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**RESEARCH ARTICLE**

# Lipid peroxidation, mitochondrial dysfunction and neurochemical and behavioural deficits in different neurotoxic models: Protective role of S-allylcysteine

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**Abstract**

Experimental evidence on the protective properties of S-allylcysteine (SAC) was collected from three models exerting striatal toxicity. In the first model, SAC (120 mg $kg^{-1} \times 5$) prevented lipoperoxidation (LP) and mitochondrial dysfunction (MD) in synaptosomal fractions from 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridinium-treated mice (30 mg $kg^{-1}$), but without complete restoration of dopamine levels. In the second model, SAC (300 mg $kg^{-1} \times 3$), prevented LP and MD in synaptosomes from rats infused with 6-hydroxydopamine (8 $\mu g$ $\mu l^{-1}$) into the substantia nigra pars compacta, but again, without total reversion of depleted dopamine levels. In the third model, SAC (100 mg $kg^{-1} \times 1$) prevented MD in synaptosomes from rats injected with 3-nitropropionic acid (10 mg $kg^{-1}$), but in contrast to the other models, it failed to prevent LP. SAC also prevented the aberrant motor activity patterns evoked by the three toxins. Altogether, the results suggest that the antioxidant properties of SAC are responsible for partial or total preservation of neurochemical, biochemical and behavioural markers, indicating that pro-oxidant reactions underlie the neurotoxicity in these models.

**Keywords:** *S-Allylcysteine, antioxidant defense, 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 3-nitropropionic acid, neurotoxicity*

## Introduction

Strengthening antioxidant defense is gaining the attention of several research groups worldwide, since it represents an accurate strategy to ameliorate and/or prevent toxic cascades further leading to neurodegeneration. The basic principles of these molecules are: (1)

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to reinforce—or even substitute—the endogenous antioxidant capacity to better resist toxic insults and preserve tissue integrity; and (2) to activate endogenous pathways at both biochemical and molecular levels to initiate lasting protective signals. Therefore, either through experimental models or clinical trials, the search for antioxidants exhibiting a broad spectrum of protective actions continues as they are promising tools for therapeutic purposes.

S-allylcysteine (SAC) is the most abundant organosulphurated molecule from the aged garlic extract. Among its many antioxidant properties described in the literature, SAC is known to scavenge superoxide ($O_2^{\cdot -}$) radical [1–3] and to neutralize hydrogen peroxide ($H_2O_2$) [2,4], thereby preventing the $H_2O_2$-induced endothelial cell damage and lipid peroxidation (LP) [4,5], as well as low-density lipoprotein oxidation [6]. Regarding its protective actions, SAC reduces oedema formation in the ischemic rat brain, probably involving mechanisms linked to the inhibition of LP [7,8], while alleviating the morphological alterations in heart and liver of mice exposed to the anti-cancer drug doxorubicin [9]. SAC also attenuates gentamicin-induced nephrotoxicity [2] and produces neuroprotective effects on the amyloid-beta peptide-induced oxidative damage and learning deficits [10], apoptosis [11], as well as neurotrophic actions on cultured rat hippocampal neurons [12]. Furthermore, SAC inhibits the $H_2O_2$-induced activation of nuclear factor kappa B [13] and regulates nitric oxide (NO) production by inhibiting the NO synthase (NOS) expression in macrophages, while increasing NO in endothelial cells, where it contributes to anti-inflammatory responses [1]. Additional positive actions of SAC include its protective and antioxidant effects on the glutamate analogue quinolinic acid [14], the neurotoxic mitochondrial inhibitor 3-nitropropionic acid [15] and the combination of both toxins in a synergic model [16]. Given all these antioxidant and protective actions, SAC is more than merely a promising agent to ameliorate toxicity in animal models and human neurodegenerative disorders, since it is also a valuable experimental tool to elucidate toxic mechanisms in different paradigms.

On the other hand, neurodegenerative events are known to be a major cause of disabled disorders. Neurodegeneration is the result of toxic processes acting in a concerted manner during the long-term to produce severe and specific patterns of brain cell damage [17,18]. In general terms, human disorders presenting neurodegeneration as a hallmark—such as Parkinson's and Huntington's diseases—have been shown to involve oxidative damage via reactive oxygen (ROS) and nitrogen (RNS) species formation, as a triggering factor for deadly cascades [18–20]. Therefore, it is desirable to adopt approaches toward the characterization of this event by means of experimental models resembling the major neurochemical features of human pathologies.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropiridinium (MPTP) administration is probably the most widely used Parkinsonian model. MPTP is a mitochondrial complex I inhibitor discovered in accidentally exposed humans [21]. This molecule easily crosses the blood–brain barrier and becomes toxic through its transformation to MPP+ by the enzyme monoamine oxidase B (MAO B). The dopamine (DA) transporter system then transports MPP+ into DA neurons to eliminate them [22]. As a result, striatal DA levels are significantly depleted. Another Parkinsonian model is associated to 6-hydroxydopamine (6-OHDA), a well known catecholaminergic neurotoxin that induces neuronal cell death and DA depletion in laboratory animals via oxidative stress after its uptake by the catecholamine transport system of DA [22], thereby producing a complex toxic pattern in animals—also known as hemi-Parkinsonism—that involves tyrosine hydroxylase loss in the substantia nigra as well as motor skill deficits. 3-Nitropropionic acid (3-NP), a mycotoxin produced by *Arthrinium spp*, is responsible of toxic events in the Central Nervous System by producing an irreversible inhibition of succinate dehydrogenase (Complex II) at the mitochondrial respiratory chain in a mechanism directly involving a disrupted energy metabolism and further recruiting excitotoxicity and oxidative stress [23]. This evidence has served to propose 3-NP as responsible for producing an experimental model of Huntington's disease in rats [24].

