Proceedings of the Nutrition Society (2002), **61**, 97–103 DOI:10.1079/PNS2001133

The Diamond Jubilee Summer Meeting of the Nutrition Society was held at the University of Sheffield on 10–12 July 2001

Postgraduate Symposium

Evidence for consistent patterns between flavonoid structures and cellular activities

F. Depeint*, J. M. Gee, G. Williamson, and I. T. Johnson
Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK

A wide variety of plant-derived compounds, including the polyphenolic flavonoids, is present in the human diet or is consumed for medicinal reasons. Epidemiological and animal studies tend to suggest a protective effect of flavonoids against cardiovascular diseases and some types of cancer. Although flavonoids have been studied for about 50 years, the cellular mechanisms involved in their biological activity are still largely unknown. Antioxidant properties of the flavonoids have been postulated as a mechanism for putative protective effect against cardiovascular disease. Nevertheless, these properties alone are not sufficient to explain the anti-carcinogenic potential of these polyphenols. The mechanisms by which the molecules interact with cells or are absorbed by them are very important for determining the intracellular concentration and distribution of the metabolites to internal organs. With the exception of the cells lining the gastrointestinal tract, all other cells in the body are only exposed to flavonoid metabolites and degradation products. No previous studies have addressed this aspect of cellular exposure, except for some methylated metabolites. Within the last decade, reports on flavonoid activities have been largely associated with enzyme inhibition and anti-proliferative activity. From our recent work on the human colon cancer cell line HT29 and comparison with published studies, structure–function relationships demonstrate that antioxidant, enzyme inhibitor or anti-proliferative activities are dependent on particular structure motifs. The present review also presents a summary of mechanistic data on a few selected compounds.

Flavonoids: Structure–activity relationship: Enzyme inhibitor activities: Anti-proliferative activities: Antioxidant activities

Flavonoids are polycyclic structures containing two aromatic rings (A and B) linked by a heterocyclic ring C. Their consumption in the diet can vary (depending on dietary choice and availability of foods) between 20 mg/d and 1 g/d (Kuhnau, 1976; Hollman & Katan, 1999). Geographical variation also accounts for wide differences in the type of flavonoids ingested, from isoflavonoid-rich food (e.g. soyabean) in Asia, to catechin derivatives (e.g. tea) in the UK. The study of flavonoid biochemistry began in the early 1950s, and by the beginning of the next decade, much of the chemistry of flavonoids (identification and determination of the mechanisms of synthesis and degradation in plants) was known. However, the analytical techniques and knowledge available at that time did not allow the study of the metabolism and cellular effects in more detail. Thousands of different flavonoids have been identified over the years, and new ones are still being isolated. For practical reasons we decided to concentrate on a defined range of subfamilies, with emphasis on the substitution pattern.

Flavonoids and health effects

Some flavonoids were originally considered pro-carcinogenic, partially because the catechol moiety in the B ring facilitated pro-oxidant reactions. However, Wattenberg (1985) was among the first researchers to suggest that flavonoids could have anti-mutagenic and anti-carcinogenic effects. A number of cohort epidemiological studies (Goldbohm *et al*. 1995; Hertog *et al*. 1995) based in The

***Corresponding author:** Flore Depeint, fax +44 160 350 7732, email flore.depeint@bbsrc.ac.uk

Netherlands (4–25 years follow-up) could not correlate flavonol intake with a reduced risk of cancer (colon, lung, stomach). The same studies, however, reported an inverse correlation with stroke and cardiovascular disease, independent of other risk factors. Identical correlations were found in studies of wine and tea consumption (for review, see Hollman & Katan, 1999). Only one cohort study in Finland (Knekt *et al*. 1997) showed a link between flavonol consumption and reduced risk of lung cancer. Other epidemiological studies showed a clear correlation between fruit and vegetable consumption and lower risk of cancer of the gastrointestinal tract (Hollman & Katan, 1999). This finding led to the suggestion that flavonoids in general (but not flavonols only) have beneficial health effects. Increased amounts of data from *in vitro* and animal studies on flavonoids demonstrate the inhibition of initiation and development of tumours, but epidemiological evidence is still lacking (Hertog & Hollman, 1996). Based on these *in vitro* and animal data, some flavonoids (flavopiridol, green-tea extracts and genistein conjugates) are undergoing clinical trials as anti-neoplastic drugs against gastrointestinal cancer, breast cancer and lymphomas respectively (Wang, 2000).

