ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

*INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH*

RESEARCH ARTICLE

Potential OrganoseleniumRadiomodifiers against Gamma Radiation Effects

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Manuscript Info

Manuscript History:

Received: 22 November 2014
Final Accepted: 23 December 2014
Published Online: January 2015

Key words:

gamma radiation; organoselenium; radiomodifying effects; biological damage

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Abstract

This study is aimed at evaluating the potential radiomodifying properties of the compounds ebselen ($C_{13}H_9NOSe$) and diphenyl diselenide ($C_6H_5Se_2C_6H_5$). Phospholipids from chicken egg yolks were gamma irradiated and their concentration of MDA were measured in order to assess radiation damage. The resulting concentrations of Malondialdehyde in the samples were found to decrease with dose, being significantly lower in most cases than the control.Ebselen and diphenyl diselenide were considered potentially effective as positive radiomodifiers.

INTRODUCTION

Exposure to ionizing radiation can produce relevant toxic effects in living cells depending on the tissue at risk. Studies towards mitigation and/or avoidance of radiation induced free radicals damage have been made in the last few years(Ramos de Andrade, Da Costa Escobar Piccoli et al. 2009; Saada, Said et al. 2009; Andrade, Cruz et al. 2011; de Freitas, Boligon et al. 2013; Robson B. de Freitas 2014). Research on food or drugs with positive protective radiomodifying action is of great interest to Public Health being potential solution even for nuclear workers and homeland defense.

Ionizing radiation can interact with atoms or molecules in the cell, particularly water, producing radicalsor more frequently called "free radicals". Interactions of the ionizing radiation with cellular water contents may produce many metabolites such as aqueous electrons, superoxide and other fragments which are able to diffuse through the cell environment over sufficient distances to harm critical targets such as DNA and membranes. This is called indirect action of radiation.

The term "radical"is used to qualify an atom or group of atoms containing an unpaired electron, which results in great chemical reactivity(Halliwell 2007). Free radicals are usually indicated by a dot ($^{\bullet}$) placed to the left or right of the chemical symbol depending on the position of the unpaired electron. Most of the radiation energy transferred to the cells is absorbed initially in water, as cells contain around 70% – 80% of water, leading to the rapid production of oxidazing and reducing reactive intermediates (the OH radical [$^{\bullet}$OH], an oxidizing agent, is probably the most damaging), which in turn can react with other molecules in the cell(Riley 1994; Halliwell 2007).

On the other hand, some recent studies have been focused on possible positive effects of radiation on critical biological targets such as fat, membranes and DNA(Peak, Ito et al. 1995; Hodgkins, Fairman et al. 1996; Ly, Aguilera et al. 2007).

Lipid peroxidation (LPO) is defined as the oxidative degradation of polyunsaturated lipids. Cell membranes are recognized as one of the most important targets of those poisoning metabolites from LPO(Halliwell 2007).Lipid peroxidation usually starts with hydrogen atom abstraction from a methylene group ($-CH_2-$), resulting in a carbon radical. Hydroxyl radical can initiate the peroxidation of fatty acids, membranes and lipoproteins, also being able to attack extrinsic carbohydrates and proteins (e.g. cell surface glycoproteins) and "head groups" of phospholipids(Riley 1994; Puthran, Sudha et al. 2009). It is very suggestive that biological material under intense radiation exposure presents increased LPO. This dangerous effect can be mitigated by the presence of OH• radical scavengers such as flavonoids, polyphenols found in foods and beverages grape-derived(Ramos de Andrade, Da Costa Escobar Piccoli et al. 2009; Saada, Said et al. 2009; Andrade, Cruz et al. 2011; de Freitas, Boligon et al. 2013; Robson B. de Freitas 2014). Flavonoids can be carried out through the cell membrane in and out of the cell environment, acting as a good radical scavenger.

