J. Pharm. Pharmacol. 1999, 51: 353–359
Received September 9, 1998
Accepted November 3, 1998

Fate of the Flavonoid Quercetin in Human Cell Lines: Chemical Instability and Metabolism

DAVID W. BOULTON*, U. KRISTINA WALLE AND THOMAS WALLE

Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC 29425, USA

Abstract

Although cell cultures are increasingly being used as models for studying the biological actions of flavonoids, no information on the fate, such as uptake and metabolism, exists for these natural products in these models.

This study examined the elimination of quercetin, one of the most abundant flavonoids, from the cultured human hepatocarcinoma cell line Hep G2 using [^{14}C]-labelled compound with HPLC and LC/MS for structure characterization. These cells showed a 9·6-fold accumulation of quercetin and the formation of an O-methylated metabolite, isorhamnetin. However, a rapid elimination of quercetin, with no unchanged compound present beyond 8 h, was mainly due to oxidative degradation. The initial intermediate reaction appears to involve peroxidation, leading to a dioxetan, as evidenced by a 32-amu increase in the molecular ion by LC/MS. Subsequently, opening of the C-ring leads to the formation of carboxylic acids, the major one identified in this study as protocatechuic acid. A separate reaction results in a polymeric quercetin product which is highly retained on a reversed-phase C18 HPLC column.

It is postulated that these degradative and metabolic changes contribute to the multiple biological actions reported for quercetin, using cell culture models. Interestingly, part of the degradative pathway could be inhibited by including nontoxic concentrations of EDTA in the cell culture medium.

Bioflavonoids constitute a large group of polyphenolic natural products, which are found in almost all foods of plant origin (Hertog et al 1992, 1993). The most prevalent member of this class of compounds is quercetin (Figure 1). Epidemiological evidence suggests that these dietary products have protective effects against coronary heart disease and stroke (Keli et al 1996; Knekt et al 1996). Proposed mechanisms for these effects are inhibition of oxidation of low density lipoprotein and inhibition of platelet aggregation. Animal studies also suggest that flavonoids have protective effects against cancer (Deschner et al 1991, 1993; Wang et al 1994; Ames et al 1995). Potential mechanisms, deduced from in-vitro studies, include effects on signal transduction pathways (Weber et al 1996; Fotsis et al 1997; Lepley & Pelling 1997) and inhibition of enzymes involved in procarcinogen bioactivation (Tsyrlov et al 1994; Walle et al 1995).

The in-vitro studies cited above as well as many others (e.g. Ranelletti et al 1992; Chi et al 1997; Csokay et al 1997; Duthie et al 1997; Kang & Liang 1997; Sato et al 1997) have used cell culture models, involving incubations over extended periods of time, to establish a wide variety of biological responses and to deduce potential mechanisms. These studies have in general not taken into account the disposition of quercetin, such as cellular uptake and metabolism, which may have an impact on its biological actions. Of even greater possible importance is chemical degradation of this labile molecule during prolonged exposure of aqueous solutions to cell culture conditions (i.e. large aerated surface areas at 37°C). Potential chemical degradation has previously been of concern in studies of the oral bioavailability of quercetin, which is low and variable (Gugler et al 1975; Hollman et al 1995).

Correspondence: T. Walle, Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA.
E-mail: wallet@musc.edu
*Present address: Dept. of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, SC 29425, USA.

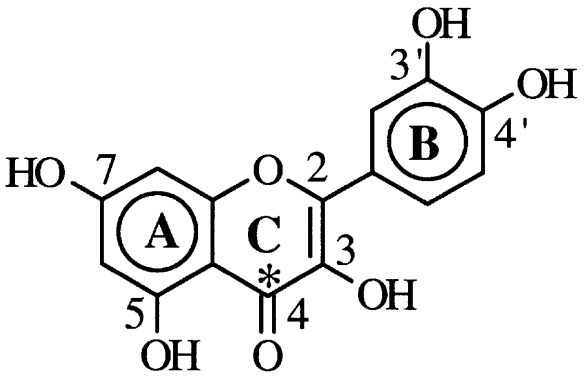

Figure 1. Chemical structure of quercetin. The asterisk denotes the site of the [^{14}C]-label.

In the present study we examined the relative contribution of chemical degradation and metabolism to the fate of quercetin in the human hepatic cell line Hep G2 and the human intestinal cell line Caco-2, using [^{14}C]-labelled quercetin. The Hep G2 line has been shown to express certain phase I and phase II drug metabolizing enzymes making it useful for the detection of mutagens and antimutagens, such as flavanoids (Knasmüller et al 1998). The Caco-2 cell line has recently been employed in studies of the trans-epithelial transport of quercetin (Walgren et al 1998). Our observations demonstrate that quercetin is highly labile in cell culture, leading to rapid degradation along with metabolism with the formation of multiple potentially biologically active products.

