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Protective role of aryl and alkyl diselenides on lipid peroxidation☆

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Abstract

The concept that selenium-containing molecules may be better nucleophiles (and therefore antioxidants) than classical antioxidants has led to the design of synthetic organoselenium compounds. In the present study we appraised the antioxidant potential, thiol peroxidase activity, and rate of dithiotreitol and reduced glutathione oxidation of simple organodiselenide compounds in rats and mice. The present results demonstrate that alkyl and aryl diselenides are antioxidant compounds. We verified that the substitution on the aromatic moiety of diphenyl diselenide or the replacement of on aryl group by an alkyl substitute on diselenides changes their antioxidant and thiol peroxidase-like properties. The diaryl diselenides $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ presented higher thiol peroxidase activity and demonstrated better antioxidant potential than the other diselenides tested. In fact, the results revealed that alkyl diselenides, at low concentrations, were prooxidants and that aryl diselenides did not present this effect. Alkyl diselenides [$(C_2H_5Se)_2$ and $(C_3H_7Se)_2$] demonstrated a higher potential for –SH group oxidation than aryl diselenides. In addition, this study demonstrated that diselenide protection against lipid peroxidation was different in mice and rats. The compounds tested acted more as antioxidants in the brains of mice than in the brains of rats.

Keywords: Diselenides; Antioxidant; Lipid peroxidation; Thiol peroxidase mimic

1. Introduction

Partially reduced derivatives of oxygen, which are produced in aerobic organisms as part of normal physiological and metabolic processes, are toxic species, oxidizing numerous biomolecules, which leads to tissue injury and cell death. These reactive oxygen species are continuously formed in the human body and removed by enzymatic and nonenzymatic antioxidant defense systems under normal conditions (Yu, 1994).

The balance between prooxidants and antioxidants is critical for survival and functioning of aerobic organisms. An imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage, has been called oxidative stress (Sies, 1986). Accumulating evidence has linked the pathogenesis of a variety of human diseases to oxidative stress (Haddad, 2002). In the pathologic condition an overproduction or scavenger diminution of these reactive oxygen species can occur. There is increasing evidence that oxygen-free radicals contribute to cerebral ischemic injury by promoting membrane lipid peroxidation and oxidative damage to DNA and proteins (Siesjo et al., 1989; Taystman et al., 1991). In fact, the generation of reactive oxygen species has been implicated in cerebral tissues damage due to central nervous system trauma, ischemia–reperfusion injury, seizures, cerebral hemorrhage, and Parkinson's disease (Bankson et al., 1993; Lynch et al., 2000; Pazdernik et al., 1992).

Thus, when the natural protective systems against reactive oxygen species are overrun, exogenous antioxidative compounds must be delivered. Consequently, the search for new antioxidants as potential drugs is an active field of medicinal chemistry (Devillers et al., 2001).

The concept that selenium-containing molecules may be better nucleophiles (and therefore antioxidants) than classical antioxidants has led to the design of synthetic organoselenium compounds (Arteel and Sies, 2001). Several reports have been published on glutathione peroxidase (GSH-px)-mimetic compounds, which, like the native enzyme, rely on the redox cycling of selenium.

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

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The first example of such a compound was Ebselen (Daiber et al., 2000; Müller et al., 1984), which has been demonstrated to exert a protective role against brain ischemia and stroke (Dawson et al., 1995; Yamaguchi et al., 1998) and, in experimental models, for glutamate exitotoxicity (Porciúncula et al., 2001; Rossato et al., 2002a, b). The mechanism underlying the neuroprotection afforded by Ebselen is still not completely understood, but it is certainly related to its antioxidant and antiinflammatory properties (Saito et al., 1998; Takasago et al., 1997).

Based on the facts that the pharmacological properties of Ebselen are related to its thiol peroxidase-like activity and that simple diorganoil chalcogenides are GSH-px-mimetic compounds, even we have investigated the pharmacological properties of diphenyl diselenide. Of particular importance is that recent data from our laboratory have demonstrated that diselenide is a secure drug when administrated acutely to mice and rats in doses that have antiinflammatory and antinociceptive activity (Nogueira et al., 2003b). Furthermore, diorganoil chalcogenides are good antioxidants in vitro (Rossato et al., 2002b). In addition, while Ebselen is a complex molecule and consequently is expensive to synthesize, diselenides are easily synthesized and structurally simpler than Ebselen.

