposure to O_3 or to O_3 with 1 mg/kg of TIB treatment. We observed that chronic exposure to O_3 decreased the retention latency in both short- and long-term memory; this decrease was statistically significant after 15 days of exposure and was maintained until 60 days of exposure to O_3 (Figs. 2a and 2b). In animals exposed to O_3 and treated with TIB, the retention latency remained at levels similar to those of the controls at 10 minutes (Fig. 2a). Although we observed a decrease in the retention latency at 24 h in animals treated with TIB, the values were higher than those of animals exposed to O_3 only (Fig. 2b).

We observed that chronic O_3 exposure decreased exploratory behavior and that this decrease was statistically significant after 30 days, whereas animals treated with TIB before exposure to O_3 retained levels of exploratory behavior similar to those observed in the controls (Fig. 3a). This result is related to the increase in freezing behavior, which was statistically significant beginning at 15 days of exposure (Fig. 3b). In animals pretreated with TIB, an increase in freezing behavior was observed, although this increase was statistically lower than that observed in animals exposed to O_3 without TIB treatment (Fig. 3b).

Finally we analyzed whether TIB may alter CA3 neuronal death provoked by O_3 exposure. Nissl staining showed O_3 induced neuronal death in CA3 region of the hippocampus (Figs. 4e, 4f, 4i). In animals previously treated with TIB, reduced CA3 neuronal death was observed (Figs. 4g, 4h, 4i) not

Figure 2. Effects of O_3 and O_3+TIB on PAT. Short-term (a) and long-term (b) memory retention latency. Mean latencies in seconds are on the ordinate and treatments on the abscissa. [a] $p < 0.05$ compared to C and C+TIB; [b] $p < 0.05$ 15d, 30d and 60d O_3+TIB compared to 15d, 30d and 60d O_3 respectively.

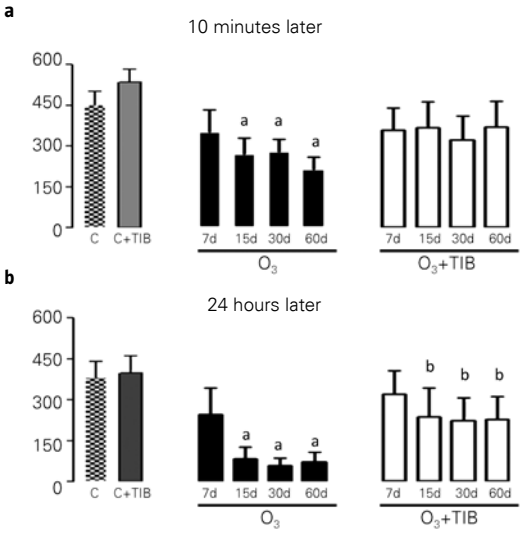
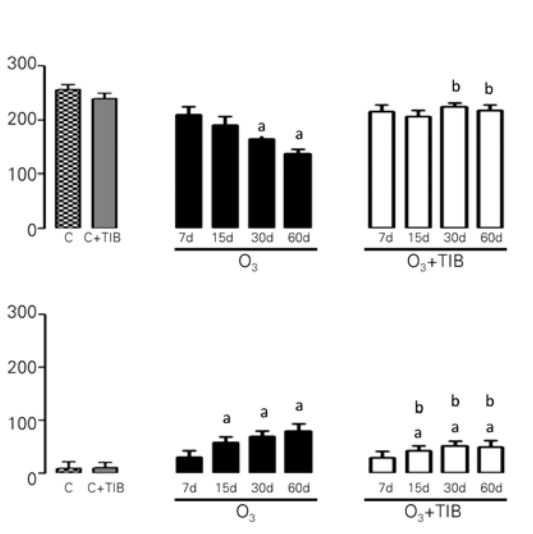


Figure 3. Effects of O_3 and O_3+TIB on exploratory and freezing behavior. Exploratory (a) and freezing (b) behaviors in mean (n) ± standard error (s) are depicted on the ordinate. The different treatments are depicted on the abscissa (n = 10 for each group). [a] $p < 0.05$ compared to C and C+TIB; [b] $p < 0.05$ 15d, 30d and 60d O_3+TIB compared to 15d, 30d and 60d O_3 respectively.



being statistically different to animals treated with air (C) (Figs. 4a, 4b, 4i) or air plus TIB (C+TIB) (Figs. 4c, 4d, 4i).

