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p,p′-Methoxyl-diphenyl diselenide-loaded polymeric nanocapsules as a novel approach to inflammatory pain treatment: Behavioral, biochemistry and molecular evidence

Marcel Henrique Marcondes Sari[a], Vanessa Angonesi Zborowski[a], Luana Mota Ferreira[b], Natália Silva Jardim[a], Allanna Valentini Barbieri[b], Letícia Cruz[b], Cristina Wayne Nogueira[a,*]

[a] *Programa de Pós-graduação em Bioquímica Toxicológica, Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Departamento de Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil*

[b] *Programa de Pós-graduação em Ciências Farmacêuticas, Laboratório de Tecnologia Farmacêutica, Departamento de Farmácia Industrial, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil*

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ABSTRACT

The current study investigated the effect of organoselenium compound *p,p′*-methoxyl-diphenyl diselenide [$(OMePhSe)_2$], free or incorporated into nanocapsules, on behavioral, biochemical and molecular alterations in an inflammatory pain model induced by complete Freund's adjuvant (CFA). Male Swiss mice received an intraplantar injection of CFA in the hindpaw and 24 h later they were treated via the intragastric route with a single $(OMePhSe)_2$ administration, in its free form (dissolved in canola oil) or $(OMePhSe)_2$ NC. The anti-hypernociceptive time- and dose-response curves were carried out using the von Frey hair test. Biochemical and histological parameters were determined in samples of injected paws and those of cerebral contralateral cortex were collected to determine immuno content of inflammatory proteins. Both $(OMePhSe)_2$ forms reduced the hypernociception induced by CFA as well as attenuated the altered parameters of the inflammatory process in the paw (paw edema, myeloperoxidase and histological). However, the $(OMePhSe)_2$ NC had a more prolonged anti-hypernociceptive action (7 h) at a lower dose (10 mg/kg) and superior effects on the paw alterations than the free compound form (4 h and 25 mg/kg). Furthermore, independent of the $(OMePhSe)_2$ form, its administration decreased the MAPKs pathway activation (JNK;$ERK_{1,2}$; p38) as well as iNOS, COX-2, Nf-κB and IL-1β protein contents in the cerebral contralateral cortex that were increased by paw CFA injection. Therefore, $(OMePhSe)_2$ NC had superior anti-inflammatory action, which possibly occurs by the inflammatory protein content modulation and also attenuates paw biochemical and histological inflammatory alterations induced by CFA injection.

1. Introduction

Despite the essential role of pain for the physiological homeostasis its endurance may lead to negative reactions, what is no longer beneficial (Olesen et al., 2012). An interplay among neuroadaptative process and distinct mediators, including inflammatory factors (Linley et al., 2010), contributes to the alteration in pain sensation, which is the most common complaint in the medical field (Sollami et al., 2015). However, the current therapy to alleviate this condition is partially effective and may be accompanied by adverse effects, which boosts the studies focusing on novel and improved pharmacological interventions (Peppin et al., 2015).

The organoselenium compounds have emerged as an important subject of research worldwide because of the pharmacological properties assigned to these molecules (Angeli et al., 2017; Heyland et al., 2013; Lu et al., 2017; Luo et al., 2013; Nogueira and Rocha, 2011; Pang

Abbreviations: $(OMePhSe)_2$, *p,p′*-methoxyl-diphenyl diselenide; $(OMePhSe)_2$ NC, *p,p′*-methoxyl-diphenyl diselenide nanocapsules; ^{13}C NMR, carbon-13 nuclear magnetic resonance; ^{1}H NMR, proton nuclear magnetic resonance; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; CONCEA, Council for Control of Animal Experiments; COX-2, cyclooxygenase-2; $ERK_{½}$, extracellular signal-regulated protein kinases; H & E, haematoxylin and eosin; i.g., intragastric; i.pl., intraplantar; IL-1β, interleukin 1β; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MPO, myeloperoxidase; NC-Br, blank nanocapsules; NF-κB, nuclear factor-κB; OD, optic density; p38, p38 mitogen-activated protein kinase; PCL, poly(ε-caprolactone); PDI, polydispersity index; *p*-$ERK_{½}$, phosphorylated extracellular signal-regulated protein kinases; *p*-p38, phosphorylated p38 mitogen-activated protein kinase; S_1, supernatant fraction; SDS, sodium dodecyl sulfate; VFH, von Frey hair

* Corresponding author at: Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil.
E-mail address: criswn@ufsm.br (C.W. Nogueira).

