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Cranberry and Its Phytochemicals: A Review of In Vitro Anticancer Studies[1–3]

Catherine C. Neto*

Department of Chemistry and Biochemistry, University of Massachusetts–Dartmouth, North Dartmouth, MA 02747

Abstract

This article reviews the existing research on the anticancer properties of cranberry fruit and key phytochemicals that are likely contributors to chemoprevention. Results from in vitro studies using a variety of tumor models show that polyphenolic extracts from *Vaccinium macrocarpon* inhibit the growth and proliferation of breast, colon, prostate, lung, and other tumors, as do flavonols, proanthocyanidin oligomers, and triterpenoids isolated from the fruit. The unique combination of phytochemicals found in cranberry fruit may produce synergistic health benefits. Possible chemopreventive mechanisms of action by cranberry phytochemicals include induction of apoptosis in tumor cells, reduced ornithine decarboxylase activity, decreased expression of matrix metalloproteinases associated with prostate tumor metastasis, and antiinflammatory activities including inhibition of cyclooxygenases. These findings suggest a potential role for cranberry as a dietary chemopreventive and provide direction for future research. J. Nutr. 137: 186S–193S, 2007.

The North American cranberry (*Vaccinium macrocarpon* Ait. Ericaceae) is of growing public interest as a functional food because of potential health benefits linked to phytochemicals in the fruit. Cranberry juice has long been consumed for the prevention of urinary tract infections, and research linked this property to the ability of cranberry proanthocyanidins to inhibit adhesion of *Escherichia coli* bacteria responsible for these infections (1). These studies, which brought to light the unique structural features of cranberry proanthocyanidins (2), have sparked numerous clinical studies probing cranberry's role in the prevention of urinary tract infections and targeting the nature of the active metabolites. Further antibacterial adhesion studies demonstrated that cranberry constituents also inhibit adhesion of *Helicobacter pylori*, a major cause of gastric cancer, to human gastric mucus (3). A subsequent randomized, double-blind human trial found significantly lower levels of *H. pylori* infection in adults consuming cranberry juice (4). As interest in cranberry consumption for disease prevention grows, it is important that we fully examine other potential health benefits.

The discovery of resveratrol and tea polyphenols has sparked increasing interest in polyphenolic compounds from foods as potential chemopreventive agents. A major goal of our research program has been to identify cranberry phytochemicals with potential anticancer activity and try to understand how they may function on a cellular and molecular level to limit the progression of this disease. Our collaborations with scientists at the University of Prince Edward Island (Robert Hurta), Universidad Peruana Cayetano Heredia (Abraham Vaisberg), University of Wisconsin, Madison (Jess Reed and Christian Krueger), and University of Massachusetts-Dartmouth (Maolin Guo and Peter Hart) use a variety of in vitro cancer models; a picture of cranberry as a potential chemopreventative is gradually emerging. This article summarizes current knowledge of the anticancer properties of cranberry and its phytochemicals. Antiinflammatory properties are also considered in the context of biological activities that may influence chemoprevention.

Antioxidant properties of cranberry fruit

Cranberry ranks high among fruit in both antioxidant quality and quantity (5) because of its substantial flavonoid content and a wealth of phenolic acids. Cranberry extracts rich in these compounds reportedly inhibit oxidative processes including oxidation of low-density lipoproteins (6,7), oxidative damage to rat neurons during simulated ischemia (8), and oxidative and inflammatory damage to the vascular endothelium (9). The antioxidant properties of the phenolic compounds in cranberry fruit may contribute to the observed antitumor activities of cranberry extracts, but recent studies suggest that cranberry's anticancer activity may involve a variety of mechanisms.

Early studies of anticancer activity

A crop grown primarily in the northeastern United States, Canada, and Wisconsin, *V. macrocarpon* belongs to the Ericaceae

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* To whom correspondence should be addressed. E-mail: cneto@umassd.edu.

family. Several groups of phytochemicals plentiful in fruit of the *Vaccinium* genus could be expected to affect cancer-related processes. The earliest report of potential anticancer activity appeared in 1996 in a University of Illinois study of several *Vaccinium* species. Extracts of cranberry, bilberry, and other fruits were observed to inhibit ornithine decarboxylase (ODC)[4] expression and induce the xenobiotic detoxification enzyme quinone reductase in vitro (10). Subsequent studies of cranberry in cellular models focused initially on breast cancer. A study by Canadian researchers reporting that cranberry juice inhibited breast tumor growth appeared in 2000 (11) and was followed by a more detailed study showing that an extract of cranberry presscake inhibited proliferation of MCF-7 and MDA-MB-435 breast cancer cells (12). The early reports of anticarcinogenic activity increased interest in cranberry's possible role in the prevention of breast and other cancers, and further studies focused on identifying active constituents.