Materials and Methods

Materials

4-[^{14}C]Quercetin dihydrate ([^{14}C]quercetin; Figure 1) was custom synthesized by Chemsyn Science Laboratories (Lenexa, KS) for the National Cancer Institute Chemical Carcinogen Reference Standard Repository. Its specific activity was 52·9 mCi mmol^{-1} and it was shown to be $\geq$98% pure by HPLC. [^{14}C]Quercetin powder (200 μCi) was dissolved in dimethyl sulphoxide (DMSO) and stored under argon at -20°C and thawed immediately before use. Unlabelled quercetin dihydrate, protocatechuic acid (3,4-dihydroxybenzoic acid) and superoxide dismutase (from bovine erythrocytes) were purchased from Sigma (St Louis, MO). Isorhamnetin was obtained from Extrasynthese (Genay, France). Solvents, including water, were of HPLC grade and all other chemicals were of analytical grade or better.

Cell culture

Human hepatocellular carcinoma Hep G2 cells and human colon adenocarcinoma Caco-2 cells were obtained from the American Type Culture Collection, Rockville, MD. Media and supplements were obtained from Sigma. Hep G2 cells were cultured in Williams' medium E supplemented with 10% foetal calf serum, 100 units mL^{-1} penicillin, 100 mg mL^{-1} streptomycin and 1% L-glutamine. The medium was changed twice weekly and stock cells subcultured weekly. Confluent Hep G2 cells in 12-well plates were used for metabolism studies when they were between 7 and 14 days post-seeding. Caco-2 cells were cultured in Minimum Essential Eagle Medium with 10% foetal calf serum, 100 units mL^{-1} penicillin, 100 mg mL^{-1} streptomycin and 1% non-essential amino-acid solution. The medium was changed every other day and stock cells subcultured at near confluency. The cells used in this study were grown in 6-well plates and used at 25 days post-seeding.

Incubations

Incubations of 5 and 15 μM [^{14}C]quercetin with Hep G2 and Caco-2 cell cultures were performed in triplicate in different batches of cells at 37°C in a humidified atmosphere of 95% air and 5% carbon dioxide. For each concentration, quercetin was added from a freshly prepared stock solution in culture medium. The final concentration of DMSO was less than 0·2%. Samples were taken at 0, 0·5, 1, 2, 4, 6 and 8 h following the addition of the stock solutions.

Samples were placed in sealable plastic tubes and an equal volume of methanol : water : glacial acetic acid (50 : 45 : 5) was immediately added to the tube to precipitate protein and to acidify the sample. Samples were frozen at -80°C until analysis. Thawed samples were centrifuged at 16 000 g for 2 min to sediment protein before injection of 200 μL of supernatant onto the HPLC. When acidified aqueous methanol was added to samples, quercetin was chemically stable for at least 24 h at room temperature as there was no significant decrease in its peak area over this time ($n = 5$, paired two-tailed Student's t-test). This sample treatment, which was necessary for the subsequent HPLC analysis as well as for protection of the samples from further chemical degradation, caused some loss of radioactivity due to the very high binding of quercetin to serum proteins (Boulton et al 1998). These losses were, however, highly reproducible ($21 \pm 5\%$; $n = 12$).

Retardation of the chemical decomposition of quercetin

To assess additives that may potentially retard the chemical decomposition of quercetin in incubation medium, Hep G2 cells were incubated as described

above for 6 h with 15 μM [^{14}C]quercetin and various concentrations of either a reducing agent (D,L-dithiothreitol, 0·1–10 mM), a superoxide scavenger (superoxide dismutase, 0·5–100 units mL^{-1}) or a metal ion chelator (ethylenediaminetetraacetic acid (EDTA), 0·01–1 mM). Samples were treated as described above and analysed by HPLC with radiochemical detection.

Cellular uptake experiments

Confluent Hep G2 cells in 6-well plates were incubated at 37°C with 5 μM [^{14}C]quercetin in 2 mL of Hanks' balanced salt solution buffered to pH 7·4 with 25 mM *N*-[2-hydroxyethyl)piperazine-N′-(2-ethanesulphonic acid)(HEPES). At 0, 1, 2, 5, 10 and 30 min the quercetin solution was removed and the cell monolayer rapidly washed 3 times with 1 mL ice-cold buffer. The cells were lysed with 0·5% sodium dodecyl sulphate in 1 M NaOH (w/v) for 2 h at 37°C. Samples of medium, washes and cell digest (after neutralizing with HCl) were subjected to liquid scintillation counting. The recovery of radioactivity was >95% in all samples. Similar incubations were carried out in 100-mm Petri dishes. After 1 h the cells were rinsed as above and scraped off the plates with a rubber policeman. The cells were collected in an Eppendorf tube, spun down at 1000 *g* for estimation of cell volume. The cells were then extracted 3 times with methanol and the combined extracts taken to dryness, reconstituted in mobile phase and subjected to HPLC analysis (see below).

