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Selol, an organic selenium donor, prevents lipopolysaccharide-induced oxidative stress and inflammatory reaction in the rat brain

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ABSTRACT

Neuroinflammation and oxidative stress are key intertwined pathological factors in many neurological, particularly neurodegenerative diseases, such as Alzheimer's and Parkinson's disorders as well as autism. The present study was conducted to evaluate the protective effects of Selol, an organic selenium donor, against lipopolysaccharide (LPS)-mediated inflammation in rat brain. The results demonstrated that the peripheral administration of LPS in a dose of 100 μg/kg b.w. evoked typical pathological reaction known as systemic inflammatory response. Moreover, we observed elevated blood levels of thiobarbituric acid-reactive substances (TBARS), a marker of oxidative stress, as well as increased concentration of tumor necrosis factor-α (TNF-α) in LPS-treated animals. Selol significantly prevented these LPS-evoked changes. Subsequently, Selol protected against LPS-induced up-regulation of proinflammatory cytokines (*Tnfa*, *Ifng*, *Il6*) in rat brain cortex. The molecular mechanisms through which Selol prevented the neuroinflammation were associated with the inhibition of oxidized glutathione (GSSG) accumulation and with an increase of glutathione–associated enzymes: glutathione peroxidase (Se-GPx), glutathione reductase (GR) as well as thioredoxin reductase (TrxR) activity and expression. Finally, we observed that Selol administration effectively protected against LPS-induced changes in the expression of brain-derived neurotrophic factor (*Bdnf*). In conclusion, our studies indicated that Selol effectively protects against LPS-induced neuroinflammation by inhibiting pro-inflammatory cytokine release, by boosting antioxidant systems, and by augmenting BDNF level. Therefore, Selol could be a multi-potent and effective drug useful in the treatment and prevention of brain disorders associated with neuroinflammation.

1. Introduction

Immune activation within the central nervous system (CNS) is involved in the pathogenesis of many neurodegenerative diseases, including Alzheimer's or Parkinson's diseases as well as various neuropsychiatric disorders, like autism (Chez et al., 2007; Leszek et al., 2016; Li et al., 2009; Pasqualetti et al., 2015). Inflammation in the brain is characterized by the activation of microglia, the resident macrophages of the CNS. They play an important role in the innate immune response and rapidly respond to damage, producing inflammatory mediators (Leszek et al., 2016; Olson and Miller, 2004). Often, the uncontrolled microglial activation may initiate and exacerbate neuronal damage. In many experimental models of neurodegenerative as well as neuropsychiatric diseases, glial overactivation followed by secretion of pro-inflammatory cytokines was documented (Chez et al., 2007; Glass et al., 2010;

Abbreviations: BDNF, brain-derived neurotrophic factor; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; HPA, hypothalamic–pituitary–adrenal; ICP-MS, the inductively coupled plasma - mass spectrometry; IL-6, interleukin-6; LD, lethal dose; LPS, lipopolysaccharide; MAPK, mitogen associated protein kinase; MDA, malondialdehyde; NF-κB, nuclear factor of kappa light polypeptide gene enhancer in B cells; NO, nitric oxide; SelP, selenoprotein P; SIR, systemic inflammatory response; TBARS, thiobarbituric acid-reactive substances; TLR, Toll-like receptor's; TNF-α, tumor necrosis factor α; TrxR, thioredoxin reductase.

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Gordon et al., 2016; Li et al., 2009; Lopategui Cabezas et al., 2014; Manu et al., 2014; Nagatsu and Sawada, 2005; Tonges et al., 2014; Whitton, 2007). Additionally, the systemic inflammation may also induce immune response in brain. Systemically administered lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria, may act on endothelial cells in the brain and induce the activation of surrounding microglia (Nava Catorce et al., 2016; Wang et al., 2016). In addition, inflammatory cells of the bloodstream, activated by LPS treatment, may enter the brain and participate in inflammation (Holman et al., 2011; Jeong et al., 2010; Su and Federoff, 2014). Inhibition of the inflammatory processes may thus represent a therapeutic target to reduce neuronal dysfunction and damage. Therefore, there is a need for finding new compounds that might inhibit, retard or reverse the complex molecular mechanisms underlying neuroinflammation and neurotransmission.