The effects on cardiovascular disease have been attributed to antioxidant properties. The antioxidant properties of flavonoids have been widely studied (for review, see Rice-Evans *et al*. 1996). However, more recently, effects on enzyme inhibition (for example, see Alcaraz & Ferrandiz, 1987; Umarova *et al*. 1998; Mutoh *et al*. 2000*a*; Shimizu *et al*. 2000) and anti-proliferative activity (for example, see Agullo *et al*. 1996; Kamei *et al*. 1996; Kawaii *et al*. 1999; Kuntz *et al*. 1999), including induction of the apoptotic pathways in tumour cells, have been observed. There is now clear evidence from animal models that a flavonoid-rich diet can protect against a range of carcinogenic insults. Caltagirone *et al*. (2000) showed that flavonoids can inhibit different phases of tumour formation in a mice melanoma model. Flavonoids in the diet at the same time as injection of the tumour cells delayed tumour growth. In addition, quercetin and apigenin, but not epigallocatechin gallate, can prevent metastasis. Conney *et al*. (1997) reviewed experiments in which curcumin could reduce the appearance of chemically-induced tumours of the skin (after topical application) and the gastrointestinal tract (after oral dose). The same effect was observed on chemically-induced colon cancer (Deschner *et al*. 1993) following a quercetin and rutin-containing diet. Mahmoud *et al*. (2000) showed that curcumin also prevent genetically-induced spontaneous tumour formation. Hayashi *et al*. (2000) finally demonstrated that quercetin chalcones can reduce tumour size in an implanted colon tumour in mice, i.e. it can affect tumour development rather than the initiation stage.

Flavonoids and carcinogenesis

It is now important to understand the mechanism by which a normal tissue can evolve into a neoplastic tumour. The carcinogenesis process is multi-stage and can span 10–20 years or more. There are three main stages. During the first or initiation stage a potential carcinogen is transformed into a mutagen product. Phase I metabolising enzymes (including cytochrome P450) catalyse the addition of a polar reactive group to a lipophilic carcinogen or xenobiotic to form a potent electrophile. They can then react with cellular neutrophiles, including DNA. Phase I metabolites can be detoxified by conjugation by phase II enzymes. The metabolites formed (e.g. sulfate or glutathione conjugates) are water soluble and easily eliminated from the body. The second stage is called the promotion stage. It can be further divided into four categories: (1) the over-expression of pro-oxidant enzymes (e.g. cyclo-oxygenase (prostaglandin-endoperoxide synthase), lipoxygenase) leading to generation of reactive oxygen species and progression towards accumulation of DNA mutations. This process is very important in colon carcinogenesis promotion where all tumours over express cyclooxygenase 2; (2) another route for carcinogenesis is via over-expression of ornithine decarboxylase. This enzyme is involved in polyamine production, and is the limiting enzyme in nucleotide synthesis. The consequences of increased ornithine decarboxylase levels are an increase in DNA synthesis and therefore increase in cell proliferation; (3) DNA synthesis can also be directly increased by over-expression or activation of DNA polymerase or topoisomerase II; (4) cell proliferation can finally be increased by expression of the enzymes involved in the tight regulation of this process. Most of the regulation pathways are through protein phosphorylation and dephosphorylation via kinases and phosphatases. They include protein kinases A and C, mitogen-activated protein kinases and cyclin-dependent kinases. The final stage of carcinogenesis is called tumour development. At this stage the mutations are fixed and cells are proliferating in an uncontrolled manner. It is this phase at which cancers are currently detected. Intervention at this stage is confined to tumour reduction, stasis or strategies to block angiogenesis (Galati *et al*. 2000).

It is clear now that carcinogenesis is the result of an imbalance in the tissue homeostasis. In a stable mature tissue the rates of replication and cell death are balanced. However, in certain circumstances the sustained rate of cell replication exceeds the rate of apoptosis, resulting in hyperplasia. Hyperplasia in itself does not imply that tumour development is inevitable. Cell proliferation is regulated by checkpoints at the major stages of the cell cycle, ensuring that the DNA sequence is correct before duplication, and that duplication and formation of the two chromatids is correct before the cell divides into two identical daughter cells. If any one of these checkpoints is overruled, the cell is prone to natural or induced (e.g. radiation or chemicals) mutations and unable to repair the damaged DNA. In mutated stem cells which escape apoptotic control, cells can become the progeny for a neoplastic cell population. This fragile balance between mitosis and apoptosis pathways is crucial.

To prevent tumour development, different stages of carcinogenesis can be targeted. To counteract the process of initiation, strategies include increasing phase II enzymes for rapid removal of the carcinogen, or redox reactions to reduce DNA damage. To regress the promotion stage there is need for enzyme inhibitors targeting ornithine decarboxylase or the different kinases involved in DNA

https://doi.org/10.1079/PNS2001133 Published online by Cambridge University Press

