Nutrient Interactions and Toxicity

Low Concentrations of Flavonoids Are Protective in Rat H4IIE Cells Whereas High Concentrations Cause DNA Damage and Apoptosis1,2

Wim Wätjen,3 Gudrun Michels,4 Bärbel Steffan,*4 Petra Niering,4 Yvonni Chovolou, Andreas Kampkötter, Quynh-Hoa Tran-Thi, Peter Proksch,* and Regine Kahl

Institute of Toxicology, Heinrich-Heine-University, 40001 Düsseldorf, Germany; and *Institute of Pharmaceutical Biology, Heinrich-Heine-University, 40225 Düsseldorf, Germany

ABSTRACT Dietary flavonoids possess a wide spectrum of biochemical and pharmacological actions and are assumed to protect human health. These actions, however, can be antagonistic, and some health claims are mutually exclusive. The antiapoptotic actions of flavonoids may protect against neurodegenerative diseases, whereas their proapoptotic actions could be used for cancer chemotherapy. This study was undertaken to determine whether a cytoprotective dose range of flavonoids could be differentiated from a cytotoxic dose range. Seven structurally related flavonoids were tested for their ability to protect H4IIE rat hepatoma cells against H2O2-induced damage on the one hand and to induce cellular damage on their own on the other hand. All flavonoids proved to be good antioxidants in a cell-free assay. However, their pharmacologic activity did not correlate with in vitro antioxidant potential but rather with cellular uptake. For quercetin and fisetin, which were readily taken up into the cells, protective effects against H2O2-induced cytotoxicity, DNA strand breaks, and apoptosis were detected at concentrations as low as 10–25 μmol/L. On the other hand, these flavonoids induced cytotoxicity, DNA strand breaks, oligonucleosomal DNA fragmentation, and caspase activation at concentrations between 50 and 250 μmol/L. Published data on quercetin pharmacokinetics in humans suggest that a dietary supplement of 1–2 g of quercetin may result in plasma concentrations between 10 and 50 μmol/L. Our data suggest that cytoprotective concentrations of some flavonoids are lower by a factor of 5–10 than their DNA-damaging and proapoptotic concentrations. J. Nutr. 135: 525–531, 2005.

KEY WORDS: • apoptosis • comet assay • fisetin • quercetin • uptake

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin; >6000 flavonoids, low-molecular-weight phenylbenzopyrones, have been identified in plant sources. This class of compounds has become increasingly popular in terms of health protection because they possess a remarkable spectrum of biochemical and pharmacologic activities (1). Flavonoids affect basic cell functions such as growth, differentiation, and apoptosis. Epidemiologic studies have suggested that flavonoids may protect against various stages of the cancer process and are associated with a reduced incidence of coronary heart disease (2,3). Flavonoids were shown to be potent antioxidants because of their radical-scavenging activity. It was also shown that flavonoids are able to complex heavy metal ions, e.g., iron and copper, which are involved in Fenton-like reactions (4). The biological actions of flavonoids have long been thought to be due to their antioxidant potential but at present, it is by no means clear whether other mechanisms of action contribute to their overall effect or are even more important than their radical-scavenging properties.

Although some flavonoids act as powerful antioxidants, it was also shown that in high concentrations, they can generate reactive oxygen species by autooxidation and redox-cycling (5–9). Consequently, many reports described adverse actions of flavonoids on a cellular level. For a number of the flavonoids used in this study, cytoprotective as well as cytotoxic and proapoptotic effects were shown in various cell culture models. Thus, protection against apoptotic stimuli was found with quercetin (10), epicatechin (11), and rutin (12) but on the other hand, induction of apoptosis was described for quercetin (13,14), fisetin (15), morin (16), myricetin (17), and rutin (16). In the comet assay, a protection against DNA strand breaks was demonstrated for quercetin (18,19), myricetin (19,20), and rutin (20,21); however, strand breaks were also induced by quercetin (18,22) and myricetin (22) in that assay. Given this wide spectrum of biological actions, it is quite understandable that numerous health claims that are in part mutually exclusive have been linked with flavonoids. Notably,
in cancer, but also in infections or autoimmune disease, a
deficiency in apoptosis is one of the key events. On the other
hand, overefficient apoptosis, as observed in fulminant liver
failure, or the long-term accumulation of apoptotic events in
neurodegenerative disorders may be equally harmful to the
organism.

