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Metabolism and growth inhibitory activity of cranberry derived flavonoids in bladder cancer cells

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Abstract

In the present study, anti-proliferative activities of cranberry derived flavonoids and some of their in vivo metabolites were evaluated using a panel of human bladder tumor cell lines (RT4, SCABER, and SW-780) and non-tumorigenic immortalized human uroepithelial cells (SV-HUC). Among the compounds tested, quercetin 3-*O*-glucoside, isorhamnetin (3'-*O*-methylquercetin), myricetin and quercetin showed strong concentration-dependent cell growth inhibitory activities in bladder cancer cells with IC₅₀ values in a range of 8-92 μ M. Furthermore, isorhamnetin and myricetin had very low inhibitory activity against SV-HUC even at very high concentrations (>200 μ M) compared to bladder cancer cells, indicating that their cytotoxicity is selective for cancer cells. To determine whether the differential cell growth inhibitory effects of isomeric flavonoids quercetin 3-*O*-glucoside (active) and hyperoside (quercetin 3-*O*-galactoside) (inactive) are related to their metabolism by the cancer cells, SW-780 cells were incubated with these compounds and their metabolism was examined by LC-MS/MS. Compared to quercetin 3-*O*-glucoside, hyperoside undergoes relatively less metabolic biotransformation (methylation, glucuronidation and quinone formation). These data suggest that isorhamnetin and quercetin 3-*O*-glucoside may be the active forms of quercetin in prevention of bladder cancer in vivo and emphasize the importance of metabolism for the prevention of bladder cancer by diets rich in cranberries.

Keywords

cranberry; quercetin; metabolites; bladder cancer; mass spectrometry; metabolism

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Conflict of interest

The authors declare that they have no conflict of interest.

Disclosure statement

The authors have nothing to disclose.

Introduction

Cranberry (*Vaccinium macrocarpon* Ait. Ericaceae), a native fruit in North America, has attracted much public interest as a functional food due to its potential health beneficial effects, including cancer prevention. Emerging evidence has suggested an *in vitro* anti-tumor activity of cranberry in breast, cervical, oral, colon and prostate cancer cell lines¹⁻³. We demonstrated that a commercially available cranberry juice concentrate can prevent urinary bladder cancers in a rat model of bladder tumorigenesis using the carcinogen, N-butyl-N-(4-hydroxybutyl)-nitrosamine (OH-BBN)⁴. In our previous studies, chronic administration of cranberry concentrate to rats resulted in detectable accumulation of quercetin and isorhamnetin in the bladder⁵. The results also revealed that cranberry components undergo rapid metabolism and elimination into the urine of rats. These observations led us to identify metabolites of cranberry that inhibit the growth of bladder cancer cells to better understand the basis for the cancer preventive activity of cranberry juice.

An inhibitory effect of cranberry polyphenols in bladder carcinogenesis is plausible since most metabolites are excreted through urine⁶. Importantly, the urinary concentration of these substances is substantially higher than in the blood, whereby the uroepithelium is exposed to high concentrations that increase their potential for anti-cancer activity. In support of our previous observation of bladder cancer prevention in rats, and to explore this idea in the context of human cancer, we report here direct growth inhibitory effects of some cranberry derived flavonoids and their *in vivo* metabolites on cultured human bladder cancer cells, but not immortalized urothelium cells. We also describe the cellular uptake and metabolism of quercetin and its analogs in the human SW-780 bladder tumor cell line.

Experimental

Materials

Standards of rutin trihydrate, quercetin 3,7,3,4 tetramethylether, peltatoside (3-arabinoglucosylquercetin), quercetin, hyperoside (quercetin 3-*O*-galactoside), quercitrin (quercetin 3-*O*-rhamnoside), isorhamnetin, myricetin, peonidin 3-*O*-glucoside, kuromanin (cyanidin 3-*O*-glucoside) chloride, ideanin (cyanidin 3-*O*-galactoside) chloride, Quercetin 3-*O*-glucoside, quercetin 3,4''di-*O*-glucoside and proanthocyanidin dimer A2 were purchased from Indofine Chemicals Inc. (Hillsborough, NJ, USA). Quercetin 3-*O*-glucuronide was purchased from Sigma Aldrich (St. Louis, MO). High Performance Liquid Chromatography (HPLC) solvents (methanol, and acetonitrile) and reagents were purchased from Fisher (Norcross, GA, USA) and were of HPLC grade.