All the toxins described above share one common characteristic: oxidative stress as an important part of their toxic mechanisms. Hence, given the evidence described above, it can be assumed that SAC, an agent exhibiting a wide range of antioxidant and protective properties, may have potential therapeutic value at experimental level in these toxic models. Therefore, the aim of this work was to investigate whether targeting the redox metabolism in nerve tissue through a single antioxidant strategy may result in neuroprotection in these paradigms that exhibit different toxic mechanisms. For this purpose, *in vivo* experiments devoted to the characterization of oxidative stress, mitochondrial dysfunction, neurochemical alterations and aberrant behaviour were performed in isolated brain synaptosomal P2 fractions and brain tissue from rodents exposed to MPTP, 6-OHDA or 3-NP. In consideration of the many positive effects that SAC have exerted in other paradigms, our experimental strategy consisted of the administration of this agent as treatment or pre-treatment to animals exposed to the different toxins. Our results revealed a strong antioxidant capacity associated to SAC, accompanied by a partial but still significant protective action on neurochemical and behavioural indexes from the tested models.

## Materials and methods

### *Reagents*

MPTP hydrochloride, 6-OHDA, 3-NP, malondialdehyde (MDA), sodium octyl sulphate, dopamine, D-amphetamine and thiazolyl blue tetrazolium bromide (MTT) were all obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were obtained from other known commercial sources. Deionized water from a Direct-Q3 UV system (Millipore, MA) was used for preparation of solutions.

### *Animals*

One hundred adult male C57 Black mice (25–30 g) and 96 adult male Wistar bred-in-house rats (270–320 g) were used throughout the study. For all experimental purposes, animals were housed five-per cage in polycarbonate box cages and provided with a standard commercial diet (Laboratory rodent diet 5001, PMI Feeds Inc., Richmond, IN) and water *ad libitum*. Housing room was maintained under constant conditions of temperature ($25 \pm 3^{\circ}$C), humidity ($50 \pm 10\%$) and lighting (12 h light/dark cycles, light on at 7:00 am). All procedures with animals were strictly carried out according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and the local guidelines on the ethical use of animals from the Health Ministry of Mexico. During the experiments, all efforts were made to minimize animal suffering.

### *Drug administration schemes*

The design for both toxins and SAC administrations can be divided into three parts, each corresponding to a different toxic model:

- *Protocol 1*: Mice (10 per group) were administered with MPTP (30 mg $kg^{-1}$, i.p.) or vehicle (PBS, pH 7.4) for 5 consecutive days, following a protocol previously reported [25]. SAC (30, 60 or 120 mg $kg^{-1}$, i.p.) or vehicle (PBS, pH 7.4) were given to animals 30 min after each MPTP administration on the same consecutive 5 days. Seven days after the last drug administration, animals were employed for the behavioural test and/or killed by decapitation for the biochemical assays.
- *Protocol 2*: Rats (10 per group) were anaesthetized with sodium pentobarbital (40 mg $kg^{-1}$, i.p.) and placed onto a Stoelting stereotaxic frame for brain surgery. Animals received a single unilateral injection of 6-OHDA (8 μg $\mu l^{-1}$, prepared in ascorbate and administered for 5 min) or vehicle into the Substantia nigra pars compacta, located at the following stereotaxic coordinates: AP −4.9, L +2.1, V −6.5 [26]. SAC (300 mg $kg^{-1}$, i.p.)—or vehicle—was given three times to animals (24 and 2 h before and 24 h after surgery). At post-lesion day 15, rats were employed for the behavioural test and immediately killed by decapitation for the biochemical assays.
- *Protocol 3*: Rats (six per group) were injected either with a single dose of 3-NP (10 mg $kg^{-1}$, i.p.) for biochemical assays or for 7 days (every 2 days at the same dose) for behavioural tests. This model served as an experimental reference since we have described protective actions of SAC on previous studies also using 3-NP. Control rats received vehicle. SAC (100 mg $kg^{-1}$, i.p.) was given 30 min before 3-NP administration, either in a single dose (biochemical assays) or every 2$^{nd}$ day (for the behavioural test). For analytical purposes, animals were decapitated 24 h (or earlier in other groups) after the single administration of 3-NP. Those animals employed for behavioural purposes were kept alive for 7 days. We decided to explore some biochemical markers at 2, 6 and mostly 24 h after the single administration of 3-NP and SAC, based on the fact that behavioural tests revealed no further protection of SAC at longer times.

