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Understanding the Mechanism of Antioxidant Potential of Organochalcogens in Rat's Brain Preparation

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Abstract

In the quest to explore mechanism of action of organochalcogens a series of them are tested for their structural-activity relationship using rat's brain preparation. Dichalcogenides are better anti-oxidants than structurally analogues mono- chalcogenides. Effects of electron donating and withdrawing groups have been explored and explained in detail. We have also proved that structural isomerisation does not influence the anti-oxidant activity of these compounds.

Keywords: Organochalcogens; Structure-Activity Relationship and Oxidative Stress

Introduction

Oxidative stress is an important biochemical condition causing several human diseases. This stress is linked to the presence of unusually high concentrations of toxic reactive species, which include reactive oxygen species (ROS) reactive nitrogen species and unbound, adventitious metal ions [1]. Most of these species are highly oxidizing, readily modifying redox sensitive proteins and enzymes, as well as attacking membranes and DNA. The living cell contains a number of important antioxidants and antioxidant catalysts. Their presence counteracts oxidative stress and also neutralizes a range of oxidizing species. Ascorbate, NADH, melatonin, trolox and GSH are common antioxidants that frequently occur in the cell. However, disruption in homeostasis can result in oxidative stress and tissue injury. Thus to respond to ROS more effectively, compounds can be envisaged that combine a range of antioxidant activities in one chemically simple molecule [2,3].

Organoselenium chemistry is a very broad and exciting field with many opportunities for research and development of applications. Organoselenium compounds have become attractive synthetic targets because of their chemio-region and stereoselective reactions [4] and their useful biological activity [5]. In fact, a variety of organoselenium compounds with potential antioxidant activity, including ebselen analogues, benzoselenazolinones, diaryl diselenides, selenamide and related derivatives have been reported in a variety of pathological situations [6-10]. The mechanism(s) underlying the toxic effect of organochalcogens are not completely understood but certainly involves the reaction of chalcogenides with endogenous thiols [11] however there is still scarcity of data about the mechanism of action of these organoselenium compounds acting as anti-oxidant agents.

Nitric oxide (NO) released from sodium nitropruside (SNP) is endogenously produced reactive specie. It is also recognized as a neurotransmitter in the central nervous system (CNS) [12,13]. It can mediate biological actions ranging from vasodilatation, neurotransmission, inhibition of platelet adherence and aggregation, and killing of pathogens mediated by macrophages and neutrophiles. High concentrations of NO are toxic and interact with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$) [12]. Peroxynitrite is a strong oxidant and, at physiological pH, is protonated to form peroxynitrous acid (HOONO), a relatively long-lived oxidant agent, which spontaneously decomposes to form another potent oxidant with the reactivity of a hydroxyl-like radical [14] which could initiate lipid peroxidation (LPO) [12,15].

Keeping in view the above stated issue we took a step in this regard and are reporting the importance of a chemically multidimensional approach towards antioxidant characterization. In this communication we will describe how chemical changes to a series of organoselenium compounds alter their biochemical rather antioxidant activities. We have tested the efficacy of these compounds against sodium nitropruside (SNP) induced thio-barbituric acid reactive species (TBARS) formation in rat's brain preparation.

Material and Methods

Synthesis of diorganyl selenides

The unsymmetrical diorganyl selenides and sulphides were synthesised using the literature procedure [16,17]. Commercially available diphenyl diselenide (CAS No: 1666-13-3), and diphenyl ditelluride (CAS No: 32294-60-3) were purchased and used for the experiments. Analysis of the ^{1}HNMR and ^{13}CNMR spectra showed that both compounds obtained presented analytical and spectroscopic data in full agreement with their assigned structures. The chemical purity of the compounds (99.9%) were determined by GC/HPLC.

Animals

Adult male wistar rats from our own breeding colony (250–350 g) were maintained in an air-conditioned room (22–25 °C) under natural lighting conditions, with water and food (Guabi, RS, Brazil) ad libitum.

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Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil.

Tissue preparation

Animals were anesthetized with ether and killed by decapitation. Brain was quickly removed, placed on ice, and homogenized within 10 min, in 10 mmol/l Tris/HCl buffer, pH 7.4 (in 10 volume). The homogenate was centrifuged at 4000 × *g* at 4 °C for 10 min to yield a low speed supernatant fraction (S1) that was used immediately for TBARS assay (Puntel et al. 2007).