synthesis. Reactive oxygen species formation can be prevented by enzyme inhibition or neutralised by the antioxidant activity of other molecules. Intervention in carcinogenesis at this early stage is clearly a preventive mechanism. Following diagnosis of cancers, the tumour is already developing, and changing the cell survival or proliferation routes is the only strategy for regression or stabilisation. There are three approaches to this strategy. First, and seemingly the easiest, is the induction of apoptosis of damaged cells, thus killing and regressing the tumour. Different strategies, depending on the product chosen and its mechanism of action, can be applied: (1) inhibition of topoisomerase would lead to random DNA strand breaks and eventually cell death. Such cell death is generally associated with arrest of the cell cycle in G2/M; (2) another strategy would be to disrupt mitochondrial membrane potential, releasing cytochrome c and procaspases, leading to a typical form of apoptosis detectable by DNA laddering. The same treatment would also lead to cytotoxicity if the respiratory chain is disrupted, ATP stocks depleted and reactive oxygen species formed, leading to cell death by oxidative stress. Thus, a fine control of this pathway is required as necrosis of the cell is not a possible option for the organism as a whole; (3) the final strategy would be by direct targeting of regulatory proteins, up regulating Bax or p21 or down regulating Bcl2 or p53 for induction of apoptosis (Galati *et al*. 2000). The second tool to prevent carcinogenesis is to slow the cell cycle, allowing for DNA repair, but this approach would only be possible at the very early stages, where the mutation level is low and a correct copy of the DNA is still available. Finally, the third possibility is to arrest the cell cycle. Tumour cells cannot then divide, but are still prone to further mutation that may escape the regulation. However, this strategy is potentially important in the context of colonocytes, where the mitotic region is located at the base of the crypt. When cells exit this compartment, they migrate up the crypt to be exfoliated at the intestinal surface. Thus, damaged cells could be eliminated by apoptotic exfoliation. Any of these strategies would lead to tumour regression.

Bioavailability of flavonoids

As stated previously, there appears to be a large discrepancy between the potential effects, as observed *in vitro*, and observed effects in human subjects. This difference could be due to the poor bioavailability of flavonoids to target tissues *in vivo*, a process that is only just beginning to be understood. To have an effect on gastrointestinal cells, or any other tissue, these molecules need to either interact with the cell membrane or be absorbed. Flavonoids are present in the diet mainly as glycosides, and the nature of the sugar and position of substitution are important factors for intestinal absorption. Aglycones are thought to be freely available by passive diffusion in the small intestine, and plasma levels peak within 2 h after ingestion. Glycosides can undergo hydrolysis by colonic bacteria, releasing the aglycone for subsequent absorption.

However, many dietary flavonoid glucosides are absorbed at the small intestine (Walle *et al*. 2000), as opposed to large intestine absorption (plasma peak at 1–2 h rather than 6–9 h after the meal). The current opinion is that flavonoid glucosides can be hydrolysed at the brush border by the enzyme lactate-phlorizin hydrolase (Day *et al*. 2000; Walle *et al*. 2000; Williamson *et al*. 2000). There is also additional indication for interaction with the neighbouring Na-dependent glucose transporter (SGLT1); (Gee *et al*. 1998, 2000; Choudhury *et al*. 1999; Walgren *et al*. 2000) followed by intracellular hydrolysis by β-glucosidase (Day *et al*. 1998). Glycosides can also be hydrolysed by the colonic microflora; the resulting aglycones and smaller degradation products are absorbed by passive diffusion. There is also recent indication (Pforte *et al*. 1999) of stomach absorption. This mechanism is unclear, as the cellular and lumen contents show the same flavonoid profile (with lower concentrations in the cells), including glycosides. It is important to note also that there is still no evidence for acid-hydrolysis in the stomach. Once inside the intestinal or colonic cells, various transformations occur very rapidly, including deglucosylation, methylation, sulfation or glucuronidation. Conjugation can also take place in the liver as part of detoxification mechanisms. The efficiency of this process is such that very little of the flavonoid recovered in plasma or urine is present as the aglycone (Walle *et al*. 2000). In addition, conjugated flavonoids (glucuronides and to a lesser extent sulfates) produced in the small intestine are rapidly excreted back to the intestinal lumen via the xenobiotic transporter MRP2 (Walle *et al*. 2000; Walgren *et al*. 2000). When the glucuronide conjugates reach the colon, they may be hydrolysed by microbial enzymes and reabsorbed for a further enterohepatic cycling (Williamson *et al*. 2000).

Walle *et al*. (2001) showed that when volunteers were fed a meal containing the flavonoid chrysin, very small amounts of flavonoid conjugates were recovered in the plasma (mainly as sulfates), about 1000 times more in the urine (as glucuronides), but the major part of the dose was recovered unchanged from the faeces, showing a very low oral bioavailability. Except for the cells lining the gastrointestinal tract, all other cells in the body are only exposed to the flavonoid metabolites and degradation products, and plasma concentrations rarely exceed 1 μM (Hollman & Katan, 1999; Scalbert & Williamson, 2000). There is, however, a possibility that some cells or tissues may accumulate flavonoid conjugates to higher, more biologically-active, concentrations due to high-affinity binding to receptors or cellular targets (Kuo, 1998; Spencer *et al*. 1999; Ader *et al*. 2000). Finally, it is interesting to consider that the conjugation profile can vary slightly depending on the diet before the feeding experiment. Manach *et al*. (1997) showed that in rats adapted to a quercetin-rich food plasma concentration could reach 20 μM compared with 1 μM following a single dose exposure. This finding suggests a possible accumulation of quercetin in the plasma, perhaps due to albumin binding or a slow rate of excretion for some flavonoids.