Selenium (Se) has been reported to play an important role in the cellular antioxidant defence and is essential for the efficient operation of the immune system in both animals and humans. Through its incorporation into selenoproteins, like glutathione peroxidases (GPxs), thioredoxin reductases (TrxRs) or selenoprotein P (SelP), Se is involved in regulating oxidative stress, redox state and other crucial cellular processes in nearly all tissues and cell types, including those involved in innate and adaptive immune responses (Dominiak et al., 2016a; Hoffmann and Berry, 2008). It was previously demonstrated that Se might improve the lymphocytes proliferation, or leukotriene B4 synthesis in macrophages, which is essential for neutrophil chemotaxis (Spallholz, 1990). Moreover, Se was shown to be involved in regulation of interleukin production in the brain by boosting antioxidant redox system (Senol et al., 2014). In turn, Se deficiency was previously shown to be responsible for the impairment of immune cells's activation, differentiation and proliferation (Huang et al., 2012) and was associated with different neurological disorders (Dominiak et al., 2016a; Gu et al., 2013; Wrobel et al., 2016). Accumulating evidence supports the protective role of Se supplementation in neuronal dysfunction, suggesting its potential role in a therapeutic approach to inflammation-related brain diseases. Up to now, different Se supplements with various pharmacological properties have been shown to effectively decrease the level of proinflammatory cytokines in various animal models (Bruning et al., 2012; Koyanagi et al., 2001; Leite et al., 2015; Xu et al., 2015). However, due to unbearable side effects the clinical application of Se supplements in therapy is greatly limited (Garousi and Farzaneh, 2015; Letavayova et al., 2008). Inorganic Se donors might be more toxic as well as have lower intestinal absorption efficacy than organic Se species (Letavayova et al., 2008). On the other hand, some organic Se donors may non-specifically incorporate into proteins like albumin or hemoglobin leading to their structural changes followed by dysfunction (Schrauzer, 2003). Concerns about safety and effectiveness of selenium therapeutic income seems to be substantiated, thus there is still a need for finding a new selenium compound.

Selol, a semi-synthetic mixture of organic selenitetriglycerides, containing Se at the +4 oxidation level, is a novel compound that exhibits low toxicity after parenteral administration, and also it does not reveal any mutagenic potential (Jastrzebski et al., 1995; Rahden-Staron et al., 2010). An additional advantage of this compound is that it undergoes rapid resorption from the digestive system, is widely distributed in the organism and it has the ability to cross the blood–brain barrier. Furthermore, it is completely eliminated from the organism after 24 h from administration, avoiding accumulation and toxic effects (Jastrzebski et al., 1997). Our previous *in vitro* data documented the high effectiveness of Selol against oxidative damage and neuronal death by adjusting free radical levels and antioxidant systems (Dominiak et al., 2016b). Therefore, the aim of the present study was to investigate whether Selol protects against inflammation and improves neuroprotective mechanisms in response to LPS treatment in rats.

2. Material and methods

2.1. Materials

Selol [(9E,11E,13E)-octadeca-9,11,13-trienoic acid 2-hexadecanoyloxy-3-{7-[5-((1E, 3E)-no-na-1,3-dienyl)-2-oxy-2λ4-[1,3,2]dioxaselenolan-4-yl]-heptanoyloxy}-propyl ester] was synthesized at the Department of Drug Analysis at Medical University of Warsaw with a declared selenium concentration of 5% (w/v). For experimental purposes Selol was diluted in sunflower oil.

LPS (from *E. coli* serotype 055:B5; toxicity 3×10^6 U/mg), DMSO, dithiotreitol, Trizol, reduced glutathione (GSH), glutathione reductase (GR), the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), sodium azide, tert-butylperoxide, 2-vinylpyridine, triethanolamine, metaphosphoric acid, bovine serum albumin (BSA), 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) and sodium nitroprusside (SNP) were purchased from Sigma (St. Louis, MO, USA). Mouse *anti*-TNF-α antibody and mouse *anti*-IL-6 antibody were purchased in R&D Systems (Minneapolis, MN, USA). Anti-mouse IgG was from GE Health Care UK (Little Chalfont, Buckinghamshire, UK). Rabbit *anti*-GAPDH and anti-rabbit IgG were from Sigma-Aldrich (St. Louis, MO, USA). Chemiluminescent reagent Clarity Western ECL Substrate was from Bio-Rad Laboratories (Hercules, CA, USA). Protease inhibitors cocktail Complete was from Roche Diagnostics GmbH (Mannheim, Germany). Glutathione Assay Kit, Thioredoxin Reductase Colorimetric Assay Kit, TBARS Assay Kit, Glutathione reductase Assay Kit were from Cayman Chemical Company (Ann Arbor, USA). Reagents for reverse transcription (High Capacity RNA-to-cDNA Master Mix) and PCR (Gene Expression Master Mix) were from Applied Biosystems (Foster City, CA, USA). TNF-α ELISA kit was from Affymetrix, (eBioscience, Vienna, Austria).

2.2. Animals

All the experiments were carried out on female, 2- to 3-month-old (200–250 g) Wistar rats, supplied from Animal House of Mossakowski Medical Research Centre PAS (Warsaw, Poland). The animals were maintained under controlled temperature and humidity conditions on a 12-h light/dark cycle. All of the experiments conducted on animals were approved by the Polish National Ethics Committee and were carried out in accordance with the EC Council Directive of 24 November, 1986 (86/609/EEC) following the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and the principles presented in the “Guidelines for the Use of Animals in Neuroscience Research” by the Society for Neuroscience. Every effort has been made to minimize the number of animals used and reduce the amount of pain and distress.

2.3. Experimental procedure

Rats were randomly divided into four groups (n = 8 each): control group supplemented with oil (control), LPS group supplemented with oil (LPS), LPS group supplemented with Selol (LPS+Selol) and a group that received only Selol (Selol).

5% Selol diluted in vegetable oil was administered *per* os in 7 consecutive days of experiment in a single dose of 5 mg Se/kg b.w., which refers to 5% of LD_{50} (LD_{50} = 100 mg Se/kg body mass) (Jastrzebski et al., 1995). Control animals were supplemented *per os* with the same volume of vegetable oil according to the same time schedule.