In these three protocols, the doses of SAC employed were fixed on the basis of previous studies of our group where we tested SAC at increasing doses and concentrations in different toxic models [10,14,15,27]. Moreover, the rationale for selecting different schemes of SAC dosage (always around those previously reported by us) was strictly according to the nature of each protocol itself (whether the exposure to the toxin in turn was acute or subchronic).

### *Preparation of brain crude synaptosomal P2 fractions*

Crude synaptosomal fractions were obtained either from the striata of both hemispheres from MPTP-treated mice and 3-NP-treated rats or from the lesioned striata from the 6-OHDA-treated animals. Synaptosomes were prepared according to a previous report [28] and used for the assessment of peroxidative damage and mitochondrial dysfunction. Briefly, the animals were killed by decapitation and their brains rapidly removed and homogenized in 10 volumes (w/v) of 0.32 M sucrose. Homogenates were centrifuged at $1{,}073 \times g$ for 10 min and the resulting supernatants recentrifuged at $17\,172 \times g$ for 15 min. Pellets were then gently resuspended in buffer-HEPES solution (0.1 M NaCl, 0.01 M $NaH_2PO_4$, 0.05 M $NaHCO_3$, 0.01 M $CaCl_2$, 0.006 M Glucose and 0.01 M HEPES; pH 7.4) and freshly used for the experiments.

*Assay of thiobarbituric acid-reactive substances (TBA-RS) formation*

Measurement of LP by TBA-RS is an accurate method to characterize oxidative damage to membrane lipids. LP was detected in brain synaptosomal fractions by measuring the formation of TBA-RS, according to a previous report [27]. Two hundred and fifty microlitre aliquots containing the synaptosomal fractions were added to 500 µl of the TBA reagent (0.75 g TBA + 15 g trichloroacetic acid + 2.54 ml HCl) and incubated at 94°C for 30 min in a boiling water bath. A pink chromophore was produced in samples in direct relation to the amount of peroxidized products. Samples were then kept on ice for 5 min and centrifuged at 3000 × g for 15 min. The optical density from the supernatants was measured in a ThermoSpectronic Genesys 8 Spectrometer at 532 nm. Final amounts of TBA-RS—mostly malondialdehyde (MDA)—were calculated by interpolation of values in a constructed MDA standard curve and corrected by the content of protein per sample [29]. Results were expressed as nmoles of MDA per mg of protein.

*Estimation of mitochondrial function by MTT reduction*

MTT reduction was measured in brain synaptosomal P2 fractions as an index of the functional status of the respiratory chain and mitochondrial function, according to a method previously described [28]. Briefly, 600 µl-aliquots containing the synaptosomes were added to 8 µl of MTT (5 mg $ml^{-1}$) and incubated at 37°C for 60 min. Samples were then centrifuged at 15 300 × g and pellets were resuspended in 1 ml of isopropanol for 1 h. A second step of centrifugation was performed at 1700 × g for 3 min. Quantification of formazan was estimated in supernatants by optical density in a ThermoSpectronic Genesys 8 Spectrometer at 570 nm. Results were expressed as the percentage of MTT reduction with respect to control values.

*Measurement of striatal DA content*

The tissue content of DA was analysed by high resolution liquid chromatography with electrochemical detection as previously described [25], in striatal samples obtained from MPTP-treated mice or 6-OHDA-treated rats. Briefly, tissue samples were obtained by dissection and immediately sonicated on ice in 300 µl of perchloric acid 0.4 N and 0.1% (w/v) sodium metabisulphite, followed by a 10 min-centrifugation step at 4000 × g at 4°C. Then, supernatants were kept frozen at −70°C until used for chromatographic analysis. A Perkin-Elmer LC-4C liquid chromatograph with a BAS CC-5 electrochemical detector was used. Peaks were integrated with a Perkin-Elmer Turbochrom Navigator 4.1 data station. Calibration curves were constructed by injecting known concentrations of the standard (prepared in perchloric acid-metabisulphite solution) into the 20 µl loop of the chromatograph. DA concentrations were obtained by interpolation on its respective standard curve. An Alltech Adsorbosphere Catecholamine (100 × 4.6 mm) column with 3 µm of particle size was used. Mobile phase consisted of an aqueous phosphate buffer solution (0.1 M, pH 3.1) containing 0.9 mM sodium octyl sulphate, 0.1 mM EDTA and 15% (v/v) of methanol. Flow rate was 1.4 ml $min^{-1}$. Potential was fixed at 0.8 V against an Ag/AgCl reference electrode. All samples were analysed in duplicate.

*Behavioural tests*

Behavioural performances were evaluated by three different tests, depending on the experimental model employed:

a. For those animals administered with MPTP, the motility patterns were estimated according to a previous report [30] and recorded in a Versamax Animal Activity Monitor and Analyser (AccuScan Instruments, Inc, Columbus, Ohio) for 1 h, 7 days after the last MPTP administration and immediately before their brains were used for biochemical analysis. Although the collected criteria from the equipment included horizontal and vertical activity, total number of movements and total distance walked, here we present only the results of the last parameter since it was the only one showing changes. Results were represented as the total distance walked (cm) in 60 min for each group.
b. Those animals intracerebrally infused with 6-OHDA were challenged with amphetamine (5 mg $kg^{-1}$, s.c.) 15 days post-lesion and separated into individual polycarbonate box cages for the observation of their motor asymmetry. The method is based on the neurochemical imbalance produced when unilateral toxin injections are practiced in rats [30]. Five minutes later, the number of ipsilateral rotations to the lesioned side was recorded every 5 min for 80 min. Each rotation was defined as a complete turn (360°). Results were expressed as the total number of turns in 80 min.
c. For those rats systemically infused with 3-NP, their motility patterns were also estimated according to the method described for MPTP-treated mice. They were recorded in a Versamax Animal Activity Monitor and Analyser (AccuScan Instruments, Inc, Columbus, Ohio) every 2nd day for 1 h, at different times after they received 3-NP. In the first day of treatment, the behavioural examination started 2 h after the first 3-NP infusion and was followed for 6, 12, 18 and 24 h. On the 3rd, 5th and 7th days, the animals received again the treatments

and were monitored in regard to their kinetic patterns. Once again, although the collected criteria from the equipment included horizontal and vertical activity, total number of movements and total distance walked, here we present only the results of the last parameter since it was the only one showing significant changes. Results were represented as the total distance walked or travelled (cm) in 60 min for each group.

*Statistical analysis*

All data are shown as mean values ± SEM. The results were analysed by one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test, using the software Prism 3.02 (GraphPad, San Diego, CA). Values of $p < 0.05$ were considered as statistically significant.

## Results

*SAC prevents the oxidative damage and reduces neurochemical and behavioural alterations induced by MPTP*

The effects of SAC on different markers of MPTP-induced toxicity in mice are shown in Figure 1. As a current index of oxidative damage, LP was assessed in synaptosomal fractions obtained from striatal tissue of MPTP-treated animals by the TBA assay (Figure 1A). LP induced by MPTP was increased by 125% above the Control, whereas SAC + MPTP-treated animals exhibited moderate or even absent oxidative damage at the two doses tested, when compared with the control group (25% and 3% at 60 and 120 mg $kg^{-1}$, respectively). Mice treated with SAC alone did not present altered LP levels.

A similar scenario occurred when MTT reduction was measured in synaptosomal fractions from striatal tissue of MPTP-treated animals as another index of oxidative damage and current marker of compromised mitochondrial function (Figure 1B). Animals receiving MPTP alone showed a significant decrease in MTT reduction capacity (47% below baseline). In contrast, animals receiving SAC (60 and 120 mg $kg^{-1}$) + MPTP presented a dose-dependent preservation of their reductive capacity (22% and 2% below the baseline, respectively). Once again, SAC alone had no effect on MTT reduction.

Striatal DA content, the neurochemical toxic marker in MPTP-treated mice (Figure 1C), was considerably depleted by the toxin (68% below Control) and partially recovered in a dose-dependent manner by SAC (60%, 53% and 34% below baseline at 30, 60 and 120 mg $kg^{-1}$, respectively). SAC alone given to mice had no effect on DA levels.

The total distance that mice walked in a box field was considered as an index of motor activity (Figure 1D). In MPTP-treated animals, this index decreased in a non-significant manner (21% below Control), suggesting a tendency to hypokinesia. SAC (60 mg $kg^{-1}$) completely prevented this trend in MPTP-treated animals (3% above Control) and when given alone had no effect on motility.

Typically known as the most employed model of Parkinsonism, the MPTP model has been explored in detail, revealing that oxidative stress and energy depletion are accounting for its toxic pattern and selective alterations of gene expression [31,32]. As the molecule is converted into the more toxic metabolite MPP+, the later is hypothesized to be responsible of the alterations in nerve tissue, also comprising major alterations in transcription factors [33] and active roles of apoptotic pathways [34], calpains [35], NADPH oxidase [36] and cyclooxygenase-2 [37], among several other signalling and effector molecules. Thus, it can be assumed that part of the toxic cascades elicited by MPTP might be silenced or at least moderated by the many positive actions of SAC and this can be suggested as other antioxidants tested in the same model, such as L-carnitine [34], metallothionein [38] and *Spirulina maxima* [39] have recently shown alleviative effects when tested against the deleterious actions of this toxin. Of course, whether SAC is acting by blocking or attenuating these mechanisms to ameliorate MPTP toxicity is a current subject of investigation of our group. In the meantime, derived from our results it seems clear that targeting oxidative stress by SAC is a relevant step for the partial preservation of major functional markers of MPTP toxicity, such as the DA levels and motor activity.

*SAC blocks the oxidative damage and ameliorates neurochemical and behavioural alterations induced by 6-OHDA*

Figure 2 summarizes the effects of SAC on different toxic markers after 6-OHDA treatment in rats. LP induced by 6-OHDA in striatal synaptosomes was significantly increased by 132% when compared with Sham animals (Figure 2A) and SAC completely prevented this effect in 6-OHDA-treated animals (3% above Sham). SAC alone exerted no effect on basal LP.

Although the mitochondrial reductive capacity was reduced by 23% in 6-OHDA-treated rats when compared with Sham (Figure 2B), this effect was not statistically significant. Nonetheless, this tendency was completely prevented by SAC in rats exposed to the toxin (2% below baseline). SAC alone did not modify MTT reduction.

