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Cancer chemopreventive effect of fractions from cranberry products

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ABSTRACT

Cancer chemopreventive properties were evaluated in HPLC fractions of different polarity obtained from two cranberry juices and three extracts isolated from frozen cranberries and pomace containing anthocyanins, water-soluble and apolar phenolic compounds, respectively. Compounds with close polarities were collected in order to obtain between three and four fractions from each juice or extract. Cranberry fractions were screened for their ability to induce the phase II xenobiotic detoxification enzyme quinone reductase (QR). The results showed that there was no cytotoxicity against the cells used in the test. All samples stimulated the quinone reductase activity except the highest concentrations of the less polar fraction of anthocyanin-rich extract from pomace, which inhibited the QR activity. The QR induction for all samples varied with the concentration and there was an optimal concentration for which the QR induction was maximal. The technological process to manufacture cranberry juice had little influence on the overall QR inducer potencies of cranberry fractions, whereas the ability of phenols in fractions to stimulate the QR activity has been reduced significantly ($P \leq 0.05$) during the technological process. Among all samples, phenolic compounds of eight fractions presented a maximum QR induction greater than 100 II(QR)/mg phenol. The phenolic compounds of the most polar fraction (rich in phenolic acids) and those of the less polar fraction (rich in proanthocyanidins) showed stronger induction than those observed with phenols from intermediate fractions.

1. Introduction

In spite of the many advances in cancer treatment, chemotherapy of solid tumors is still greatly limited by the lack of selectivity of anticancer drugs and by the recurrence of drug resistant tumors. Finding a source of novel chemotherapeutics continues to be a focus of effort. Diets rich in grains, fruits, and vegetables are known to reduce cancer risk, implicating edible plants as potential sources of anticancer agents. A variety of compounds produced by edible plants have demonstrated anticancer activity (Waladkhani & Clemens, 1998); many of these belong to the flavonoid family (Chen, Schell, Ho & Chen, 1998; Choi et al., 2001; Katsube, Iwashita, Tsushida, Yamaki & Kobori, 2003). Berries, including cranberry (*Vaccinium macrocarpon* Ait. Ericaceae), are a rich source of many flavonoids. The anticancer properties of cranberry and the nature of the compounds that provide protection against tumor promotion and proliferation have not been fully investigated (Côté, Caillet, Doyon, Sylvain & Lacroix, 2010a). Extracts of cranberry were also shown to inhibit the proliferation of tumor cell lines *in vitro* (Katsube et al., 2003; Yan, Murphy, Hammond, Vinson & Neto, 2002). Reports of two cranberry extracts that inhibited proliferation of MCF-7 and MDA-MB-435 breast cancer cells (Yan et al., 2002) did not identify the active constituents. However, a study demonstrated that a flavonoid fraction from cranberry presscake (the material left after the juice is squeezed out of cranberries) was able to inhibit proliferation of 8 human tumor cell lines of multiple origins (Ferguson, Kurowska, Freeman, Chambers & Koropatnick, 2004). Also, triterpene hydroxycinnamates with *in vitro* antitumor activity were isolated from whole cranberry fruit (Murphy et al., 2003). Bomser, Madhavi, Singletary and Smith (1996) reported that extracts from berries of *Vaccinium* species were able to inhibit the induction of ornithine decarboxylase (ODC), an enzyme involved in tumor proliferation, and induce quinone reductase, an enzyme that can inactivate certain carcinogens. Proanthocyanidin-rich cranberry extracts containing other flavonoids were reported to inhibit ODC induction in epithelial cells (Kandil et al., 2002); recent studies also report on anti-angiogenic properties of the berries (Roy et al., 2002). In addition, In addition, preliminary evidence from our group demonstrated the cancer chemopreventive effect of cranberry and the effect of juice processing on this property (Caillet, Côté, Doyon, Sylvain & Lacroix, 2011a).