Lipid peroxidation assay

Tissue homogenate was prepared by homogenization as described above. An aliquot of 100 µl of S1 was incubated for 1 h at 37ºC in the presence of organochalcogens (final concentrations range of (0–100 µM), with and without the prooxidant i.e. sodium nitropruside (SNP) at final concentration of 10µM. Production of TBARS were determined as described by method of Ohkawa et al. [18] except that the buffer of color reaction have a pH of 3.4. The color reaction was developed

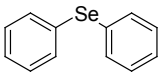
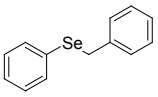
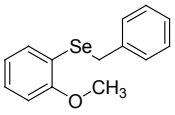
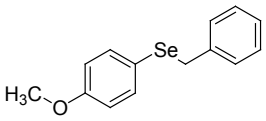
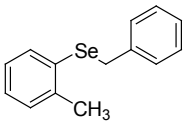
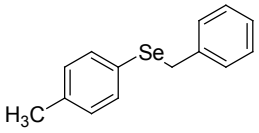
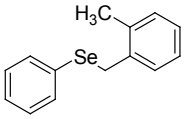
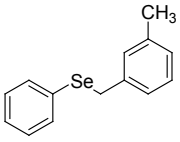
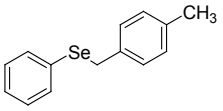
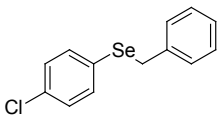
#	R	R'	X	Product	nmol of MDA (TBARS)					
					Control (0)	1	5	10	50	100
C-1	Ph	Ph	Br		542± 21	538±22	540±23	563±22	523±12	511±21 [a]
C-2	Ph	$PhCH_2$	Cl		542±19	549±19	550±21	564±31	526±19	509 ±12 [a]
C-3	o-$MeOC_6H_4$	$PhCH_2$	Cl		542±19	541±21	559±12	547±18	535±21	443 ±23 [b]
C-4	p-$MeOC_6H_4$	$PhCH_2$	Cl		542±34	511±15	523±28	565±18	484±22	307 ±32 [c]
C-5	o-MeC_6H_4	$PhCH_2$	Cl		542±12	547±23	548±32	534±32	548±32	469 ±13 [b]
C-6	p-MeC_6H_4	$PhCH_2$	Cl		542±21	561±12	545±31	555±21	507±12	348 ±23 [c]
C-7	Ph	o-$MeC_6H_4CH_2$	Br		542±23	541±31	551±12	533±12	496±12	440 ±21 [b]
C-8	Ph	m-$MeC_6H_4CH_2$	Br		542±32	551±23	568±17	546±23	498±18	459 ±14 [b]
C-9	Ph	p-$MeC_6H_4CH_2$	Br		542±12	527±24	536±16	541±31	482±21	448 ±31 [b]
C-10	p-ClC_6H_4	$PhCH_2$	Cl		542±32	736±21	701±32	730±18	746±23	780 ±22 [e]

Table 2: Effect of Diphenyl Diselenide (DPDS) and Diphenyl Ditelluride (DPDT) on TBARS production in rat's brain preparation. TBARS are expressed as nmol of MDA/g of tissue. Data are presented as mean ± S.E.M. (*n* = 5). Asterisk presents the significant effect of SNP while different letters represent significant effect of the tested compounds.

by adding 200 µl 8.1% SDS to S1, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% of thiobarbituric acid (TBA). This mixture was incubated at 95ºC for 1 h. TBARS produced were measured at 532 nm and the absorbance was compared to that of a standard curve obtained using malondialdehyde (MDA).

Results and Discussion

Table 1-2 shows the anti-oxidant behavior of all tested compound in brain homogenate. It is considered that the electron donating groups increase the electronic density on selenium atom and theoretically it can increase the anti-oxidant activity. To prove the hypothesis we introduced an electron donating (mesomerically i.e. methoxy group) on C-2. We further managed to synthesize two isomers. First we introduced a methoxy group at ortho position (C-3). The introduction of the electron donating group at ortho position significantly improved the anti-oxidant behavior. In the same way when a methoxy group was introduced at para position (C-4) the resulting compound showed significantly higher antioxidant potential than C-3. This result proves that para isomer is a better anti-oxidant than ortho isomer. To verify the position effect, another electron donating group, this time inductively electron donating group i.e. methyl (CH_3) group was introduced. And as expected the ortho substituted (CH_3) group (C-5) showed significantly higher anti-oxidant behavior than C-1 & C-2. Similarly (C-6) with para (CH_3) group displayed higher antioxidant activity than (C-5). The results demonstrate that the antioxidant activity significantly depend on the electronic effects of the substituents on the aromatic ring.