The striatal content of DA was dramatically depleted by 6-OHDA (88% below Sham) (Figure 2C). In contrast, those animals pre-treated with SAC and exposed to the toxin exhibited a partial prevention of DA loss (only 53% of total neurotransmitter

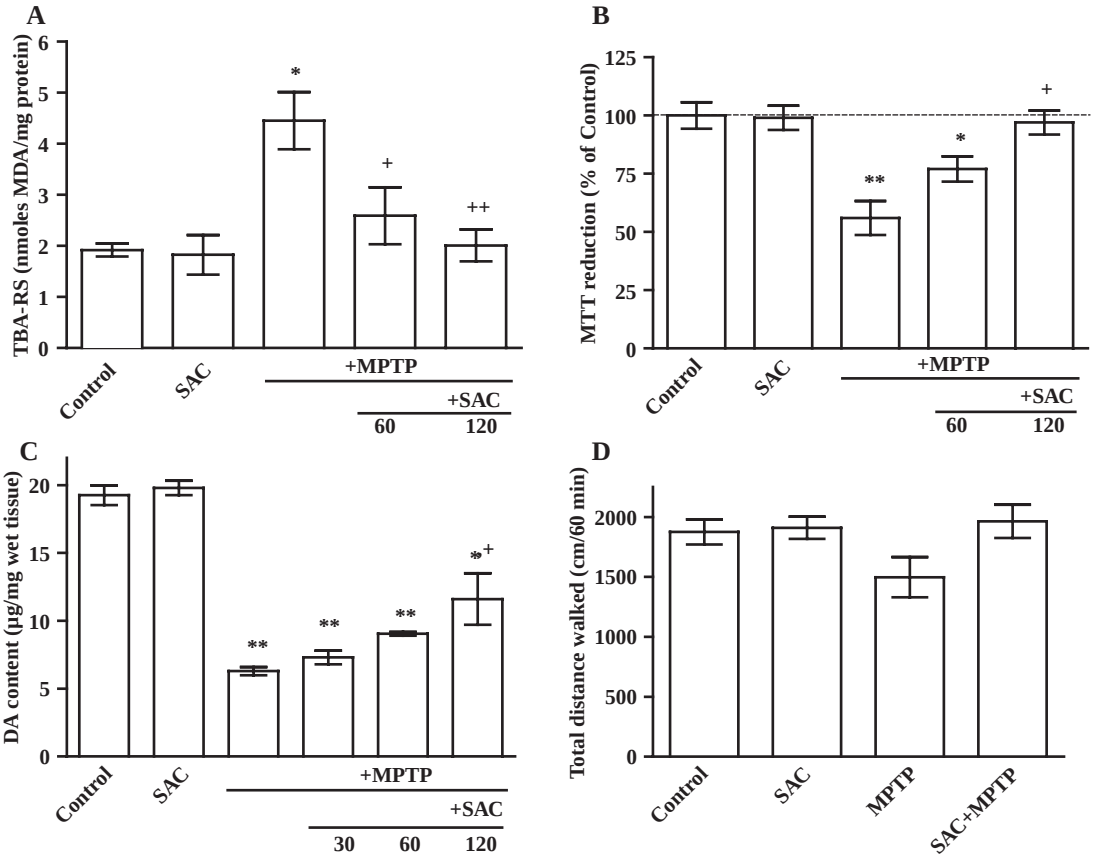


Figure 1. Effect of S-allylcysteine (SAC) on different markers of 1-methyl-4 phenyl-1,2,3,6-tetrahydropiridinium (MPTP)-induced striatal toxicity. SAC (30, 60 or 120 mg $kg^{-1}$, i.p.) was administered to mice for 5 consecutive days, 30 min before the administration of MPTP (30 mg $kg^{-1}$, i.p.). In (A) SAC prevented the MPTP-induced lipoperoxidation in mice synaptosomal fractions in a concentration-dependent manner; (B) SAC ameliorated the MPTP-induced disrupted mitochondrial reductive capacity in mice synaptosomal fractions in a concentration-dependent manner; (C) SAC attenuated the MPTP-induced dopamine depletion in mice striatal tissue in a concentration-dependent manner; (D) SAC reduced the MPTP-induced low motility pattern in mice. All markers were evaluated 7 days after the last MPTP administration. For all graphics, mean values ± SEM of 9–10 experiments per group are presented. * $p < 0.05$ and ** $p < 0.01$, statistically different of Control; + $p < 0.05$ and ++ $p < 0.01$, different of MPTP alone; one-way ANOVA followed by Tukey's test.

depletion). The baseline of DA levels remained unchanged when the animals received SAC alone.

Circling behaviour evoked by 6-OHDA augmented more than 150-fold the number of rotations observed in Sham rats (Figure 2D), whereas SAC-pretreated 6-OHDA-lesioned animals almost completely returned the rotations down to basal levels (14-fold of Sham). SAC alone produced a pattern of rotations similar to Sham.