In the present study, we appraised the antioxidant potential, thiol peroxidase activity, and rate of dithiotreitol and reduced glutathione oxidation of simple organodiselenide compounds in rats and mice. Diselenides are good candidates for antioxidant agents because they have some chemical and biochemical characteristics is common with Ebselen, i.e., they possess glutathione peroxidase-like activity and are able to react with –SH groups (Wilson et al., 1989).

2. Material and methods

2.1. Materials

Diaryl diselenides [$(PhSe)_2$, $(p\text{-}CH_3PhSe)_2$, $(p\text{-}CH_3OPhSe)_2$, $(p\text{-}ClPhSe)_2$, $(o\text{-}H_2NPhSe)_2$, $(m\text{-}F_3CPhSe)_2$], Ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]-one), and dialkyl diselenides [$(C_2H_5Se)_2$, $(C_3H_7Se)_2$, $(C_4H_9Se)_2$, $(OHC_6H_{13}Se)_2$] were synthesized by previously described methods (Engman, 1989; Paulmier, 1986). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2. Animals

Male adult albino Wistar rats (150–200 g) and male adult Swiss albino mice (25–35 g) from our own breeding colony were used. The animals were kept in separate animal rooms, on a 12-h light/dark cycle, at a room temperature of 22°C, and with free access to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Medicine, Veterinary, and Animal Science of the University of Sao Paulo, Brazil.

2.3. Lipid peroxidation and thiobarbituric acid reactions

Animals were decapitated and whole brain tissue was rapidly homogenized in 50 mM Tris–Cl, pH 7.5 (1/10, w/v), and centrifugated at 4000*g* at 4°C for 10 min. An aliquot of 200 μL of homogenized brain of mice or rats was incubated at 37°C in the presence of dialkyl diselenides [$(C_2H_5Se)_2$, $(C_3H_7Se)_2$, $(C_4H_9Se)_2$, and $(HOC_6H_{13}Se)_2$] or diaryl diselenides [$(PhSe)_2$, $(p\text{-}ClPhSe)_2$, $(o\text{-}H_2NPhSe)_2$, and $(m\text{-}F_3CPhSe)_2$] at different concentrations (0.1–100 μM) for 1 h. TBARS was determined as described by Ohkawa et al. (1979).

2.4. Oxidation of dithiotreitol and reduced glutathione

The rate of thiol oxidation was determined in the presence of 50 mM Tris–Cl, pH 7.5, and 100 μM dialkyl diselenides [$(C_2H_5Se)_2$, $C_3H_7Se)_2$, $(C_4H_9Se)_2$, and $(HOC_6H_{13}Se)_2$], diaryl diselenides [$(PhSe)_2$, $(p\text{-}ClPhSe)_2$, $(o\text{-}H_2NPhSe)_2$, $(p\text{-}CH_3PhSe)_2$, $(p\text{-}CH_3OPhSe)_2$], or Ebselen. The rate of thiol oxidation was evaluated by measuring the disappearance of –SH groups. Free –SH groups were determined according to Ellman (1959). Incubation at 37°C was initiated by the addition of the thiol compounds GSH (1.0 mM) or dithiothreitol (DTT) (0.5 mM). Aliquots of the reaction mixture (200 μL) were checked for the amount of –SH groups at 412 nm.

2.5. Thiol peroxidase activity

The catalytic effects of alkyl and aryl diselenides on the reduction of H_2O_2 by reduced glutathione were assessed using the rate of GSH oxidation. Free –SH groups were determined according to Ellman (1959). Alkyl [$(C_2H_5Se)_2$, $C_3H_7Se)_2$, and $(C_4H_9Se)_2$], aryl diselenides [$(PhSe)_2$, $(p\text{-}ClPhSe)_2$, $(o\text{-}H_2NPhSe)_2$, $(p\text{-}CH_3PhSe)_2$, and $(p\text{-}CH_3OPhSe)_2$], or Ebselen at 30 μM was incubated in the medium containing GSH (1.0 mM) with and without H_2O_2 (0.3 mM). At 0, 10, 20, and 30 min, aliquots of the reaction mixture (200 μL) were checked for the amount of GSH.

