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Short communication

Assessment of the ability of seaweed extracts to protect against hydrogen peroxide and *tert*-butyl hydroperoxide induced cellular damage in Caco-2 cells

A.M. O'Sullivan [a], Y.C. O'Callaghan [a], M.N. O'Grady [a], B. Queguineur [b], D. Hanniffy [b], D.J. Troy [c], J.P. Kerry [a], N.M. O'Brien [a,*]

[a] School of Food and Nutritional Sciences, University College, Cork, Ireland
[b] Irish Seaweed Centre, Martin Ryan Institute, National University of Ireland Galway, Ireland
[c] Teagasc, Ashtown Food Research Centre, Ashtown, Dublin, Ireland

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ABSTRACT

The ability of brown seaweed extracts, *Ascophyllum nodosum*, *Laminaria hyperborea*, *Pelvetia canaliculata*, *Fucus vesiculosus* and *Fucus serratus* to protect against *tert*-butyl hydroperoxide (*tert*-BOOH) induced stress in Caco-2 cells was investigated. Oxidative stress was determined by measuring alteration in the enzymatic activity of catalase (CAT) and superoxide dismutases (SOD) and cellular levels of glutathione (GSH). *L. hyperborea*, *P. canaliculata* and *F. serratus* significantly protected against *tert*-BOOH induced SOD reduction but did not protect against the reduction in CAT activity or the increased cellular levels of GSH. The ability of *F. serratus* and *F. vesiculosus* to protect against H_2O_2 and *tert*-BOOH induced DNA damage was also assessed. The DNA protective effects of the two seaweed extracts was compared to those of three metal chelators; deferoxamine mesylate (DFO), 1,10-phenanthroline (o-phen) and 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis (BAPTA-AM). *F. serratus* and *F. vesiculosus* significantly protected ($P < 0.05$) against H_2O_2 (50 μM) induced DNA damage but not *tert*-BOOH induced damage.

1. Introduction

Seaweed consumption has been associated with a range of health benefits, such as anticancer, immunomodulatory, antiviral, antibacterial and antioxidant activities which are attributed to seaweed constituents, such as sulphated polysaccharides, carrageenans, fucoidans, terpenoids, polyphenols and polyunsaturated fatty acids (Smit, 2004). In addition, studies indicate that seaweed extracts prevent lipid peroxidation and therefore may be a useful alternative to synthetic antioxidants in food systems such as meat, fish and dairy (Wang, Jónsdóttirb, & Ólafsdóttira, 2009). Phlorotannins are phenolic compounds found in brown seaweed which have demonstrated protective effects against oxidant induced cellular antioxidant enzyme depletion and DNA damage in human cells (Heo et al., 2009).

Compounds with the capacity to sequester metal ions can protect against cell damage induced by oxidants such as hydrogen peroxide (H_2O_2)- and *tert*-butyl hydroperoxide (*tert*-BOOH) (Sestili et al., 2002). Polyphenols have been shown to reduce or prevent H_2O_2-mediated DNA damage in *Escherichia coli* cells by binding iron (Perron, Hodges, Jenkins, & Brumaghim, 2008). In addition, polyphenols such as quercetin and rutin have been shown to reduce *tert*-BOOH-induced damage in Caco-2 cells (Aherne & O'Brien, 2000). Green seaweed such as *Halimeda macroloba* can contain high levels of polyphenols such as epigallocatechin and catechol, both of which have exhibited potent iron binding and DNA protective capabilities against H_2O_2-induced damage (Perron et al., 2008; Yoshie, Wang, Hsieh, & Suzuki, 2002). Seaweed also contains significant levels of vitamin C, vitamin E and carotenoids, all of which have been shown to protect against oxidant induced DNA damage (Cemelia, Baumgartnera, & Anderson, 2009).