The hemi-Parkinsonian model induced by 6-OHDA—typically produced by ROS formation [40] and lately described to involve oxidative modification and altered expression of thioredoxin-dependent peroxidases [41]—has been shown to be positively sensitive to the use of antioxidants, such as astaxanthin [42], melatonin and its modulation of tyrosine hydroxylase expression [43], green tea polyphenols and the inhibition of ROS-nitric oxide pathway [44], salvianolic acid B and its prevention of 6-OHDA-induced apoptotic activity [45] and protection by flavonoids derived from plant extracts [46]. Similarly to the MPTP model, SAC might be acting not only as a free radical scavenger, but probably also by modulating the metabolic and transcription pathways accounting for protection of neuronal dopaminergic cells. Again, this should be mechanistically characterized in further studies.

### *SAC failed to decrease the 3-NP-induced peroxidative damage, but prevents mitochondrial dysfunction and ameliorates the associated behavioural deficit*

Figure 3 shows the effects of SAC on kinetic and biochemical markers of 3-NP-induced neurotoxicity in rats. The motor activity produced by a daily administration of 3-NP remained unchanged for the first 18 h (Figure 3A), but a hypokinetic pattern started at 24 h and lasted up to 7 days (52–60% below Control, respectively). Interestingly, SAC prevented the affected motility of 3-NP-injected animals only at 24 h (6% above Control), but failed to improve motor activity at longer times tested. No effect of SAC alone was observed.

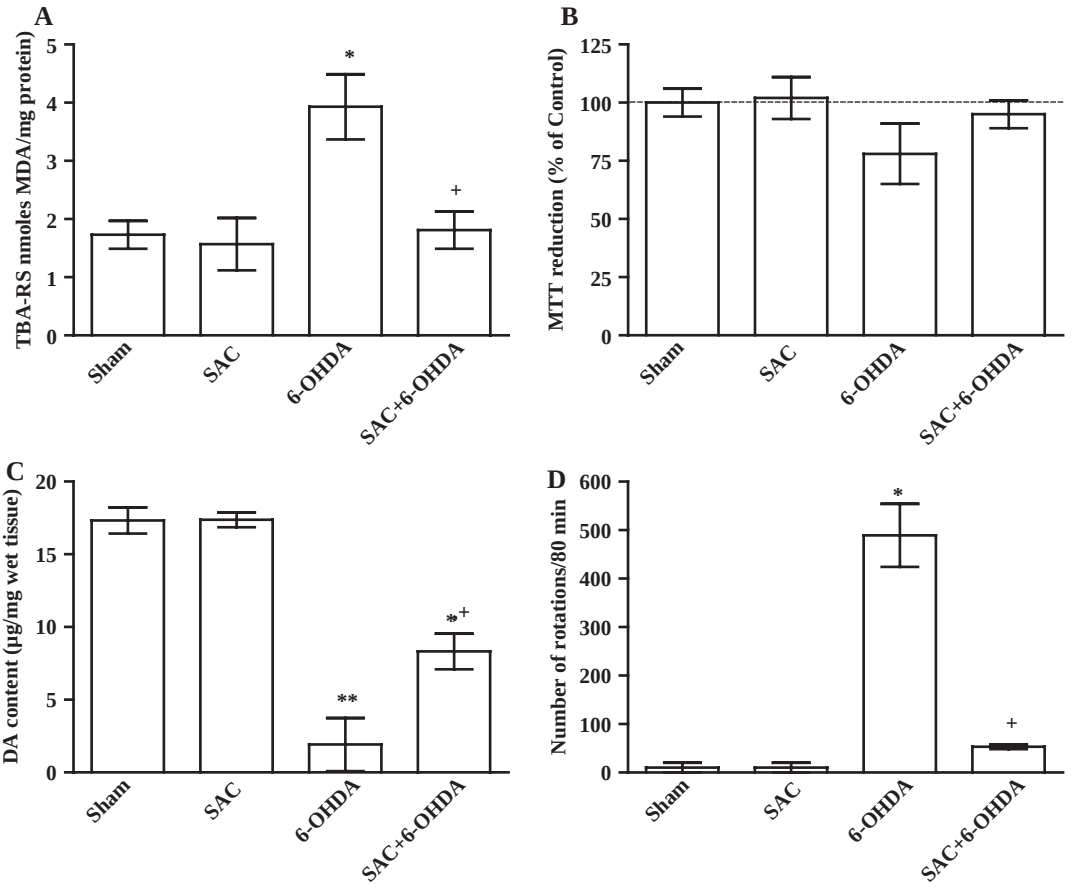


Figure 2. Effect of S-allylcysteine (SAC) on different markers of 6-hydroxydopamine (6-OHDA)-induced striatal toxicity. SAC (300 mg $kg^{-1}$, i.p.) was administered three times to rats (24 and 2 h before and 24 h after surgery). A single unilateral injection of 6-OHDA (8 µg $\mu l^{-1}$) was done into the left SNc. In (A) SAC prevented the 6-OHDA-induced lipoperoxidation in rat synaptosomal fractions; (B) SAC attenuated the 6-OHDA-induced disrupted mitochondrial reductive capacity in rat synaptosomal fractions; (C) SAC ameliorated the 6-OHDA-induced dopamine depletion in rat striatal tissue; (D) SAC reduced the 6-OHDA-induced rotational pattern in rats. All markers were evaluated 15 days post-lesion. For all graphics, mean values ± SEM of 7–10 experiments per group are presented. * $p < 0.05$ and ** $p < 0.01$, statistically different of Control; + $p < 0.05$, different of 6-OHDA alone; one-way ANOVA followed by Tukey's test.