Cranberry phytochemicals and chemoprevention

Cranberry fruit has a diverse phytochemical profile that includes 3 classes of flavonoids (flavonols, anthocyanins, and proanthocyanidins), catechins, hydroxycinnamic and other phenolic acids, and triterpenoids. Several groups of researchers examined activity of whole polyphenolic extracts of the fruit or spray-dried juice. Our group developed a bioassay-guided fractionation approach to identification of antitumorigenic compounds while researching plants used in traditional Peruvian medicine (13). Our strategy used a simple NCI tumor growth inhibition assay (14) to screen for activity in several tumor cell lines. We examined antitumor activities of not only whole cranberry fruit and juice extracts but also individual compounds and groups of compounds to identify active constituents. A discussion of the major phytochemicals occurring in cranberry fruit and their biological activities observed by our group and by other researchers follows.

Studies of cranberry polyphenolic extracts

Cranberry flavonoids, like those from other food sources, can be expected to play a role in chemoprevention and may act synergistically. In 2002 a University of Illinois study revealed that extracts of whole cranberry containing proanthocyanidins and other flavonoids inhibited ODC activity in mouse epithelial (ME-308) cells (15). Characterization of an active subfraction revealed the presence of dimers and oligomers of catechin-epicatechin, monomeric catechins, and quercetin glycosides. ODC has an important role in the biosynthesis of polyamines involved in cellular proliferation. A UCLA study showed that water-soluble cranberry phenolic extracts prepared from commercial cranberry powder effectively inhibited proliferation of several human tumor cell lines (16). A total polyphenol extract containing a variety of flavonoids inhibited proliferation of 2 oral cancer cell lines (CAL27 and KB), 4 colon cancer cell lines (HT-29, HCT-116, SW480, and SW620), and 3 prostate cancer cell lines (RWPE-1, RWPE-2, and 22Rv1). Anthocyanin and proanthocyanidin subfractions were less effective in the oral and colon cell lines than the total polyphenolic extract but showed strong inhibition in the prostate cell lines.

[4] Abbreviations used: COX, cyclooxygenase; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MMP, matrix metalloproteinase; ODC, ornithine decarboxylase; TNF-α, tumor necrosis factor; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

Quercetin's antitumor properties

Cranberries are one of the leading fruit sources of quercetin on a weight basis. Analyses in our laboratory have found total flavonol content of cranberry fruit usually falls in the range of 20–30 mg/100 g fresh fruit weight, with ~75% of the flavonols being quercetin glycosides. Both quercetin and myricetin occur mainly as monoglycosides in the fruit (**Fig. 1**), with quercetin galactoside the most abundant form (4). In vivo, quercetin glycosides are usually metabolized to sulfates or gluconurides. Tumor growth inhibition assays in our laboratories found that quercetin inhibited the growth of MCF-7 human breast adenocarcinoma, HT-29 human colon adenocarcinoma, and K562 human chronic myelogenous leukemia cell lines with GI_{50} in the range of 15–60 mg/L (17).

Among the flavonoids, quercetin is one of the most extensively studied with regard to anticancer activity because of its prevalence among fruits and vegetables. There are numerous reports of quercetin's ability to inhibit proliferation of cancer cell lines in vitro, including breast, colon, pancreas, and leukemia (18,19). Its mechanisms of chemopreventive action include induction of apoptosis, observed in HepG2 hepatoma and colorectal cells, with arrest of the HepG2 cell cycle in G_1 phase (19–21); inhibition of epidermal growth factor receptor expression and associated tyrosine kinase activity (18,21); reduced expression of Ras protein in colon cancer cells and primary colorectal tumors (22); increased expression of endogenous inhibitors of matrix metalloproteinases (23); and phytoestrogenic activity involving interaction with the estrogen α- and β-receptors of human mammary MCF-7 cells (24).

Much of the observed anticancer activity of whole cranberry extracts is likely to result in part from quercetin's activities. Quercetin's bioavailability and activity in vivo have been investigated in recent years. In a study comparing the ability of 4 herbal flavonoids (quercetin, curcumin, rutin, and silymarin) to suppress aberrant crypt foci formation in an azoxymethane-induced rat colon cancer model, a quercetin-enriched diet decreased the number of aberrant crypt foci formations 4-fold compared with control. Western blot analysis of colon scrapings suggested that quercetin induced apoptosis by a mitochondrial pathway involving modulation of Bax and Bcl-2 protein expression (25).