Given that both beneficial and adverse effects can in prin-
ciple be caused by flavonoids, it must be assumed that in
addition to the cell type or tissue involved and to the presence
or absence of a stressor, dose determines which action prevails.
Dose dependency, however, has not been studied systemati-
cally. The importance of the chemical structure of the fla-
vonoids in relation to their biological activity is also far from
obvious. Dietary flavonoids are predominantly present in a
glycosidic form, e.g., in fruits (23), although free flavonoids
also occur, e.g., myricetin in red wine. The basic structure of
flavonoids consists of an O-heterocyclic ring (A) fused to an
aromatic ring (A) with a third ring system (B) attached at C2
of the heterocyclic ring. Out of the great variety of structures,
we selected 7 representative flavonoids to investigate their
pro- and antioxidative effects and their pro- and antiapoptotic
properties (Fig. 1). Quercetin, a major flavonoid in the diet,
can be regarded as the lead structure. The structures of the
other flavonoids differ from quercetin in the number and
position of hydroxy substituents (fisetin, morin, myricetin),
the C2-C3 double bond (taxifolin) or the existence of a
glycoside moiety (rutin). In the case of (±)-catechin, the only
flavanol analyzed, no 4-keto oxygen or C2-C3 double bond is
present.

Because flavonoids are not marketed as drugs, they have
seldom been subjected to the stringent pharmacologic and
toxicological testing protocols of drug authorization. Instead,
flavonoids are marketed as components of functional food and
as flavonoid-containing food supplements, thus avoiding tox-
icological testing. The aim of this study was to collect data on
the dose-response relation of protective and adverse effects of
flavonoids in a cell culture model to test the hypothesis that
adverse effects occur at higher dose levels than protective
effects. This effort was undertaken to contribute to the ur-
gently needed risk assessment for the use of high-dose fla-
vonoid food supplements.

MATERIALS AND METHODS

Chemicals. Quercetin, fisetin, rutin, catechin, and taxifolin were
purchased from Sigma, morin was obtained from Merck, and myric-
etin was purchased from Calbiochem; caspase substrates were obtained
from ICN Biomedicals.

Cell culture. The metabolically active and differentiated rat
H4IE hepatoma cell line was used as a model system for further
experiments in vivo on the influence of flavonoids on enzyme acti-
vation in rat liver. Cells were grown in DMEM (4.5 g/L glucose, 2
mmol/L L-glutamine, 100 kU/L penicillin and 100 mg/L streptom-
cin, and 10% fetal calf serum) in a humidified atmosphere at 37°C
with 5% CO₂.

Cellular uptake of flavonoids. We used HPLC (Eurosphere C-18,
photodiode array detector) to investigate intracellular concentra-
tions of flavonoids as described earlier (24). HPLC-MS-MS was performed
to analyze the formation of flavonoid metabolites. The intracellular
distribution of quercetin was analyzed by fluorescence microscopy
(excitation: 450–490 nm, emission: 515 nm). Nuclei were stained
with Hoechst 33342 (100 μmol/L) and 10% fetal calf serum in a humidified atmosphere at 37°C
with 5% CO₂.

Apoptotic DNA-fragmentation. DNA was isolated using phenol:
chloroform extraction and oligonucleosomal fragmentation of DNA
was analyzed electrophoretically as described earlier (24).

Caspase activity. Colorimetrically labeled substrates for
VDVADase activity (caspase-2), DEVDase activity (caspase-3/7),
and LEHDase activity (caspase-9) were used according to the
manufacturer’s protocol (Calbiochem).

