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Antiproliferative activity of Ontario grown onions against colorectal adenocarcinoma cells

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ABSTRACT

Cancer is the leading cause of mortality in Canada and other industrialized nations; the development of new/improved cancer therapies is desperately needed and continues to be a major focus of cancer research. Flavonoids, which are found in high levels in onions, have been shown to exert antiproliferative and potentially anti-cancer activities. To test their therapeutic potential, we assessed the antiproliferative, cytotoxic, apoptosis-inducing, and anti-migratory activities of five onion varieties grown in Ontario against human adenocarcinoma (Caco-2) cells. The properties of onion extracts were compared to pure extracts of flavonoids known to exhibit antiproliferative effects (quercetin, myricetin, and kaempferol). We compared more than one variety of onion, as agronomic and genetic factors influence the composition, as well as the quality of phytochemicals (e.g. flavonoids) in plant cultivars. We found that all onion varieties exhibited antiproliferative activity similar to purified flavonoids. The cytotoxic effects of the Stanley and Fortress onion varieties were strongest among the selected cultivars, as determined via lactate dehydrogenase (LDH) assays, while Safrane extracts showed the weakest activity. The Stanley and Lasalle cultivar extracts also had strong anti-migratory effects. Altogether these onion extracts may contain one or more compounds that may be effective anti-cancer therapies, while the Stanley extract showed the most comprehensive biological activities against Caco-2 cells.

1. Introduction

Increasing evidence from clinical and epidemiological studies supports an association between a diet rich in fruits and vegetables and a reduced incidence of chronic diseases, such as cancer (Hui et al., 2013; He, Jin, Gong, Zhang, & Zhou, 2014; Liu et al., 2014; Guercio, Turati, La Vecchia, Galeone, & Tavani, 2016). In 2012, there were 14.1 million newly reported cases of cancer reported globally (Ferlay et al., 2015), of which malignant neoplasms were the mainly leading cause of mortality in Canada (Statistics Canada, 2012). After resection, mainstay cancer therapeutics remain radiation and chemotherapy, despite their often severe side effects and mixed successes. Therefore, researchers continue to search for alternative therapeutics, which ideally may include lifestyle changes or other non-invasive mitigation strategies for fighting this prevalent and chronic disease. One reason a diet rich in fruits and vegetables may be beneficial in preventing cancer is that these foods are an excellent source of phytochemicals. Phytochemicals are bioactive compounds, classified by their chemical structures and divided into several classes, some of which are more prevalent in a wider range of fruits and vegetables (Schreiner & Huyskens-Keil, 2006). Phytochemicals commonly found in plant-based foods include carotenoids, phenolic acids, organosulfides, polyphenols and flavonoids.

Flavonoids represent the most prevalent phytochemical in plants. The health-promoting effects of dietary flavonoids continue to be an active area of research (Caridi et al., 2007). Flavonoids can exert antioxidant radical scavenging activities, limiting the lipid peroxidation of cell membranes (Peng & Kuo, 2003). Additionally, flavonoids have been demonstrated to have anti-cancer properties against several cancer types, including colorectal (He et al., 2014; Linsalata, Orlando, Messa, Refolo, & Russo, 2010), upper digestive tract (Guercio et al., 2016), breast (Yamazaki, Miyoshi, Kawabata, Yasuda, & Shimoi, 2014), and liver cancers (Yang, Meyers, Heide, & Liu, 2004). Dietary sources of flavonoids include berries, onions, garlic, citrus fruits, apples, and leeks (Manach, Scalbert, Morand, Rémésy, & Jime, 2004). Onions are one of the most widely produced and consumed vegetables (Sellappan & Akoh, 2002; Slimestad, Fossen, & Vågen, 2007), representing one of the most common sources of flavonoids in the human diet (Suleria, Butt, Anjum, Saeed, & Khalid, 2015). Three well studied flavonoids, kaempferol, myricetin, and quercetin, are found in onions, while quercetin is the most abundant among these in onions (Sellappan & Akoh, 2002).

Agronomic and genetic factors can influence the quality of phytochemicals among cultivars (Tomas-Bareberan & Espin, 2001),

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negatively impacting the health-promoting benefits that these nutrients provide. Therefore, it is possible that the benefits reported in onions grown outside of Canada may not necessarily apply to the locally grown varieties, as the cultivars and growing conditions are different between locals. Accordingly, an investigation on the health promoting properties of Ontario grown onions, specifically in regards to their cytotoxic and cytostatic bioactivity, is merited. Caco-2 as our cell line of interest because it is extensively used as a model for human intestinal epithelium and is the work horse of the pharmaceutical industry for in-vitro toxicology studies (Sambuy et al., 2005; Yamazaki et al., 2014). Therefore, we investigated the bioactive properties of five Ontario grown varieties of onion and assessed their capacity to exert cytostatic/cytotoxic effects on a relevant human colorectal cancer cell line.

2. Materials and methods

2.1. Materials

Quercetin (purity: pharmaceutical secondary standard), myricetin (purity ≥96%), kaempferol (purity > 98%), MEM (minimum essential medium), FBS (fetal bovine serum), L-glutamine, and D-glucose were purchased from Sigma Aldrich (St Louis, MO). Phenol free DMEM (Dulbeccos' modified eagle's medium) and 100× penicillin-streptomycin solution were obtained from GE Healthcare Life Sciences (Logan, UT). Lactate dehydrogenase (LDH) cytotoxicity and MTS cell proliferation assay kits were purchased from BioVision Inc. (Milipitas, CA). A TrypLE express and Image-iT® lipid peroxidation kit was purchased from Invitrogen (Carlsbad, CA). The ORIS™ cell migration assay kit was acquired from Platypus Technologies (Madison, WI). Five Ontario grown onion varieties were graciously donated by the Holland Marsh Growers' Association (Bradford, Ontario).