Discussion

Our team developed a well-established noninvasive OS induced by O_3 exposure. These OS damage can lead impaired brain plasticity, resulting in learning, memory and motor activity deficits [18,21], by increasing LPO levels [23].

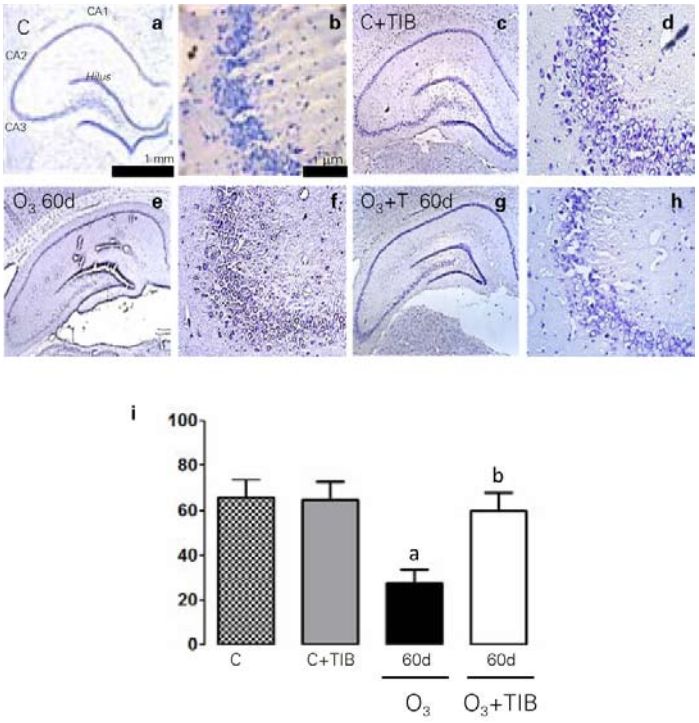
We observed that chronic inhaled exposure of O_3 increases 4-HNE and NT in the rat hippocampus after 15 days of exposure and that these levels are maintained until 60 days (Fig. 1a). These results are in agreement with ours previous works in which we reported that chronic exposure to O_3 increases LPO [23]. The brain is especially vulnerable to LPO by ROS because it consumes approximately one fifth of a human's oxygen intake, has a relative paucity of antioxidant systems and contains high concentrations of polyunsaturated fatty acids (PUFAs) [42]. 4-NHE is a highly reactive aldehyde generated by the exposure of polyunsaturated fatty acids to peroxide and ROS and is a biomarker for the occurrence and extent of OS in neurodegenerative diseases [43,44].

As discussed above, E_2 exerts antioxidant activity, donating hydrogen atoms from its phenolic hydroxyl structure to lipid peroxy radicals, ROS and other damaging radicals, which can inhibit LPO and block memory deterioration [23,26].

In the last few years, the CNS effects of TIB have been extensively researched [35-41,45-50]. Although the cognitive and neuroprotective aspects have not been fully elucidated, studies in animals and humans suggest that TIB could be helpful against damage induced by long-term OVX [35], menopause [46], leuprolide acetate administration [36] and aging [38]. TIB has been proposed as a neuroendocrine modulator [45] and as a neurotransmitter modulator [35], and it reduces the formation of paired helical filaments (PHF) by increasing the proportion of dephosphorylated Tau [41].

On our study, we observed that treatment with TIB maintains the levels of these markers of OS at values similar to those of the controls along the same time-course as O_3 treatment (Figs. 1a and 1b). These results are in agreement with previous reports in which the administration of TIB reduced the LPO induced by aging in rat hippocampus [38].

Figure 4. TIB partially reduced the CA3 pyramidal neuron loss induced by chronic O_3 exposure. Left: Nissl stained coronal sections of the dorsal hippocampus of rats subjected either to airstream free of O_3 exposure (C; a, b) or airstream free of O_3 exposure + 1 mg/kg of TIB for 60 days (C+TIB; c, d), or to O_3 exposure for 60 days (O_3 60d; e, f) or to O_3 exposure + 1 mg/kg of TIB for 60 days (O_3+T 60d; g, h), obtained at the last day of O_3 exposure. Down (i): number (mean ± standard error) of pyramidal neurons in the CA3 in three experimental groups. Scale bars: 1 mm (hippocampus), 100 µm (CA3). Tukey test: [a] $p < 0.05$ vs. C , C+TIB and O_3+TIB; [b] $p < 0.05$ vs. O_3 60d.