Cell Culture

Non-tumorigenic immortalized human urothelium cells (SV-HUC), transitional bladder papilloma cells (RT-4), transitional carcinoma cells (SW-780), and squamous carcinoma cells (SCABER) were obtained from ATCC and were sub-cultured every 3-4 days in RPMI-1640 supplemented with 10% FBS and 2 mM glutamine and incubated at 37°C with 5% CO₂, and maintained at sub-confluent density⁷⁻¹⁰. Prior to each experiment, cells were trypsinized, and centrifuged to remove media, and suspended in complete medium. Cells

(5,000/well) were plated and cultured with or without test compounds with a final concentration range of 0.03-200 μ M. Compounds were dissolved in DMSO and diluted in growth medium maintaining a final concentration of 0.2% DMSO in both experimental and vehicle control samples. Sulindac sulfide amide (10 μ M) was used as positive control. After 72 h incubation, assay plates were allowed to cool to room temperature for 10 min prior to addition of 100 μ L per well Promega CellTiterGlo reagent, followed by an additional 10 min incubation at room temperature. The resulting luminescence was quantitated using a PerkinElmer Victor 3V multimode microplate reader. Percent growth inhibition was calculated as follows, where 100 represents the viability of vehicle treated control samples (concentration = 0): $100 - [100 \times (\text{sample}) / \text{vehicle control}]$. Thus, on average, growth inhibition of vehicle control samples equals zero. Potency of compounds was determined using the Non Linear Dose Response algorithm (4-parameter logistic fit) with GraphPad Prism 5 software. For intracellular metabolite analysis, cells were treated with test compounds (50 μ M). Cell free control incubations were performed with DMSO at a final concentration of 0.5% in the medium. Following a 3, 6, 9, 24 and 72 h incubation, the culture medium was removed, and the cells washed with ice cold PBS, detached and lysed with methanol.

LC-MS/MS analysis

Analysis of cranberry metabolites in SW-780 cells was performed by LC-MS/MS consisting of a model SIL-HT refrigerated Shimadzu autosampler, an HPLC instrument (Shimadzu Scientific Instruments, Inc. Columbia, MD, USA), and an API 4000 mass spectrometer (Sciex, Concord, Ontario, Canada). Chromatography was carried out on a reverse-phase Phenomenex Fusion C18 column (150 $\times$ 2.0 mm i.d.) (Torrance, CA, USA) with the mobile phase consisting of solvent A (0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid). A 11 min gradient was established from 10% to 80% over first five minutes and reached to 100% B at 5.5 min. It was maintained for further 1 min and the system was returned to the initial 10%B at 7.0 min.

The column effluent was introduced into the mass spectrometer using electrospray ionization (ESI) in the negative and positive ion modes. Nitrogen was used as nebulizer and curtain gas. The nebulizer current and temperature were 5 A and 300 $^{\circ}$ C, respectively. The collision gas (N_2) was set at high and collision energy was 30-86 eV (negative ion mode) with electron multiplier voltage (1900 V). The following mass transitions were used for multiple reaction monitoring (MRM) analysis: quercetin m/z 301/151, isorhamnetin m/z 315/151 and 315/300, quercetin monoglucuronide m/z 477/301, methylquercetin glucuronide m/z 491/315, quercetin glucoside/galactoside m/z 463/301, quercetin glucoside/galactoside glucuronide m/z 639/463, quercetin quinone m/z 302/153, and quercetin glucuronide quinone m/z 478/302 (positive ion mode). The LC-MS-MS system was controlled by BioAnalyst 1.4.2 software.