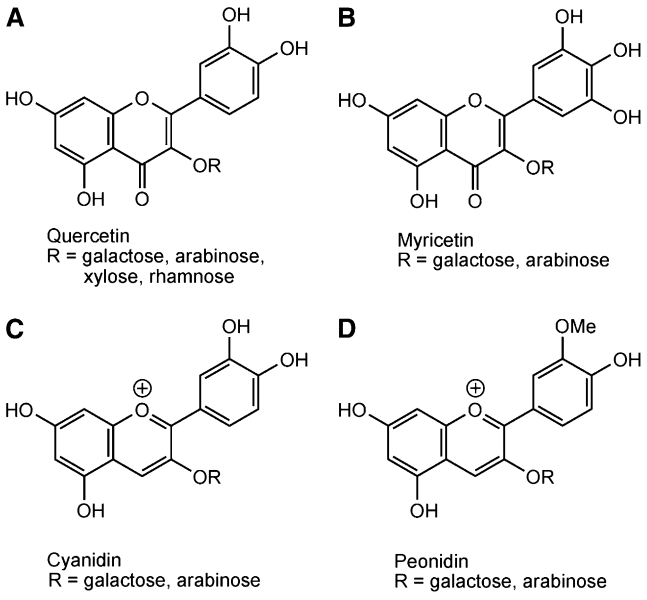

Figure 1 Flavonol and anthocyanin monoglycosides in cranberry fruit.

Cranberry anthocyanins

The major anthocyanins in cranberry (Fig. 1) are galactosides and arabinosides of cyanidin and peonidin (26). *Vaccinium* fruits are among the most plentiful food sources of anthocyanin. Content varies widely among cranberry cultivars, averaging 25–65 mg/100 g of ripe fruit at harvest (27), with reports of anthocyanin content as high as 100 mg/100 g fresh fruit weight (28). Fruit of the Early Black cultivar is significantly higher in anthocyanins and proanthocyanidins than most other cranberry cultivars (29).

Because of their superior antioxidant efficacy, cranberry anthocyanins may be expected to inhibit oxidative processes linked to tumorigenesis. Compared with other compounds in the fruit, the anthocyanins have shown little direct antiproliferative or growth-inhibitory properties in our in vitro models. Purified cyanidin-3-galactoside was evaluated by us in 8 tumor lines in vitro using the SRB assay. In all cell lines, GI_{50} values were >250 mg/L (17). Similarly, a mixed anthocyanin fraction demonstrated little tumor growth inhibition. In a multi-cell-line study at UCLA using a luminescent cell viability assay (16), an anthocyanin subfraction of cranberry limited growth in 3 prostate tumor lines (RWPE-1, RWPE-2 and 22Rv1) by 50–70% but did not significantly inhibit oral or colon tumor cell line proliferation.

Anthocyanins including those from cranberry have, however, been implicated in the observed antiangiogenic properties of mixed berry extracts (30,31). Mixed anthocyanin-rich extracts inhibited the induction of vascular endothelial growth factor by both hydrogen peroxide and tumor necrosis factor (TNF-α) and also resulted in decreased hemangioma formation and tumor growth (32). This suggests that the antioxidant and antiinflammatory properties of these compounds may limit angiogenesis.

Cranberry proanthocyanidins in cancer models

The potential roles of proanthocyanidins in chemoprevention by dietary cranberry are gradually coming to light. Studies reporting in vitro antiproliferative activity of flavonoid-rich extracts from cranberry in KB and CAL-27 oral; RWPE-1, RWPE-2, and 22Rv1 prostate; and HT-29, HCT-116, and SW-620 colon cancer cell lines (16) as well as MCF-7 and MDA-MB-435 breast cancer lines (12) have implicated proanthocyanidins as contributing to these activities. ODC inhibition in epithelial cells by cranberry was also linked to a proanthocyanidin-rich fraction (15).

A proanthocyanidin fraction from whole cranberry fruit was observed to selectively inhibit the growth of H460 human large cell lung carcinoma, HT-29 colon adenocarcinoma, and K562 chronic myelogenous leukemia cells in our panel of 8 tumor cell lines. A subfraction with improved activity over the parent fraction in those 3 cell lines was isolated and characterized by us using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The proanthocyanidin subfraction contained oligomers composed primarily of 4–7 epicatechin units with at least 1 or 2 A-type linkages between the units (33).