To our knowledge, this is the first published report of protein oxidation levels upon chronic treatment with TIB. We observed maintenance in the values of the oxidative and nitrosative stress biomarkers as normalized to the control rats' values, even with chronic exposure. Previous studies have reported that chronic administration of TIB reduces the LPO produced by OVX in the rat brain and in the blood of menopausal women. These results might be due to the property of TIB to donate hydrogens from its A ring with a resulting increased capacity of scavenging oxyradicals, and/or by stimulating direct and indirect mechanisms of antioxidants (e.g. tocopherol) within increased plasma concentrations of them as observed in previous references [38,50].

Our results indicate that TIB reduces the oxidation of hippocampal lipids and proteins in this model. However, the mechanisms through which TIB performs this action are unknown and deserve further investigation.

The PAT is a Pavlovian fear-motivated avoidance task useful for evaluating the effect of novel chemical entities on learning and memory as well as studying the mechanisms involved in cognition.

In our study, chronic O_3 exposure decreased the retention latency in short- and long-term memory. These results are in agreement with previous reports, which demonstrated that chronic O_3 exposure can lead to impaired brain plasticity and produce learning and memory deficits [18,21]. In contrast, we observed that pretreatment with TIB, retention latency remained at levels similar to the controls at the 10-minute time point (Fig. 2a). Despite the decrease in retention latency at 24 h in animals treated with TIB, the values are higher than those of animals treated with O_3 only (Fig. 2b). Similarly [38], have reported that E_2 valerate and TIB affect memory differently in females in a dose- and age-dependent manner. In a previous study, our group reported that TIB improved learning, modulating the hippocampal cholinergic and serotonergic systems [35]. All these results indicate that the chronic administration of TIB could be helpful against some types of damage that induce memory impairment.

In addition, we observed that chronic O_3 exposure decreases exploratory behaviors and increases freezing behaviors. These results are in agreement with previous reports of impairments in motor activity after different periods and concentrations of O_3 exposure [9,18,21]. In animals treated with TIB before exposure to O_3, exploratory behavior levels remained similar to those observed in the controls (Fig. 3a). Although there was also an increase in freezing behavior, this increase was statistically lower than in animals exposed to O_3 without TIB treatment (Fig. 3b).

A previous report indicated that O_3 inhalation produced impaired nigral cell morphology and a loss of dopamine neurons in (OVX) rats. This effect was counteracted after 60 days of 17β-E_2 treatment, when blood levels were highest [24]. Other study reported, that treatment with 17β-E_2 significantly attenuated both motor impairment and the lipid peroxidation process, and it restored the levels of antioxidants in a neurotoxicity model induced by ethanol [51]. All these results indicate that TIB attenuated the motor activity impairment induced by OS in the O_3 exposure model could be by its estrogen activity on the CNS [34,41].

In respect to the histological assessment we observed neuronal death induced by O_3 after 60 days of exposure. These results reinforces our previous

reports where we found O_3 induced neuronal death and loss of brain repair in the hippocampus of adult rats [1]. We also observed that TIB pretreatment reduces the number of neurons death by chronic O_3 exposure probably because of that antioxidant ability discussed before [38,50].

This reduce of neuronal death provides a possible explanation of why motor activity impairment was also diminished. The same effect observed on hippocampus might be happening in the substantia nigra with a lowering loss of dopaminergic neurons and restore of dopaminergic communication. Moreover, further studies on that region have to be performed to confirm such hypothesis.

