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Dicholesteroyl diselenide: Cytotoxicity, genotoxicity and mutagenicity in the yeast *Saccharomyces cerevisiae* and in Chinese hamster lung fibroblasts

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ABSTRACT

The organoselenium compound, dicholesteroyl diselenide (DCDS) is a structural analogue of diphenyl diselenide (DPDS) and may be considered as a promising antioxidant drug *in vivo*. Nevertheless, little is known about the toxicological properties of DCDS. In the present study we evaluated the cytotoxic, genotoxic and mutagenic properties of DCDS in Chinese hamster lung fibroblasts (V79) and in strains of the yeast *Saccharomyces cerevisiae*, proficient and deficient in several DNA-repair pathways. The results with V79 cells show that DCDS induced cytotoxicity, GSH depletion and elevation of lipid peroxidation at lower concentrations than did DPDS. DCDS also generated single- and double-strand DNA breaks in V79 cells, both in the presence and in the absence of metabolic activation, as revealed by alkaline and neutral comet assays. Moreover, the induction of oxidative DNA base-damage was demonstrated by means of a modified comet assay with formamidopyrimidine-DNA glycosylase and endonuclease III. Treatment with DCDS also induced micronucleus formation in V79 cells as well as point and frame-shift mutations in a haploid wild-type strain of *S. cerevisiae*. Yeast mutants defective in base excision-repair proteins were the most sensitive to DCDS. Pre-incubation with *N*-acetylcysteine reduced DCDS's oxidative, genotoxic and mutagenic effects in yeast and in V79 cells. Our findings indicate that the presence of cholesteroyl substituents in DCDS results in elevation of its cytotoxic and genotoxic potential compared with that of DPDS in yeast and in V79 cells. However, due to dose-dependent contrasting behaviour of organoselenium compounds and differences in their toxicity in *in vitro* and *in vivo* systems, further studies are needed in order to establish the non-toxic concentration range for treatment in mammals.

1. Introduction

A number of new synthetic selenium compounds have been proposed as potential pharmacological agents, because they show a variety of biological activities as antioxidants, anti-tumour agents, neuro-protectors and immuno-modulators [1–3]. Selenium (Se) is an essential trace element for maintenance of many cellular processes in mammalian organisms [4,5]. However, depending on the concentration, Se compounds behave differently [6–8]. At higher concentrations, Se may induce oxidative stress by thiol depletion and generation of reactive oxygen species (ROS), leading to cellular damage [3,8]. At low levels, Se enhances the cellular antioxidant capacity [8,9] and also stimulates DNA repair [8,10]. Selenium is a component of the essential amino acid selenocysteine, which plays a role as structural component of selenoproteins, including glutathione peroxidase and thioredoxin reductase, both key enzymes in the control of redox homeostasis [3,4,11].

The synthetic organoselenium (OS) compound diphenyl diselenide (DPDS) (Fig. 1A) has been widely studied as a potential antioxidant and chemopreventive agent due to its high thiol-peroxidase activity and its ability to mimic the enzyme glutathione

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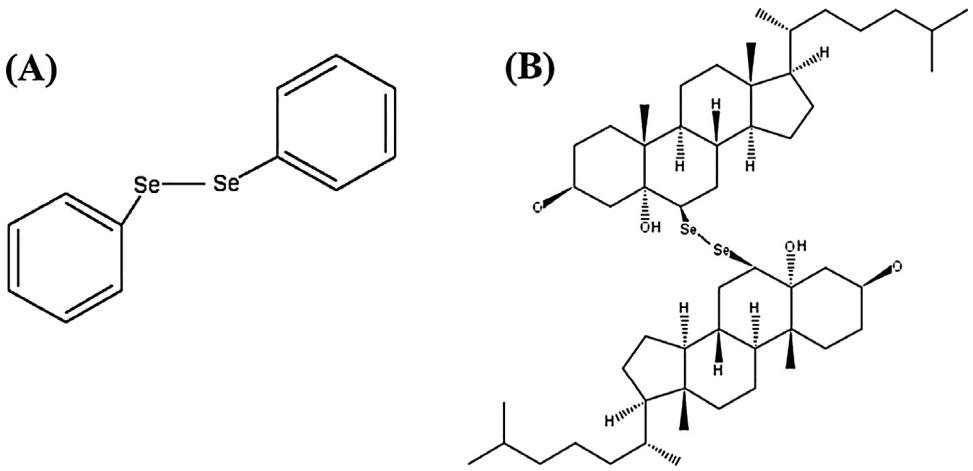

Fig. 1. (A) Chemical structure of diphenyl diselenide (DPDS) and (B) dicholesteroyl diselenide (DCDS).

peroxidase [12–15]. Studies by our group showed that low concentrations of DPDS protect against cytotoxicity induced by methyl methanesulfonate, UVC radiation and hydrogen peroxide, and reduce the DNA damage and chromosome breaks in Chinese hamster lung fibroblasts (V79 cells) by increasing glutathione peroxidase activity [13]. Moreover, hepatoprotective, neuroprotective, anti-inflammatory, and antinociceptive effects of DPDS have been reported [13,14,16,17].

