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Studies on the antioxidant effect and interaction of diphenyl diselenide and dicholesteroyl diselenide with hepatic δ-aminolevulinic acid dehydratase and isoforms of lactate dehydrogenase

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ABSTRACT

Studies on the interaction of dicholesteroyl diselenide (DCDS) and diphenyl diselenide (DPDS) with hepatic δ-aminolevulinic acid dehydratase (ALA-D) and different isoforms of lactate dehydrogenase (LDH) from different tissues were investigated. In addition, their antioxidant effects were tested *in vitro* by measuring the ability of the compounds to inhibit the formation of hepatic thiobarbituric acid reactive species (TBARS) induced by both iron (II) and sodium nitroprusside (SNP). The results show that while DPDS markedly inhibited the formation of TBARS induced by both iron (II) and SNP, DCDS did not. Also, the activities of hepatic δ-aminolevulinic acid dehydratase (ALA-D) and different isoforms of lactate dehydrogenase (LDH) were significantly inhibited by both DPDS and DCDS. Moreover, we further observed that the *in vitro* inhibition of different isoforms of lactate dehydrogenase by DCDS and DPDS likely involves the modification of the groups at the NAD^+ binding site of the enzyme. Since organoselenides interacts with thiol groups on proteins, we conclude that the inhibition of different isoforms of lactate dehydrogenase by DPDS and DCDS possibly involves the modification of the thiol groups at the NAD^+ binding site of the enzyme.

1. Introduction

Physiologically, selenium is an essential element that participates in the antioxidative defense systems (Mugesh et al., 2001; Nogueira et al., 2004). Essentially, there are claims that the organoselenium compounds such as ebselen and diorganyl diselenide exhibit both glutathione peroxidase (GSH-Px)-mimetic and thioredoxin peroxidase mimetic activities (Arteel and Sies, 2001; Zhao and Holmgren, 2002) and these properties of organoselenium compounds may be related in parts to their observed *in vitro* antioxidative activity making them promising candidates as medicaments in the management of a number of degenerative diseases in which oxidative stress have been implicated in their aetiology (Nogueira et al., 2001, 2004). Hence, the synthesis and evaluation of pharmacological potency of selenium and its organo-compounds have continued to attract the attention of researcher for some decades (Nogueira et al., 2004). Ebselen, the first reported synthetic organoselenium compound, is a complex molecule and consequently expensive to synthesize. Hence synthesis and biological testing of other simpler and inexpensive forms of organoselenium compounds such as the diorganyl diselenide: diphenyl diselenides (DPDS), akyl and aryl diselenides with promising pharmacological potency have been given attention in recent times (Mugesh et al., 2001). In fact, we recently described the possible pharmacological potency of a novel diorganyl diselenide derived from two cholesterol steroidal molecules, dicholesteroyl diselenide (DCDS) which was recently synthesized in our laboratory (Kade et al., 2008, Scheme 1).

Although selenium compounds generally hold promise as potent antioxidant drugs, they are generally poisonous to mammalian systems (Nogueira et al., 2003), and in fact, despite considerable efforts at understanding precise mechanism involved in toxicity elicited by selenium compounds (Nogueira et al., 2004; Mugesh et al., 2001), chemistry of selenium poisoning is still an enigma: the toxic symptoms are complex and the mechanism of the poisoning is still obscure. Some investigators believe that selenium compounds exert their toxic effect through interference with certain enzyme systems in the living organism, particularly through mercaptide formation with important sulphydryl enzymes with

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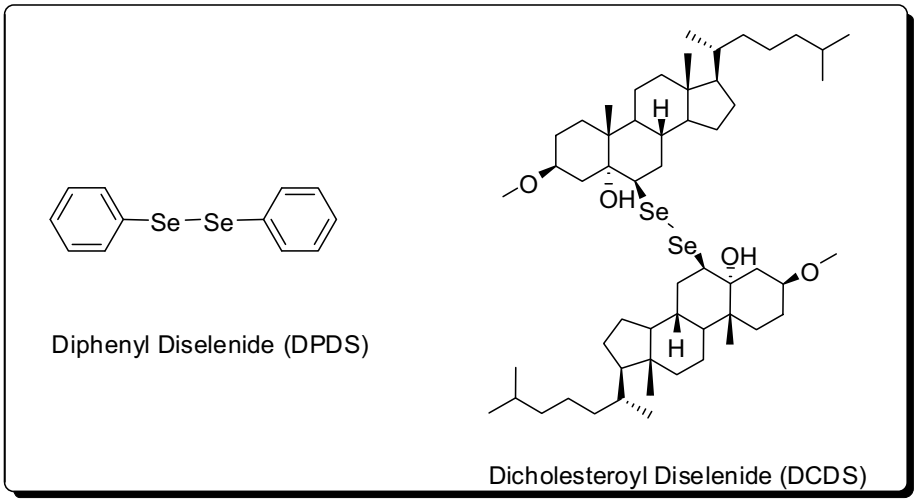

Scheme 1. Structure of diphenyl diselenide and dicholesteroyl diselenide.

physiological importance in biological systems (Nogueira et al., 2004). Two of these sulphydryl enzymes are δ-aminolevulinate dehydratase (δ-ALA-D) and lactate dehydrogenase.