References

1. Rivas-Arancibia S, Guevara-Guzmán R, López-Vidal Y, Rodríguez-Martínez E, Zanardo-Gomes M, Angoa-Pérez M, et al. Oxidative stress caused by ozone exposure induces loss of brain repair in the hippocampus of adult rats. Toxicol Sci 2010; 113: 187-97.
2. Creţu DI, Sovrea A, Ignat RM, Filip A, Bidian C, Creţu A. Morpho-pathological and physiological changes of the brain and liver after ozone exposure. Rom J Morphol Embryol 2010; 51: 701-6.
3. Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. J Toxicol 2011; 2011: 48707.
4. Kadiiska M, Basu S, Brot N, Cooper C, Saari Csallany A, Davies M, et al. Biomarkers of oxidative stress study V: ozone exposure of rats and its effect on lipids, proteins, and DNA in plasma and urine. Free Radic Biol Med 2013; 61C: 408-15.
5. Mustafa MG. Biochemical basis of ozone toxicity. Free Radic Biol Med 1990; 9: 245-65.
6. Cross CE, Reznick AZ, Packer L, Davis PA, Suzuki YJ, Halliwell B. Oxidative damage to human plasma proteins by ozone. Free Radic Res Commun 1992; 15: 347-52.
7. Sunil V, Vayas K, Massa C, Gow A, Laskin J, Laskin D. Ozone-induced injury and oxidative stress in bronchiolar epithelium are associated with altered pulmonary mechanics. Toxicol Sci 2013; 133: 309-19.
8. Groves A, Gow A, Massa C, Laskin J, Laskin D. Prolonged injury and altered lung function after ozone inhalation in mice with chronic lung inflammation. Am J Respir Cell Mol Biol 2012; 47: 776-83.
9. Dorado-Martínez C, Paredes-Carbajal C, Mascher D, Borgonio-Pérez G, Rivas-Arancibia S. Effects of different ozone doses on memory, motor activity and lipid peroxidation levels, in rats. Int J Neurosci 2001; 108: 149-61.
10. Martínez-Lazcano JG, Del Carmen Rubio M, Franco-Pérez J, Custodio V, Hernández-Cerón M, Livera C, et al. The effects of ozone exposure and associated injury mechanisms on the central nervous system. Rev Neurosci 2013; 24: 337-52.
11. Gackière F, Saliba L, Baude A, Bosler O, Strube C. Ozone inhalation activates stress-responsive regions of the CNS. J Neurochem 2011; 117: 961-72.
12. Zhong Z, Zeng T, Xie K, Zhang C, Chen J, Bi Y, et al. Elevation of 4-hydroxynonenal and malondialdehyde modified protein levels in cerebral cortex with cognitive dysfunction in rats exposed to 1-bromopropane. Toxicology 2013; 306: 16-23.
13. Rivas-Arancibia S, Vázquez-Sandoval R, González-Kladiano D, Schneider-Rivas S, Lechuga-Guerrero A. Effects of ozone exposure in rats on memory and levels of brain and pulmonary superoxide dismutase. Environ Res 1998; 76: 33-9.
14. Ávila-Costa MR, Colín-Barenque L, Fortoul TI, Machado-Salas JP, Espinosa-Villanueva J, Rugerio-Vargas C, et al. Motor impairments in an oxidative stress model and its correlation with cytological changes on rat striatum and prefrontal cortex. Int J Neurosci 2001; 108: 193-200.
15. Ávila-Costa MR, Colín-Barenque L, Fortoul TI, Machado-Salas P, Espinosa-Villanueva J, Rugerio-Vargas C, et al. Memory deterioration in an oxidative stress model and its correlation with cytological changes on rat hippocampus CA1. Neurosci Lett 1999; 270: 107-9.
16. Colín-Barenque L, Ávila-Costa MR, Fortoul T, Rugerio-Vargas C, Machado-Salas JP, Espinosa-Villanueva J, et al. Morphologic alteration of the olfactory bulb after acute ozone exposure in rats. Neurosci Lett 1999; 274: 1-4.
17. Pereyra-Muñoz N, Rugerio-Vargas C, Angoa-Pérez M, Borgonio-Pérez G, Rivas-Arancibia S. Oxidative damage in substantia nigra and striatum of rats chronically exposed to ozone. J Chem Neuroanat 2006; 31: 114-23.
18. Rivas-Arancibia S. Effect of acute ozone exposure on locomotor behavior and striatal function. Pharmacol Biochem Behav 2003; 74: 891-900.
19. Kovacic P, Somanathan R. Redox processes in neurodegenerative disease involving reactive oxygen species. Curr Neuropharmacol 2012; 10: 289-302.
20. Jones B. Neurodegenerative disease: Oxidative stress in cells near amyloid plaques linked to neuronal death. Nat Rev Neurol 2013; 9: 300.
21. Rivas-Arancibia S, Dorado-Martínez C, Borgonio-Pérez G, Hiriart-Urdanivia M, Verdugo-Diaz L, Durán-Vázquez A, et al. Effects of taurine on ozone-induced memory deficits and lipid peroxidation levels in brains of young, mature, and old rats. Environ Res 2000; 82: 7-17.
22. Guerrero AL, Dorado-Martínez C, Rodriguez A, Pedroza-Ríos K, Borgonio-Pérez G, Rivas-Arancibia S. Effects of vitamin E on ozone-induced memory deficits and lipid peroxidation in rats. NeuroReport 1999; 10: 1689-92.
23. Guevara-Guzmán R, Arriaga V, Kendrick KM, Bernal C, Vega X, Mercado-Gómez OF, et al. Estradiol prevents ozone-induced increases in brain lipid peroxidation and impaired social recognition memory in female rats. Neuroscience 2009; 159: 940-50.
24. Angoa-Pérez M, Jiang H, Rodríguez A, Lemini C, Levine R, Rivas-Arancibia S. Estrogen counteracts ozone-induced oxidative stress and nigral neuronal death. Neuroreport 2006; 17: 629-33.
25. Behl C, Moosmann B, Manthey D, Heck S. The female sex hormone oestrogen as neuroprotectant: activities at various levels. Novartis Found Symp 2000; 230: 221-34.
26. Bednarek-Tupikowska G. Antioxidant properties of estrogens. Ginekol Pol 2002; 73: 61-7.
27. Conner P, Christow A, Kersemaekers W. A comparative study on breast cell proliferation during HRT effects of tibolone and continuous combined estrogen progestogen treatment. Climacteric 2004; 7: 50-8.
28. Kenemans P. Postmenopausal hormone therapy and breast cancer: what is the problem? Maturitas. 2005; 15: 75-82.
29. Reed MJ, Kloosterboer HJ. Tibolone: a selective tissue estrogenic activity regulator (STEAR). Maturitas 2004; 48: S4-6.
30. Campisi R, Marengo FD. Cardiovascular effects of tibolone: a selective tissue estrogenic activity regulator. Cardiovasc Drug Rev 2007; 25: 132-45.
31. Kloosterboer HJ. Tibolone: a steroid with a tissue-specific mode of action. J Steroid Biochem Mol Biol 2001; 76: 231-8.
32. Verheul HAM, Van Iersel MLPS, Delbressine LPC, Kloosterboer HJ. Selective tissue distribution of tibolone metabolites in mature ovariectomized female cynomolgus monkeys after multiple doses of tibolone. Drug Metab Dispos 2007; 35: 1105-11.
33. Verheul HAM, Timmer CJ, Van Iersel MLPS, Delbressine LPC, Kloosterboer HJ. Pharmacokinetic parameters of tibolone and metabolites in plasma, urine, feces, and bile from ovariectomized cynomolgus monkeys after a single dose or multiple doses of tibolone. Drug Metab Dispos 2007; 35: 1112-8.
34. Verheul HAM, Kloosterboer HJ. Metabolism of exogenous sex steroids and effect on brain functions with a focus on tibolone. J Steroid Biochem Mol Biol 2006; 102: 195-204.