Results

Cranberry derived flavonoids and their anti-proliferative effects

Anti-proliferative activities of some cranberry derived *flavonoids* and their metabolites were evaluated in a panel of human bladder cancer cell lines. As shown in Table 1, quercetin 3-O-glucoside, 3'-O-methylquercetin (isorhamnetin), quercetin, and myricetin showed moderately potent activities with IC₅₀ values ranging from 8.7 to 92.4 μ M in bladder cancer cells. A limited structure-activity relationship study was performed to define the structural requirement for quercetin-induced growth inhibition in a variety of human bladder cancer cells. For this, we compared the anti-proliferative activity of quercetin with its glycoside analogs and phase II conjugated metabolites (methylated and glucuronidated) and other flavonoids. Compared to the aglycone quercetin, glycoside analogs of quercetin such as peltatoside, quercetin 3-4'-di-O-glucoside and rutin trihydrate (Fig. 1) showed no growth inhibitory activity. However, quercetin-3-O-galactoside and quercetin-3-O-rhamnoside showed weaker activity than quercetin 3-O-glucoside (Table 1). These indicate that the nature and number of sugar residues play important role for anti-proliferative activities.

While isorhamnetin showed a slightly improved activity compared to quercetin, methylation at 3,7,3',4' positions lost the activity (data not shown), indicating the importance of hydroxyl groups in the rings A, B and C for growth inhibitory activity. Furthermore, isorhamnetin and myricetin revealed very low inhibitory activity in normal human uroepithelial SV-HUC cells (IC₅₀ >200 μ M) compared to bladder cancer cells (Table 1 and Fig. 2), while the rest of compounds showed similar values of anti-proliferative effect between cancer and normal cells. These results indicate that the methylated metabolite (isorhamnetin), but not the parent compound (quercetin) may be the active metabolite in the prevention of bladder cancer.

We also examined the activity of quercetin 3-O-glucuronide - the major phase II metabolite of quercetin. It showed no growth inhibitory activity even with the highest concentration (200 μ M) in SW-780 bladder tumor cell line. Although cranberry proanthocyanidins have previously been shown to have anti-proliferative activity in various tumor cell lines¹¹, proanthocyanidin dimer (proanthocyanidin A2), one of the major proanthocyanins of cranberry fruit juice, did not show appreciable activity in the present study. Similarly, anthocyanins- cyanidin 3-O-galactoside, cyanidin 3-O-glucoside and peonidin 3-O-glucoside showed weak effects on bladder SW-780 cancer cells (Table 1).

Cellular metabolism of quercetin and its analogs

To investigate the role of cellular metabolism of cranberry polyphenols in cancer prevention, quercetin, quercetin galactoside, quercetin glucoside and phase II metabolites of quercetin were studied in SW-780 cells using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Following 3, 6, 9, 24 and 72 h exposure, cell supernatants were removed, cells were washed with ice cold PBS, detached, lysed with methanol, and then analyzed by LC-MS/MS operated in the MRM mode.

MRM analysis of cell extracts and cell supernatants showed that levels of quercetin and its phase II metabolites (glucuronidated and methylated products) change with the time of

incubation. The levels of quercetin gradually decreased over time in both supernatant (Fig. 3) and cell extract samples, indicating its conversion to O-methylated and glucuronidated metabolites. A single peak at Rt 6.30 min corresponding to 3'O-methylquercetin was observed at 3 h after incubation; however, an additional peak, possibly 4'O-methylquercetin observed at Rt 6.12 min after 24 h exposure to quercetin (Fig. 3). Methylquercetin reached the highest level after 9 h incubation in both cell supernatant and lysate. While glucuronides of quercetin gradually decreased in the cell lysates over time, the cell supernatant collected at 24 h showed more than five-fold increase in its concentration, compared to 3h (Fig. 4).