High-pressure liquid chromatography

The HPLC systems used were from Waters (Waters Corp., Milford, MA) and consisted of Model 510 pumps, a Model U6K manual injector or a Model 717 Plus autosampler. The column used was a 3·9 × 150 mm C18 analytical column (Symmetry, Waters) with a C18 pre-column insert (μBondapak, Waters) and the mobile phase consisted of 35% methanol, 5% glacial acetic acid and 60% distilled deionized water at a flow rate of 0·9 mL min^{-1}.

On-line spectral analysis of the HPLC peaks employed a photodiode array detector (Model 996, Waters) set to scan from 200 to 500 nm. Chromatographic data analysis was performed with Millennium Chromatography Manager software (Waters).

For quantitative analysis, detection was performed with a flow scintillation analyser (Radio-Matic Series A-500 (Packard, Meriden, CT) with FLO-ONE for Windows software. The scintillation fluid (Ultima Flo M, Packard) was set at a 3 : 1 ratio with mobile phase. The [^{14}C] counting efficiency was 90%. The intra-day coefficient of variation of the radiochemical assay was 8·1% and 3·5% at concentrations of 5 and 35 μM, respectively (n = 5). The limit of detection (signal to noise ratio 3 : 1) was 0·08 μM.

HPLC-mass spectrometry

Electrospray ionization mass spectrometry (ESI MS) was performed on an LCQ LC/MS system (Finnegan Corp, San Jose, CA), which was coupled to a Model 1100 Series HPLC system (Hewlett Packard GmbH, Waldbrom, Germany) with a variable wavelength UV detector set at 375 nm. The HPLC was operated under the conditions described above and one-third of the effluent was directed into the electrospray ionization (ESI) source. The ion spray voltage was set to 5 kV and the interface temperature was 200°C. The scan range was set from 100 to 2000 mass units with detection in the positive ion mode. MS/MS data were acquired using helium as the collision gas with a collision energy of 30 eV.

Statistics

For experiments designed to examine the retardation of the chemical decomposition of quercetin, changes in the amounts of compounds present in incubates containing various amounts of additive were compared with control solutions with no additives. Changes were assessed by the paired Student's *t*-test.

Results and Discussion

The fate of quercetin in cultured Hep G2 cells was studied with [^{14}C]-labelled quercetin in combination with HPLC separation of potential metabolites and degradation products and radiometric detection. After incubation, samples of the medium were prepared for analysis by addition of methanol and acetic acid and centrifugation. This treatment effectively precipitated proteins and stabilized quercetin and its products. When the medium was analysed after a 6-h incubation with 15 μM [^{14}C]quercetin, the HPLC tracing in Figure 2A was obtained. Five distinct radioactive peaks were observed, peak 3 being unchanged [^{14}C]quercetin. Peak 5 was observed only after the mobile phase was changed from 35% to 100% methanol. When the incubation was performed in the absence of Hep G2 cells, the HPLC tracing in Figure 2B was obtained. The tracing was identical to that in Figure 2A except for the absence of peak 4. These observations suggested that peak 4 is a metabolite of quercetin, whereas peaks 1, 2 and 5 are likely to be products of chemical degradation.

Figure 3 summarizes the quantitative fate of quercetin, both at 5 and 15 μM, in the Hep G2 cell

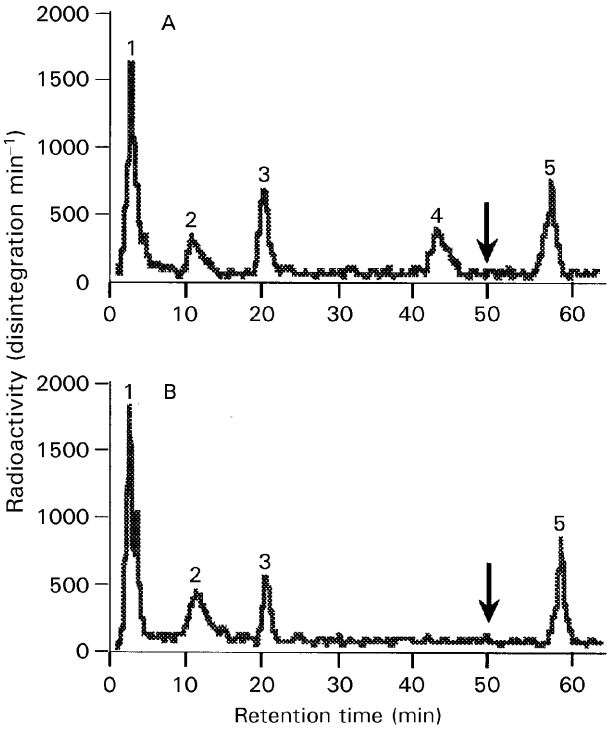

Figure 2. Reversed-phase HPLC of culture media after 6-h incubations of 15 μM [^{14}C]quercetin with Hep G2 cells (A) and cell-free wells (B). The arrow indicates where the mobile phase (35% methanol and 5% glacial acetic acid in water) was changed to 100% methanol. Peak 3 represents unchanged quercetin.