On the other hand, high doses of DPDS caused adverse toxic effects in mouse brain, liver and kidney, as well as teratogenic effects [18–20]. DPDS was clearly genotoxic and mutagenic in V79 cells and in several organs of mice, due to its ability to deplete GSH. The observed effects could be attributed to adduct formation with GSH and thiol-containing proteins, leading to induction of the pro-oxidant status, as confirmed by an increase in thiobarbituric acid-reactive species (TBARS) and DNA damage [18]. In a study with microorganisms, DPDS induced frame-shift mutations in *Salmonella typhimurium* and in the haploid yeast strain *Saccharomyces cerevisiae* [21].

In view of the pharmacological potential of OS compounds as well as their contrasting dose-dependent behaviours, the development of novel derivatives with low DNA-damaging property seems to be important. Modification in the molecular structure of OS compounds can result in significant changes in their pharmacological and biological effects [3,22]. It is also known that compounds containing steroidal moieties could possess pharmacological potency [23]. These observations triggered the synthesis of a novel OS compound, *i.e.* dicholesteroyl diselenide (DCDS), with DPDS as a prototype (Fig. 1B). This compound possesses two cholesterol molecules as organic component [24].

In contrast with its structural analogue DPDS, little is known about the biological and toxicological properties of DCDS. The possible pharmacological potential of DCDS has been described by Kade et al. [24]. This derivative significantly increased the levels of GSH and vitamin C in mice, which is indicative of an antioxidant effect [25]. DCDS possesses weak reactivity towards thiols and also a weak peroxidase-mimetic activity, compared with DPDS and, consequently, DCDS had a weak inhibitory effect on thiol-containing proteins such as Na^+/K^+-ATPase and δ-ALA-D [24,25]. In contrast, DCDS significantly inhibited all isoforms of lactate dehydrogenase (LDH), and it showed pro-oxidant effects in rat brain [24–26]. It has been suggested that the different activities of DPDS and its derivative may be related to the influence of DCDS's organic component [24].

Considering that only a few data about the biological potential of DCDS are available, the aim of the present study was to investigate the effects of DCDS on cellular redox status and cell viability and its genotoxic and mutagenic activity in cultured V79 mammalian cells and in the yeast *S. cerevisiae*. The cytotoxicity of DCDS was measured by means of the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay and the lactate dehydrogenase (LDH) leakage test in V79 cells, and in a survival assay employing repair-deficient yeast strains. Alterations in the cellular redox status were evaluated by assessing the levels of GSH and lipid peroxidation in V79 cells. DCDS-induced DNA damage in V79 cells was estimated by use of the comet assay. The mutagenic potential of DCDS was clear from micronucleus formation in V79 cells and induction of frame-shift mutation in the haploid *S. cerevisiae* strain XV185-14c. The effect of pre-treatment with *N*-acetylcysteine on DCDS toxicity was also evaluated. This study is relevant to human health since it evaluates the genotoxic properties of DCDS in order to permit safe pharmacological applications and to estimate how changes in the structure of OS compounds could modify their reactivity.

2. Materials and methods

2.1. Chemicals

DCDS was provided by Dr. Antônio Braga, Chemistry Department, Federal University of Santa Catarina, Brazil. The ^{1}H NMR and ^{13}C NMR spectra showed that the analytical and spectroscopic data of DCDS fully agreed with the assigned structure. The chemical purity of DCDS (99.9%) was determined by gas chromatography/high-performance liquid chromatography [24,27]. Yeast extract, bacto-peptone and bacto-agar were obtained from Difco Laboratories (Detroit, MI, USA). Dulbecco's modified Eagle Medium (DMEM), foetal bovine serum (FBS), trypsin-EDTA, L-glutamine, and antibiotics were purchased from GIBCO BRL (Grand Island, NY, USA). L-histidine, L-threonine, L-methionine, L-tryptophan, L-leucine, L-lysine, nitrogenous bases (adenine and uracil), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, reduced glutathione (GSH), oxidized glutathione (GSSG), NADPH, glutathione reductase, thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrolyzed 1,1,3,3-tetramethoxypropane (TMP), *N*-acetylcysteine, 5,5′-dithionitrobenzoic acid (DTNB), cytochalasin-B (Cyt-B), methyl methanesulfonate (MMS), 4-nitroquinoline-oxide (4-NQO), cyclophosphamide (CP), hydrogen peroxide (H_2O_2), phenylmethylsulfonyl fluoride (PMSF), and 4-vinyl-pyridine were purchased from Sigma (St. Louis, MO, USA). Low melting-point agarose and normal agarose were obtained from Invitrogen (Carlsbad, CA, USA). Formamidopyrimidine DNA-glycosylase (Fpg, also known as MutM) and endonuclease III (Endo III, also known as Nth) were obtained from New England BioLabs (Ipswich, MA, England). The S9 fraction, prepared from the livers of Sprague-Dawley rats pre-treated with the polychlorinated biphenyl mixture Aroclor 1254, was purchased from Moltox (Annapolis, MD, USA).

2.2. Assays in V79 cells

2.2.1. V79 cell culture and treatments

V79 cells were cultured under standard conditions in DMEM supplemented with 10% heat-inactivated FBS, 0.2 mg/mL L-glutamine, 100 IU/mL penicillin, and