Two peaks corresponding to quercetin glucuronide (mass transition m/z 477/301 in negative ion mode) appeared at Rt 4.99 and 5.28 min (Fig.3). While four monoglucuronides of quercetin (7-,3-, 4' - and 3' -glucuronides) are possible, the two peaks observed in the present study are likely to be 3- and 3' or 7-glucuronides of quercetin because these products have been reported to be formed with highest efficiency¹². Quercetin 3-O-glucoside and its galactoside analog not only showed differential activities on cell proliferation, but also in terms of their metabolism. Since both compounds were observed intact in significant amounts in cell lysates, the hydrolysis of the glycosidic bond does not seem to be crucial for their absorption into the cells. However, the level of glucuronide of quercetin 3-O-glucoside appeared to be more than twice that of quercetin 3-O-galactoside (Fig. 5). Although the rate of glucuronidation of the galactoside standard was found to be lower than that of the glucoside standard in these cells, most of the glucuronide originating from cranberry in vivo would likely come from the galactoside or rhamnoside, as they are present in much higher starting concentrations than the glucoside.

Several intracellular metabolites with quercetin quinone/quinone methide-like structures are formed in SW-780 bladder tumor cells following treatment for 24 h. LC-MS/MS analysis confirmed the formation of glucuronides of quercetin m/z 479 and its methylated analog m/z 493 and their quinone/quinone methide analogs m/z 478 and 492 (one mass unit less than their parent compounds). Our observations provide support for previous reports concluding that the formation of quinone species increase significantly over time^{13,14}. For example, quercetin glucuronide quinone species increased more than 100 fold (3-24 h) in the cell supernatants treated with quercetin (Fig. 6). When a neutral loss scan of 176 Da was performed to detect all glucuronides of quercetin, ions corresponding to quercetin glucuronide (m/z 479), quercetin glucuronide of quinone (m/z 478), and their monomethylated products (m/z 493 and 492, respectively) were also detected (data not shown).

Quinone analogs of quercetin have characteristic product ions in their MS/MS spectra. Quercetin glucuronide m/z 479 in LC-MS/MS appeared at Rt 5.2 min with a product ion m/z 303.1 whereas its quinone analog appeared at Rt 5.1 min with a product ion m/z 302. MS/MS of the ions m/z 302.0, and 303 and m/z 316 and 317 showed slightly different fragmentation patterns and retention times. The ion m/z 316 was previously reported as a proton-stabilized quinone species¹³.

Formation of quercetin-quinone like metabolites is markedly different in the cell supernatant samples exposed to quercetin, quercetin 3-O-glucoside and quercetin 3-O-galactoside for 72

h (Fig. 7). Compared to quercetin glycosides [7B and C], quinone species derived from aglycone quercetin [7A] in the cells were detected at relatively higher levels in peak intensities using the MRM assay. Cells exposed to quercetin 3-O-galactoside [7C] showed about five-fold less quinone species than that of quercetin 3-O-glucoside. These results, for the first time, demonstrate that quercetin 3-O-glucoside undergoes more glucuronidation reaction and oxidative metabolism than its galactose analog.

The formation of a quercetin glutathione (GSH) adduct was indicated by the presence of ion m/z 608 $[M+H]^+$ in positive ion mode. MS/MS of the ion m/z 608 further confirmed the formation of glutathionyl conjugate of quercetin (Fig. 8). The loss of the glycine moiety from the glutathionyl conjugate of quercetin ion m/z 608 resulted in product ion m/z 533. Hong and Mitchell¹⁵ found glutathionyl quercetin (m/z 608) and glutathionyl quercetin (quinone) m/z 606 in positive ion mode. In their investigations, MS/MS of m/z 608 and 606 showed product ions m/z 335 and 333, respectively. However, we observed an intense product ion m/z 333 upon MS/MS of the ion m/z 608, indicating that the product ion m/z 333 could exist in a quinone form as shown in Fig. 8.