We used clonogenic soft agar assays to assess the ability of cranberry extracts and fractions to inhibit tumor colony formation in HT-29 and HCT-116 colon tumor cell lines. Over 2 wk the appearance of new tumor colonies decreased in these cell lines in a dose-dependent manner when they were treated with a whole-cranberry polyphenolic extract and by a proanthocyanidin fraction, both prepared from Early Black variety cranberry fruit (34). MALDI-TOF MS characterization of this proanthocyanidin fraction revealed that it was composed primarily of trimers through hexamers of epicatechin with both A- and B-type linkages. HCT-116 tumor colony formation was reduced more effectively by the proanthocyanidins than the whole extract, with >50% inhibition of tumor colony formation observed at a concentration of <10 mg/L. The effect was more pronounced in HCT-116 than in HT-29 cells. Seeram et al. (16) also report that HCT-116 cells were more susceptible than HT-29 to total polyphenolic extract of cranberry, with proliferation of HCT-116 cells reduced by 92% at 200 mg/L.

Linking proanthocyanidin structure and activity

As a class, proanthocyanidins can be complex in structure and composition, featuring various flavan-3-ols (most commonly catechin, epicatechin, and galloylated catechins) linked together in different ways. Cranberry proanthocyanidins are primarily dimers, trimers, and larger oligomers of epicatechin. Typically, these molecules contain 2 types of linkages between epicatechin units: the 4$\beta\rightarrow$ 8 (B-type) linkage commonly found in proanthocyanidins from sources other than *Vaccinium* fruit (apples, grape seed, cacao), and the less common A-type linkage featuring both 4$\beta\rightarrow$ 8 and 2$\beta\rightarrow$O$\rightarrow$7 interflavanoid bonds (**Fig. 2**). The combination of linkages provides diversity of 3-dimensional structure within this group of molecules, even among the smaller proanthocyanidins. For example, proanthocyanidins reported to inhibit adherence of P-fimbriated *E. coli* include at least 3 different trimer structures (2). MALDI-TOF MS analysis of proanthocyanidin oligomers from whole cranberry fruit with tumor antiproliferative activity ranged in size up to 12 degrees of polymerization with as many as 4 A-type linkages. Most contained exclusively epicatechin units, but some epigallocatechin unit masses were detected (33).

Proanthocyanidins from grape seeds are more widely studied and were reported to inhibit the growth of breast cancer cells both in vitro (35,36) and in vivo (37). Recently, grape seed proanthocyanidins were reported to inhibit proliferation of a highly metastatic mouse mammary carcinoma cell line (4T1) both in vitro and in a mouse model (38). Metastasis to the lungs was significantly inhibited. An extract from grape seed powder containing primarily procyanidin dimers also inhibited carcinogenesis in a 12-O-tetradecanoyl phorbol-13-acetate (TPA)-promoted SENCAR mouse skin model (39). With regard to events preceding

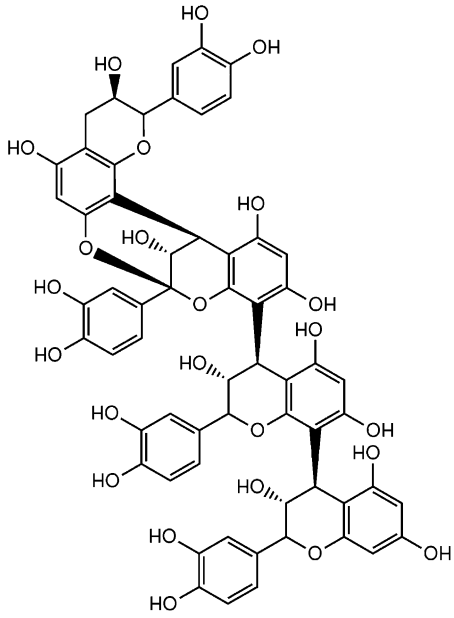

Figure 2 Structure of a typical cranberry proanthocyanidin tetramer composed of epicatechin units with one A-type linkage.

colon cancer, dietary grape seed proanthocyanidins reduced formation of aberrant crypt foci in the colons of rats by 72–88% (40). Grape seed proanthocyanidins differ from cranberry proanthocyanidins in that they are more likely to contain galloylated flavan-3-ols, and the monomers are primarily B linked (41).

The presence of A-type linkages may influence tumor inhibitory and selectivity properties of proanthocyanidins. In a screening of a variety of smaller polyflavan-3-ols from different plants against GLC4 lung and COLO 320 colon carcinomas, a trimer with an A-type linkage was more cytotoxic than dimers with A-type linkages and trimers with only B-type linkages (42). Wild blueberry proanthocyanidins containing A-type linkages reportedly inhibited growth of androgen-sensitive LNCaP prostate cancer cells to a greater degree than androgen-insensitive DU145 cells (43). These findings are consistent with our observations that cranberry proanthocyanidins with A-type linkages show antiproliferative activity in selected cell lines (33). It is reasonable to expect that the biological properties of cranberry proanthocyanidins will resemble those of other condensed tannins in some ways but differ in others, depending on structural similarity and also on how these compounds are metabolized in vivo.