1.1 Malondialdehyde (MDA) and TBARS assay

Malondialdehyde arises largely from peroxidation of polyunsaturated fatty acids (PUFAs). It exists in several forms, depending on pH, reacting with DNA bases leading to mutagenic lesions(Puthran, Sudha et al. 2009). TBARS assay is one of the oldest and most frequently used methods for measuring lipid peroxidation. The method consists in the analysis of the end products of LPO, which by reacting with 2-thiobarbituric acid (TBA) form colored complexes characterized by spectrophotometry(Gonzalez-Flecha and Demple 1994).

1.2 Organoselenium compounds

Selenium is an essential trace element, whose nutritional essentiality was demonstrated in 1957 in rats(Schwarz and Foltz 1999). This chalcogen presents antioxidant property (Nogueira, Zeni et al. 2004). The concept that selenium-containing molecules may be better nucleophiles (antioxidants) than classical antioxidants have led to the development of synthetic organochalcogens(Arteel and Sies 2001).As a consequence, anefforthasbeenmade for describingtheantioxidantactivityof ebselen anddiphenyl diselenideaccordingtodifferent experimental models(Nogueira, Zeni et al. 2004).

The present study is focused on evaluating the potential radiomodifying activity of the ebselen and diphenyl diselenide against radiation-induced reactive species in phospholipids"in vitro".

2. MATERIALS AND METHODS

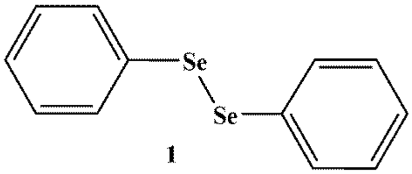
2.1Phospholipid cross-section analysis

It is very important that organoselenium compounds show water-like behavior.This assure that all free radicals coming up from gamma radiation interactions are from phospholipids.Provide that simulations using cross sections theory were performed. Atomic cross sections and attenuation coefficients for the phospholipids were calculated by using a web program called XCOM.Mayneord's formulawas employed to compute the effective atomic number, which is used in the XCOM calculations(Goodsitt, Christodoulou et al. 2011).The effective atomic number (Z_{eff}) for water, diphenyl diselenide and ebselen are presented in table 1.

2.2Irradiation and phospholipid TBARS

Irradiation was performed with the Theratron 780C ^{60}Co gamma ray (average photon energy of1.25MeV) facility for radiotherapy located at the University Hospital of Santa Maria. Doses ranged from 0 (non-irradiated) up to 50 Gy.TBARS production from phospholipid was determined using Ohkawa's Method(Ohkawa, Ohishi et al. 1979). Chicken egg yolk,which is very rich in phospholipids,was used as an intermediate alternative way to avoid using animals.

3. RESULTS AND DISCUSSION



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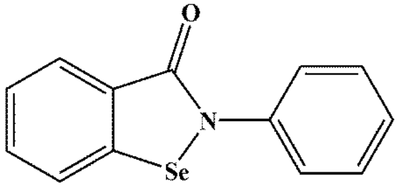

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Fig. 1 - Ebselen (C13H9NOSe) and DiphenylDiselenide (C6H5Se2C6H5) structure

Table 1 - Effective atomic number (Z_{eff}) from Mayneord's formula for water, diphenyl diselenide and ebselen

	Diphenyl diselenide ($C_6H_5Se_2C_6H_5$ – CAS Number 1666-13-3)	Ebselen ($C_{13}H_9NOSe$ – CAS Number 60940-34-3)	Water (H_2O)
Z_{eff}	26,04	21,36	7,42

Figure 2 (A to C) shows the photoelectric cross section for the substances used in the experiment, as a function of incident radiation energy compared to water (figure 2A). It should be noticed that photon peaks appear around 10^{-2} MeV, which is smaller than the energy used biological stressor (1.25 MeV) by a factor of 10^4. Also, the same pattern of cross section curve is followed by all substances with no differences when compared to water. It suggests that there will beno contribution from ebselen and diphenyl diselenide to LPO of phospholipids.