DNA strand breaks ("comet assay"). Cells (3 × 10⁶) were
seeded out in a 6-well dish and incubated 24 h later with either
flavonoid alone for 3 h or with 500 μmol/L H₂O₂ (2 h) in
the presence or absence of a preincubation (1 h) with flavonoids.
For determination of DNA strand breaks, the comet assay (28) was used.

Microscopic analysis of nuclear fragmentation. To investigate
nuclear fragmentation as a further feature of apoptotic cell death,
fluorescent staining with Hoechst 33342 (100 μmol/L) was used as
described earlier (24).

TEAC assay. Trolox equivalent antioxidative capacity (TEAC)
was determined as described earlier (29), and absorption was mea-
sured after 4 min of mixing the substances with the ABTS solution.

Protein. Protein concentration was determined spectrophotome-
trically according to Bradford (30) using bovine serum albumin as
the standard.

Statistics. Data are given as means ± SEM of at least 3 inde-
pendent experiments. The significance of differences was assessed

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5 Abbreviations used: EC₅₀, 50% effective concentration; LSD, least signifi-
cant difference; MDA, malondialdehyde; TEAC, Trolox equivalent antioxidative
capacity.
using 1-way ANOVA followed by the LSD test (Analyze-it). Differences were considered significant at \( P < 0.05 \).

**RESULTS**

**Antioxidative effects of flavonoids in a cell-free system.** Myricetin (3 hydroxyl groups in the B-ring) showed the strongest antioxidative effect in the TEAC assay. Quercetin, fisetin, morin, and catechin (2 hydroxyl groups in the B-ring) also exhibited good antioxidative properties. The capacities of taxifolin and rutin to decolorize the ABTS radical were the lowest of the flavonoids analyzed but were in the same range as that of the synthetic antioxidant Trolox itself which was used as the reference compound (Table 1).

**Effects of flavonoids on \( \text{H}_2\text{O}_2 \)-induced DNA strand breakage in H4IIE cells.** Hydrogen peroxide produced a dose- and time-dependent increase in DNA strand breakage as measured by alkaline single-cell electrophoresis. The increase reached a plateau at concentrations >500 \( \mu \text{mol/L} \), a concentration at which the DNA of almost every cell was fragmented (data not shown). Incubation with 500 \( \mu \text{mol/L} \text{H}_2\text{O}_2 \) for 2 h increased the image length from 14.01 ± 0.26 \( \mu \text{m} \) (control, no “comet-tail”) to 56.07 ± 4.7 \( \mu \text{m} \). The order of protective potency against \( \text{H}_2\text{O}_2 \)-induced DNA strand break formation of the 7 flavonoids tested differed markedly from the order of their antioxidative effect in the TEAC assay. In preincubation experiments, the flavonoids quercetin and fisetin protected against \( \text{H}_2\text{O}_2 \)-mediated DNA strand breaks in a dose-dependent manner. Preincubation with 10 \( \mu \text{mol/L} \) quercetin or 25 \( \mu \text{mol/L} \) fisetin decreased the \( \text{H}_2\text{O}_2 \)-induced strand breaks by ~50% (Fig. 2A). Preincubation with other flavonoids at a concentration of 50 \( \mu \text{mol/L} \) caused only minor (taxifolin, myricetin) or no reduction (morin, rutin, catechin) in \( \text{H}_2\text{O}_2 \)-mediated DNA strand breakage (Table 1).