Cancer chemoprevention has been defined as the use of dietary and pharmacological interventions with specific natural or synthetic agents, designed to prevent, suppress, or reverse the process of carcinogenesis before the development of malignancy (Hong & Sporn,

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1997). Recently, increasing evidence based on *in vitro* and *in vivo* investigations have been linked to a decreased cancer risk with the consumption of certain dietary photochemicals (Bachmeier, Killian, Pfeffer & Nerlich, 2010). Among the multiple mechanisms underlying the chemopreventive potential of these constituents, xenobiotic-transforming and antioxidant defense systems are widely viewed as affording protection from cancer through the detoxification of potential carcinogens and mitigation of oxidative stress (Calabrese et al., 2008). It is generally accepted that carcinogenesis is a multistage process that may be caused by carcinogen-induced genetic and epigenetic damage in susceptible cells (Sporn, 1996). The first stage of the carcinogenic process, tumor initiation, involves exposure of normal cells to electrophilic carcinogen metabolites or reactive oxygen species (Prochaska & Talalay, 1991). The induction of phase II enzymes was proposed as a major mechanism of cellular protection against the toxic and reactive chemical species (Begleiter, Leith, Curphey & Doherty, 1997), and neoplastic effects of carcinogens (Wattenberg, 1992). One of the major mechanisms to protect against the toxic electrophilic metabolites of carcinogens and reactive oxygen is induction of phase II detoxification enzymes such as glutathione *S*-transferases, UDP-glucuronosyltransferases, and NAD(P)H: quinone reductase (NQOl) (Gutierrez, 2000; Kang & Pezzuto, 2004). NAD(P)H:quinone reductase (NQO1), one of the phase II drug-metabolizing enzymes, plays an important role in the mechanism of cancer chemoprevention, presumably at the initiation stage of carcinogenesis. The induction of phase II enzymes can offer protection against toxic and reactive chemical species (Prochaska, Santamaria & Talalay, 1992; Talalay, 2000). Several studies have shown that elevation of phase II enzymes correlates with protection against chemical-induced carcinogenesis in animal models (Kang & Pezzuto, 2004). Enzyme inducers are of two types: monofunctional and bifunctional (Prochaska & Talalay, 1988). Bifunctional inducers increase phase II enzymes as well as phase I enzymes such as aryl hydrocarbon hydroxylase, and bind with high affinity to the aryl hydrocarbon receptor (Yang, Smith & Hong, 1994). Monofunctional inducers induce phase II enzymes selectively and are independent of the aryl hydrocarbon receptor. Since phase I enzymes can activate procarcinogens to their ultimate reactive species, monofunctional agents that induce phase II enzymes selectively would theoretically appear to be more desirable candidates for cancer chemoprotection (Talalay, 2000). The elevation of phase II detoxification enzymes through the antioxidant response element by dietary factors is considered more beneficial than coordinative induction of both phase I (cytochrome P450s) and phase II enzymes. In addition, selective phase II enzyme inducers would be anticipated to serve as anticarcinogens early in the process of carcinogenesis. Bearing in mind the importance of phase II enzyme induction in cancer chemoprevention, methods to determine phase II enzyme inducer potencies of pure compounds and extracts of natural products are necessary. Quinone reductase is inducible in the cells of many tissues such as those from the liver, lung, and colon (Wang, Liu, Higuchi & Chen, 1998), and induction provides a reasonable biomarker for the cancer. The murine hepatoma cell line Hepa 1c1c7 contains inducible quinone reductase that is easily measurable and provides a reliable, high-throughput system for the detection of induction (Prochaska et al., 1992). This cell line has been used for the discovery of novel natural product anticarcinogens (Talalay, 2000). Measuring the induction of quinone reductase activity may provide an efficient approach to searching the potential chemopreventive phytochemicals from plants, and this may lead to further understanding the chemopreventive mechanisms behind their actions.