The quercetin quinone species is chemically reactive and scavenged by GSH. Previous studies have shown that glutathionyl quercetin adducts are formed in cells and subsequently excreted into the incubation medium¹⁴. In the present study, the attachment of glutathione moiety to quercetin was unknown. Although 6- and 8-glutathionyl-quercetins were as previously reported¹⁶, glutathione conjugation could possibly occur on the ring B of quercetin via an O-quinone form. Therefore, the structure of m/z 608 is tentatively assigned as shown in Fig. 8.

Discussion

Cranberry contains a number of flavonoids in which quercetin 3-O-galactoside is the most abundant in fruit powder⁴. Quercetin has been extensively investigated for its anti-cancer properties^{13,14}. While quercetin 3-O-glucoside showed potent anti-proliferative activity in a number of bladder cancer cell lines, quercetin 3-O-galactoside failed to show significant growth inhibitory activity in our studies. Previous studies also showed that quercetin 3-O-glucoside is a superior cytotoxic flavonoid in vivo compared to its other analogs¹⁷. Addition of rhamnose and disaccharide rutinose to quercetin also showed attenuated activities. These glycoside conjugates of quercetin showed similar trends in previous studies in human promyeloleukemic HL-60 cells¹⁸.

To determine whether the differential cell growth inhibitory effects of isomeric quercetin 3-O-glucoside (active) and quercetin 3-O-galactoside (inactive) are related to their metabolism in the cancer cells, SW-780 cells were incubated with these compounds and their metabolism was examined by LC-MS/MS. Compared to quercetin 3-O-glucoside, quercetin 3-O-galactoside undergoes relatively little metabolism such as hydrolysis to aglycone, glucuronidation and oxidative metabolism. Although hyperoside (quercetin-3-O-galactoside) is the major compound in cranberry and relatively inactive in in vitro assay, cranberry's preventive effects against bladder carcinogenesis is likely to be combinatorial effects of aglycone quercetin and its conjugated analogs. Combination of quercetin and hyperoside has

previously been shown to exhibit anticancer effects in renal cancer cells through inhibition of oncogenic microRNA-27a¹⁹.

The uptake, metabolism, pharmacokinetics and biological effects of quercetins depend on their chemical structures and biological systems being tested. Quercetin glucuronides are the main circulating phase II metabolites in humans. In vitro studies have shown that glucuronidated metabolites of polyphenols are more hydrophilic, less active or inactive and better eliminated than their aglycone counterparts²⁰.

In our studies, quercetin 3-O-glucuronide did not show potent cell growth inhibitory effects on bladder cancer cells. Perhaps, as opposed to other cell types, bladder cells are exposed to essentially all toxic metabolites with this modification, it is reasonable to expect that they are equipped to exclude/ efflux them.

Previous studies have indicated that the action of quercetin and its metabolites *in vivo* is mediated by intracellular metabolites¹³. Quercetin is more likely to exist as a semi-quinone radical form and quinoidal product and they may be responsible for the anti-proliferative activity of quercetin²¹. In the present studies, quercetin and quercetinquinone-like metabolites may inhibit bladder tumor cell growth in quercetin treated cells. By contrast, the growth inhibitory effects of quercetin 3-O-glucoside does not seem to be related to its cellular metabolites as it produced much less quinone-like compounds, compared to aglycone quercetin. Such metabolites may interact with a membrane-bound or extracellular target to exert its anti-proliferative activity^{13, 22}.

Generally, the uptake of flavonoid O-glycosides is preceded by hydrolysis and followed by re-conjugation (glucuronidation/sulfation and methylation) in which the metabolism are controlled by the specificity and distribution of enzymes that catalyze the reaction^{23,24}. However, high levels of intact quercetin 3-O- galactoside and quercetin 3-O- glucoside were detected in the cell lysates, indicating that the hydrolysis of O-linked glycoside is not essential for cellular uptake (Fig. 5). The entry of quercetin's into cells may be facilitated by membrane lipids and proteins by making it more soluble in an aqueous environment ²⁵.