δ-ALA-D is an important sulfhydryl containing enzyme (Rocha et al., 1995) that catalyses the condensation of two δ-aminolevulinic acid (δ-ALA), yielding porphobilinogen (PBG) which is a heme precursor (Jaffe, 1995). Inhibition of δ-ALA-D can block this pathway causing accumulation of heme intermediates, including its substrate, δ-ALA, which has been reported to posses some pro-oxidant activity under physiological conditions (Bechara, 1996). Earlier studies in our laboratory (Barbosa et al., 1998) have shown that the inhibition of δ-ALA-D by selenium compounds involves the oxidation of essential SH groups of the enzyme, since thiol protecting groups such as DTT and reduced glutathione (GSH) can reverse this inhibition. In addition, we also observed that the mechanism underlying the inhibitory effect of these compounds on δ-ALA-D is related to the oxidation of essential cysteinyl residues located at the active site of the enzyme.

On the other hand, lactate dehydrogenase (LDH), another thiol containing enzyme reversibly converts pyruvate and NADH into lactate and NAD^+. Generally, the isoenzymes of LDH are tetramers formed from two types of monomers (Koslowski et al., 2002). The two isoforms are labeled H (heart) and M (muscle), (formerly referred to as B and A, respectively) and their ratio varies between cell types. The LDH isoforms ratio has long been proposed to indicate the metabolic state of cells: it is believed that the M isoform favors lactate production while the H isoform favors pyruvate production (Stambaugh and Post, 1966). Therefore, LDH isoform ratio can serve as an indicator of the relative flux through aerobic/anaerobic gycolytic pathways. The enzyme contains two main domains: the coenzyme (nicotinamide adenine dinucleotide, reduced [NADH] or nicotinamide adenine dinucleotide [NAD^+] binding domain, which is highly conserved in the dehydrogenases (the "Rossmann fold"), and the substrate binding domain (Kutzenko et al., 1998). All isoforms of lactate dehydrogenases contain zinc as a functional component of the active site (Vallee and Wacker, 1956) and can be inhibited by sulfhydryl-binding reagents, such as *p*-chloromercuribenzoato (Neilands, 1954).

In our recent report (Kade et al., 2008), we observed that DCDS in comparison with DPDS have a weak glutathione peroxidase (GSH-Px)-mimetic activity and also a weak ability to oxidize both mono (glutathione and cysteine) and dithiols (DMPS, DMSA and DTT), explaining the reasons for the observed weak antioxidant effect and inhibition of thiol containing enzymes (cerebral ALA-D and Na^+/K^+-ATPase) by DCDS. However, since we earlier observed that the non-selenium moiety of the organochalcogens can have a profound effect on their antioxidant activities and also exert differential effects in their reactivities towards –SH groups from low molecular weight molecules and proteins (Kade et al., 2008), the present study therefore, sought to compare the antioxidant potential as well as the interaction of DPDS and DCDS with another two thiol containing proteins, namely hepatic δ-ALA-D and different isoforms of LDH.

2. Materials and methods

2.1. Chemicals

DPDS and DCDS (Scheme 1) were synthesized according to literature methods (Paulmier, 1986). These drugs were dissolved in 99% ethanol. Analysis of the ^{1}HNMR and ^{13}CNMR spectra showed that all the compounds obtained presented analytical and spectroscopic data in full agreement with their assigned structures. The purity of the compounds were assessed by high resonance mass spectroscopy (HRMS) and was higher that 99.9%. All other chemicals used were of analytical grade and obtained from Sigma–Aldrich, FLUKA, BDH and other standard commercial suppliers.

2.2. Animals

Male adult Wistar rats (200–250 g) from our own breeding colony were used. Animals were kept in separate animal rooms, on a 12 h light: 12 h dark cycle, at a room temperature of 22–24 °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, the Federal University of Santa Maria, RS, Brazil.

2.3. Preparation of tissue homogenate for (TBARS) assay

Rats were decapitated under mild ether anesthesia and the liver tissues was rapidly dissected, placed on ice and weighed. Tissues were immediately homogenized in cold 10 mM Tris–HCl, pH 7.5 (1/10, w/v) with 10 up-and-down strokes at approximately 1200 rev/min in a Teflon-glass homogenizer. The homogenate