2.6. IC_{50} determination

The IC_{50} was calculated by the method of Dixon and Webb (1964). Data are the means of four to six experiments.

2.7. Statistical analysis

Statistical significance was assessed by analysis of variance, followed by Duncan's test when appropriate. A value of $P<0.05$ was considered significant.

3. Results

3.1. Effect of aryl and alkyl diselenides on lipid peroxidation in mice

Diaryl diselenides [$(PhSe)_2$, (p-$ClPhSe)_2$), and (m-$F_3CPhSe)_2$] protected against lipid peroxidation at 10 μM, whereas (o-$H_2NPhSe)_2$ was only effective at 100 μM (Fig. 1A). In contrast, alkyl diselenides $(C_2H_5Se)_2$ and $(C_3H_7Se)_2$ increased lipid peroxidation at 10 μM (Fig. 1B). Dialkyl diselenides [$(C_4H_9Se)_2$, $(C_2H_5Se)_2$, and $(C_3H_7Se)_2$] reduced lipid peroxidation at 40 μM ($P<0.05$ by Duncan's multiple-range test). However, the $(HOC_6H_{13}Se)_2$ compound displayed this effect at a concentration as 70 μM.

3.2. Effect of aryl and alkyl diselenides on lipid peroxidation in rats

Diaryl diselenides [$(PhSe)_2$, (p-$ClPhSe)_2$, (m-$F_3CPhSe)_2$] and dialkyl diselenide $(C_4H_9Se)_2$ protected against lipid peroxidation at 10 μM in rats (Figs. 2A and B). Diselenides [$(C_2H_5Se)_2$, $(C_3H_7Se)_2$, $(HOC_6H_{13}Se)_2$, and $(H_2NPhSe)_2$] decreased lipid peroxidation only at the high concentration of 100 μM. In contrast, $(C_2H_5Se)_2$, $(C_3H_7Se)_2$, and $(HOC_6H_{13}Se)_2$ at 10 μM increased lipid peroxidation (Fig. 2B).

3.3. Comparative effect of diselenides on lipid peroxidation in rats and mice

The IC_{50} data (Table 1) demonstrated that compounds $(C_2H_5Se)_2$, $(C_3H_7Se)_2$, $(C_4H_9Se)_2$, and