Usually, polyphenol aglycones may be active compounds *in vivo*^{24,26}. However, quercetin 3-O-glucoside showed the most potent activity among the flavonoids tested. As glucuronidation is widely regarded as detoxification step in quercetin metabolism²⁷, quercetin glucuronide showed no growth inhibitory activity in bladder tumor cell lines.

Chemically, quinones are oxidants and electrophilic in nature and exposure to these chemicals are known to be cytotoxic. Biological activities of polyphenols may be mediated by intracellular metabolites quinone/quinone methide. Quercetin at higher concentrations (>100 μ M) generates toxic quinones that show cytotoxic and apoptotic effects²⁸. Thus, quercetin may act as a pro-oxidant and toxic polyphenol. However, quinone/quinone methides are reactive and rapidly react with GSH to produce reversible GSH adduct. The stability of these thiol adducts may range from minutes and hours and therefore, they are relatively unstable ²⁹.

We previously demonstrated that cranberry components undergo rapid metabolism and elimination into the urine of rats and quercetin and 3'-O-methylquercetin are present in the urinary bladder tissue potentially allowing them to inhibit urinary bladder carcinogenesis⁵. Results from this study showed that growth inhibitory effect of the methylated metabolite of quercetin (3'-O-methylquercetin) is stronger than quercein, whereas quercetin 3-O-glucuronide didn't show effects. These data further suggest that the effect of quercetin could be mediated by its methylated conjugates, apart from quinones. It also emphasizes the importance of metabolism of polyphenols for the prevention of bladder cancer by diets rich in cranberries.

In addition to the upper intestinal metabolism, the major part of uptake and metabolism of polyphenols, including proanthocyanidins take place in the colon, where the resident microbiota produce metabolites that can undergo further metabolism upon entering the systemic circulation. The microbial metabolism may result into major structural transformations on polyphenolics such as cleavage of the glycosidic bond, reduction of double bond, dehydroxylation, demethylation, decarboxylation and ring-fission³⁰. Future studies should be directed to testing the microbial metabolites for their anti-cancer effects.

Conclusion

Cranberry components quercetin 3-O-glucoside, isorhamnetin, myricetin and aglycone quercetin show dose-dependent cell growth inhibitory effects against bladder cancer cells and therefore could be responsible for the cancer preventive activity of cranberry as we reported using a rat model of chemical-induced bladder tumorigenesis⁴. While quercetin 3-O-glucoside undergoes more glucuronidation reaction and oxidative metabolism than its galactoside analog, the underlying mechanism accounting for the tumor cell growth inhibitory effects of quercetin 3-O-glucoside is less clear cut. A comprehensive in vitro study on anti-cancer effects of upper intestinal and microbial metabolites of these polyphenols is further warranted.

Acknowledgements

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List of abbreviations

OH-BBN	N-butyl-N-(4-hydroxybutyl)-nitrosamine
HPLC	High Performance Liquid Chromatography
ATCC	American type culture collection
SVHUC	non-tumorigenic immortalized human uroepithelial cells

RT-4	transitional bladder papilloma cells
SW-780	transitional carcinoma cells
SCABER	squamous carcinoma cells
DMSO	dimethyl sulfoxide
PBS	phosphate buffered saline
LC-MS/MS	liquid chromatography tandem mass spectrometry