2.2. Phytochemical extraction using pressurized low polarity water

Low polarity water extractions of five Ontario grown onion varieties (Lasalle, Fortress, Safrane, Stanely, and Ruby Ring) were performed. Chopped onions were lyophilized, and 5 g of freeze-dried onion powder was mixed with 80 mL of 0.1% formic acid in milliQ water (v/v). An automated Speed SFE NP model 7100 instrument (Applied Separation Inc., Allentown, PA) equipped with a module 7100 pump and a 10 mL thick-walled stainless cylindrical extractor vessel was used for the extractions. Phytochemical extraction of the onions using pressurized low polarity water extraction was performed under the following conditions: 60 °C at 150 bar for 60 min.

2.3. Total flavonoid content

The total flavonoid content assay was performed by aluminum chloride colorimetric assay. 0.5 mL of the total extracted onion test samples were mixed with 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M potassium acetate. Followed by the addition of 2.8 mL of deionized water. The test samples were then kept for incubation at room temperature for 30 min. The optical density (OD) was then measured using 96 well plate by a plate reader (ELISA plate reader (Amersham Biosciences Corp., USA) at 415 nm. The total flavonoid content was expressed as mg quercetin equivalent (QE)/g dry plant sample.

2.4. Cell culture

The Caco-2 strain, a human colorectal adenocarcinoma cell line, was purchased from the American Type Culture Collection (Rockville, MD). Cells were maintained in MEM media supplemented with 4 mM L-glutamine, 1% non-essential amino acids, 10% heat inactivated fetal bovine serum, and 1% 100× penstrep solution (final concentration of 100 IU/mL penicillin and 100 μg/mL streptomycin). D-glucose was supplemented at a final concentration of 4 g/L. Caco-2 cells were propagated in a 75-cm^2 culture flask at 37 °C and 5% CO_2 in a humidified atmosphere. Media was refreshed three times per week, and cells were passaged at approximately 80% confluency. Cells were harvested using Tryple Express and then collected using centrifugation at 200 × *g* for 5 min. Cells at passage numbers 18–20 were used for the experiments.

2.5. Colorimetric quantification of Caco-2 cell proliferation

The cytostatic capacities of the onion extracts were determined by measuring Caco-2 proliferation using the MTS colorimetric assay. Briefly, Caco-2 cells were seeded in a tissue culture-treated 96-well plate at a density of 1.5×10^4 cells per well. Cells were left undisturbed for 24 h to allow for attachment. After 24 h, a total of 100 μL of the sample extracts containing the growth media were subsequently added. Flavonoids quercetin, myricetin, and kaempferol were used as positive controls to assess the cytostatic properties of the onion extracts. The concentration of pure flavonoids used for the present study was 100 μM. The flavonoids were dissolved in DMSO and further diluted in cell culture media. The final concentration of DMSO was 0.5% (v/v). The 96-well plate was incubated for 72 h and was gently washed with PBS prior to the addition of the MTS dye. Absorbance was measured at 490 nm using an xMark spectrophotometer (Bio-rad; Hercules, CA) after a 3 h incubation period with MTS dye.

2.6. Determination of cytotoxicity and cytolysis

Lactate dehydrogenase (LDH) supernatant levels were assessed as an indicator of Caco-2 cell death and were used as a measure of cytotoxicity activity. Caco-2 cells were seeded into a tissue culture-treated, 96-well plate at a density of 1.5×10^4 cells per well. The plate was undisturbed for 24 h to allow for cell attachment. The media was then aspirated and replaced with media containing onion extracts at different concentrations. LDH activity was determined 72 h post-treatment. The plate was centrifuged at 600 × *g* for 10 min to precipitate any cells and cellular debris. Ten μL of the debris free medium was transferred to a 96-well plate. To assess LDH levels, 100 μL of a reaction mixture solution containing a water soluble tetrazolium salt dye was added to the supernatant samples. Absorbance was read using an xMark spectrophotometer (Bio-rad; Hercules, CA) after a 30 min incubation period at 450 nm.

2.7. Induction of apoptosis in Caco-2 cells

Expression of the executioner caspases, caspases 3 and 7, was used to determine the presence of bioactive properties capable of inducing apoptosis. Briefly, cells were seeded in a black, clear bottom 96 well plate at 1.5×10^4 cells per well. The plate was left undisturbed for 48 h, after which the media was aspirated. Media containing the various extract treatments was added as previously described. After 24 h treatment incubation, the treatment medium was replaced with 100 μL of phenol free RPMI:F12 (1:1 ratio) medium supplemented with 5% FBS, 2 mM L-glutamine, and the CellEvent™ Caspase-3/7 Green Detection Agent at a final concentration of 7.5 μM. Cells were incubated for 30 min. High content imaging was performed using a Cytation 5 multimode plate reader (Biotek, Winooski, VT).

2.8. Cell migration

Measurements of cell motility were used to determine the efficacy of extract treatments in inhibiting the invasive progression of Caco-2 cells. The Oris™ cell migration assay kit consists of a 96-well plate with round bottom silicone stoppers which create a 2 mm diameter exclusion zone. Caco-2 cells were seeded at 1×10^5 cells/well and were allowed to attach for 24 h. After the incubation period, the silicone stoppers were gently removed, and the plate was imaged to capture the exclusion zone at time 0. Media containing the extract treatments was then added to the growth media. The plate was incubated for another 72 h,