Cranberry triterpenoids: properties of ursolic acid and its phenolic esters

The peel of cranberry fruit contains a substantial amount of pentacyclic triterpenoid ursolic acid. Quantitative analysis of cranberry fruit and products using LC-MS shows that the ursolic acid content of whole cranberry fruit of different cultivars is 60–110 mg/100 g fresh fruit (44), and a similar content is found in sweetened, dried fruit. Considerably less ursolic acid is detected in jellied cranberry sauce. None was detected in commercial cranberry juice. Apple peels also contain ursolic acid, as do highbush blueberries, from which ursolic acid was isolated and shown to inhibit growth of several leukemia cell lines and A-549 human lung carcinoma (45).

Using a bioactivity-guided fractionation approach, we determined that an ethyl acetate extract of whole cranberry fruit inhibited growth of several tumor cell lines (6). We subsequently isolated 2 phenolic esters of ursolic acid from whole cranberry fruit. The esters inhibited the growth of several types of tumor cells in vitro, particularly MCF-7 but also HT-29 colon, DU-145 prostate, H460 lung, ME180 cervical, and K562 leukemia cell lines (17). Our previous studies showed that ursolic acid and derivatives isolated from another plant source (*Polylepis racemosa*) inhibited tumor growth in the same panel of cell lines (13). The esters were effective at lower concentrations than those observed for ursolic acid, with GI_{50} for the esters of 11–28 mg/L depending on cell line. LC-MS analysis of various cultivars found the hydroxycinnamate esters are present in whole cranberry fruit in quantities averaging ~15–20 mg/100 g fresh fruit (44).

Clonogenic assays using soft agar to assess effects on tumor colony formation show that ursolic acid inhibits tumor colony formation in a dose-dependent manner in both HT-29 and HCT-116 models of colon cancer (34). Inhibition was slightly more pronounced in the HCT-116 cell line, although ursolic acid inhibited HT-29 tumor colonies more effectively than the cranberry proanthocyanidin fraction did. TUNEL assays for apoptosis in MCF-7 breast tumor cell line show that ursolic acid induces a high rate of apoptosis (L. Griffin, S. Rego, E. Correiro, C. Neto, and P. Hart, unpublished results).

Ursolic acid occurs in many plants and is a constituent of several herbal medicines marketed in Asia and worldwide for inflammatory conditions (46). As a potential functional food phytochemical, ursolic acid has received relatively scant attention, perhaps because little is known about its oral bioavailability. Few in vivo cancer studies of ursolic acid appear in the literature. A single model study reported the effect of ursolic acid administered by intravenous injection to C3H mice bearing either FSaII murine fibrosarcoma or MCaIV murine mammary adenosarcoma. A dose of 100 mg/kg significantly inhibited FSaII tumor growth and reduced tumor interstitial fluid pressure (47).

Numerous reports of ursolic acid's in vitro antitumor activity have appeared in the literature (48), and several suggest possible mechanisms of action for tumor inhibition. Ursolic acid inhibited the proliferation of mouse melanoma cell line B16 and MCF-7 breast tumor cells by exerting an early cytostatic effect on the cell cycle at G_1 (49,50). The cytotoxic and cytostatic effects are likely to involve apoptosis. Ursolic acid induced apoptosis in HL-60 human leukemia cells (51), an activity thought to involve enhancement of intracellular Ca^{2+} signaling (48). Ursolic acid induced apoptosis in HepG2 human hepatoblastoma cells in a dose-dependent manner, with DNA fragmentation, enhanced release of cytochrome *c*, and activation of caspase-3. Expression of $p21^{WAF1}$ was increased, indicating possible involvement in mediating cell-cycle arrest (52). In a human prostate cancer model, ursolic acid induced apoptosis by activating several caspase enzymes. Down-regulation of c-IAPs (inhibitor of apoptosis proteins) that normally block apoptotic signaling of caspases was also observed (53). Proliferation of HT-29 colon cells was decreased by ursolic acid in a dose-dependent manner, with apoptosis induced by activation of caspases 3, 8, and 9 (54). Ursolic acid may also inhibit invasion and metastasis by decreasing matrix metalloproteinase (MMP) expression as observed in a study showing that ursolic acid decreased MMP-9 expression in HT1080 human fibrosarcoma cells (55). Thus, ursolic acid could be expected to contribute to the antitumor properties of cranberry fruit.