A previous study conducted in our laboratory found that extracts of *Fucus vesiculosus* (*F. vesiculosus*) and *Fucus serratus* (*F. serratus*) reduced H_2O_2-mediated DNA damage and *Pelvetia canaliculata* (*P. canaliculata*) extract protected against H_2O_2-mediated superoxide dismutase (SOD) depletion in Caco-2 cells (O'Sullivan et al., 2011). The objective of the present study was to establish if methanolic extracts of brown seaweeds (*Ascophyllum nodosum* (*A. nodosum*), *Laminaria hyperborea* (*L. hyperborea*), *P. canaliculata*, *F. vesiculosus* and *F. serratus*) protect against *tert*-BOOH-induced oxidative stress in Caco-2 cells by measuring catalase, SOD and glutathione. Caco-2 cells have been widely used as an in vitro model for the small intestine and are a well established model for investigating the

* Corresponding author. Tel.: +353 21 4902884; fax: +353 21 4270244.
E-mail address: nob@ucc.ie (N.M. O'Brien).

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antioxidant effect of bioactive compounds (Phelan, Aherne, Wong, & O'Brien, 2009). In addition, the ability of extracts of *F. serratus* and *F. vesiculosus* to protect against H_2O_2 and *tert*-BOOH induced DNA damage was examined using the comet assay. The DNA protective effects of seaweed extracts were compared to those of three metal chelators; deferoxamine mesylate (DFO) which chelates iron, 1,10-phenanthroline (o-phen) which chelates ferrous and copper ions, and 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis (BAPTA-AM) which chelates calcium. These chelators have been used in several studies to investigate the mechanism involved in DNA damage (Aherne & O'Brien, 2000; Barbouti, Doulias, Zhu, Frei, & Galaris, 2001).

2. Materials and methods

2.1. Materials

Human colon adenocarcinoma cells (Caco-2) were acquired from the European Collection of Animal Cell cultures (Salisbury, UK), Foetal Bovine Serum (FBS) was purchased from Invitrogen (Paisley, Scotland). Cell culture plastics were purchased from Cruinn Diagnostics (Greiner Bio-One, Frickenhausen, Germany). Calbiochem SOD Assay Kit II was purchased from Merck Chemicals Ltd. (Nottingham, UK). All other cell culture chemicals and reagents were purchased from Sigma Chemical Co. (Dublin, Ireland).

2.2. Preparation of extracts and chelators

Seaweed samples were collected and prepared as previously described by O'Sullivan et al. (2011). The seaweed extracts were reconstituted in 10 ml distilled water resulting in a final concentration of 10 mg/ml for addition to cells. The metal chelators DFO, o-phen and BAPTA-AM were dissolved in cell culture media, DMSO and H_2O, respectively and were added to the cells at concentrations ranging from 2.5–15 mM, 25–1000 μM and 0.5–10 μM, respectively.

2.3. Cell culture

Human colon adenocarcinoma, Caco-2 cells were grown in antibiotic-free Dulbecco's modified Eagle's medium (DMEM) supplemented with FBS (10% v/v) and non-essential amino acids (1% v/v). Cells were incubated in an atmosphere of CO_2–air (5:95, v/v) at 37 °C. Caco-2 cells were plated at a density of 1×10^5 or 2×10^5 cells/ml for the comet and antioxidant assays, respectively.