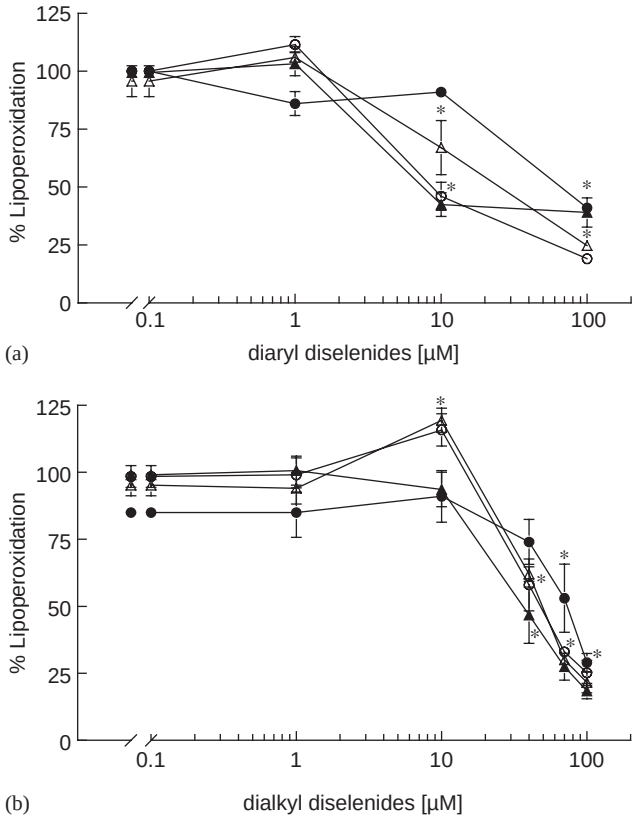

Fig. 1. Effects of aryl and alkyl diselenides on lipid peroxidation in mice brains. (A) Diaryl diselenides ○, $(PhSe)_2$; △, (p-$ClPhSe)_2$, ▲, ($F_3CPhSe)_2$; and ●, ($H_2NPhSe)_2$ at different concentrations (0.1–100 μM). (B) Dialkyl diselenides △, $(C_2H_5Se)_2$, ○, $(C_3H_7Se)_2$, ▲, $(C_4H_9Se)_2$; and ●, $(HOC_6H_{13}Se)_2$ at 0.1, 1, 10, 40, 70, and 100 μM for TBARS determination. The results are represented as lipid peroxidation percentage of vehicle DMSO. $P<0.05$ by Duncan's tests. Data are reported as means ± SEM of five experiments.

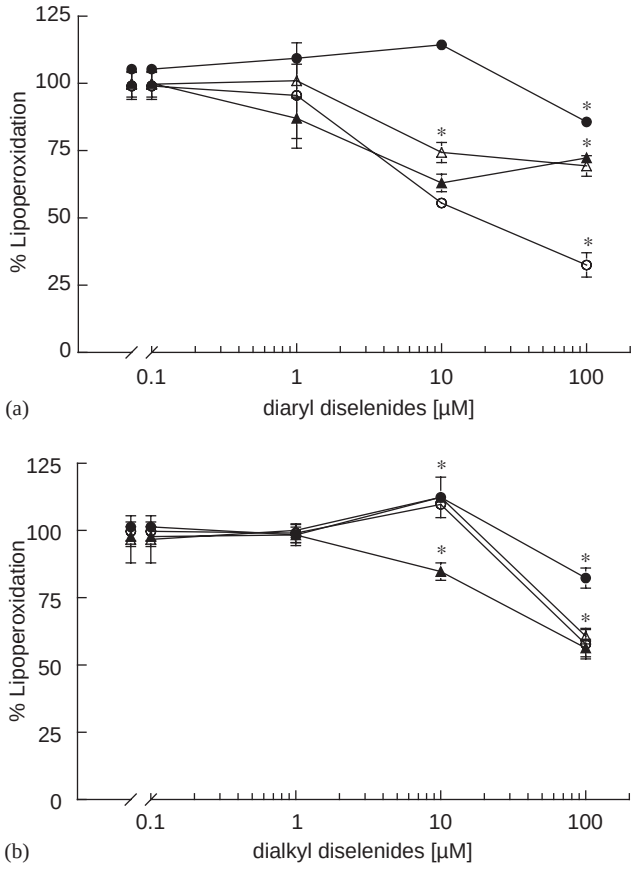

Fig. 2. Effects of aryl and alkyl diselenides on lipid peroxidation in rats. (A) Diaryl diselenides ○, $(PhSe)_2$; △, (p-$ClPhSe)_2$; ▲, ($F_3CPhSe)_2$; and ●, ($H_2NPhSe)_2$ at different concentrations (0.1–100 μM). (B) Dialkyl diselenides △, $(C_2H_5Se)_2$; ○, $(C_3H_7Se)_2$; ▲, $(C_4H_9Se)_2$; and ●, $(HOC_6H_{13}Se)_2$ (0.1, 1, 10, 40, 70, and 100 μM) for TBARS determination. The results are represented as the lipid peroxidation percentage of vehicle DMSO. $P<0.05$ by Duncan's tests. Data are reported as means ± SEM of five experiments.

Table 1
IC_{50} of diselenides on the lipid peroxidation in brains of mice and rats

Compounds	Lipid peroxidation	
	Mice (μM)	Rats (μM)
$(C_2H_5Se)_2$	60.6±17.0	>100
$(C_3H_7Se)_2$	56.8±23.7	>100
$(C_4H_9Se)_2$	41.7±22.4	>100
$(HOC_6H_{13}Se)_2$	>100	>100
$(PhSe)_2$	28.5±1.5	33.5±26.5
$(p\text{-ClPhSe})_2$	35.3±1.5	>100
$(m\text{-}F_3CPhSe)_2$	>100	>100
$(o\text{-}H_2NPhSe)_2$	>100	>100

IC_{50} was calculated according to Dixon and Webb (1964). The data are expressed as means with SE from four to six experimental animals.