Possible chemopreventive mechanisms

Numerous in vitro studies have focused on determining plausible mechanisms of tumor inhibition by dietary phytochemicals. Tea and grape polyphenols are among the most well-studied (56,57) because of the high worldwide consumption of tea and grape-derived products including wine and supplements. Studies of cranberry's mechanisms of activity are still in the early stages. Tumor inhibition by cranberry is likely to involve synergistic activities between the cranberry phytochemicals discussed above, including the flavonols (quercetin being the major flavonol), proanthocyanidins, and ursolic acid. Some possible mechanisms of action supported by in vitro evidence include induction of apoptosis in cancer cells, decreased invasion and metastasis as a result of inhibition of MMPs, inhibition of ornithine decarboxylase expression and activity, and inhibition of inflammatory processes including cyclooxygenase (COX) activity. A discussion of the evidence supporting these mechanisms follows.

Cranberry induces apoptosis in breast tumor cells

Many dietary phytochemicals have been observed to limit proliferation by inducing apoptosis. Resveratrol, epigallocatechin gallate, and quercetin (a major flavonoid in cranberry fruit) are examples of phytochemicals capable of inducing apoptosis. Evidence is emerging for a key role of apoptosis in cranberry's anticancer activity. This property may be linked to the content of quercetin and other compounds in the fruit. In vitro studies using breast tumor models have reported dose-dependent induction of

apoptosis by cranberry. An antiproliferative fraction from cranberry presscake induced apoptosis in MDA-MB-435 breast tumor cells as determined by annexin-V staining (12), and cells were arrested in both G_1 and G_2 phases. An 80% aqueous acetone extract of whole cranberry fruit increased apoptosis of MCF-7 cells by 25% at a concentration of 50 g/L with significant arrest in the G_1 phase (58).

We used a fluorescent TUNEL assay to evaluate the apoptotic effects of a whole polyphenolic extract of cranberry fruit on tumorigenic (MCF-7) vs. nontumorigenic (MCF-10A) breast cell lines. At the highest concentration (250 mg/L), the cranberry extract increased baseline apoptosis rate to 92% in MCF-7 cells while not increasing apoptosis in MCF10A cells significantly (L. Griffin, S. Rego, E. Correiro, C. Neto, and P. Hart, unpublished results). Comparison of the whole fruit extract with desugared, freeze-dried organic 100% cranberry juice showed that both the whole fruit extract and the juice extract increased rates of apoptosis in MCF-7 breast tumor cells, with the juice extract showing slightly greater efficacy (C. C. Neto, E. Domingues, and P. Hart, unpublished data). At a treatment concentration of 25 mg/L, well below the cytotoxic level, rates of apoptosis in MCF-7 cells doubled when treated with whole-cranberry polyphenolic extract and increased further when cells were treated with juice extract. Differences in composition that may account for these observations are the subject of ongoing study. MALDI-TOF MS analysis of a proanthocyanidin fraction prepared from the juice extract detected the presence of novel anthocyanin-epicatechin oligomers in addition to the primarily epicatechin-based oligomers found in the fractions prepared from whole-fruit extract. Whether these compounds arose through processing or occur naturally in juice is unknown.

The exact pathways involved in the induction of apoptosis by cranberry phytochemicals are unknown. Cranberry polyphenolics, like other dietary polyphenolics, may induce apoptosis in breast tumor cells via activation of the mitochondrial apoptosis pathway. The possible effects of cranberry on expression of genes controlling steps in the mitochondrial apoptosis pathway (cytochrome C, APAF1) are currently under investigation.

Invasion and metastasis: inhibition of matrix metalloproteinases

Proanthocyanidins and flavonoids from cranberry and other *Vaccinium* berries show some promise toward limiting processes involved in tumor invasion and metastasis. They may function by blocking the expression of MMPs involved in remodeling the extracellular matrix (59). We found that whole cranberry polyphenolic extract inhibits the expression of MMP-2 and MMP-9 in the DU-145 prostate tumor cell line in a dose-dependent manner. A cranberry proanthocyanidin fraction also showed MMP inhibition in DU-145 cells; its activity was somewhat less than that of the whole fruit extract (33), suggesting that other flavonoids in the fruit also contribute to the observed inhibition. A flavonoid-rich extract of highbush blueberry (*V. angustifolium*) was observed to inhibit MMP expression in this model (60), and this activity was attributed in large part to the proanthocyanidins. Purified ursolic acid and hydroxycinnamate esters from cranberry fruit were also evaluated by us (**Fig. 3**). These compounds strongly inhibited expression of both MMP-2 and MMP-9 at micromolar concentrations (61), a finding that is consistent with the observed ability of ursolic acid to inhibit MMP expression in fibrosarcoma cells (55).