**Effects of quercetin and fisetin on \( \text{H}_2\text{O}_2 \)-mediated apoptosis and cytotoxicity.** \( \text{H}_2\text{O}_2 \) caused apoptosis in H4IIE cells. Incubation with 1000 \( \mu \text{mol/L} \) \( \text{H}_2\text{O}_2 \) increased caspase-3 activity (1497 ± 235% of control value) after an incubation time of 15 h; after this peak, activity decreased again likely due to secondary necrotic events (data not shown). Quercetin and fisetin at a concentration of 25 \( \mu \text{mol/L} \) reduced the increase in caspase-3 activation caused by 1000 \( \mu \text{mol/L} \text{H}_2\text{O}_2 \) by ~50% (Fig. 2B). Quercetin and fisetin at 10 \( \mu \text{mol/L} \) provided ~50% protection against the loss of cell viability induced by 150–250

### TABLE 1

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>TEAC ( \mu \text{mol/L} )</th>
<th>% of control</th>
<th>( \text{H}_2\text{O}_2 )-induced caspase-3 activity</th>
<th>( \text{H}_2\text{O}_2 )-induced DNA strand breaks (nmol/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0.90 ± 0.036^a</td>
<td>85 ± 4^c</td>
<td>100 ± 33.72^a</td>
<td>56.07 ± 4.7^a</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.26 ± 0.055^c</td>
<td>161 ± 10^b</td>
<td>41.03 ± 12.8^b</td>
<td>13.6 ± 0.4^d</td>
</tr>
<tr>
<td>Fisetin</td>
<td>0.27 ± 0.054^c</td>
<td>258 ± 31^a</td>
<td>33.90 ± 21.07^b</td>
<td>22.51 ± 0.4^c</td>
</tr>
<tr>
<td>Myricetin</td>
<td>0.16 ± 0.038^c</td>
<td>ND</td>
<td>119.76 ± 40.01^a</td>
<td>47.8 ± 6.8^b</td>
</tr>
<tr>
<td>Morin</td>
<td>0.39 ± 0.046^c</td>
<td>84 ± 7^c</td>
<td>89.79 ± 24.06^a</td>
<td>54.73 ± 6.5^a</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>0.52 ± 0.066^b</td>
<td>88 ± 11^c</td>
<td>100.83 ± 32.78^a</td>
<td>47.67 ± 4.9^b</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.54 ± 0.063^b</td>
<td>82 ± 7^c</td>
<td>78.68 ± 17.59^a</td>
<td>56.67 ± 7.7^a</td>
</tr>
<tr>
<td>Catechin</td>
<td>0.29 ± 0.048^c</td>
<td>73 ± 7^c</td>
<td>110.99 ± 17.59^a</td>
<td>59.19 ± 4.4^a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, \( n > 3 \). Means in a column without a common letter differ, \( P < 0.05 \).
2 \( \mu \text{mol/L} \) Trolox: absorption (734 nm) = 0.545 ± 0.034.
3 DMSO, dimethyl sulfoxide; ND, not determined.
μmol/L H₂O₂. Other flavonoids showed no protective effect against H₂O₂-mediated caspase activation (Table 1).

**Uptake of flavonoids.** To investigate whether the difference of in vitro and in vivo potency of the flavonoids was due to differences in cellular uptake, we analyzed their intracellular concentration by HPLC at different incubation times (0.5–24 h). Quercetin, fisetin, morin, taxifolin, and myricetin were detected in the cells after 1 h of incubation although to a very different extent (quercetin > fisetin > morin > taxifolin > myricetin, Table 1) which might explain why only quercetin and fisetin are protective in the cells. Rutin and catechin (up to 500 μmol/L) were not taken up by H4IIE cells even after an incubation of 24 h. Incubation with 50 μmol/L quercetin led to an intracellular concentration of 0.82 ± 0.14 nmol/10⁶ cells. Quercetin uptake was not saturated at high concentrations [3.5, 9.7, 33.4 ± 10.7, and 109 ± 21 nmol quercetin/10⁶ cells after incubation (1 h) with 250, 500, and 750 μmol/L quercetin, respectively].