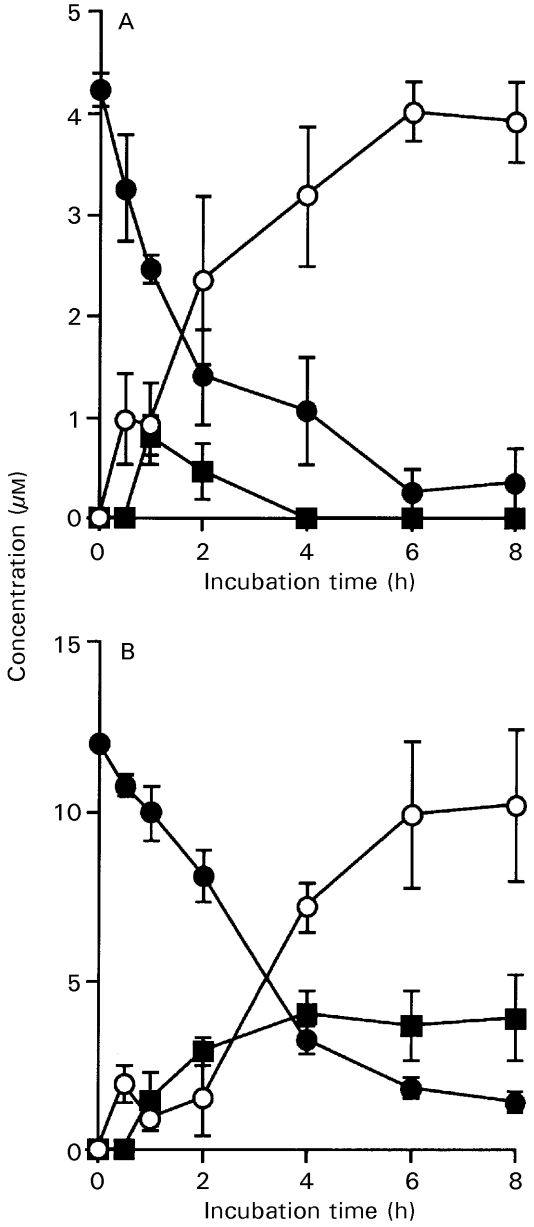

Figure 3. Fate of quercetin during incubation with Hep G2 cells. A. 5 μM [^{14}C]quercetin, B. 15 μM [^{14}C]quercetin. ● quercetin; ○ degradation products peak 1, 2 and 5; ■ metabolite peak 4 (isorhamnetin). The results shown are the mean values of 3 experiments (with separate batches of cells) ± s.e.m.

culture, with respect to metabolism (peak 4) and decomposition (peaks 1, 2 and 5). At the lower concentration, decomposition clearly predominated with very little metabolite peak 4 formed. At the higher concentration, it appears that the decomposition had been partially saturated. The rate of loss of quercetin is slower and the rate of formation of peak 4 is substantially increased.

The observation that degradation of quercetin dominates over metabolism in the Hep G2 cell culture might in part be due to poor cellular uptake of quercetin. This prompted a study of the accumulation kinetics for quercetin. As shown in Figure 4, maximum uptake of 5 μM [^{14}C]quercetin appeared to occur at 10 min at 37°C. Similar uptake experiments were performed with large culture dishes, permitting an estimate of Hep G2 cell volume. These experiments demonstrated a 9·6-fold concentration ratio (mean of 2 determinations) for quercetin between cells and medium. Thus, the Hep G2 cells are capable of concentrating quercetin.

Peak 4 in Figure 2 was confirmed to be a metabolite of quercetin using liquid chromatography–mass spectrometry (LC/MS) with electrospray ionization. Whereas peak 3, unchanged quercetin, had a distinct (M + 1) ion of m/z 303, peak 4 had an (M + 1) ion of m/z 317 (Figure 5A). This is consistent with methylation of one of the hydroxyl groups and was confirmed by the MS/MS of m/z 317 (Figure 5B), which showed a distinct loss of 15 amu. The fragment ion at m/z 285 is likely derived from the loss of two oxygen atoms. This finding is consistent with previous observations, demonstrating that the catechol structure of quercetin is a very good acceptor of a methyl group from catechol-O-methyltransferase with formation of isorhamnetin (Zhu et al 1994).