References

1. Seeram NP, Adams LS, Hardy ML, Heber D. J. Agric. Food Chem. 2004; 52:2512–2517. [PubMed: 15113149]
2. Neto C. J. Nutr. 2007; 137:186S–193S. [PubMed: 17182824]
3. Guthrie N. Proc Exper Biology Conf. 2000; 53:13.
4. Prasain JK, Jones K, Moore R, Barnes S, Leahy M, Roderick R, Juliana MM, Grubbs CJ. Oncology Reports. 2008; 19:1565–1570. [PubMed: 18497966]
5. Rajbandhari R, Peng N, Moore R, Arabshahi A, Wyss JM, Barnes S, Prasain JK. J. Agric. Food Chem. 2011; 59:6682–8. [PubMed: 21634376]
6. Iswaldi I, Arráez-Román D, Gómez-Caravaca AM, Contreras MM, Uberos J, Segura-Carretero A, Fernández-Gutiérrez A. Food Chem Toxicol. 2013; 55:484–92. [PubMed: 23395719]
7. Christian BJ, Loretz LJ, Oberley TD, Reznikoff CA. Cancer Res. 1987; 47:6066–73. [PubMed: 2822239]
8. Kyriazis AA, Kyriazis AP, McCombs WB 3rd, Peterson WD Jr. Cancer Res. 1984; 44:3997–4005. [PubMed: 6744315]
9. Reitmair A, Shurland DL, Tsang KY, Chandraratna RA, Brown G. Int J Cancer. 2005; 115:917–23. [PubMed: 15729717]
10. Masters JRW, Hepburn PJ, Walker L, Highman WJ, Trejdosiowicz LK, Povey S, Parkar M, Hill BT, Riddle PR, Franks LM. Cancer Research. 1986; 46:3630–36. [PubMed: 3708594]
11. Neto CC, Krueger CG, Lamoureux TL, Kondo M, Vaisberg AJR, Hurta, Curtis S, Matchett MD, Yeung H, Sweeney MI, Reed JD. J Sci Food Agric. 2006; 86:18–25.
12. Chen YK, Chen SQ, Li X, Zeng S. Xenobiotica. 2005; 35:943–54. [PubMed: 16393854]
13. Spencer JP, Kuhnle GG, Williams RJ, Rice-Evans C. Biochem. J. 2003; 372:173–81. [PubMed: 12578560]
14. Awad HM, Boersma MG, Boeren S, van der Woude H, van Zanden J, van Bladeren PJ, Vervoort J, Rietjens IM. FEBS Letters. 2002; 520:30–4. [PubMed: 12044865]
15. Hong YJ, Mitchell AE. Chem. Res. Toxicol. 2006; 19:1525–32. [PubMed: 17112241]
16. Boersma MG, Vervoort J, Szymusiak H, Lemanska K, Tyrakowska B, Cenas N, Segura-Aguilar J, Rietjens IM. Chem. Res. Toxicol. 2000; 13:185–91. [PubMed: 10725115]
17. Park SH, Kim HJ, Yim SH, Kim AR, Tyagi N, Shen H, Kim KK, Shin BA, Jung DW, Williams DR. J Nat Prod. 2014; 77:2389–96. [PubMed: 25397870]
18. Shen SC, Chen YC, Hsu FL, Lee WR. J Cell Biochem. 2003; 89:1044–55. [PubMed: 12874837]
19. Li W, Liu M, Xu YF, Feng Y, Che JP, Wang GC, J.H. Oncol Rep. 2014; 31:117–24. [PubMed: 24173369]
20. González-Sarriás A, Giménez-Bastida JA, Núñez-Sánchez MÁ, Larrosa M, García-Conesa MT, Tomás-Barberán FA, Espín JC. Eur J Nutr. 2014; 53:853–64. [PubMed: 24077694]
21. Metodieva D, Jaiswal AK, Cenas N, Dickanaité E, Segura-Aguilar J. Free Radic. Biol. Med. 1999; 26:107–16. [PubMed: 9890646]
22. Hung H. Forum Nutr. 2007; 60:146–57. [PubMed: 17684411]
23. Murakamia A, Ashidab H, Teraoc J. Cancer Letters. 2008; 269:315–325. [PubMed: 18467024]

