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Semiquinone glucoside derivative (SQGD) isolated from *Bacillus* sp. INM-1 protects against gamma radiation-induced oxidative stress

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ABSTRACT

In the present study, radioprotective potential of Semiquinone glucoside derivative (SQGD) isolated from radioresistant bacterium *Bacillus* sp. INM-1 was evaluated. γ-Radiation induced protein carbonylation, plasmid DNA damage, enzyme functional impairment, lipid peroxidation, HO• radicals generation and their protection by SQGD was assessed. As a result of SQGD treatment, significant inhibition ($p < 0.05$) in protein carbonylation was observed with BSA. SQGD treatment was found to restore supercoiled (~70 ± 3.21%) form of irradiated plasmid DNA against γ-irradiation. SQGD protects enzymes (EcoR1 and BamH1) against radiation-induced dysfunctioning. SQGD significantly inhibited ($p < 0.05$) lipid peroxidation in liposomes, brain and liver homogenate. Higher HO• radicals-averting activity of SQGD was observed in the serum and liver homogenate of C57BL/6 mice against H_2O_2-induced oxidative stress. In conclusion, SQGD demonstrates excellent radical-scavenging activity towards bio-macromolecules in irradiated environment and can be developed as an ideal radioprotector against radiation-induced oxidative stress in future.

1. Introduction

Ionizing radiation induces oxidative stress *via* generation of free radical species i.e. hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot}$), singlet oxygen (O), hydrogen peroxide (H_2O_2) and nitric oxide ($NO^{\cdot}$) radicals (Sadani and Nadkarni, 1997; Mishra et al., 2013; Kumar et al., 2011a) in cellular environment. Free radicals-mediated oxidative stress induces lipid peroxidation in the biological membranes and oxidation in signalling proteins, enzymes and DNA, which is considered deleterious to the cell fate, if not repaired (Dalle-Donne et al., 2003). Carbonylation of proteins and inactivation of enzymes are considered as the most sensitive markers of radiation-induced oxidative stress (Chevion et al., 2000; Sacter, 2000). Formation of protein carbonyl groups has also been reported in several diseases including Alzheimer's, diabetes, inflammatory bowels disease and arthritis and responsible for their pathological conditions (Smith et al., 1991; Grattagliano et al., 1998; Telci et al., 2000). Radiation-induced oxidation of enzymes particularly at their catalytic sites, affects their catalytic functions (Raaphorst et al., 1993). Interaction of free radicals with proteins or

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enzymes leads to cleavage of disulphide bonds and thus significantly impairs their regulatory and catalytic activities (Simic et al., 1986; Manning et al., 1989). DNA and RNA molecules are exceptionally labile to oxidative stress caused by ionizing radiation. Oxidative stress induced structural modifications in cellular DNA lead to transformations, mutations and cell death, if not repaired (Hansama, 2001). Irradiation to supercoiled plasmid DNA induces double strand breaks and converts it into circular or linear form. γ-Irradiation also induces conformational relaxation in plasmid DNA leading to loss of their self-replication properties *in vivo* (Smialek et al., 2009).

Hydroxyl radicals ($OH^{\bullet}$) generated by water radiolysis is considered as a rapid initiator of lipid peroxidation. $OH^{\bullet}$ radicals are known to remove electron from unsaturated fatty acids (Rollet-Labelle et al., 1998). $OH^{\bullet}$ radicals also react with polyunsaturated fatty acids and produce lipid radicals ($L^{\bullet}$), which in turn react with molecular oxygen and form lipid peroxyl radicals ($LOO^{\bullet}$). The $LOO^{\bullet}$ can abstract electron from the neighbouring fatty acid to produce lipid hydro-peroxides ($LOOH^{\bullet}$) and secondary lipid radicals (Catala, 2006). This destructive chain reaction alters integrity, fluidity, permeability and functionality of bio-membranes. Oxidative lipid chain reaction also damages membrane bound enzymes and converts low density lipoprotein (LDL) to pro-atherogenic and pro-inflammatory forms and generates potential toxic (mutagens and carcinogens) lipid peroxidation products (Laughton et al., 1989; West and Marnett, 2006; Greenberg et al., 2008). Hence, it is important to search for protective agents which may prevent radiation-induced lipid peroxidation.