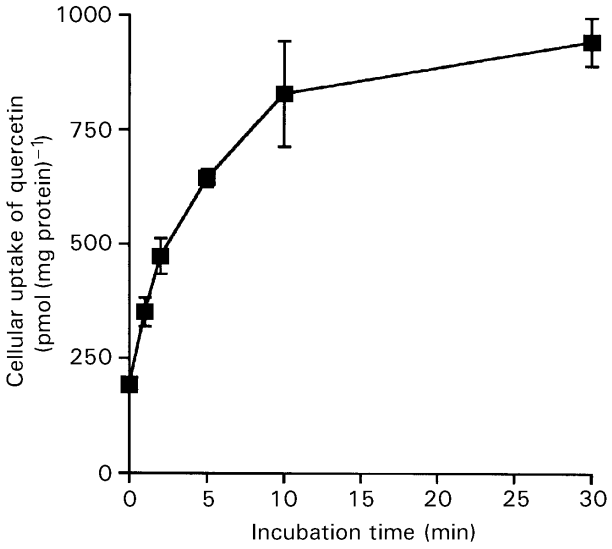

Figure 4. Hep G2 cell uptake of 5 μM [^{14}C]quercetin at 37°C. The results shown are the mean values of 3 experiments (with separate batches of cells) ± s.e.m.

Peaks 1, 2 and 5 could not be conclusively identified by LC/MS, either because of interferences or due to their instability under the LC/MS conditions used. The first of these peaks formed during incubations appeared to be peak 2. It had a UV spectrum identical to quercetin (maxima at 238 and 365 nm). LC/MS suggested an (M + 1) ion of m/z 335, which might be an oxidized form of quercetin, possibly a dioxetan or a peroxide (Nishinaga & Matsuura 1973; Rajananda & Brown 1981). Interestingly, peak 2 was not present in late samples, suggesting that it was an intermediate to the early eluting peak 1. This early peak most likely consisted of several polar degradation products of quercetin, presumably carboxylic acids (Nishinaga & Matsuura 1973). When samples of the medium were subjected to solid-phase extraction, evaporated to dryness and methylated with diazomethane, a major fraction of the very early radioactivity could be observed as a distinct HPLC peak at a retention time of 3·8 min. Its M + 1 ion by LC/MS of m/z 169, together with the identical retention time of authentic standard, strongly suggested this peak to be methylated protocatechuic acid (3,4-dihydroxy-benzoic acid). This is in agreement with findings from previous chemical studies (Nishinaga & Matsuura 1973). Thus, it is proposed that one route of quercetin degradation is peroxidation followed by opening of the C-ring to form acidic products.

Peak 5, which required 100% methanol for its elution from the HPLC column, was also a degradation product of quercetin. It had a UV spectrum identical to that of quercetin. It could not be further