Grape seed proanthocyanidins were recently observed to inhibit MMP-2 and MMP-9 expression in a dose-dependent manner in a prostate carcinoma cell line. This activity was associated with inhibition of activation of the mitogen-activated protein kinase cascade (62). Grape seed proanthocyanidins also inhibited metastasis to the lungs of a highly metastatic mouse mammary carcinoma (4T1) in vivo (38), which may be linked to decreased MMP activity. More studies are needed to determine the efficacy of cranberry and its proanthocyanidins against tumor metastasis compared with those of grape seed.

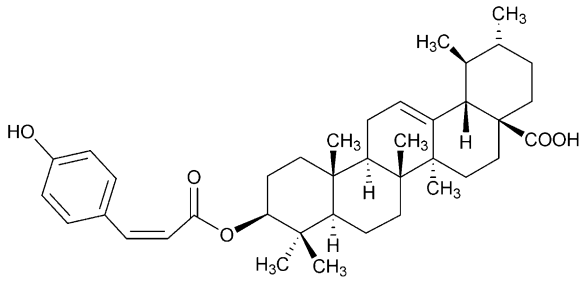

Figure 3 *Cis*-hydroxycinnamoyl ester of ursolic acid from cranberry fruit.

Ornithine decarboxylase: induction and inhibition

Polyamines such as spermidine and spermine are important participants in the process of cell growth and proliferation, and their biosynthesis and metabolism are controlled by enzymes including ODC and spermidine/spermine N^1-acetyltransferase. Overexpression of these enzymes is observed in models of cancer in which ODC can play a regulatory role in transformation, invasion, and angiogenesis (63). ODC activity can be induced by proinflammatory agents including lipopolysaccharides and tumor promotors such as TPA. A fraction isolated from cranberry fruit containing proanthocyanidin oligomers and other flavonoids inhibited the activity of ODC in a mouse epidermal cell line (ME-308) as determined by an assay measuring conversion of substrate (15). Cranberry also influences the expression of ODC. Lipopolysaccharides were used to induce ODC expression in an H-ras–transformed mouse fibroblast model. When treated with whole cranberry polyphenolic extract, a dose-dependent inhibition of lipopolysaccharide-induced ODC expression was observed. Concentrations of 100 mg/L or less reduced ODC expression relative to control, and induction by lipopolysaccharides was completely abolished (64).

Cranberries and inflammation

Evidence is growing that cranberry possesses antiinflammatory properties. These effects could be linked to the presence of several key phytochemicals in the fruit. Much of what is currently known about the potential antiinflammatory actions of cranberry has come from studies that do not directly involve cancer models. A common theme is the inhibition of lipopolysaccharide-induced inflammatory response. Lipopolysaccharide is a proinflammatory mitogen, often produced by bacterial pathogens during infection. Evidence presented at a recent scientific meeting suggests that cranberry polyphenolics inhibit COX-2 expression in lipopolysaccharide-stimulated macrophages (J. D. Reed, personal communication, 2005). A high-molecular-weight cranberry fraction inhibited lipopolysaccharide-induced production of inflammatory cytokines IL-1β, IL-6, and IL-8 and TNF-α in macrophages in a study evaluating the use of cranberry for reducing inflammation caused by periodontopathogens (65). Observations that cranberry polyphenolics mediate lipopolysaccharide-stimulated events are consistent with recent studies by our group (described above) showing that cranberry inhibits lipopolysaccharide-induced ODC expression in H-ras–transformed mouse fibroblasts (64).

Inhibition of cyclooxygenase activity

COX-2 overexpression is thought to play a role in promoting certain cancers; thus, inhibition of COX-2 activity or expression presents another potential route to chemoprevention. Inhibition of cyclooxygenase activity by cranberry extracts was noted in a study by Seeram et al. (66) in which anthocyanin fractions isolated from cherries and berries were evaluated for COX-1 and COX-2 inhibitory activity using an assay measuring oxygen uptake on conversion of arachidonic acid in microsomal preparations by either isoform. Cranberry anthocyanins inhibited COX-1 and COX-2 activity by approximately the same degree, reducing activity by ~10% at 125 mg/L. Pure cyanidin was more effective in this assay, showing greater inhibition of COX-2 (47%) than COX-1 (37%). Other studies by this group have found cyanidin superior to most anthocyanins and catechins, including epigallocatechin gallate, for inhibition of COX-2 activity (67).