3-NP significantly increased the lipoperoxidative action in synaptosomal fractions (33% above Control), but in contrast to the other experimental models SAC was unable to prevent this effect (27% above Control) (Figure 3B). SAC alone slightly decreased the basal LP (14% below Control). Oxidative damage was also assessed at earlier times (2 and 6 h), but, as expected, there were no significant changes in LP in synaptosomes from 3-NP rats when compared with Control (data not shown), presumably due to the low dose employed for the toxin.

In regard to the reductive capacity of mitochondria in synaptosomes (Figure 3C), 3-NP significantly depleted this function by 38% compared with Control and SAC recovered this effect in 3-NP treated rats (13% below the Control). SAC alone had no effect on MTT reduction. In addition, no changes in MTT reduction were detected at shorter times (2 and 6 h) in synaptosomes from 3-NP-treated rats (data not shown).

3-NP represents one of the toxic paradigms replicating Huntington's disease phenotype in experimental animals [47]. In this model, we found protective actions of SAC on the hypokinetic pattern produced by the toxin at 24 h after its administration, as well as on the mitochondrial dysfunction evidenced by MTT reduction. Interestingly, we did not find a protective effect of SAC on the lipoperoxidative action of 3-NP in synaptosomal fractions. While the first two markers responded in accord with previous observations of our own group [15], the later finding is contrasting with the same report in which the 3-NP-induced LP was decreased by SAC. The reason of this apparent discrepancy is the doses of SAC used: In this project SAC was administered at 100 mg $kg^{-1}$, whereas in the previous report the dose was 300 mg $kg^{-1}$. In light of this consideration, the lack of effect of SAC on LP in this protocol could be easily explained by considering that this dose is not sufficient to prevent oxidative damage. This simple finding revealed an important possible mechanism: the primary target of the protective actions of SAC in this specific model could be the preservation of mitochondrial functional integrity, instead of the scavenging of ROS. Nonetheless, this