Structure–function relationships

We have focused our studies on colonocytes, as they are one of the primary sites of exposure to flavonoids in the body, and because previous reports of the effect of flavonoids on

https://doi.org/10.1079/PNS2001133 Published online by Cambridge University Press

these cells have been limited. In addition to the principle aglycones, we have included metabolites in our studies to assess systemic exposure, and natural or synthetic prenylated compounds for more information on substitution patterns. Screening was carried out using a cell viability assay in which neutral red dye is incorporated into lysosomes of live cells. The intensity of the dye is assessed by spectrophotometry and the data is proportional to the number of viable cells. Results are expressed as a percentage of the control sample (the same conditions as for the test compounds, but cells were exposed to solvent only). The threshold for an active compound is set to a statistically significant inhibition of cell growth, with a value < 90 % cell population compared with the control (also referred to as percentage survival) and $P < 0{\cdot}01$. The ninety-six-well plate format allows high throughput screening of a large number of structures, and in all sixty-nine were tested. Although the assay measures total viable cells, and can therefore suggest changes in apoptotic or mitotic rates, it cannot differentiate between the causative mechanisms. It does, however, indicate where further investigation is warranted.

Structure–function relationships, mainly related to the core flavonoid skeleton, have been postulated. Our findings are broadly in agreement with the limited number of previously published data available on colon cells (Cushman & Nagarathnam, 1991; Agullo *et al*. 1996; Kuntz *et al*. 1999). We found that only flavonoids with a given core structure suppress viability in the ninety-six-well plate assay. This group of compounds includes all flavones and flavonols (also identified by Agullo *et al*. 1996 and Kawaii *et al*. 1999) with inter-structure variations, irrespective of hydroxylation pattern. The results for these compounds range from 46 % survival for quercetin to 82 % survival for kaempferol. Of the other subclasses of flavonoids tested, flavanones, anthocyanidins, chalcones and isoflavones are not effective (see structures in (Fig. 1). These findings seem in disagreement with those of Kuntz *et al*. (1999), who postulate that at least some flavonoids from each of the subclasses are anti-proliferative. However, the authors also indicated that the concentrations necessary for such activity could reach 200 μM, which is considerably greater than the concentrations we have tested. The results of Agullo *et al*. (1996) and ours, however, indicate a very crucial role of the heterogenous ring C, as any variation of its structure leads to reduced activity. Such changes included: (1) saturation or opening of the ring and loss of the planar structure in the case of flavanones and chalcones respectively; (2) loss of the carbonyl group as in anthocyanins; (3) position of attachment of the aromatic ring B as in isoflavones. In addition, flavonoid glycosides are generally ineffective, irrespective of the nature or position of the sugar substitution. This factor is not as crucial, as intestinal and