The intracellular concentration of quercetin decreased exponentially with time (15.9 ± 4.7, 9.7 ± 3.5, and 1.7 ± 0.6 mmol quercetin/10⁶ cells after 2, 4, and 8 h, respectively) due to its metabolism; 3 peaks at 13.53, 13.78, and 15.61 min were identified as glucuronidated metabolites of quercetin due to spectral characteristics and MS fragmentation spectra. The m/z of [M-H]-ions was 477, which were fragmented to [M+H]-ions with an m/z of 301. We further analyzed the intracellular distribution of quercetin in the cells by fluorescence microscopy. After 1 h of incubation with 50 μmol/L quercetin, a bright nuclear fluorescence was observed, suggesting that the flavonoid accumulates in the nucleus (Fig. 3).

**Cytotoxicity of flavonoids.** The intrinsic cytotoxicity of the 7 flavonoids differed greatly. Although quercetin [50% effective concentration (EC₅₀) = 35 ± 4 μmol/L] and fisetin (EC₅₀ = 48 ± 3 μmol/L) were relatively toxic, taxifolin, rutin, and catechin (up to 500 μmol/L) did not reduce cell viability (no EC₅₀ determined). The order of cytotoxic potential in H4IIE cells was quercetin > fisetin > myricetin > morin > taxifolin = catechin = rutin using the MTT assay and thus roughly resembled the order of cellular uptake. With the neutral red assay, quercetin and morin exhibited a lower

toxicity than in the MTT assay, whereas the toxicity of the other flavonoids remained almost the same (Table 2), suggesting that mitochondria are a more sensitive target of these 2 flavonoids. After 3 h of incubation, the time at which the comet assay was performed, no EC50 could be determined for all flavonoids tested up to concentrations of 500 μmol/L (data not shown).

Contribution of oxidative stress to flavonoid-induced cytotoxicity. Preincubation of H4IIE cells with antioxidants (50 μmol/L α-tocopherol, 500 μmol/L ascorbic acid, 500 μmol/L N-acetylcysteine, 1000 μmol/L glutathione) or the metal chelator desferoxamine (25 μmol/L) did not protect against quercetin-induced cytotoxicity. In the case of ascorbic acid, a further increase in flavonoid-mediated cytotoxicity occurred (data not shown). Incubation of H4IIE cells with high concentrations of flavonoids (up to 500 μmol/L) for 24 h did not increase the formation of MDA, a marker of oxidative stress (Table 2).

Induction of DNA strand breaks by flavonoids. Incubation of H4IIE cells with quercetin increased comet formation in a time- and concentration-dependent manner. A dose-response curve was found with saturation at 250 μmol/L (500 μmol/L quercetin: image length, 36.0 ± 5.7 μm). Fisetin also induced DNA breakage (Fig. 4). There was a slight increase in DNA strand breaks after incubation with morin but no increase in DNA “comet” formation after incubation with taxifolin, rutin, catechin, or myricetin (Table 2).

Induction of apoptosis by flavonoids. The ability of flavonoids to induce apoptotic cell death was investigated first by analyzing oligonucleosomal-sized DNA fragmentation (DNA ladder formation). Although myricetin and taxifolin induced apoptosis only at high concentrations (500, 1000 μmol/L), quercetin induced DNA ladder formation between 100 and 500 μmol/L and fisetin was the most potent apoptosis-inducing flavonoid with effective concentrations as low as 50 μmol/L (Fig. 5A). Induction of apoptosis by quercetin and fisetin was confirmed by measurement of caspase activation. Incubation with 250 μmol/L quercetin and fisetin for 24 h increased caspase-2 activity, caspase-3 activity, and caspase-9 activity by 100–500% (Fig. 5B). Apoptotic cell death was further confirmed for quercetin by the demonstration of cellular blebbing and nuclear fragmentation (Fig. 5C). Quercetin (500 μmol/L) increased condensed and fragmented nuclei with an apoptotic index of 32.4 ± 1.47 (control: 1.8 ± 0.56).

FIGURE 4 Generation of DNA strand breaks by quercetin and fisetin in H4IIE rat hepatoma cells incubated with quercetin or fisetin (3 h). Values are means ± SEM, n > 3. *Different from control, P < 0.05.