To prove the hypothesis that electron donating group present on the phenyl ring which has a direct bond with selenium would be a better anti-oxidant. We synthesized three- isomers i.e. C-7, C-8 and C-9 where the same electron donating group i.e. (CH_3) was introduced on benzyl ring (This ring does not have a direct bond with selenium). All resulting compound showed significantly lower antioxidant activity than C-6 where methyl (CH_3) group was directly attached to phenyl ring (bonded directly with selenium). These structural isomeric effects confirm the supposition that isomerisation have a profound effect on the anti-oxidant activity of organoselenium compounds. We took another step in this regard and introduced an electron withdrawing group directly attached to phenyl ring i.e. C-10. The results indicated that C-10 does not posses any anti-oxidant activity rather at highest concentration it showed pro-oxidant behavior.

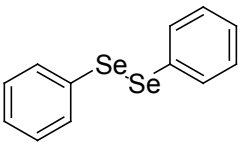
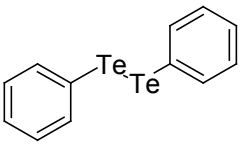
Concentration (µM)	nmol of MDA (TBARS)
Diphenyl Diselnide (DPDS) 	
Basal (B)	218±16
SNP –Induced [a*]	351±31*
SNP + 4µM [a]	334±30
SNP + 10µM [b]	196±12
SNP + 40µM [b]	154±19
SNP + 100µM [b]	67±21
Diphenyl Ditelluride (DPDT) 	
Basal (B)	274 ±21
SNP –Induced [a*]	536 ±34*
SNP + 1 µM [a]	503 ±25
SNP + 1.2 µM [b]	341 ±19
SNP + 1.4 µM [c]	213 ±17
SNP + 1.6 µM [e]	44 ±7

Table2: Effect of Diphenyl Diselenide (DPDS) and Diphenyl Ditelluride (DPDT) on TBARS production in rat's brain preparation. TBARS are expressed as nmol of MDA/g of tissue. Data are presented as mean ± S.E.M. (n = 5). Asterisk presents the significant effect of SNP while different letters represent significant effect of the tested compounds.

Earlier studies have indicated that photodegradation of SNP ultimately produces NOd, $[(CN)\ 5\text{-}Fe]^{3+}$ and $[(CN)4\text{-}Fe]^{2+}$ species [19,20]. NO is a molecule that is regarded as a universal neuronal messenger in the central nervous system, in the pathophysiology of such disorders as Alzheimer's and Parkinson's diseases, stroke, trauma, seizure disorders, etc. [21,22]. The result presented in (Table 1-2) indicated that organoselenium exerted an antioxidant effect on in vitro SNP induction of lipid peroxidation in brain homogenate. The NO released from SNP added in the incubation medium can undergo reaction with superoxide radicals to afford peroxynitrite. Peroxynitrite is a potent free radical and is capable of inducing oxidative damage to several biomolecules, including membrane phospholipids [19]. Thus, organoselenium might be conferring its protective effect by decomposing lipid hydroperoxides resulted from lipid peroxidation chain reaction caused by NO released from SNP. Another possible explanation might be the direct interaction between organoselenium and SNP or its derivatives. It should be noted that the organoselenium anti-oxidant activity was not modified by change in pro-oxidant as apparent from results.

It would be important to mention that when the diselenide bond of diphenyl diselenide is disrupted, two selenols can be yielded, differently from monoselenides, improving the catalytic reaction that is of particular significance to living cells. This reflects in the better anti-oxidant potential of diphenyl diselenide (a diselenide) (Table 2) than all mono selenides (Table 1). The anti-oxidant potency of DPDT (Table 2) can be explained by the fact that organotellurium compounds are readily oxidized from the divalent to the tetravalent state. This property makes them attractive as scavengers of reactive oxidizing agents such as hydrogen peroxide, hypochlorite, and peroxyl radicals, and as inhibitors of lipid peroxidation in chemical and biological systems [23]. This study also suggests that diorganoyl ditelluride are more reactive than structurally related diorganoyl mono/diselenide compounds. The higher potency of DPDT can be explained, essentially due to the higher electro negativity in relation to carbon associated with a larger atomic volume of the tellurium atom.

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