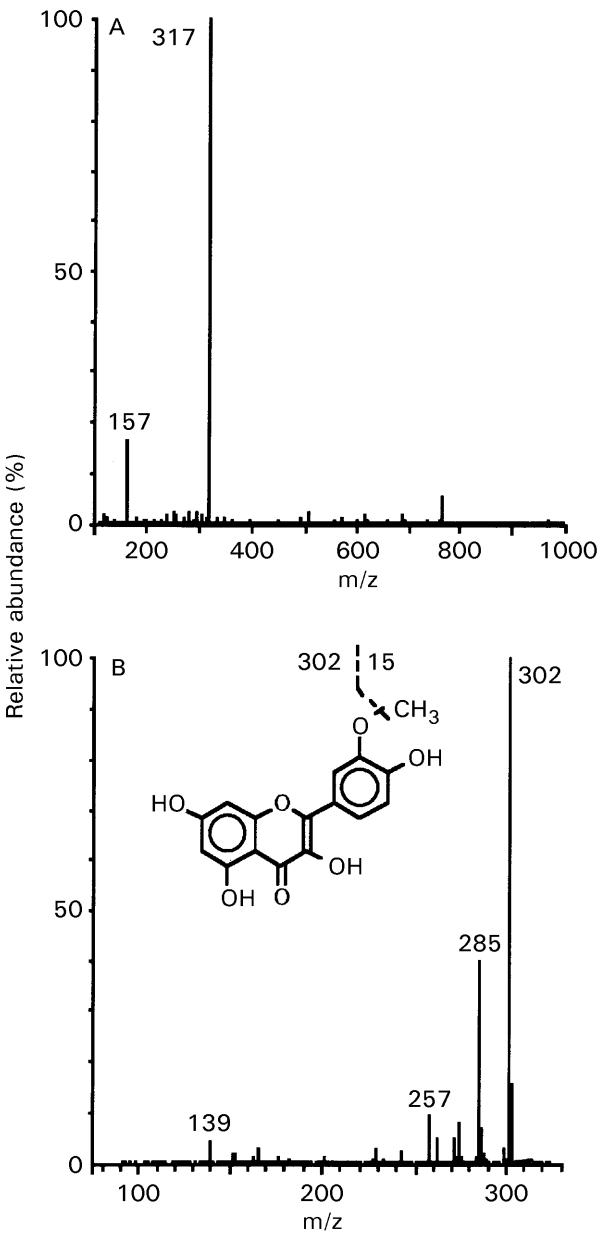

Figure 5. HPLC/MS of metabolite peak 4 (see Figure 2A). A. Full mass spectrum, B. MS/MS of the ion of m/z 317 as well as the proposed structure of this metabolite.

resolved by other mobile-phase compositions, showing it to be a single compound. Its LC/MS spectrum was complex, with the most abundant ion at m/z 625, suggesting that the compound may be an oxidized polymer of a kind previously suggested from chemical studies (Nordström 1968). This compound, together with peaks 1 and 2, accounted for the total loss of quercetin by degradation.

With the intestinal Caco-2 cells, experiments identical to those done with the Hep G2 cells yielded data very similar to those in Figure 2B. Thus, in contrast to the Hep G2 cells, there was no clearly detectable O-methylation of quercetin by

Caco-2 cells, only chemical degradation. Also, when incubations were performed in the absence of foetal bovine serum, the degradation of quercetin was accelerated such that no unchanged quercetin could be detected after 4 h. These observations indicate that serum proteins, presumably due to their high binding to quercetin (Boulton et al 1998), have a protective effect by reducing the amount of free quercetin in solution.

Drugs and other bioactive molecules are commonly assumed to lose their activities after chemical degradation. However, this is not true for at least one of the degradation products of quercetin. Protocatechuic acid, for example, has recently been shown to inhibit tumorigenesis induced by 7,12-dimethylbenz[a]anthracene (Ohnishi et al 1997) and 12-*O*-tetradecanoylphorbol-13-acetate (Tseng et al 1998) and to protect against oxidative damage (Tseng et al 1996). For the proposed dioxetan (peak 2) and the polymer (peak 5) with retentions of the quercetin structure (identical UV absorption), biological activity would not be surprising.

To be able to protect quercetin from chemical degradation, knowledge of the mechanism(s) should be helpful. The nature of oxidation of quercetin has previously been of great concern with respect to understanding quercetin's mutagenic potential (Hatcher & Bryan 1985; Vrijsen et al 1990; Rueff et al 1992; Cai et al 1997). However, it still appears unclear which oxidative species is most important. In our own preliminary studies (unpublished) quercetin was very rapidly degraded by hydrogen peroxide in the presence of ferrous chloride (Fenton reaction), whereas quercetin was much less rapidly broken down by xanthine oxidase, i.e. superoxide radicals.

Based on these observations we carried out protection experiments with the Hep G2 cells, using incubation conditions that did not affect the viability of the cells. These experiments used either a reducing agent (D,L-dithiothreitol), a superoxide scavenger (superoxide dismutase) or a metal ion (e.g. Fe^{2+}) chelator (EDTA). Of these agents only EDTA had a protective effect (Figure 6). As shown in Figure 6, for an EDTA concentration of 0·5 mM, the formation of degradation products 1 and 2 (see Figure 2) was almost completely inhibited during a 6-h incubation with 15 μM quercetin. As a result, unchanged quercetin increased. However, a more dramatic increase was observed for peak 4 (isorhamnetin), demonstrating that the Hep G2 cells were metabolically active. Interestingly, peak 5, the proposed quercetin polymer, was unaffected by EDTA, indicating that this degradation product is independent of Fe^{2+}.