35. Espinosa-Raya J, Neri-Gómez T, Orozco-Suárez S, Campos MG, Guerra-Araiza C. Chronic administration of tibolone modulates anxiety-like behavior and enhances cognitive performance in ovariectomized rats. Horm Behav 2012; 61: 76-83.
36. Palomba S, Orio F, Falbo A, Oppedisano R, Tolino A, Zullo F. Tibolone reverses the cognitive effects caused by leuprolide acetate administration, improving mood and quality of life in patients with symptomatic uterine leiomyomas. Fertil Steril 2008; 90: 165-73.
37. Qiu J, Bosch MA, Rønnekleiv OK, Kloosterboer HJ, Kelly MJ. Tibolone rapidly attenuates the GABAB hypothalamic neurones response in hypothalamic neurones. J Neuroendocrinol 2009; 20: 1310-8.
38. Aguiar R, Dickel O, Cunha R. Estradiol valerate and tibolone: effects upon brain oxidative stress and blood biochemistry during aging in female rats. Biogerontology 2008; 9: 285-98.
39. Aguiar R, Dickel O, Cunha R. Estradiol valerate and tibolone: effects on memory. Pharmacol Biochem Behav 2006; 85: 689-96.
40. Ceylan U, Akhan S, Bastu E, Gungor-Ugurlucan, F Iyibozkurt A, Topuz S. Comparison of tibolone and 17beta-estradiol administration on the expression of zonula occludens-1, occludin, glial fibrillary acidic protein and c-fos levels in the brain cortex and hippocampus of female rats. Neuro Endocrinol Lett 2012; 33: 505-10.
41. Pinto-Almazán R, Calzada-Mendoza CC, Campos-Lara MG, Guerra-Araiza C. Effect of chronic administration of estradiol, progesterone, and tibolone on the expression and phosphorylation of glycogen synthase kinase-3β and the microtubule-associated protein tau in the hippocampus and cerebellum of female rat. J Neurosci Res 2012; 90: 878-86.
42. Lovell MA, Markesbery WR. Oxidative damage in mild cognitive impairment and early Alzheimer's disease. J Neurosci Res 2007; 85: 3036-40.
43. Uchida K. 4-hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog Lipid Res 2003; 42: 318-43.
44. Perluigi M, Coccia R, Butterfield D. 4-hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neurodegenerative diseases: a toxic combination illuminated by redox proteomics studies. Antioxid Redox Signal 2012; 17: 1590-609.
45. Gibbs RB, Edwards D, Lazar N, Nelson D, Talameh J. Effects of long-term hormone treatment and of tibolone on monoamines and monoamine metabolites in the brains of ovariectomised, cynomologous monkeys. J Neuroendocrinol 2006; 18: 643-54.
46. Celik O, Erdem G, Hascalik S. Resonance spectroscopic comparison of the effects of resveratrol (3, 4', 5-trihydroxy stilbene) to conjugated equine estrogen, tibolone and raloxifene on ovariectomized. Eur J Obstet Gynecol Reprod Biol 2005; 120: 73-9.
47. Genazzani AR, Pluchino N, Bernardi F, Centofanti M, Luisi M. Beneficial effect of tibolone on mood, cognition, well-being, and sexuality in menopausal women. Neuropsychiatr Dis Treat 2006; 2: 299-307.
48. Genazzani A, Bernardi F, Pluchino N. Effect of tibolone administration on central and peripheral levels of allo-pregnanolone and -endorphin in female rats. Menopause 2006; 13: 57-64.
49. Fluck E, File SE, Rymer J. Cognitive effects of 10 years of hormone-replacement therapy with tibolone. J Clin Psychopharmacol 2002; 22: 62-7.
50. Vural P, Akgül C, Canbaz M. Effects of menopause and tibolone on antioxidants in postmenopausal women. Ann Clin Biochem 2005; 42: 220-3.
51. Ramezani A, Goudarzi I, Lashkarbolouki T, Ghorbanian M, Salmani M, Abrari K. Neuroprotective effects of the 17β-estradiol against ethanol-induced neurotoxicity and oxidative stress in the developing male rat cerebellum: biochemical, histological and behavioral changes. Pharmacol Biochem Behav 2011; 100: 144-5.