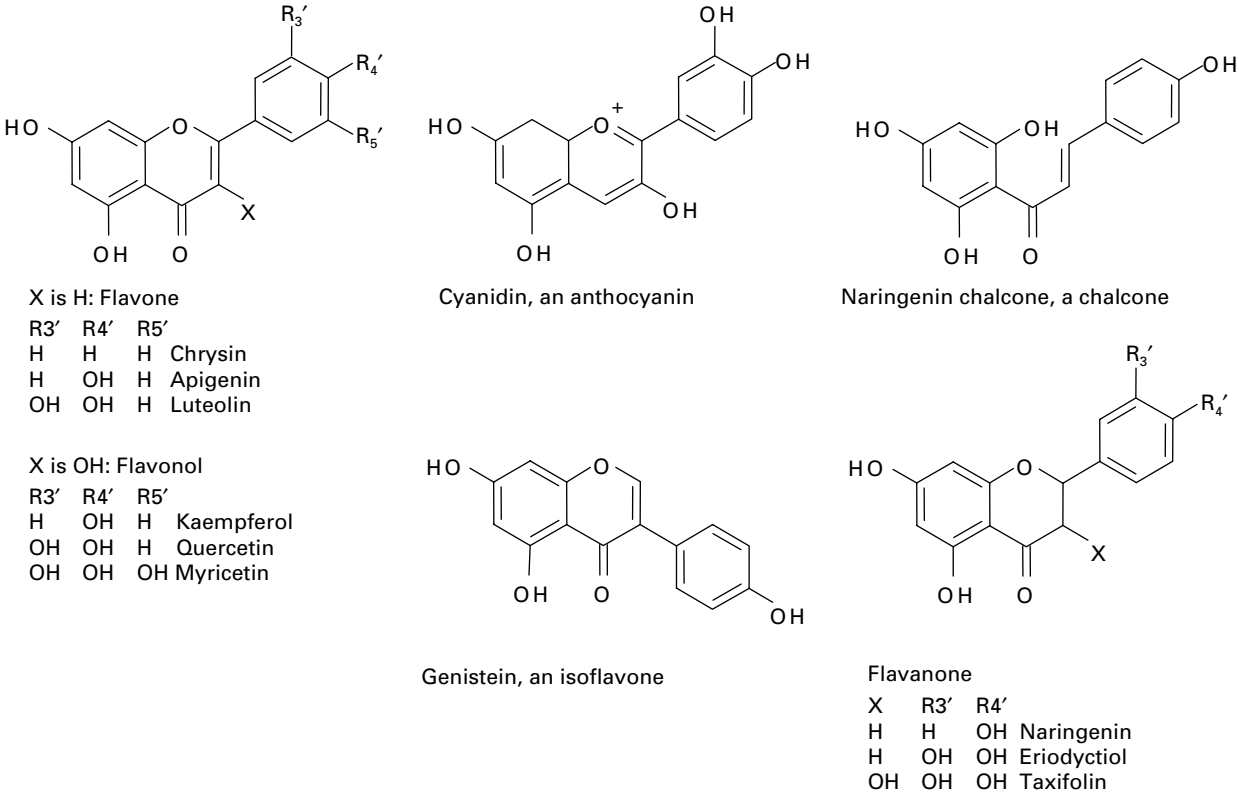

Fig. 1. Flavonoid structures. Twelve flavonoid aglycones have been tested for anti-proliferative activity, the structures of which are shown. Of these compounds, only the flavones and flavonols were positive for cell inhibition. The order of potency of flavonols is quercetin (46 % survival), myricetin (68 %) and kaempferol (82 %), whilst for flavones the order of potency is apigenin (55 %), luteolin (60 %) and chrysin (77 %). Survival data for inactive compounds vary between 92 % (naringenin) and 101 % (eriodictyol). Thus, there is no possible ambiguity between active and inactive compounds.

https://doi.org/10.1079/PNS2001133 Published online by Cambridge University Press

colonic cells are exposed principally to hydrolysed products, following lumen microbial transformations or cytosolic processing.

Some structures have emerged as being consistently 'required' for flavonoids to be active as an antioxidant (for review, see Rice-Evans *et al*. 1996), enzyme inhibitor (Wheeler & Berry, 1986; Austin *et al*. 1992; Harborne & Williams, 2000; Mutoh *et al*. 2000*b*; Shimizu *et al*. 2000), or anti-proliferative agent. Flavonoids can also affect the expression of some target genes such as cycloxygenase 2 (Kong *et al*. 2000; Mutoh *et al*. 2000*a*,*b*; Skibola & Smith, 2000). Requirements for these functionalities include either an unsaturated heterocyclic ring C or the presence of a carbonyl group at C-4. Structures such as specific flavonoid variants (chalcones, isoflavones or catechins) seem to have a more restricted range of activity, being an antioxidant but a poor anti-proliferative agent. Isoflavones have oestrogen-like structures and can mimic the hormone effects. It is important to note, however, that certain molecules or pathways are cell or tissue specific. Thus, caution must be observed when comparing results from cells of different origins.

Of the metabolites of quercetin tested, only quercetin-7-sulfate appears to be active against the human colon cancer cell line HT29, having an anti-proliferative potential within the same range as the aglycone. None of the glucuronides or monomethylated derivatives had detectable activity. As sulfation in position 7 is a rare metabolite in human plasma, it would seem that colonocytes are more likely to be affected by luminal exposure rather than systemic exposure to the flavonoids.

By screening a large number of prenyl substitutions of the aglycone chrysin, we showed that the position and nature of the substituent are important for efficacy. No substitutions at position C-7 (except sulfate conjugate) induced any effect, while substitution at the C-5 position appeared to be highly potent. Position C-6 substitutions were generally less effective than the aglycone, whilst position C-8 substitutions were generally as, or more, effective than chrysin. The most active of prenylated flavonoids (5,7-dihydroxy, 8(1,1)-dimethylallyl, 3,3′,4′-trimethoxyflavone) reach 10 % survival (or 90 % cell growth inhibition) within 6 h. This result suggests a different mechanism of action compared with the aglycone, which may include fast apoptotic response as well as cytotoxicity and necrotic death. Additional comparisons showed that aromatic substitutions were less effective than aliphatic substitutions. Finally, within the aliphatic substitutions, the longer chains were more effective than the shorter ones.

By a preliminary screening of the selected flavonoids we were able to corroborate previously published data (Cushman & Nagarathnam, 1991; Agullo *et al*. 1996; Kuo *et al*. 1997; Thompson *et al*. 1998; Kuntz *et al*. 1999; Richter *et al*. 1999; Mutoh *et al*. 2000*a*,*b*; Ranelletti *et al*. 2000) and provide additional data on the effect of metabolites and substitution patterns. We are currently investigating specific intracellular pathways to identify the target mechanisms in relation to cell proliferation. This investigation is being done on a subsection of seventeen structures that demonstrated anti-proliferative activity in the viability screening. Published data on effective compounds show a consistent induction of apoptosis for all those tested, related mainly to a cell cycle arrest in G1/S (flavones and flavonols) or G2/M (isoflavones).