24. Scalbert A, Williamson G. J. Nutr. 2000; 8:2073S–2085S.
25. Moridani MY, Galati G, O'Brien PJ. Chem Biol Interact. 2002; 139:251–64. [PubMed: 11879815]
26. Allred CD, Twaddle NC, Allred KF, Goepfinger TS, Churchwell MI, Ju YH, Helferich WG, Doerge DR. J. Agric Food Chem. 2005; 53:8542–50. [PubMed: 16248551]
27. A O'Leary K, J Day A, W Needs P, S Slyd W, M O'Brien N, Williamson G. FEBS Lett. 2001; 503:103–106. [PubMed: 11513863]
28. Dajas F, Abin-Carriquiry JA, Arredondo F, Blasina F, Echeverry C, Martínez M, Rivera F, Vaamonde L. Neurochem Int. 2015; 89:140–8. [PubMed: 26160469]
29. Awad HM, Boersma MG, Boeren S, Van Bladeren PJ, Vervoort J, Rietjens IM. Chem Res Toxicol. 2003; 16:822–31. [PubMed: 12870884]
30. Aura A. Phytochemistry Reviews. 2008; 7:407–429.

1.
Structures of quercetin and its conjugated analogs.

2.

Growth inhibitory effects of isorhamnetin and myricetin against normal uroepithelial and transitional carcinoma cells. Three-fold serial dilutions of experimental compounds were added to four replicate wells in each cell line, along with vehicle control (0.2% DMSO). Cells were incubated with the compounds for 72 h. Growth inhibition was measured with the CellTiterGlo® reagent. IC_{50} values and dose-response curves were defined using GraphPad Prism 5.

3.

MRM chromatograms showing conversion of quercetin into methylated and glucuronidated quercetins in quercetin treated cell supernatant. [A] standards; [B] after 3 h incubation; [C] 24 h incubation in SW-780 cells.

4.

Change in concentration of quercetin (Q), 3'-O-methylquercetin (Me-Q) and quercetin 3-O-glucuronide (Q-3-O-Glu) inside and outside cells after 3, 6, 9 and 24 h incubation of quercetin in SW-780 cells. Y axis shows abundance ratios relative to 3 h time point.

5. MRM chromatograms showing the difference in formation of glucuronides in the cell lysates when SW-780 cells were exposed to quercetin 3-O-glucoside [A], and quercetin 3-O-galactoside [B] for 72 h. Mass transitions were m/z 301/151 (quercetin); 463/301 (quercetin 3-O-glucoside or quercetin 3-O-galactoside), 639/463 (glucuronide of quercetin 3-O-glucoside or quercetin 3-O-galactoside).

6. MRM chromatograms showing quercetin conversion into quercetin quinone and quercetin glucuronide quinone like metabolites in different time points in SW-780 cell supernatants. Mass transition m/z 302/153 (quercetin quinone) and 478/302 (quercetin glucuronide quinone) were used.

7.

MRM chromatograms showing that the formation of quercetin-quinone like metabolites is markedly different in the cell supernatant samples exposed to quercetin [A], [B] quercetin 3-O-glucoside and [C] quercetin 3-O-galactoside for 72 h. Mass transition m/z 302/153 was used.

8. MS/MS spectrum of quercetin glutathione m/z 608.0 $[M+H]^+$ in the quercetin treated cell supernatant after 72 h incubation.

Table 1Growth inhibitory effects of cranberry derived flavonoids in human bladder tumor cell lines^{*}

Compound	IC ₅₀ (μM)			
	RT4	SCABER	SV-HUC1	SW780
Quercetin	92.39	24.25	81.18	43.43
Isorhamnetin	34.26	43.21	>200	12.51
Myricetin	72.68	30.26	>200	20.94
Quercetin-3-O-glucoside	20.11	8.66	12.60	20.13
Quercetin-3-O-galactoside	ND	ND	ND	135.7
Quercetin-3-O-rhamnoside	ND	ND	ND	189.5
Cyanidin-3-O-galactoside	ND	ND	ND	84.49
Cyanidin-3-O-glucoside	ND	ND	ND	169.3
Peonidin 3-O-glucoside	ND	ND	ND	137.0

^{*} only compounds with IC₅₀s, are listed in this Table. ND = not determined.