Efecto neuroprotector de la tibolona contra el estrés oxidativo inducido por la exposición a ozono

Introducción. El estrés oxidativo aumenta la lipoperoxidación, produce déficits de memoria y de actividad motora así como una neurodegeneración progresiva en el sistema nervioso central. La tibolona es un tratamiento para los síntomas de la menopausia que disminuye los niveles de peroxidación de lípidos y mejora la memoria y el aprendizaje.

Objetivo. Estudiar el efecto de la tibolona sobre la peroxidación de lípidos, los déficits de memoria y motor en el modelo de estrés oxidativo inducido por la exposición crónica al ozono.

Materiales y métodos. Se dividieron aleatoriamente 100 ratas adultas Wistar en 10 grupos: control (C), que recibió aire durante 60 días; (C + tibolona), aire más 1 mg/kg de tibolona durante 60 días; los grupos 3-6, ozono durante 7, 15, 30, y 60 días; y los grupos 7-10, 1 mg/kg de tibolona durante 7, 15, 30 y 60 días previo a la exposición al ozono. Se realizaron pruebas de memoria y motoras y se determinó el contenido del 4-hidroxinonenal y de la nitrotirosina por *Western blot*, así como la muerte neuronal en el hipocampo.

Resultados. La administración de tibolona disminuyó el contenido de lípidos peroxidados, la oxidación de proteínas y la muerte neuronal en el hipocampo; mejoró la memoria y previno las alteraciones motoras en los animales expuestos a ozono.

Conclusión. Nuestros resultados indican un posible papel neuroprotector de la tibolona como un tratamiento útil para prevenir la neurodegeneración inducida por el estrés oxidativo.

Palabras clave. 4-hidroxinonenal. Aprendizaje. Estrés oxidativo. Hipocampo. Lipoperoxidación. Memoria. Ozono. Neurodegeneración. Nitrotirosina. Tibolona.