FIGURE 5 Induction of apoptosis by quercetin and fisetin in H4IIE rat hepatoma cells. (A) Effects of quercetin and fisetin on oligonucleosomal fragmentation (24 h). (B) Effects of quercetin, fisetin (250 μmol/L, 24 h) and dimethyl sulfoxide (DMSO) on activation of caspases 2, 3 and 9 (VDVADase, DEVDase, LEHDase activity). Values are means ± SEM, n > 3. *Different from DMSO control, P < 0.05. (C) Induction of nuclear fragmentation (Hoechst staining) by quercetin (250 μmol/L, 24 h): (1: cell morphology, control), 2: cell morphology, quercetin, 3: Hoechst staining, control, 4: Hoechst staining, quercetin).

DISCUSSION

It was the major aim of our study to determine the margin of exposure for cytoprotective and cytotoxic actions of flavonoids in a cell culture system. We achieved this with 2 of the compounds tested, quercetin and fisetin, which were readily taken up by the cells. Protection against H2O2-induced cytotoxicity, DNA strand breaks, and caspase-3 activation were detected at 10–25 μmol/L of quercetin and fisetin. On the other hand, these compounds induced cytotoxicity, DNA strand breaks, oligonucleosomal DNA fragmentation, and
All flavonoids tested proved to be good antioxidants in a cell-free assay; their potency equaled that of the standard antioxidant, Trolox. However, the pharmacologic activity of the flavonoids tested did not correlate with their antioxidant potential measured in this in vitro assay. The data suggest that this might be due to differences in cellular uptake. There was a convincing relation between cellular uptake and both cytoprotective and proapoptotic actions indicating that these events require the presence of the compounds in the cell. Intrinsic cytotoxicity of the flavonoids, however, does not fit into this scheme. Morin and myricetin in particular had only low intracellular concentrations, but their cytotoxicity was comparable to that of quercetin and fisetin. Various explanations for the cytotoxicity of flavonoids have been proposed, including the inhibition of enzymes such as protein kinase C, cGMP-dependent protein kinase, adenylyl cyclase, DNA topoisomerases, or glutathione S-transferase (1,23), leading to disturbances in cell cycle, for example, followed by apoptotic or necrotic events. There are several ways in which a flavonoid could exert pharmacologic activity in the absence of cellular uptake, e.g., extracellular H₂O₂ formation may occur (31,32). Alternatively, the attack may take place at a membrane structure, e.g., a growth hormone receptor. For the green tea polyphenol, epigallocatechin gallate, the existence of a cell surface receptor was suggested (33). Myricetin and morin caused growth inhibition in HT29 cells at concentrations very similar to those that led to cytotoxicity in our study (47 or 117 μmol/L, respectively) and myricetin activated caspase-3 in these cells but not in MCF-7 cells and LLC-PK1 cells (17). In our study the apoptosis-inducing potential of myricetin in H4IIE cells was negligible.

Controversy exists concerning whether the cytotoxic and DNA-damaging effects of flavonoids involve the formation of reactive oxygen species. In our study, we did not find evidence for increased lipid peroxidation even at high concentrations of flavonoids. Also, no protection against quercetin- and fisetin-induced cytotoxicity was achieved by bathocuproine, a copper-specific chelator. We investigated the effects of desferoxamine on quercetin-induced cytotoxicity but again found no protective effects.

Only limited conclusions can be derived from our results with respect to the structure–activity relation of the compounds tested. We demonstrated that the antioxidative potential in vitro of the flavonoids increased with the number of their phenolic hydroxyl groups because myricetin, which has the highest number of hydroxyl groups, exhibited the strongest antioxidant action in the TEAC assay. In view of the correlation between intracellular concentration and the range of pharmacologic activity that includes protective, DNA-damaging, and apoptotic effects, structural hindrance is likely to occur at the level of cellular uptake. It was shown previously that the rutinoside moiety of flavonoids inhibits membrane crossing (35); therefore the inactivity of rutin was not unexpected. It cannot be excluded that in addition to uptake, the intracellular actions of the flavonoids are also influenced by structural differences. Current results do not allow us to distinguish these 2 levels of effect. In any case, it appears that the double bond in the ring C causing the planarity of the ring is supportive but not sufficient for uptake and/or biological activity.

