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In vitro antioxidant activity and *in vivo* antidepressant-like effect of α-(phenylselanyl) acetophenone in mice

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ABSTRACT

In this study, the antioxidant and antidepressant-like effects of α-(phenylselanyl) acetophenone (PSAP), an organoselenium compound, were investigated. To assess the *in vitro* antioxidant properties, PSAP was evaluated in four test systems (DPPH, ABTS, FRAP and inhibition of lipid peroxidation). PSAP (100–500 μM) showed potent antioxidant activity and protected against lipid peroxidation. Additionally, we investigated whether PSAP, when administered in mice (100, 200 and 400 mg/kg, per oral, p.o.), could cause acute toxicity. Our results demonstrated that PSAP did not cause the death of any animal, significantly reduce body weight or cause any oxidative tissue stress following treatment. This study also evaluated the effect of PSAP (0.1–10 mg/kg, p.o) on mice in a forced swim test (FST) and tail suspension test (TST), assays that are predictive of depressant activity and motor activity in the open-field. PSAP (5–10 mg/kg) significantly reduced immobility time in the FST and TST without affecting motor activity. In addition, the antidepressant-like effect caused by PSAP (5 m/kg, p.o) in mice during the TST was dependent on an interaction with the serotonergic system (5-HT_{1A} receptors), but not with the noradrenergic, dopaminergic or adenosinergic system. Together, these results suggest that PSAP possesses antioxidant and antidepressant-like properties and may be of interest as a therapeutic agent for the treatment of depressive disorders.

1. Introduction

Depression is a common and debilitating life-threatening illness with a high incidence. The World Health Organization predicts that depression will be the second cause of loss in human disability-adjusted life years worldwide. Although the mechanisms provoking depression have not been clearly elucidated, oxidative stress, in the form of free radical production may play an important role in its pathophysiology (Eren et al., 2007). It is likely that oxidative stress is primarily or secondarily involved in the pathogenesis of major depression. Maes et al. (2000), along with a growing number of other investigators (Tsuboi et al., 2006; Sarandol et al., 2007) have established the co-existence of increased oxidative stress with depressive symptoms in patients, as evidenced by defective plasma antioxidant defenses in association with enhanced susceptibility to lipid peroxidation. Moreover, preclinical studies have suggested that antioxidants in the form of radical scavengers may have antidepressant properties (Eren et al., 2007; Zafir et al., 2009). It appears reasonable to propose that exogenous antioxidants may be effective in treating depression. Thus, drugs with potential antioxidant action could be attractive targets for the treatment of depressive disorders.

In this context, selenium is an essential trace element nutritionally important to mammals, with physiological roles as a structural component of several antioxidant enzymes (Ursini and Bindoli, 1987; Rayman, 2000). In addition, considerable evidence suggests that low selenium levels lead to depressed mood and anxiety (Sher, 2000, 2007; Rayman, 2000) while high dietary selenium or selenium supplementation may improve mood and depression status (Benton and Cook, 1991). Thus, a number of novel pharmaceutical agents that are selenium-based or that target specific aspects of selenium metabolism are under development, and a variety of organoselenium compounds possess pharmacological activity (Nogueira et al., 2004; Nogueira and Rocha, 2011). Among them, diphenyl diselenide, ebselen, 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene, *m*-trifluoromethyl-diphenyl diselenide and bis selenide have been documented as promising pharmacological agents in a number of experimental models of oxidative stress that predict depressant activity

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(Savegnago et al., 2008; Gay et al., 2010; Brüning et al., 2011; Jesse et al., 2010, 2011).

In this context, arylselanyl acetophenones are a class of organoselenium compounds that have a number of interesting biological activities. Studies have shown that α-(phenylselanyl) acetophenone (PSAP) exhibits glutathione peroxidase-like activity (Cotgreave et al., 1992; Engman et al., 1994; Nikolic, 2006) and has the capacity to inhibit tumor promoter-induced downregulation of intercellular communication between liver epithelial cells via gap junction (Hu et al., 1995). However, to our knowledge, there has not yet been a study on the antioxidant and antidepressant-like activities of this compound.

Therefore, the aim of this study was to evaluate antioxidant activity *in vitro* and antidepressant-like effects of PSAP *in vivo*. We also investigated the possible mechanism responsible for the antidepressant-like effects of PSAP. To investigate the potential future application of the compound as a novel therapeutic drug, we also evaluated its acute toxicity.

2. Material and methods

2.1. Drugs

α-(Phenylselanyl) acetophenone (PSAP, Fig. 1) was prepared and characterized in our laboratory according to the method described by Victoria et al. (2009). An analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with their assigned structures. Chemical purity was determined by GC/HPLC and was found to be 99.9%. All other chemicals were of analytical grade and obtained from standard commercial suppliers. PSAP was dissolved in dimethylsulfoxide (DMSO) for the *in vitro* experiments and in canola oil for the *in vivo* experiments. The compound was administered by oral route (p.o.) at different doses for the *in vivo* experiments at a constant volume of 10 ml/kg body weight. Appropriate vehicle-treated groups were also simultaneously assessed.