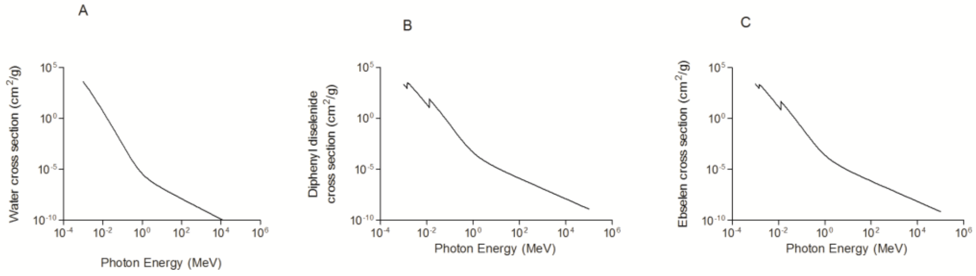

Fig. 2 - Photoelectric effect cross sections for ebselen and diphenyldiselenide, for photons beam ranged from 1 keV up to 10 MeV

Figure 3A and 3B show results from TBARS measurements. Figures 4A and 4B show the relative protection observed in different radiation doses for the organoselenium compounds, potential radiomodifiers.

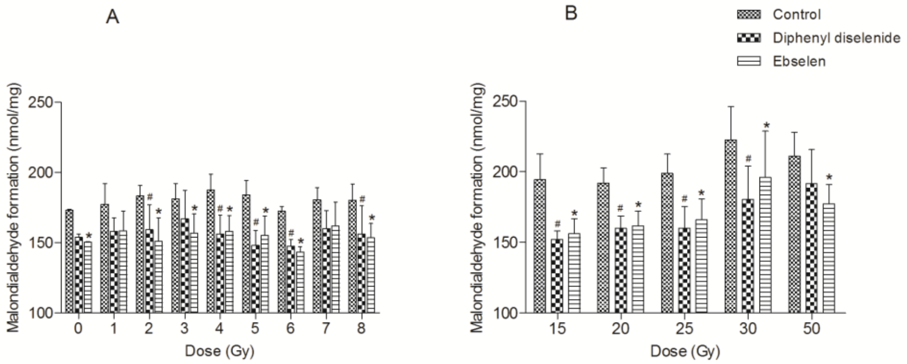

Fig. 3 – TBARS test results. Amount of MDA present in samples after irradiation with doses ranging from 0 (non-irradiated) to 50 Gy.**#** means significant difference between diphenyl diselenide and control and, ***** means significant difference between control and ebselen. There is no significant difference between diphenyl diselenide and ebselen

Results from figures 3A and 3B suggests thatorganoselenium compounds acts as positive radiomodifiers, mitigating radiation damage to phospholipids at all radiation doses tested. The overall radiomodifying effect was apparently the same for both compounds, even though relative differences between diphenyl diselenide and ebselen were found. Also, it is suggestive that the increasingly absorbed radiation dose is not necessarily followed by an increase in the MDA formation. This may suggests that the MDA formation is not dose-dependent linear function.

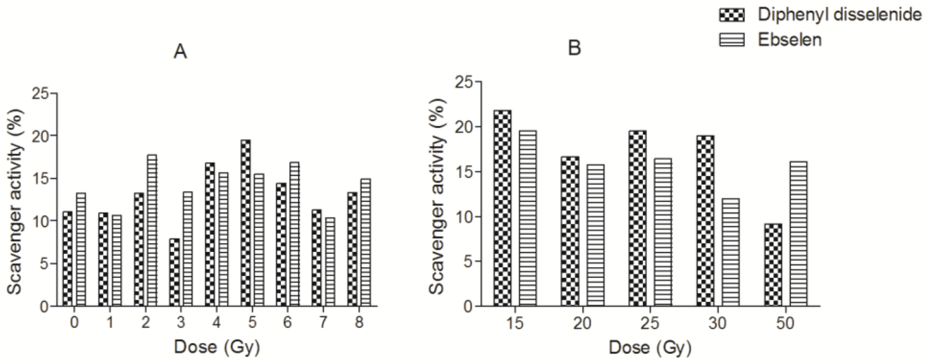

Fig. 4 – Relative scavenging activity (%) of diphenyl diselenide and ebselen over irradiated phospholipids, in comparison with controls. Figure 4A for doses below 10 Gy and fig. 4B for doses above 10 Gy

Results from figure 4A and 4B show the relative radiomodifying action of organoseleniumcompounds.The relative efficiency of the compounds was higher at 15-Gy dose.However, this dose is quite high, out of the range for that current treatments allow appreciable human survival.