Our investigations on quercetin have confirmed a cell cycle arrest in G1/S that is dose dependent, but no clear proof of apoptosis so far. This finding is in agreement with those of Russo *et al*. (1999), who reported that quercetin without CD95 could not trigger apoptosis (external signal-induced apoptosis). Yoshida *et al*. (1990) also confirmed that quercetin reduced DNA synthesis, with no mention of apoptosis. In addition, Agullo *et al*. (1994) studied the effect of quercetin on colon cells, and observed a selective effect on growing non-differentiated cells. Cells went into a cell cycle arrest associated with reduced energy metabolism (ATP production or glycolysis) and apoptosis. Csokay *et al*. (1997), however, showed another interesting aspect of quercetin activity. They showed that quercetin, depending on the concentration, could induce both apoptosis and cell differentiation in the same cell line. Apoptosis was triggered by down-regulation of the phosphatidyl-inositol 4-phosphate kinase pathway as well as c-myc and Ki-ras proliferation oncogenes. Kang & Liang (1997) showed similar results of quercetin inhibiting phosphatidylinositol 4-phosphate kinase in leukaemic cells. Quercetin also targets other kinases such as protein-tyrosine kinase (membrane-bound but not the cytosolic fraction) and protein kinase C (cytosolic but not the membrane-bound fraction).

Another highly studied group of flavonoids are green-and black-tea extracts. Achiwa *et al*. (1997) showed that catechin gallates from tea were anti-proliferative agents with weak ornithine decarboxylase inhibition potential (slow DNA synthesis), but that they induced apoptosis in leukaemic cells. This observation was confirmed by Chen *et al*. (1998), who also showed a higher sensitivity of tumour *v.* normal cells to epigallocatechin gallate. The causative mechanism was reported to be apoptosis, induced within 4 h without cytotoxicity.

Normal and tumour cells have been shown to behave differently, but the exact reason for this differential effect is not fully understood. Choi *et al*. (1999) demonstrated that different flavonoids can arrest the cell cycle at different stages, depending on their cellular targets and the mechanisms involved. These authors also showed that the same flavonoid could arrest the cell cycle at different stages, depending on the cell line under investigation. This finding suggests a very specific mechanism of action that is tissue specific.

It is suggested, in view of the lack of activity of the metabolites, that the anti-proliferative effect of flavonoids on colonic cells is primarily due to luminal exposure rather than systemic exposure. This explanation is further confirmed in the experiments of Haza *et al*. (2000), in which they showed that exposing cultured colonic cells to a bacterial-free colonic lumen content, recovered after flavonoid-rich meal, is sufficient to induce apoptosis in the cells.

Conclusions

In the present review we have emphasised the importance of regulation of cell proliferation in the prevention and

https://doi.org/10.1079/PNS2001133 Published online by Cambridge University Press

regression of carcinogenesis, with a particular interest in colon cancer. As part of our daily diet, flavonoids may be important factors in this process. They have been shown to have potent antioxidant properties, which are important in the prevention of the early stages of carcinogenesis, i.e. preventing DNA mutations. In addition to these well-studied processes, flavonoids are also good anti-proliferative agents. This aspect of flavonoid protection may be due in part to a large range of enzyme inhibition (e.g. kinases, pro-oxidant enzymes), and also apoptosis and cell cycle regulation. In our studies we have tested sixty-nine flavonoid structures for anti-proliferative activity and correlated the results with previously published data. New data has also emerged on the role of human metabolites found in plasma. With the exception of the 7-sulfate, all other conjugates tested were inactive against the human colon cancer cell line HT29. This finding suggests a luminal rather than systemic anti-proliferative effect of flavonoids in the colon. These results also put into perspective experimental designs in which flavonoid glycosides and aglycones are tested on cell lines derived from other tissues, because these cell lines are unlikely to be exposed to such forms of the flavonoid. A subset of seventeen flavonoids is currently being investigated to identify in more detail the mechanisms involved in cellular anti-proliferation.

Acknowledgements

We would like to acknowledge Professor Denis Barron and Jean Baptiste Daskiewicz (Laboratoire des Produits Naturels, Lyon, France) for the synthesis of most compounds (forty-eight) tested in the current study, mainly for the prenylated and sulphate compounds. We would like to thank as well Paul Needs and Susan DuPont (Institute of Food Research, Norwich, UK) for the synthesis of quercetin glucuronides. Our work is sponsored by a EU grant under the POLYBIND Project (QLK1-1999–00505).

References

Achiwa Y, Hibasami H, Katsuzaki H, Imai K & Komiya T (1997) Inhibitory effects of persimmon (*Diospyros kaki*) extract and related polyphenol compounds on growth of human lymphoid leukemia cells. *Bioscience Biotechnology Biochem* **61**, 1099–1101.

Ader P, Wessmann A & Wolffram S (2000) Bioavailability and metabolism of the flavonol quercetin in the pig. *Free Radical Biology and Medicine* **28**, 1056–1067.

Agullo G, Gamet L, Besson C, Demigne C & Remesy C (1994) Quercetin exerts a preferential cytotoxic effect on active dividing colon carcinoma HT29 and Caco-2 cells. *Cancer Letters* **87**, 55–63.

Agullo G, Gamet-Payrastre L, Fernandez Y, Anciaux N, Demigne C & Remesy C (1996) Comparative effects of flavonoids on the growth, viability and metabolism of a colonic adenocarcinoma cell line (HT29 cells). *Cancer Letters* **105**, 61–70.

Alcaraz MJ & Ferrandiz ML (1987) Modification of arachidonic metabolism by flavonoids. *Journal of Ethnopharmacology* **21**, 209–229.