2.4. Antioxidant assays

Caco-2 cells were supplemented with 100 μg/ml seaweed extracts for 24 h in 6-well plates with a final volume of 2 ml media containing reduced FBS (2.5% v/v). The cells were subsequently incubated with *tert*-BOOH for 30 min in serum free media. Cells were harvested and homogenised to release intracellular enzymes for the determination of the cellular antioxidant status. In the superoxide dismutases (SOD) assay, cells were exposed to 1000 μM *tert*-BOOH or 200 μM H_2O_2 and SOD activity was determined using a Calbiochem SOD Assay Kit II (Merck Chemicals Ltd., Nottingham, UK). Catalase (CAT) activity was determined following exposure of cells to 100 μM *tert*-BOOH using a Calbiochem catalase colorimetric activity kit (Merck Chemicals Ltd., Nottingham, UK). The glutathione (GSH) content was measured according to the method of Hissin and Hilf (1976) following exposure to 2000 μM *tert*-BOOH. Briefly, perchloric acid (15%, v/v) was added to the cell sonicates and samples were centrifuged at 14000 rpm for 30 min at 4 °C. The final GSH assay mixture contained 100 μl sample, 1.8 ml sodium phosphate-ethylenediamine tetraacetic acid buffer, pH 8, and 100 μl o-phthaldialdehyde (1 mg/ml). Fluorescence was detected at 430 nm following excitation at 360 nm using a Spectrofluor plus platereader (Tecan, Männedorf, Switzerland).

SOD, catalase and GSH were expressed relative to the protein content (mg), as determined by the bicinchoninic acid (BCA) method (Smith et al., 1985). SOD and catalase were expressed as units mg^{-1} protein where one unit of SOD is defined as the amount of enzyme needed to induce 50% dismutation of the superoxide radical and one unit of catalase activity is defined as the amount of enzyme required to produce 1 nM formaldehyde min^{-1} at 25 °C. GSH were expressed as nmol mg^{-1} protein.

2.5. Determination of DNA damage (Comet assay)

Caco-2 cells were supplemented with 100 μg/ml seaweed extracts for 24 h in 6-well plates with a final volume of 2 ml media, containing reduced FBS (2.5% v/v). Following incubation, the cells were treated with or without 50 μM H_2O_2 or 200 μM *tert*-BOOH for 30 min. Cells were harvested, embedded in low melting point (LMP) agarose and placed on microscope slides. The comet assay was conducted as previously described in O'Sullivan et al. (2011). Komet 5.5 image analysis software (Andor Technology, Belfast, Northern Ireland) was used to measure the level of DNA damage which was expressed as percentage tail DNA.

2.6. Statistical analysis

Data represent the means of four independent experiments ± standard error (SE), which are represented by vertical bars. Statistical analysis was conducted using repeated measures ANOVA followed by Dunnett's (Prism 4.0, GraphPad Inc, San Diego, CA, USA). The level of statistical significance was taken at $P < 0.05$ or $P < 0.01$.

3. Results and discussion

3.1. Enzymatic antioxidant activity

The bioactive compounds investigated in the present study were extracted from the seaweed samples using methanol and water which has previously been demonstrated as an effective method for the extraction of bioactives such as phlorotannins. Extracts may also contain salts, saponins, mucus, glycosides and organic acids (Cho et al., 2007; Hagerman, 1988).

The first objective of the present study was to determine if the seaweed extracts protected against *tert*-BOOH induced oxidative stress in Caco-2 cells. The addition of 1000 μM *tert*-BOOH to Caco-2 cells reduced the SOD activity to 73.9% that of the control (Fig. 1). The addition of 100 μg/ml of *L. hyperborea* ($P < 0.05$), *P. canaliculata* ($P < 0.05$) and *F. serratus* ($P < 0.01$) to Caco-2 cells significantly protected against the *tert*-BOOH mediated depletion in SOD activity, increasing activity from 73.9% to 100.1%, 97.0% and 108.0%, respectively. Extracts of *A. nodosum* and *F. vesiculosus* did not significantly protect against the reduction in SOD activity. We previously found that all of the seaweed extracts examined in the present study significantly protected against the H_2O_2-mediated depletion of SOD activity (O'Sullivan et al., 2011).

A possible mechanism for the protective effects exhibited by the seaweed extracts against H_2O_2 and *tert*-BOOH induced SOD depletion may be copper chelation. Copper chelation has demonstrated a protective effect against oxidant induced SOD fragmentation (Kang et al., 2002). Therefore, the copper chelator, o-phen, was used to determine if the chelation of copper was effective against the SOD depletion induced by *tert*-BOOH and H_2O_2. However, no