Quercetin is one of the most abundant flavonoids in the diet; it is also considered to be one of the most promising members of this class for both general chemoprevention and cancer chemotherapy. For this reason, the pharmacokinetics of quercetin were studied repeatedly in rats as well as in humans. Manach et al. (36) reported that rats fed quercetin (single meal, 0.2%) exhibited constant plasma concentrations of quercetin metabolites of ~50 μmol/L for at least 16 h. Rats adapted to quercetin (0.2%) maintained plasma concentrations of ~100 μmol/L (quercetin + metabolites). The authors suggested that the elimination of quercetin metabolites is low and that high plasma concentrations are easily maintained with a regular supply of quercetin in the diet. More data on the pharmacokinetics of quercetin in humans are also available. Both the aglycone and the glucosides can enter the body via the small intestine, whereas other conjugates can be taken up only after hydrolysis by colonic bacteria (37,38). One study with 4 doses (250 mg/day of quercetin administered as capsules resulted in plasma concentrations of 1.5 μmol/L (39); however, later studies using quercetin glucosides yielded relatively higher plasma concentrations, i.e., 7 μmol/L after quercetin-4’-O-glucoside equivalent to 100 mg quercetin (40), 4.5 μmol/L after 150 mg quercetin 4’-glucoside, and 5 μmol/L after 150 mg quercetin 3-glucoside (41). When administered in onions (a plant source that contains quercetin as glucosides) the following plasma concentrations of total quercetin as related to dose were reported: 0.05 μmol/L after 15 mg (42), 0.63 μmol/L after 68–94 mg (43), 7.65 μmol/L after 100 mg (40), and 4 μmol/L after 300 mg (44). Half-lives between 10 and 30 h were reported (40,41,45), suggesting that continuous daily intake will result in a steady-state concentration. All kinetic variables measured in humans were measured in plasma, and no information on flavonoid concentrations in different tissues is available. In some studies, no free quercetin was found in blood samples [e.g., (43)], whereas in others, free quercetin was detected (46). Isorhamnetin is the only phase I metabolite described (40). It is unclear at present whether the metabolites still possess pharmacologic activity. Data suggest that flavonoids can be deglucuronidated at the site of action, e.g., in endothelial cells (47,48). In contrast to quercetin, data on fisetin plasma concentrations in humans are lacking, probably because it is minimally present in the diet. Fisetin, like quercetin, is methylated in human liver (49). Taken together, the data on quercetin pharmacokinetics in humans suggest that a dietary supplement of 1–2 g of quercetin, an amount proposed by supplement makers, may result in plasma concentrations exceeding 10 μmol/L but probably not exceeding 50 μmol/L if taken properly.

In summary, we found that quercetin and fisetin were readily taken up into H4IIE cells and protected against H₂O₂-induced cytotoxicity, DNA strand breaks, and apoptosis at concentrations of 10–25 μmol/L; however, these compounds themselves induced cytotoxicity, DNA strand breaks, oligonucleosomal DNA
fragmentation and caspase activation at concentrations between 50 and 250 μmol/L. The other flavonoids tested were good antioxidants in a cell-free assay; their pharmacologic activity did not correlate with in vitro antioxidant potential but rather with cellular uptake. Our data suggest that cytotoxic and genotoxic effects of some flavonoids are lower by a factor of 5–10 than their DNA-damaging and proapoptotic concentrations. These results have implications also for humans in terms of risk assessment and in the modulation of isolated food constituents; they should be carefully studied because flavonoids are used increasingly in dietary supplements.

ACKNOWLEDGMENT

We thank Sandra Ohler for excellent technical assistance.

LITERATURE CITED