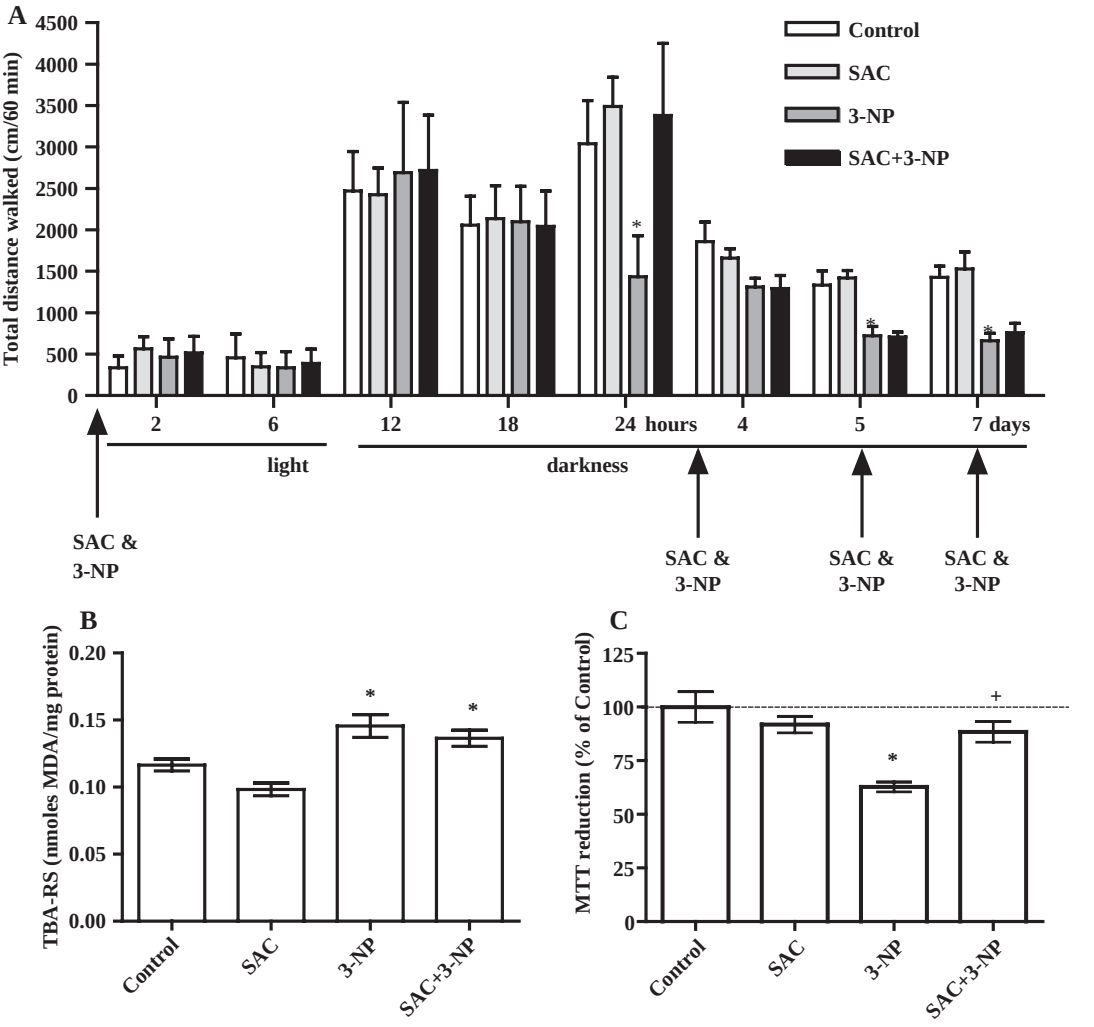


Figure 3. Effect of S-allylcysteine (SAC) on different markers of 3-nitropropionic acid (3-NP)-induced striatal toxicity. For behavioural purposes, SAC (100 mg $kg^{-1}$, i.p.) was administered four times to rats (every 2 days) 30 min before each 3-NP administration (10 mg $kg^{-1}$, i.p.), given for 7 days. All other markers were evaluated 24 h after a single administration of SAC and 3-NP at the same doses mentioned above. In (A) SAC inhibited the 3-NP-induced hypokinetic pattern in rats only at 24 h; (B) SAC failed to prevent the 3-NP-induced lipoperoxidation in rat synaptosomal fractions; (C) SAC ameliorated the 3-NP-induced disrupted mitochondrial reductive capacity in rat synaptosomal fractions. For all graphics, mean values ±SEM of six experiments per group are presented. * $p < 0.05$, statistically different of Control; + $p < 0.05$, different of 3-NP alone; one-way ANOVA followed by Tukey's test.

possibility will require further demonstration. Several other groups have demonstrated ameliorative and/or protective actions of other antioxidants against the 3-NP toxic model, giving support to our findings. Recent evidence validates the use agents exhibiting not only antioxidant properties, but also neurotrophic, energetic or anti-apoptotic features, such as melatonin [48] and L-carnitine [30,49,50].

## Discussion

Findings of this work describe for the first time the protective effects of SAC in two models of Parkinsonism evoked by MPTP and 6-OHDA, also emphasizing its positive actions in an experimental model resembling the deficient energy metabolism typical of Huntington's disease produced by 3-NP. This feature is also relevant for the Parkinsonian models, as it has been demonstrated that several neurodegenerative disorders, including Parkinson's disease, course with mitochondrial dysfunction and oxidative stress [51,52].

The data from this study demonstrate the capability of SAC to preserve the redox condition at basal levels upon different toxic conditions, protecting most of the time the major physiological functions, such as the neurochemical and behavioural parameters. How can these protective actions of SAC be explained in models exhibiting quite different mechanisms of toxicity? Although the most plausible explanation for the neuroprotective actions produced by SAC seems to be related with its antioxidant

properties (as judged by the consistent inhibition that SAC exerted specifically on those markers of oxidative stress in the models tested), we have also to consider the potential contribution of other protective actions inherent to this agent. Besides the many properties of SAC as antioxidant, wide-spectrum free radical scavenger, anti-apoptotic, neurotrophic and neuromodulatory profiles, reinforcing evidence has recently demonstrate that SAC plays a major role as a protector in several pathological situations: (1) Preventing ischemia-reperfusion injury [53,54]; (2) Alleviating noxious effects on ethanol-induced liver injury through anti-inflammatory actions [55]; (3) Showing anti-amyloidogenic and destabilizing activities in Alzheimer's beta-amyloid fibrils [56]; (4) Stabilizing mitochondrial and lysosomal enzymes in isoproterenol-induced cardiac toxicity [57]; and (5) Eliciting anti-apoptotic effects on chromium (VI)-induced damage through the activation of Nrf2 in hepatocytes [58]. Whether some of these mechanisms exerted by SAC could act in concert in nerve tissues in the tested models to produce protection remains to be elucidated in future studies. Of further consideration, we have recently characterized an additional protective property of SAC involved in the preservation of the nerve tissue function: SAC significantly reduces the oxidative damage produced by a model of combined excitotoxicity and energy deficit even under conditions of deprived or sequestered extracellular $Ca^{2+}$, thereby suggesting that this agent is not only a strong antioxidant in nerve tissue preparations involving $Ca^{2+}$-induced excitotoxic events, but also a possible anti-excitotoxic and energy-preserving agent by itself [16].

### *Final remarks*

Altogether, the results of this work demonstrate that SAC exerts antioxidant and neuroprotective properties in different models exhibiting specific toxic mechanisms. Several mechanisms have been suggested to explain the protective effects of SAC: antioxidative activity and free-radical scavenging, neurotrophic, transcriptional and anti-apoptotic signalling, etc. Although most of the protective actions of SAC reported in models of Parkinsonism and Huntington's disease might be attributable to its potent antioxidant nature, it is also likely that all the properties of this agent may be acting in a concerted manner to produce antioxidative and neuroprotective frames in different paradigms, thus supporting the therapeutic potential of this and other garlic-derived compounds. In summary, if the use of SAC—and other protective agents—in these models prevents biochemical markers of damage and ameliorates neurochemical deficits and altered motor patterns, its choice as therapeutic tool is promising as it can be inducing a considerable level of nerve tissue protection, thus allowing the preservation of major integrative behavioural tasks.

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