Recent studies in our laboratories examined the effect of whole-cranberry polyphenolic extract and cranberry proanthocyanidin fraction from Early Black fruit on the activity of COX-1 and COX-2 enzymes. Using a commercial kit to measure in vitro prostaglandin $H_{2\alpha}$ production from arachidonic acid by enzyme immunoassay, we evaluated inhibition of COX-1 and COX-2 activity. Whole-cranberry polyphenolic extract showed some inhibition of both COX-1 (IC_{50} = 170 mg/L) and COX-2 (IC_{50} = 270 mg/L). The proanthocyanidin fraction strongly inhibited COX-1 (IC_{50} = 20 mg/L), but very little inhibition of COX-2 activity by proanthocyanidins was observed (Y. Wei, J. Amoroso, C. Neto, and M. Guo, unpublished results). The effects of other cranberry fractions are under investigation.

Effects on expression of proinflammatory factors by phytochemicals in cranberry

Although cranberry inhibits COX enzyme activity in vitro, the question of whether cranberry can decrease the expression of COX-1 or COX-2 in cellular models remains to be answered. Current studies by our group are targeted toward determining whether the observed antiproliferative activity of cranberry phytochemicals toward colon tumor cells correlates with a decrease in COX expression in these cell lines. To date, no published studies have evaluated the effects of cranberry on COX expression in cancer models. Inhibition of COX-2 expression, if it is observed, could be an important mechanism in light of evidence linking COX-2 inhibition to decreased tumorigenesis, particularly in the colon (68,69). Recent studies of foods rich in polyphenolics, such as pomegranates, have shown that TNF-α–induced COX-2 expression can be suppressed in colon cancer cells (70).

Circumstantial evidence suggests that cranberry's antiinflammatory activities would also be likely to involve modulation of COX-2 expression and associated pathways. Cranberry constituents including ursolic acid and quercetin are established inhibitors of COX expression in cells. The antiinflammatory actions of triterpenes have long been known, and structure-activity relationships have been reviewed (71). Several studies of ursolic acid reported antiinflammatory activities in vivo, including reduced inflammation in mouse-ear edema models (72,73). In vitro studies have been used to examine the effects of ursolic acid on proinflammatory pathways including inhibition of COX-2–catalyzed prostaglandin biosynthesis (74). Subbaramaiah et al. (75) reported that ursolic acid inhibits COX-2 transcription in a human mammary oncogenic epithelial cell line (184B5/HER) and that the observed suppression of gene expression involves the protein kinase C signal transduction pathway. Other possible antiinflammatory mechanisms for ursolic acid include induction of NF-κB–mediated expression of inducible nitric oxide synthase and TNF-α in macrophages, implying a possible anticarcinogenic mechanism involving enhanced nitric oxide production (76).

Many studies reported antiinflammatory activities of quercetin, most citing decreased cytokine production in macrophages and similar models. In cancer cell models, quercetin reduced COX-2 mRNA expression in Caco-2 colon cancer cells. Quercetin and metabolite quercetin-3′-sulfate also inhibited COX-2 activity (77). A study of quercetin metabolites showed decreased COX-2 expression in lymphocytes in vitro but not in lymphocytes isolated from human subjects fed a high-quercetin diet (78), suggesting that bioavailability is an important issue to be considered. Other potential antiinflammatory mechanisms for quercetin include mediation of NF-κB. Quercetin dose-dependently inhibited TNF-α–dependent NF-κB activation in a study of rutin's effects on colitis (79). Whether cranberry extracts mediate NF-κB activation remains to be seen.

Future directions for cranberry research

Emerging evidence suggests that cranberry phytochemicals (particularly proanthocyanidins, quercetin, and ursolic acid) are likely to have a mitigating effect on certain types of tumors by inducing apoptosis, inhibiting proliferation and colony formation, and limiting their ability to invade and metastasize. COX-2 inhibition by cranberry phytochemicals, particularly anthocyanins, may also contribute to a decreased risk of the development of some cancers. It will be important to continue to examine the roles of cranberry phytochemicals in regulating cellular processes related to apoptosis, inflammation, and proliferation, including the expression of key genes in these pathways, so that we may begin to understand how this unique blend of phytochemicals may best work.

Cranberry's efficacy against tumor development in vivo will depend largely on the bioavailability of its phytochemicals to the various tissues, a topic that is yet to be thoroughly researched. An effort should be made to design studies that examine the effect of dietary cranberry on animal models of breast, gastric, and colon cancer as well as those that examine prostate tumor growth and metastasis. Design of such studies should pay close attention to chemical composition to maximize the diversity of available phytochemicals because several compounds in the fruit may be capable of producing complementary biological effects.

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