2.2. Animals

Adult male Swiss albino mice (25–35 g; 2–3 months of age) were obtained from our own breeding colony. The animals were maintained in an air conditioned room (20–25 °C) under a 12 h light/dark cycle. All experimental procedures were conducted according to the guidelines of the Committee of Ethics in Research of the Federal University of Pelotas and Federal University of Pampa, Brazil. For *in vivo* assays, the mice were acclimatized in the laboratory for at least 1 h prior to testing, and each mouse was used only once during the experiments. The experimental procedures were performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). After the experiments, the animals were euthanized by cervical displacement. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

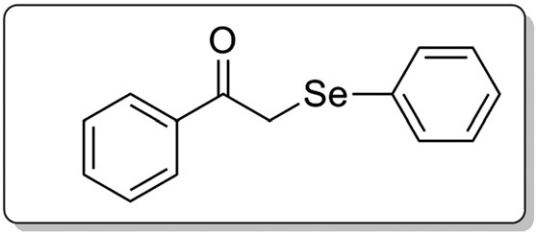

Fig. 1. Chemical structure of α-(phenylselanyl) acetophenone (PSAP).

2.3. Determination of in vitro antioxidant activities of PSAP

2.3.1. Tissue preparation

Mice were euthanized and the cerebral tissue of the whole brain was rapidly removed, placed on ice and weighed. Tissues were immediately homogenized in cold 50 mM Tris–HCl, pH 7.4 (1/5, weight/volume [w/v]). The homogenate was centrifuged for 10 min at 2000 rpm to yield a pellet that was discarded and a low-speed supernatant (S_1) was obtained and used to determine the effects of different concentrations of PSAP on lipid peroxidation levels and δ-ALA-D activity. The S_1 mainly contained water, proteins, lipids, DNA and RNA (Belle et al., 2004).

2.3.2. Lipid peroxidation assay

To investigate the antioxidant effects of PSAP, sodium nitroprusside (SNP) was used as a classical inductor of lipid peroxidation (Rauhala et al., 1998). Lipid peroxidation was estimated by the measurement of malondialdehyde (MDA) levels. MDA is an end product of lipid peroxidation, and its level was determined spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) assay, as previously described by Ohkawa et al. (1979). For this purpose, 100 μl of homogenate (S_1) was added to the reaction mixture containing 50 μM SNP and PSAP at different concentrations (10–100 μM). The mixture was subsequently pre-incubated at 37 °C for 1 h. The reaction product was determined using 500 μl of thiobarbituric acid (TBA, 0.8%), 200 μl of sodium dodecyl sulfate (SDS, 8.1%), and 500 μl of acetic acid (pH 3.4) after incubation at 95 °C for 2 h. MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex which is measured as % inhibition related to induction.

2.3.3. δ-Aminolevulinate dehydratase (δ-ALA-D) activity

δ-ALA-D is a sulfhydryl-containing enzyme that can be inhibited in different pro-oxidant situations and can be used as a marker of toxicity (Folmer et al., 2003). Cerebral δ-ALA-D activity was assayed according to the method described by Sassa (1982) by measuring the rate of product (porphobilinogen) formation, with the following modification; 84 mM potassium phosphate buffer (pH 6.4) and 2.5 mM δ-aminolevulinic acid were used. All experiments were carried out after 10 min of pre-incubation (100 μl of S_1 and 10 μl of the drug at concentrations ranging from 0.1 to 10 μM), meaning that the reaction was started 10 min after the addition of the enzyme preparation by adding the substrate. Incubations were carried out for 3 h at 37 °C. The reaction product was determined using modified Ehrlich's reagent at 555 nm, with a molar absorption coefficient of $6.1 \times 10^4\ cm^{-1}\ M^{-1}$ for the Ehrlich-porphobilinogen salt.

2.3.4. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

The DPPH radical is a stable free radical commonly used as a substrate to evaluate *in vitro* antioxidant activity. Antioxidants can scavenge radicals by hydrogen donation, which causes a decrease in DPPH absorbance (Ancerewicz et al., 1998). Radical-scavenging activity was determined by the reaction of the stable DPPH radical with the compounds, in accordance with the method of Choi et al. (2002) with some modifications. Different concentrations of compound (10–500 μM) were mixed with a methanolic solution containing the DPPH radical, resulting in a final concentration of 85 μM DPPH. The mixture was incubated for 30 min at 30 °C, and the absorbance was measured at 517 nm. The values are expressed as the percentage of inhibition of DPPH absorbance (% inhibition) in relation to the control values without the compound, as calculated from the following equation:

$$I\%_{DPPH} = [(A_c - A_s / A_c) \times 100]$$

where I = DPPH inhibition, A_c is the absorbance of the control reaction mixture excluding the test compound and A_s is the absorbance of the test compound in different concentrations.