Austin CA, Patel S, Ono K, Nakane H & Fisher LM (1992) Site-specific DNA cleavage by mammalian DNA topoisomerase II induced by novel flavone and catechin derivatives. *Biochemical Journal* **282**, 883–889.

Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, Aiello FB & Piantelli M (2000) Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. *International Journal of Cancer* **87**, 595–600.

Chen ZP, Schell JB, Ho CT & Chen KY (1998) Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. *Cancer Letters* **129**, 173–179.

Choi SU, Ryu SY, Yoon SK, Jung NP, Park SH, Kim KH, Choi EJ & Lee CO (1999) Effects of flavonoids on the growth and cell cycle of cancer cells. *Anticancer Research* **19**, 5229–5233.

Choudhury R, Srai SK, Debnam E & Rice-Evans CA (1999) Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration. *Free Radical Biology and Medicine* **27**, 278–286.

Conney AH, Lou YR, Xie JG, Osawa T, Newmark HL, Liu Y, Chang RL & Huang MT (1997) Some perspectives on dietary inhibition of carcinogenesis: studies with curcumin and tea. *Proceedings of the Society for Experimental Biology and Medicine* **216**, 234–245.

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

Cushman M & Nagarathnam D (1991) Cytotoxicities of some flavonoid analogues. *Journal of Natural Products* **54**, 1656–1660.

Day AJ, Canada FJ, Diaz JC, Kroon PA, McLauchlan R, Faulds CB, Plumb GW, Morgan MR & Williamson G (2000) Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Letters* **468**, 166–170.

Day AJ, DuPont MS, Ridley S, Rhodes M, Rhodes MJ, Morgan MR & Williamson G (1998) Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Letters* **436**, 71–75.

Deschner EE, Ruperto JF, Wong GY & Newmark HL (1993) The effect of dietary quercetin and rutin on AOM-induced acute colonic epithelial abnormalities in mice fed a high-fat diet. *Nutrition and Cancer* **20**, 199–204.

Galati G, Teng S, Moridani MY, Chan TS & O'Brien PJ (2000) Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. *Drug Metabolism and Drug Interactions* **17**, 311–349.

Gee JM, DuPont MS, Day AJ, Plumb GW, Williamson G & Johnson IT (2000) Intestinal transport of quercetin glycosides in rats involves both deglycosylation and interaction with the hexose transport pathway. *Journal of Nutrition* **130**, 2765–2771.

Gee JM, DuPont MS, Rhodes MJ & Johnson IT (1998) Quercetin glucosides interact with the intestinal glucose transport pathway. *Free Radical Biology and Medicine* **25**, 19–25.

Goldbohm RA, van't Veer P, van den Brandt PA, van't Hof MA, Brants HA, Sturmans F & Hermus RJ (1995) Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *European Journal of Clinical Nutrition* **49**, 420–429.

Harborne JB & Williams CA (2000) Advances in flavonoid research since 1992. *Phytochemistry* **55**, 481–504.

Hayashi A, Gillen AC & Lott JR (2000) Effects of daily oral administration of quercetin chalcone and modified citrus pectin. *Alternative Medicine Review* **5**, 546–552.

Haza AI, Glinghammar B, Grandien A & Rafter J (2000) Effect of colonic luminal components on induction of apoptosis in human colonic cell lines. *Nutrition and Cancer* **36**, 79–89.

Hertog MG & Hollman PC (1996) Potential health effects of the dietary flavonol quercetin. *European Journal of Clinical Nutrition* **50**, 63–71.

Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F *et al*. (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine* **155**, 381–386.

Hollman PC & Katan MB (1999) Dietary flavonoids: intake, health effects and bioavailability. *Food Chemistry and Toxicology* **37**, 937–942.

Kamei H, Kojimo T, Koide T, Hasegawa M, Umeda T, Teraba K & Hashimoto Y (1996) Influence of OH group and sugar bonded to flavonoids on flavonoid-mediated suppression of tumor growth in vitro. *Cancer Biotherapy and Radiopharmacology* **11**, 247–249.

Kang TB & Liang NC (1997) Studies on the inhibitory effects of quercetin on the growth of HL-60 leukemia cells. *Biochemical Pharmacology* **54**, 1013–1018.

Kawaii S, Tomono Y, Katase E, Ogawa K & Yano M (1999) Antiproliferative activity of flavonoids on several cancer cell lines. *Bioscience, Biotechnology and Biochemistry* **63**, 896–899.

Knekt P, Jarvinen R, Seppanen R, Hellovaara M, Teppo L, Pukkala E & Aromaa A (1997) Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *American Journal of Epidemiology* **146**, 223–230.

Kong AN, Yu R, Chen C, Mandlekar S & Primiano T (2000) Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Archives of Pharmacal Research* **23**, 1–16.

Kuhnau J (1976) The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Review of Nutrition and Dietetics* **24**, 117–191.

Kuntz S, Wenzel U & Daniel H (1999) Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. *European Journal of Nutrition* **38**, 133–142.

Kuo SM (1998) Transepithelial transport and accumulation of flavone in human intestinal Caco-2 cells. *Life Sciences* **63**, 2323–2331.