Table 2
Thiol peroxidase-like activity of diselenides

Compounds	Time (min)		
	10	20	30
Ebselen	100	100	100
$(PhSe)_2$	158.7	82.6	88.2
$(p\text{-ClPhSe})_2$	195	154.5	80.8
$(p\text{-}CH_3PhSe)_2$	117.5	86	63
$(o\text{-}H_2NPhSe)_2$	47.5	31.4	34
$(p\text{-}CH_3OPhSe)_2$	—	—	—
$(C_2H_5Se)_2$	203	88	—
$(C_3H_7Se)_2$	—	—	—
$(C_4H_9Se)_2$	41.25	30	—

Thiol peroxidase-like activity was determined according to the method of Ellman (1959). Data are the percentage of Ebselen thiol peroxidase-like activity. The data are expressed as means with SE from four to six experimental animals.

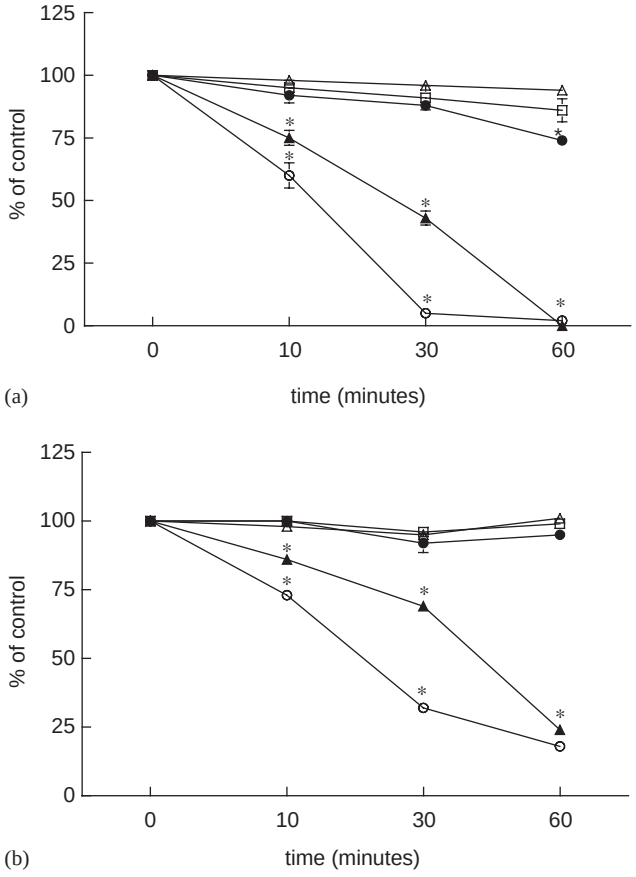

Fig. 3. Effects of alkyl diselenides ○, $(C_2H_5Se)_2$; ▲, $(C_3H_7Se)_2$; ●, $(C_4H_9Se)_2$; and □, $(HOC_6H_{13}Se)_2$ on the rates of DTT (A) and GSH (B) oxidation. The rate of oxidation was evaluated at different times (0, 10, 30, and 60 min). Data are the means of five to seven independent experiments. SEM was less than 10% of the respective mean. *Significant difference of control (△) DMSO.

$(p\text{-ClPhSe})_2$ were effective against lipid peroxidation and showed more antioxidant potential in mice than rats. Conversely, $(PhSe)_2$ was the most antioxidative compound and had similar activity in mice and rats (IC_{50} 28.5 and 33.5 μM, respectively). The compounds $(HOC_6H_{13}Se)_2$, $(H_2NPhSe)_2$, and $(m\text{-}F_3CPhSe)_2$ did not present good antioxidant profiles in rats and mice ($IC_{50}>100$ μM).

3.4. Effect of aryl and alkyl diselenides and Ebselen on thiol peroxidase-like activity

Compounds $(p\text{-ClPhSe})_2$, $(PhSe)_2$, and $(C_2H_5Se)_2$ demonstrated thiol peroxidase-like activity higher than that of, Ebselen, but this activity decreased in the reaction course. The thiol peroxidase-like activity of $(p\text{-}CH_3PhSe)_2$ was similar to that of Ebselen in the first 10 min (Table 2). Diselenides $(o\text{-}H_2NPhSe)_2$ and $(C_4H_9Se)_2$ showed thiol peroxidase-like activity lower than that of Ebselen. Diselenide compounds $(p\text{-}CH_3OPhSe)_2$ and $(C_3H_7Se)_2$ did not present a catalytic effect in this reaction (Table 2).