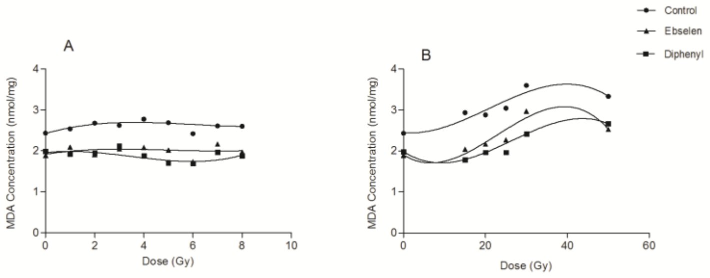

Fig. 5 – Adjusted curves for experimental data of scavenging activity for diphenyl diselenide and ebselen. Fig. 5A being for doses below 10 Gy and fig. 5B for doses above 10 Gy.

Results from figure 5 shows functions that fit the experimental data. Those functions are smoother for doses below 10 Gy (Fig. 5A). The smooth behavior of the curves suggests that, for doses below 10 Gy, the scavenging phenomenon is not strongly dependent on radiation dose. Likewise, from figure 5B, the scavenging phenomenon seems to be dependent on radiation dose. Such behavior may be associated with an increasingly free radical production, which is dose-dependent, suggesting a saturation limit for the scavenging activity.

A reasonable explanation for the organoselenium compound activity could be based on the bond energy exchanges involved in the radical scavenging phenomena (see Fig.7). The mechanism of selenol (6) generation of ebselen (2) in vivo is initiated by a thiol group, usually glutathione (GSH) in its reduced form. An explanation for the reaction invokes the enthalpy variation, based on the bond energies involved in the process which is about (-)7 kcal/mol. The process is thus slightly favorable in terms of enthalpy variation. The selenol (6) produced is then able to react with reactive oxygen species, as hydroxyl radical ($^{\bullet}$OH) and hydrogen peroxide (H_2O_2), acting like a peroxidase. Diphenyl diselenide (2) acts similarly, although its conversion to phenyl selenol(4) occurs in the presence of cysteine instead of glutathione(Kerr 1966).

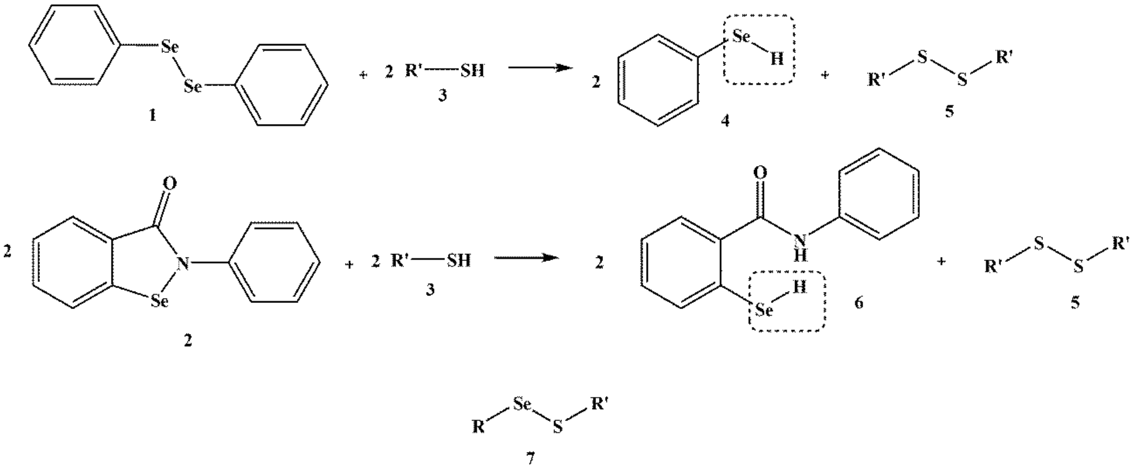

Fig. 6 – Overall process of selenols (4)and (6) formed from (1)and (2)