Kuo SM, Morehouse HF Jr & Lin CP (1997) Effect of antiproliferative flavonoids on ascorbic acid accumulation in human colon adenocarcinoma cells. *Cancer Letters* **116**, 131–137.

Mahmoud NN, Carothers AM, Grunberger D, Bilinski RT, Churchill MR, Martucci C, Newmark HL & Bertagnolli MM (2000) Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. *Carcinogenesis* **21**, 921–927.

Manach C, Morand C, Demigne C, Texier O, Regerat F & Remesy C (1997) Bioavailability of rutin and quercetin in rats. *FEBS Letters* **409**, 12–16.

Mutoh M, Takahashi M, Fukuda K, Komatsu H, Enya T, Matsushima-Hibiya Y, Mutoh H, Sugimura T & Wakabayashi K (2000*a*) Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure-activity relationship. *Japanese Journal of Cancer Research* **91**, 686–691.

Mutoh M, Takahashi M, Fukuda K, Matsushima-Hibiya Y, Mutoh H, Sugimura T & Wakabayashi K (2000*b*) Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. *Carcinogenesis* **21**, 959–963.

Pforte H, Hempel J & Jacobasch G (1999) Distribution pattern of a flavonoid extract in the gastrointestinal lumen and wall of rats. *Nahrung* **43**, 205–208.

Ranelletti FO, Maggiano N, Serra FG, Ricci R, Larocca LM, Lanza P, Scambia G, Fattorossi A, Capelli A & Piantelli M (2000) Quercetin inhibits p21-RAS expression in human colon cancer cell lines and in primary colorectal tumors. *International Journal of Cancer* **85**, 438–445.

Rice-Evans CA, Miller NJ & Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20**, 933–956.

Richter M, Ebermann R & Marian B (1999) Quercetin-induced apoptosis in colorectal tumor cells: possible role of EGF receptor signaling. *Nutrition and Cancer* **34**, 88–99.

Russo M, Palumbo R, Tedesco I, Mazzarella G, Russo P, Iacomino G & Russo GL (1999) Quercetin and anti-CD95(Fas/Apo1) enhance apoptosis in HPB-ALL cell line. *FEBS Letters* **462**, 322–328.

Scalbert A & Williamson G (2000) Dietary intake and bioavailability of polyphenols. *Journal of Nutrition* **130**, 2073S–2085S.

Shimizu K, Kondo R & Sakai K (2000) Inhibition of tyrosinase by flavonoids, stilbenes and related 4-substituted resorcinols: structure-activity investigations. *Planta Medica* **66**, 11–15.

Skibola CF & Smith MT (2000) Potential health impacts of excessive flavonoid intake. *Free Radical Biology and Medicine* **29**, 375–383.

Spencer JP, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK & Rice-Evans C (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Letters* **458**, 224–230.

Thompson MA, Rosenthal MA, Ellis SL, Friend AJ, Zorbas MI, Whitehead RH & Ramsay RG (1998) c-Myb down-regulation is associated with human colon cell differentiation, apoptosis, and decreased Bcl-2 expression. *Cancer Research* **58**, 5168–5175.

Umarova FT, Khushbactova ZA, Batirov EH & Mekler VM (1998) Inhibition of Na^+, K(+)-ATPase by flavonoids and their inotropic effect. Investigation of the structure-activity relationship. *Membrane and Cell Biology* **12**, 27–40.

Walgren RA, Lin JT, Kinne RK & Walle T (2000) Cellular uptake of dietary flavonoid quercetin 4′-beta-glucoside by sodium-dependent glucose transporter SGLT1. *Journal of Pharmacology and Experimental Therapeutics* **294**, 837–843.

Walle T, Otake Y, Walle UK & Wilson FA (2000) Quercetin glucosides are completely hydrolyzed in ileostomy patients before absorption. *Journal of Nutrition* **130**, 2658–2661.

Walle T, Otake Y, Brubaker JA, Walle UK & Halushka PV (2001) Disposition and metabolism of the flavonoid chrysin in normal volunteers. *British Journal of Clinical Pharmacology* **51**, 143–146.

Walle UK, French KL, Walgren RA & Walle T (1999) Transport of genistein-7-glucoside by human intestinal CACO-2 cells: potential role for MRP2. *Research Communications in Molecular Pathology and Pharmacology* **103**, 45–56.

Wang HK (2000) The therapeutic potential of flavonoids. *Expert Opinion on Investigational Drugs* **9**, 2103–2119.

Wattenberg LW (1985) Chemoprevention of cancer. *Cancer Research* **45**, 1–8.

Wheeler EL & Berry DL (1986) In vitro inhibition of mouse epidermal cell lipoxygenase by flavonoids: structure-activity relationships. *Carcinogenesis* **7**, 33–36.

Williamson G, Day AJ, Plumb GW & Couteau D (2000) Human metabolic pathways of dietary flavonoids and cinnamates. *Biochemical Society Transactions* **28**, 16–22.

Yoshida M, Sakai T, Hosokawa N, Marui N, Matsumoto K, Fujioka A, Nishino H & Aoike A (1990) The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. *FEBS Letters* **260**, 10–13.