3.5. Effect of aryl and alkyl diselenides and Ebselen on oxidation of DTT and GSH

Alkyl diselenides $(C_2H_5Se)_2$ and $(C_3H_7Se)_2$ at 100 μM significantly increased the rate of DTT (Fig. 3A) and GSH oxidation (Fig. 3B). $(C_4H_9Se)_2$ oxidized DTT but did not alter GSH oxidation. In contrast, in the presence of $(HOC_6H_{13}Se)_2$ the rate of DTT and GSH oxidation did not change.

The most active DTT oxidant, $(p\text{-}CH_3OPhSe)_2$, was 10 times more oxidant than Ebselen. Aryl compounds $(o\text{-}H_2NPhSe)_2$, $(PhSe)_2$, and $(p\text{-}CH_3PhSe)_2$, were two times more DTT oxidant than Ebselen. In contrast, $(p\text{-ClPhSe})_2$ was less DTT oxidant than the other diaryl diselenides tested (Fig. 4A).

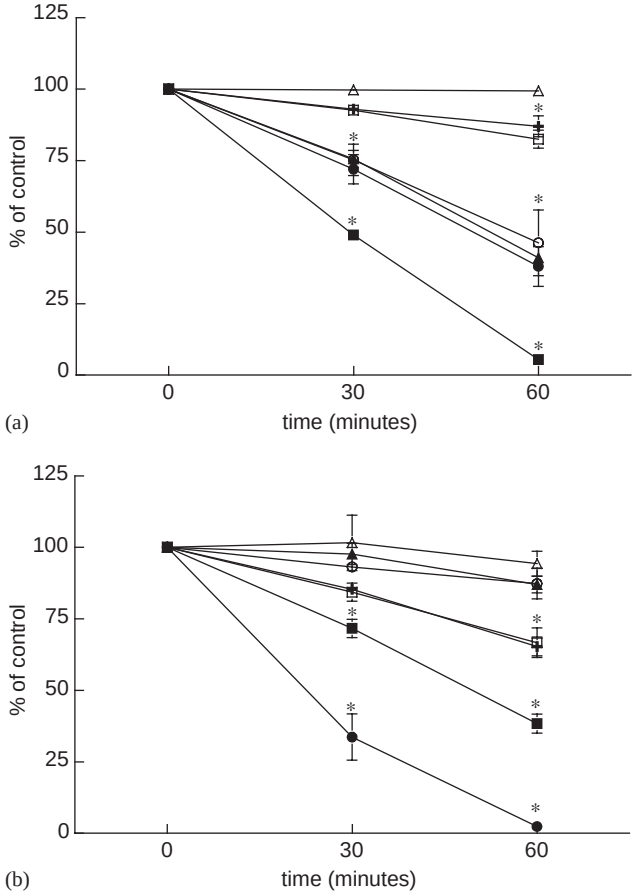

Fig. 4. Effects of (+) Ebselen and aryl diselenides ○, $(PhSe)_2$; ●, $(H_2NPhSe)_2$; □, $(p\text{-}ClPhSe)_2$; ▲, $(p\text{-}CH_3PhSe)_2$; ■, $(p\text{-}CH_3OPhSe)_2$ on the rate of DTT (A) and GSH (B) oxidation. The rate of oxidation was evaluated at different times for (0, 30, and 60 min) DTT oxidation and (0, 30, and 120 min) GSH oxidation. Data are the means of five to seven independent experiments. SEM was less than 10% of the respective mean. *Significant difference of control (△) DMSO.

$(o\text{-}H_2NPhSe)_2$ was more GSH oxidant than $(p\text{-}CH_3OPhSe)_2$, Ebselen and $(p\text{-}ClPhSe)_2$. $(PhSe)_2$ and $(p\text{-}CH_3PhSe)_2$ did not demonstrate GSH oxidant potential at 30 and 120 min (Fig. 4B).