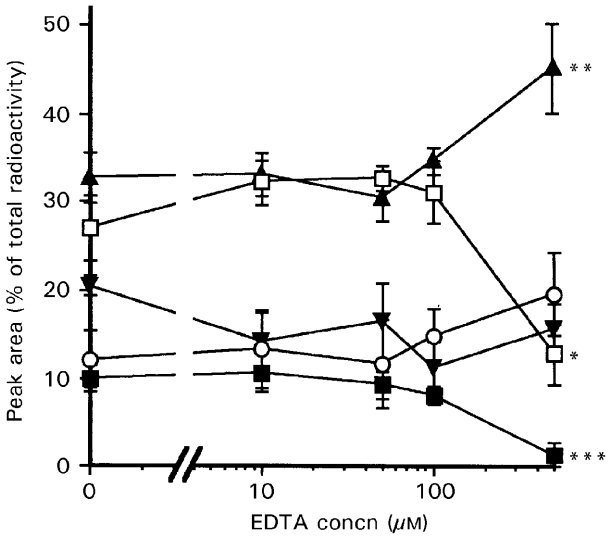

Figure 6. Effect of EDTA on metabolism (peak 4) and degradation (peaks 1, 2 and 5) of quercetin (peak 3) in Hep G2 cell culture. The cells were incubated with 15 μM quercetin for 6 h. ○ quercetin; □ peak 1; ■ peak 2; ▲ peak 4; ▼ peak 5. The results shown are the mean values of 3 experiments (with separate batches of cells) ± s.e.m. $*P < 0{\cdot}05$; $**P < 0{\cdot}02$ and $***P < 0{\cdot}001$ compared with no EDTA.

In summary, this represents the first study attempting to establish the fate of quercetin in a cell culture model. Although cellular uptake and metabolism through O-methylation occurs, a very large fraction of added quercetin, particularly at low concentrations, is chemically degraded to multiple labile products. This degradation will likely occur also with other cell types and must be taken into consideration when evaluating biological properties of this and other bioflavonoids in cell cultures and possibly also in-vivo. Our observations in this study also suggest that the main pathway of chemical degradation is dependent on the presence of ferrous ions. Addition of EDTA to complex such iron inhibited the main pathway of chemical degradation, leading to increased cell-mediated metabolism.

Acknowledgements

This work was supported by the National Institutes of Health grants CA69138 and GM55561 and by a Medical University of South Carolina Postdoctoral Fellowship Award. The use of the Medical University of South Carolina Core Mass Spectrometry Facility in the structure identification work is also acknowledged.

References

Ames, B. N., Swirsky Gold, L., Willett, W. C. (1995) The causes and prevention of cancer. Proc. Natl Acad. Sci. USA 92: 5258–5265

Boulton, D. W., Walle, U. K., Walle, T. (1998) Extensive binding of the bioflavonoid quercetin to human plasma proteins. J. Pharm. Pharmacol. 50: 243–249

Cai, Q., Rahn, R. O., Zhang, R. (1997) Dietary flavonoids, quercetin, luteolin and genistein, reduce oxidative DNA damage and lipid peroxidation and quench free radicals. Cancer Lett. 119: 99–107

Chi, C.-W., Chang, Y.-F., Ou, Y.-R., Hsieh, C.-C., Lui, W.-Y., P'Eng, F.-K., Liu, T.-Y. (1997) Effect of quercetin on the in vitro and in vivo growth of mouse hepatoma cells. Oncol. Rep. 4: 1021–1024

Csokay, B., Prajda, N., Weber, G., Olah, E. (1997) Molecular mechanisms in the antiproliferative action of quercetin. Life Sci. 60: 2157–2163

Deschner, E. E., Ruperto, J., Wong, G., Newark, H. L. (1991) Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis 12: 1193–1196

Deschner, E. E., Ruperto, J., Wong, G., Newark, H. L. (1993) The effects of dietary quercetin and rutin on AOM-induced acute colonic epithelial abnormalities in mice fed a high-fat diet. Nutr. Cancer 20: 199–204

Duthie, S. J., Collins, A. R., Duthie, G. G., Dobson, V. L. (1997) Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidised pyrimidines) in human lymphocytes. Mutation Res. 393: 223–231

Fotsis, T., Pepper, M. S., Aktas, E., Breit, S., Rasku, S., Adlercreutz, H., Wähälä, K., Montesano, R., Schweigerer, L. (1997) Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res. 57: 2916–2921

Gugler, R., Leschik, M., Dengler, H. J. (1975) Disposition of quercetin in man after single oral and intravenous doses. Eur. J. Clin. Pharmacol. 9: 229–234

Hatcher, J. F., Bryan, G. T. (1985) Factors affecting the mutagenic activity of quercetin for Salmonella typhimurium TA98: metal ions, antioxidants and pH. Mutation Res. 148: 13–23

Hertog, M. G. L., Hollman, P. C. H., Katan, M. B. (1992) Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J. Agr. Food Chem. 40: 2379–2383

Hertog, M. G. L., Hollman, P. C. H., van de Putte, B. (1993) Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. J. Agr. Food Chem. 41: 1242–1246

Hollman, P. C. H., de Vries, J. H. M., van Leeuwen, S. D., Mengelers, M. J. B., Katan, M. B. (1995) Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am. J. Clin. Nutr. 62: 1276–1282

Kang, T., Liang, N. (1997) Studies on the inhibitory effects of quercetin on the growth of HL-60 leukemia cells. Biochem. Pharmacol. 54: 1013–1018

Keli, S. O., Hertog, M. G. L., Feskens, E. J. M., Kromhout, D. (1996) Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Arch. Int. Med. 156: 637–642