Radioresistant bacteria may have the capability to synthesize novel biomolecules which may provide protection to their own DNA, proteins, enzymes and membrane lipids during irradiation and post irradiation recovery periods. With this background, a semiquinone glucoside derivative (SQGD) was isolated and purified from a radioresistant bacterium *Bacillus* sp.INM-1 (Deposited in NCBI gene bank with an accession number EU 240544.1). Antioxidant, immune-stimulatory properties (Kumar et al., 2011a, 2011b) and radioprotective efficacy of SQGD towards reproductive, gastrointestinal (Patel *et al.*, 2012a, 2012b) and renal system have been evaluated (Mishra et al., 2013) in the murine model. The present study was focused to find out the radioprotective efficacy of SQGD against γ-radiation-induced oxidative damage to the biomolecules in *in vitro* and *ex vivo* models.

2. Materials and method

2.1. Material

pUC19 plasmid, BamH1, EcoR1and lambda DNA were procured from Fermentas, Vilnius, Lithuania. Cholesterol, soya lecithin, and agarose were purchased from Sigma-Aldrich, USA. Tris–base, acetic acid, ethylene-diamine-tetra-acetic acid (EDTA), 2,4-dinitrophenylhydrazine, guanidine-HCl, trichloroacetic acid, ethyl acetate and thiobarbituric acid were procured from Calbiochem, Dermstadt, Germany. Ethanol was purchased from Haymann Chemicals, Essex, England. While, HORAC Assay kit was procured from Cell Biolabs, USA.

2.2. Methods

2.2.1. Isolation and characterization of semiquinone glucoside derivative (SQGD)

Isolation and chemical characterization of semiquinone glucoside derivative (SQGD) were carried out using solvent fractionation and extraction procedures:column chomatography, UV–visible spectroscopy, Fluorescence spectroscopy, FTIR spectroscopy, ^{H}NMR spectroscopy, LC/MS spectroscopy and electron-paramagnetic resonance spectroscopy as described in our previous reports (Kumar et al., 2011a, 2011b).

2.2.2. In vitro detection and quantification of protein carbonylation in irradiated Bovine Serum Albumin (BSA) and its modulation by SQGD treatment

Levine method was used (Levine, 1990) to quantify the degree of carbonylation in Bovine Serum Albumin (BSA) and its protection against radiation-induced damage by SQGD treatment. In brief, aqueous solution (1.0 mg/ml) of BSA was irradiated (Gamma Cell-5000 ^{60}Co source; Board of Radioisotope technology, Mumbai, India) with different doses (400–1000 Gy) of γ-radiation in the presence of SQGD (1.0 mg/ml). In control groups, BSA (1.0 mg/ml) was irradiated with the same doses (400–1000 Gy) of γ-radiation but in the absence of SQGD. 250 μl of 10 mM 2,4-Dinitrophenylhydrazine (DNPH) in 2.5 M HCl was added to test and control groups. In the negative untreated control group, DNPH was replaced with 2.5 M HCl and all sets were incubated in the dark for 15 min with regular vortexing every 5 min. 125 μl Trichloroacetic acid (TCA) (50% w/v) was added to all samples and incubated at 20 °C for 15 min. The samples were then centrifuged at 9000 × *g* for 15 min at 4 °C. Supernatant was discarded without disturbing the pellet. Pellet was then washed thrice with ice cold ethanol/ethyl-acetate (1:1) by centrifugation for 2.0 min at 9000 × *g* and supernatant was discarded. Pellet was dissolved in 6 M guanidine-HCl and absorbance was recorded at 370 nm (Powerwave XS2, Biotek, USA) taking 6 M guanidine–HCl alone as a blank.

2.2.3. Determination of γ-radiation-induced functional impairment in restriction enzymes and its protection by SQGD treatment

The effect of γ-irradiation on restriction endonucleases' (i.e. EcoRI and BamH1) functional activity and its modulation by SQGD treatment were evaluated by slight modification in the method described by Daly et al. (2010). In brief, one unit of either restriction enzyme (i.e. EcoRI and BamH1) was mixed with equal volume of SQGD (1.0 mg/ml) solution and incubated at 37 °C for 30 min. After incubation, reaction mixtures were irradiated using Gamma Cell-5000 (Board of Radio Isotope Technologies, Mumbai, India) with different doses (800–1400 Gy; dose rate of 1.41 kGy/hour) of γ-radiation. Another set of restriction enzymes were irradiated with similar doses of γ-radiation but in the absence of SQGD. Un-irradiated normal control restriction endonuclease enzyme was referred to as positive control. Following irradiation, restriction digestion reaction was performed by incubating the endonuclease enzymes with 300 ng λ DNA in 10× restriction buffer at 37 °C for 60 min. Upon completion of restriction digestion reaction, entire reaction mixture was analyzed on 1% agarose gel electrophoresis (Bio-Rad, USA).