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et al., 2017; Sancineto et al., 2015; Sancineto et al., 2016). Of particular importance, preclinical studies demonstrated that such compounds have potential antinociceptive and anti-inflammatory actions (Bruning et al., 2015; Jesse et al., 2009; Nogueira and Rocha, 2011; Sari et al., 2017). The *p,p′*-methoxyl-diphenyl diselenide [$(OMePhSe)_2$] is a diaryl diselenide class representative that has low toxicological potential in comparison to other diselenides and a multi-target antinociceptive action (Jesse et al., 2009; Marcondes Sari et al., 2017). It has been reported the $(OMePhSe)_2$ effectiveness in different chemical and thermal murine models of acute nociception (Jesse et al., 2009; Pinto et al., 2008) as well as in a pain-depression dyad induced by reserpine in rats (Oliveira et al., 2016). However, the high lipophilic characteristic of this compound limits its solubility in the physiologic fluids and may reduce its bioavailability (Prigol et al., 2013).

Recently, our research group developed $(OMePhSe)_2$ polymeric nanocapsules in order to circumvent the aforementioned physicochemical issue of this organoselenium compound (Marcondes Sari et al., 2017). The polymeric nanocapsules, which consist of a polymeric involucre around an oily core (Fessi et al., 1989), are a versatile nanocarrier system that provides photo- or chemical protection to the incorporated molecules; besides, greatly prolongs and/or increases the pharmacological action of drugs improving their bioavailability (Frank et al., 2015). This nanotechnological approach expanded the $(OMePhSe)_2$ tissue biodistribution profile without causing toxicity (Marcondes Sari et al., 2017). Moreover, a comparative behavioral study demonstrated that the $(OMePhSe)_2$ antinociceptive action was prolonged and increased by its nanoencapsulation (Sari et al., 2017).

Considering the $(OMePhSe)_2$ potential pharmacological actions and the urgency for novel effective pharmaceutical interventions to pain treatment, this study evaluated the $(OMePhSe)_2$ anti-inflammatory effect, with the substance in its free form and incorporated into polymeric nanocapsules, on behavioral and molecular alterations in an inflammatory pain model induced by complete Freund's adjuvant (CFA).

2. Material and methods

2.1. Chemicals and reagents

The $(OMePhSe)_2$ was synthesized in our laboratory based on a previous published methodology (Paulmier, 1986). The ^1H NMR and ^{13}C NMR spectra analysis showed analytical and spectroscopic data in full agreement with its assigned structure. Besides, the compound chemical purity (99.9%) was determined by gas chromatography-mass spectrometry association (Shimadzu QP2010PLUS GC/MS). The poly(ε-caprolactone) (MW:80 kDa [PCL]), Span 80® (sorbitan monooleate), CFA, *N,N,N′,N′*-tetramethylbenzidine, celecoxib, bovine serum albumin (BSA), protease inhibitor cocktail, tris(hydroxymethyl)aminomethane, acrylamide, bis-acrylamide, sodium dodecyl sulfate (SDS), pre-stained molecular weight standard and bromophenol blue were obtained from Sigma-Aldrich (São Paulo, Brazil). Medium chain triglycerides and Tween 80® (polysorbate 80) were bought from Delaware (Porto Alegre, Brazil). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2. Animals

The study was carried out using male adult Swiss mice (25–35 g) obtained from the Federal University of Santa Maria breeding colonies. All experiments were approved by the Ethical Research Committee of Federal University of Santa Maria (#**1446300315/2015**) affiliated to the Council for Control of Animal Experiments (CONCEA) and in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The animals were housed in plastic boxes receiving standard food and water ad libitum (GUABI, RS, Brazil) under a 12 h light/dark cycle (7 AM to 7 PM) and temperature at 22 ± 2 °C. Before testing, in order to eliminate stress effects, the animals were acclimatized in an appropriate behavioral room with controlled temperature (25 °C) and humidity (60%) suitable brightness and soundproofing.