Thus, the aim of the present study was to evaluate the potential cancer chemopreventive effect of fractions of different polarity obtained from two cranberry juices (clarified juice and juice concentrate) and three extracts (anthocyanins, water-soluble and apolar phenolic compounds) from cranberry fruits and pomace. In this work, an HPLC method was established to achieve the separation of different fractions and cranberry fractions were screened for their ability to induce the phase II xenobiotic detoxification enzyme quinone reductase (QR).

2. Materials and methods

2.1. Raw material and cranberry processing

Frozen cranberries (*V. macrocarpon*) and three main cranberry processing products (pomace, clarified juice and juice concentrate (final product)) were provided by Atoka Cranberries Inc. (Manseau, QC, Canada) and were stored at −80 °C until used. The initial processing step to make juice involves reducing frozen cranberries to a mash using a fruit mill. Then, the raw juice recovery from mash was done using a fruit press at 1.90 bar. During the juice pressing step, high amounts of press cake were obtained: cranberry pomace is the main byproduct of the cranberry processing industry. It is composed primarily of skin, seeds, and stems left over after pressing the fruit for juice. During the filtering process, a cross-flow membrane filtration was used to remove colloids and generate a clear juice from raw juice. Then the clarified juice was concentrated by evaporation to obtain a juice concentrate at 50°Brix.

2.2. Extraction of phenolic compounds and sample preparation

The extraction conditions employed were as mild as possible to avoid oxidation, thermal degradation and other chemical and biochemical changes in the sample. Extraction of phenolic compounds from frozen cranberries and pomace was achieved according to three methods using solvents of different graded polarity for the recovery of specific classes of phenols which have different solubility. The most water soluble phenolic compounds were extracted with water/methanol (85:15, v/v) (Seeram, Adams, Hardy & Heber, 2004), the most apolar phenolic compounds (flavonols, flavan-3-ols and proanthocyanidins) were extracted with acetone/methanol/water (40:40:20, v/v), modified from a method described by Neto et al. (2006), and the anthocyanins were extracted with methanol/water/acetic acid (85:15:0.5, v/v/v) as described by (Wu & Prior, 2005). Frozen cranberries or pomace were crushed at 4 °C for 40 s in a Waring commercial blender (Waring Laboratory, Torrington, CT) to obtain a fine powder. Immediately after crushing fruit, extractions have been performed at 4 °C under agitation and nitrogen for 40 min by macerating of 300 g of the fruit powder with the extracting solvents. Three successive extractions in each extracting solvent were performed using the same procedure. The first extraction was done using 700 mL of solvent, but for the two last ones, 500 mL was used instead. The solvent containing the phenolic compounds was recuperated after each extraction and the solvents from the successive extractions were combined, then filtered on Whatman paper no. 4 (Fisher Scientific, Nepean, ON, Canada). The filtrate was concentrated by evaporation of solvent using the SpeedVac Automatic evaporation system (Savant System, Holbrook, NY), then dry matter was determined by freeze-drying the extracts for 48 h with a Virtis Freeze mobile 12 EL (The Virtis Co., Gardiner, N.Y), and stored at −80 °C until used.

2.3. HPLC-DAD fractionation of extracts and juices

The HPLC analyses were performed on a ProStar 230 (Varian Canada Inc., Mississauga, ON, Canada), equipped with a ternary pump delivery system, a Rheodyne injection valve (500 µL capacity, Waters Ltd., Dorval, QC, Canada) and a ProStar 330 diode-array UV–Vis detector (Varian); integration and data elaboration were performed using Star Chromatography Workstation software (Varian). A Zorbax SB-C_{18}, 5 µm, 9.4 × 250 mm (Agilent Technologies Canada Inc, Mississauga, ON, Canada) column was used. All solvents were filtered with a 0.45 µm Millipore (Millipore Canada Ltd., Etobicoke, ON, Canada) filter disk and degassed with helium. A gradient elution was carried out using the following solvent systems: mobile phase A, double-distilled water/acetic acid (97/3, v/