The Se—Se bond found in diphenyl diselenide (1) presents dissociation enthalpy of around 79 kcal/mol while Se—N bond existing in ebselen (2) presents 90 kcal/mol. Therefore, the radical form of diphenyl diselenide (1) is probably preferably formed from ebselen (2) instead of selenol. As a result, under the experimental conditions, organoselenium compounds can react directly with the hydroxyl radical to yield seleninic acids (8) and (9). Likewise, hydroxyl scavenging can also include the reaction between TBA and organoselenium compounds to yield selenols(4) and (6) in addition to forming the S-Se bond, which in turn is the activation process that normally occurs in vivo (see figure 7).

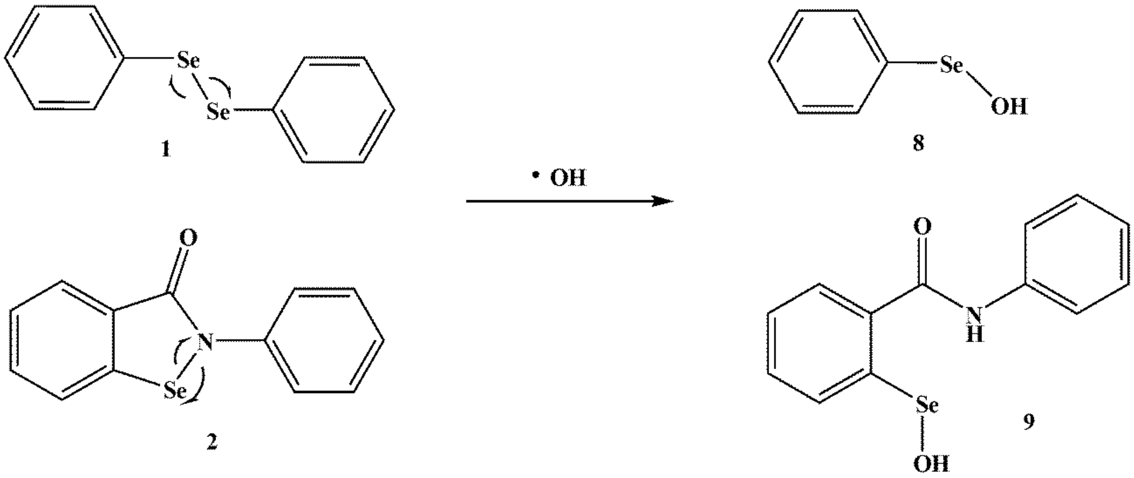

Fig. 7 – Hypothesis for formation of seleninic acids from diphenyl diselenide (1) and ebselen (2) directly from hydroxyl radical

3.1 Statistical Analysis

The software package GraphPad®Prism 5 was used for data analysis. Significant differences between means were evaluated by analysis of variance (Two-way ANOVA) and in the case of significance an appropriated *post-hoc* test was also applied. Differences were considered significant when $p \leq 0.05$.

4. CONCLUSIONS

The findings suggest that diphenyl diselenide and ebselen are effective in mitigating the indirect radiation damage, lipid peroxidation, expected in phospholipids for the type of radiation and energy ranges tested. Data analysis suggests that both compounds have approximately the same efficiency. Further studies should be performed in order to explore the mechanism of scavenging the hydroxyl radical. In the near future these organoselenium compounds may be employed in investigations aimed at obtaining: (a) more accurate data on toxicity for humans when coupled with other drugs, (b) the experimental photoelectric cross section, (c) results from experiments based on “in silicon”, “in vivo” and “ex vivo” environments, (d) the efficiency of organoselenium compounds in radiotherapy, and (e) an optimized experimental design that would also account for physiological effects and interactions with other metabolic processes. Finally, it can be concluded that the present findings are consistent with the hypothesis that the synthesis of organoselenium compounds is a potential source of new radiomodifiers.

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