2.3. $(OMePhSe)_2$-loaded polymeric nanocapsules

The $(OMePhSe)_2$[$(OMePhSe)_2$ NC] nanocapsules suspension was prepared according to Fessi et al. (1989) by the interfacial deposition of preformed polymer methodology at a $(OMePhSe)_2$ concentration of 2.5 mg/mL (Marcondes Sari et al., 2017). Briefly, an organic phase containing $(OMePhSe)_2$ (0.025 g), Span® 80 (0.077 g), PCL (0.1 g), medium chain triglycerides (330 μL) and acetone (27 mL) was kept under magnetic stirring for 60 min at 40 °C. Following, this phase was injected into the aqueous phase (Tween® 80 [0.077 g]; 53 mL of water) and the system was maintained under moderate magnetic stirring during 10 min. The excessive solvent was removed by evaporation under low pressure to achieve a 10 mL of final volume. For comparison purposes, a nanocapsule suspension without the compound (NC-Br) was prepared following the same protocol. The formulations were stored in amber glass flasks for ultraviolet ray protection and were used within a 30-day period.

All nanocapsules suspension parameters were determined soon after their preparation as previously described (Marcondes Sari et al., 2017). As expected, the formulations had appropriate colloidal system characteristics, particle diameter in the nanometric range, PDI values lower than 0.2, negative values of zeta potential, pH around the neutrality range and a compound content close to the theoretical value (98%) (*Data not shown*).

2.4. Animal model of inflammatory pain

The $(OMePhSe)_2$ anti-inflammatory action was investigated in the acute pain model induced by the injection of CFA in a hindpaw, which is a well characterized experimental protocol for screening novel compounds for inflammatory pain (Ren and Dubner, 1999).

2.4.1. Experimental schedule and general procedures

This study was divided in two different experimental sets applying the same animal model (Fig. 1). The first protocol was carried out to establish the $(OMePhSe)_2$ time- and dose-response curves in the mechanical hypernociceptive behavior induced by the CFA injection using the von Frey hair (VFH) test (Fig. 1.I). Another set of animals was treated to study the biochemical and molecular mechanisms associated with the $(OMePhSe)_2$ action (Fig. 1.II).

The formulation or compound was administered by the intragastric (i.g.) route in a single regimen and in a 10 mL/kg constant dosing volume. The compound was dissolved in canola oil ($(OMePhSe)_2$ Free). The formulations ($(OMePhSe)_2$ NC and NC-Br) were prepared as previous described (Section 2.3) and used as obtained or diluted in distilled water to achieve lower formulation doses (10 and 5 mg/kg of $(OMePhSe)_2$ NC). The animals were randomly assigned in five groups each one receiving the following treatment:

I) **Control** – the animals received a subcutaneous intraplantar saline injection in the hindpaw and were treated with canola oil or NC-Br (10 mL/kg);
II) **Induced** – the animals received a subcutaneous intraplantar CFA injection in the hindpaw and were treated with canola oil or NC-Br (10 mL/kg);
III) **$(OMePhSe)_2$ Free** – the animals received a subcutaneous intraplantar CFA injection in the hindpaw and were treated with $(OMePhSe)_2$ dissolved in canola oil (10 mL/kg);
IV) **$(OMePhSe)_2$ NC** – the animals received a subcutaneous intraplantar CFA injection in the hindpaw and were treated with $(OMePhSe)_2$ incorporated into the NCs suspension (10 mL/kg);
V) **Celecoxib** – the animals received a subcutaneous intraplantar CFA injection in the hindpaw and were treated with celecoxib at a dose