Knasmüller, S., Parzefall, W., Sanyal, R., Ecker, S., Schwab, C., Uhl, M., Mersch-Sundermann, V., Williamson, G., Hietsch, G., Langer, T., Darroudi, F., Natarjan, A. T. (1998) Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens. Mutation Res. 402: 185–202

Knekt, P., Järvinen, R., Reunanen, A., Maatela, J. (1996) Flavonoid intake and coronary mortality in Finland: a cohort study. Br. Med. J. 312: 478–481

Lepley, D. M., Pelling, J. C. (1997) Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin. Mol. Carcinogenesis 19: 74–82

Nishinaga, A., Matsuura, T. (1973) Base-catalyzed autoxidation of 3,4′-dihydroxyflavone. J. Chem. Soc. Chem. Comm. 9–10

Nordström, C. G. (1968) Autoxidation of quercetin in aqueous solution. An elucidation of the autoxidation reaction. Suomen Kemistilehti B41: 351–353

Ohnishi, M., Yoshimi, N., Kawamori, T., Ino, N., Hirose, Y., Tanaka, T., Yamahara, J., Miyata, H., Mori, H. (1997) Inhibitory effects of dietary protocatechuic acid and costunolide on 7,12-dimethylbenz[a]anthracene-induced hamster cheek pouch carcinogenesis. Jpn. J. Cancer Res. 88: 111–119

Rajananda, V., Brown, S. B. (1981) Mechanism of quercetin oxygenation – a possible model for haem degradation. Tetrahedron Lett. 22: 4331–4334

Ranelletti, F. O., Ricci, R., Larocca, L. M., Maggiano, N., Capelli, A., Scambia, G., Benedetti-Panici, P., Mancuso, S., Rumi, C., Piantelli, M. (1992) Growth-inhibitory effect of quercetin and presence of type-II estrogen-binding sites in human colon-cancer cell lines and primary colorectal tumors. Int. J. Cancer 50: 486–492

Rueff, J., Laires, A., Gaspar, J., Borba, H., Rodrigues, A. (1992) Oxygen species and the genotoxicity of quercetin. Mutation Res. 265: 75–81

Sato, M., Miyazaki, T., Kambe, F., Maeda, K., Seo, H. (1997) Quercetin, a bioflavonoid, inhibits the induction of interleukin 8 and monocyte chemoattractant protein-1 expression by tumor necrosis factor-a in cultured human synovial cells. J. Rheumatol. 24: 1680–1684

Tseng, T. H., Wang, C. J., Kao, E. S., Chu, H. Y. (1996) Hibiscus protocatechuic acid protects against oxidative damage induced by tert-butylhydroperoxide in rat primary hepatocytes. Chem.-Biol. Interactions 101: 137–148

Tseng, T. H., Hsu, J. D., Lo, M. H., Chu, C. Y., Chou, F. P., Huang, C. L., Wang, C. J. (1998) Inhibitory effect of Hibiscus protocatechuic acid on tumor promotion in mouse skin. Cancer Lett. 126: 199–207

Tsyrlov, I. B., Mikhailenko, V. M., Gelboin, H. V. (1994) Isoenzyme- and species-specific susceptibility of cDNA-expressed CYP1A P-450s to different flavonoids. Biochim Biophys Acta 1205: 325–335

Vrijsen, R., Michotte, Y., Boeyé, A. (1990) Metabolic activation of quercetin mutagenicity. Mutation Res. 232: 243–248

Walgren, R. A., Walle, U. K., Walle, T. (1998) Transport of quercetin and its glucosides across human intestinal epithelial Caco-2 cells. Biochem. Pharmacol. 55: 1721–1727

Walle, T., Eaton, E. A., Walle, U. K. (1995) Quercetin, a potent and specific inhibitor of the human P-form phenol-sulfotransferase. Biochem. Pharmacol. 50: 731–734

Wang, Z. Y., Huang, M.-T., Lou, Y.-R., Xie, J.-G., Reuhl, K. R., Newmark, H. L., Ho, C.-T., Yang, C. S., Conney, A. H. (1994) Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz-[a]anthracene-initiated SKH-1 mice. Cancer Res. 54: 3428–3435

Weber, G., Shen, F., Prajda, N., Yeh, Y. A., Yang, H., Herenyiova, M., Look, K. Y. (1996) Increased signal transduction activity and down-regulation in human cancer cells. Anticancer Res. 16: 3271–3282

Zhu, B. T., Ezell, E. L., Liehr, J. G. (1994) Catechol-O-methyltransferase-catalyzed rapid O-methylation of mutagenic flavonoids – metabolic inactivation as a possible reason for their lack of carcinogenicity in vivo. J. Biol. Chem. 269: 292–299