4. Discussion

An antioxidant defense is mounted multiples strategies and at various levels. However, it is clear that selenium and GSH contribute significantly to the defense of the organism. Several attempts have been made to synthesize low-molecular-weight antioxidant compounds that utilize the redox activity of selenium (Cotgreave et al., 1992; Müller et al., 1984; Rossato et al., 2002b; Sies, 1993). The present results demonstrate that alkyl and aryl diselenides can be considered potential antioxidant compounds.

Previous data have demonstrated that the substitution on an aromatic moiety of diphenyl diselenide or the replacement of an aryl group by an alkyl substitute on diselenides changes their effects (Nogueira et al., 2003a). Here we also verified that the substitution on an aromatic moiety of diphenyl diselenide or the replacement of an aryl group by an alkyl substitute on diselenides changes their antioxidant and thiol peroxidase like-properties. In fact, the diaryl diselenides $(PhSe)_2$ and $(p\text{-}ClPhSe)_2$ were the most potent antioxidants (IC_{50} 28.5 ± 1.5 and $35.3 \pm 1.5\,\mu M$, respectively) in mice. Conversely, alkyl diselenides such as $(C_4H_9Se)_2$, $(C_3H_7Se)_2$, and $(C_2H_5Se)_2$ presented the same antioxidant potential, but their antioxidant potential is different from that of $(HOC_6H_{13}Se)_2$ ($IC_{50} > 100\,\mu M$).

Recent results from our group suggest that the selenide effect depends on the species (rats or mice) (Nogueira et al., 2003a). Similarly, this study has also demonstrated that the protective effect of diselenides against lipid peroxidation is different in mice and rats. The compounds tested were more antioxidant in the brains of mice than in those of rats. When the IC_{50} data were considered, only diphenyl diselenide demonstrated similar antioxidant activity for rats and mice. Compare the IC_{50} of 28.5 ± 1.5 for mice and the IC_{50} of $33.5 \pm 26.5\,\mu M$ for rats.

The thiol peroxidase-like activity of diorganyl calchogenides can explain, at least in part, the in vitro antioxidant properties of these compounds (Müller et al., 1984; Parnham and Graf, 1991; Schewe, 1994; Sies, 1993; Wendel et al., 1984). $(p\text{-}ClPhSe)_2$ and $(PhSe)_2$ presented higher thiol peroxidase activity and demonstrated better antioxidant potential than the other diselenides tested. For the other aryl and alkyl diselenides we did not find a relationship between thiol peroxidase and antioxidant properties.

Although the peroxidase-like activity of diselenides may account for their antioxidant properties, the thiol–diselenide exchange catalyzed by chalcogenides may contribute to their toxicological properties by oxidizing relevant thiol-containing metabolites and proteins without consuming toxic substances such as peroxides. Selenides can react with –SH groups, forming selenosulfide or –SeH and disulfides (Wilson et al., 1989). In fact, alkyl diselenides [$(C_2H_5Se)_2$ and $(C_3H_7Se)_2$] demonstrated higher potential for –SH group oxidation than aryl diselenides. In addition, the present results verified that alkyl diselenides at low concentrations were prooxidants, in contrast, aryl diselenides did not present this effect. Some reports have suggested that the anticarcinogenic property of selenium compounds is likely due to the known toxicity of selenium compounds found in animals and humans (Spallholz, 1994, 1997). This property could be related to –SH groups oxidation and to selenium's ability to generate superoxide (Spallholz et al., 2001).

As noted above, the results of the present investigation provide very useful information about the importance of the synthetic organic selenium compounds that mimic the antioxidative activity of natural selenoprotein glutathione peroxidase and the potential role of these organic selenium compounds in reducing and preventing the toxic effect of peroxides. However, detailed toxicological studies of simple organoselenides are still scarce in the literature (Bolzan et al., 2002; Farina et al., 2001; Jacques-Silva et al., 2001; Maciel et al., 2000; Meotti et al., 2003; Nogueira et al., 2001a, b, 2003b; Parnham and Graf, 1991; Rossato et al., 2002a, b) and must be considered before suggesting a pharmacological use for these compounds.

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